

Abstract

Wallace, Jeff Thomas. An Enzymatic Fiber Modification Method for Enhancing Tissue Properties. (Under the direction of Dr. Joel J. Pawlak).

Retail consumers of tissue products desire the highest softness at the lowest cost. The softness of a product can be broken down into two components, the bulk (i.e. structural) softness and the surface softness. Traditionally, papermakers have used a variety of tools, such as refining, beating, chemical addition, etc., to enhance the softness of paper. The use of enzymatic treatment to enhance the softness of paper is less common, but offers a unique way to modify the chemical composition and ultra-structure of the papermaking fibers.

Procter and Gamble has developed a patented process for enzymatically modifying and degrading cellulose fibers to produce softer tissue grade pulps. The principle of this technology is that by selectively degrading a cellulose fiber, the bulk softness of the resulting fiber structure can be increased. The main goal of this research is to investigate the fundamental interactions of enzymes with cellulosic substrates in order to better understand the fiber modification process and the potential for new properties and products created by this technology.

A duplication of the Procter and Gamble patented technology was carried out in the research labs using different enzyme concentrations and incubation times. From these duplication experiments, it was found that the patent claims could be reproduced in our laboratories. Furthermore, the enzyme dosage and incubation time could be optimized.

An investigation into localized fiber degradation produced by the enzymatic treatment of the fibers was carried out. This investigation was broken into three parts and the first part was to characterize enzyme absorption on softwood fibers by investigating the location of enzyme activity on the fiber using microscopic and image analysis techniques. The results along with the patent duplication experiments relates back to how enzymes affect the fibers to create weaker and more compressible fibers as seen in the microscopic and the zero-span tensile strength results. The results proved that notch/dislocation areas of the fiber are more accessible areas for enzymes to degrade. The results also showed that as incubation time increased, the fibers began to break up and fragment at dislocation areas for enzyme concentrations of 0.5% and higher.

The second part of the localized fiber modification study was to study enzyme attachment to softwood fibers. This was tested functionally by attempting to first absorb the enzymes onto fibers and then incubate the fibers with untreated fibers. The sheet properties of jointly incubated fibers and separately incubated fibers were compared. The results indicated that enzymes absorb and desorb from the fiber, reattaching and redistributing to other fibers.

The third part of this investigation was to study the effect of enzyme treatment on wet fiber flexibility for refined softwood fibers. The effect on wet fiber flexibility relates back to how enzymes affect the compressibility, bending stiffness and modulus of the sheet as a whole, which is important for improving bulk softness. From the results, it can be concluded that enzymatic degradation affects fiber flexibility as a function of incubation time up to 4.5 hours at 0.5% enzyme concentration. Beyond this time flexibility begins to decrease due to fiber length reduction.

Also, two other studies are included in the appendices sections of this thesis (Appendices F & G). The first report is a softwood fiber defects quantification and assessment study. This study attempted to create a method to quantify different types of defects (kinks, dislocations, curls, etc.). The second report is a study on pre-enzyme treatment of fibers to introduce fiber defects with Hobart kneading. It was found that Hobart kneading does not create new defects, but does enhance existing defects and also introduces curl and kink into the fiber.

An Enzymatic Fiber Modification Method for Enhancing Tissue Properties

by

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Dedication

I am dedicating this thesis to the loving memory of my Nanny Wallace and Papa Farmer.

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Preface

Retail consumers of tissue products desire the highest softness at the lowest cost. The softness of a product can be broken down into two components, the bulk (i.e. structural) softness and the surface softness. The surface softness is controlled by factors such as coefficient of friction, surface roughness, and surface compressibility [Ramasubramanian, 2001]. The bulk softness, which is controlled primarily by the structure of the sheet, is related to the bending stiffness, compressibility and modulus of the structure as a whole.

Traditionally papermakers have used a variety of tools, such as refining, beating, chemical addition, etc., to enhance the softness of paper. The use of enzymatic treatment to enhance the softness of paper is less common, but offers a unique way to modify the chemical composition and ultra-structure of the papermaking fibers. Enzyme technology is a versatile tool for modifying the fibers, since it has a tendency to degrade specific components [Buchert, J., et al., 1996].

The technology underlying this project is that by selectively degrading a cellulose fiber, the bulk softness of the resulting fiber structure can be increased. Selective enzymatic treatments affect the chemical and physical properties of the fiber ultra-structure such as the ratio of crystallinity, pore size distribution, elastic modulus, and fiber strength. The selective enzymatic degradation of the fibers results in fibers that have significantly reduced strength as a result of localized defect creation or enhancement without significant reduction in the coarseness of the fibers or the bulk of the fiber network. Low-density tissue products have fiber strength values much higher than the network strength, and thus controlled reduction of fiber strength does not lower the network strength.

Procter and Gamble has patented a technology for enzymatically modifying and degrading cellulose fibers to produce a softer tissue sheet. The main goal of this research

was to investigate the fundamental interactions of enzymes with cellulosic substrates in order to better understand the fiber modification process. In order to achieve this goal, three major tasks were completed. The first goal was to conduct a thorough literature review and patent search to provide a baseline of the state of the art in the areas of fiber modification with enzymes. This literature review provided extensive knowledge and understanding of enzyme terminology and chemistry and different methods of measuring softness.

The second task was to duplicate the Procter and Gamble patented technology in our research labs using different enzyme concentrations and incubation times. This provided a firm foundation for the development of laboratory procedures for process evaluation. From these experiments, it was found that it was possible to reproduce the patent claims. Also, the appropriate concentration and incubation time for repeating these claims was determined. Zero-span tensile strength and normal tensile strength test were used in these duplication experiments. The change in zero-span tensile strength results from enzyme treatment relates to the change in fiber strength and hence it's effect on compressibility of the sheet as a whole.

The third task was to investigate the localized fiber modifications produced by the enzymatic treatment of the fibers. This task was broken into three parts and the first part was to characterize the enzyme absorption on softwood fibers by investigating the isolation of enzyme activity to fiber defect (notch/kink) areas using polarized optical microscopy and image analysis techniques. The results from this experiment along with the patent duplication experiments relates back to how enzymes affect the fibers to create weaker and more compressible fibers as shown by the microscopic and the zero-span tensile strength results. As mentioned earlier, fiber network compressibility is a major component of bulk softness. The second part of this task was to study the permanence of enzyme attachment to softwood fibers. This experiment was to answer the question of whether the enzyme attachment to the fibers persists throughout the degradation process or whether desorption and redepositon takes place. It was found that enzymes do desorb from fiber and will redeposit on other fibers. The third part of the task was to investigate

the effect of enzyme treatment on wet fiber flexibility for refined softwood fibers. The effect on wet fiber flexibility relates back to how enzymes affect the bending stiffness and modulus of the sheet as a whole, which influences bulk softness.

Chapter 1

Literature Review

1.1 Enzyme Basics and Their Influence

As mentioned earlier in the introduction, papermakers use a variety of tools to enhance the papermaking characteristics of fibers. These tools include: different methods of pulping, refining, beating, high consistency kneading, chemical addition, etc. The use of enzymatic treatment to enhance the papermaking characteristics of fibers is less common, but offers a unique way of modifying the chemical composition and ultra-structure of the fiber. To fully understand how enzyme technology can be used to alter fibers and create softer tissue paper, a review of basic enzyme chemistry is first needed.

Enzymes are biological catalysts that increase the rate of chemical reactions and the reactants of enzymes are termed substrates [Palmer, 1991]. There are many types of enzymes, but all enzymes are proteins. Without the presence of non-protein component called a cofactor, many enzyme proteins lack catalytic activity. Some of the common terminology used for enzyme chemistry includes apoenzyme, holoenzyme, coenzyme and prosthetic group [Palmer, 1991]. An apoenzyme is the inactive protein component of an enzyme. The holoenzyme is the active enzyme, including cofactor. A coenzyme is a cofactor that is an organic molecule. And the prosthetic group is a cofactor that is so tightly bound that it is difficult to remove without damaging the enzyme [Palmer, 1991].

All reactions catalyzed by enzymes are reversible to some degree and the classification which would be given to enzymes for the catalysis of the forward reaction would not be the same for the reverse reaction. Also, enzymes exhibit group specificity in that they may act on several different, though closely related, substrates to catalyze a reaction involving a particular chemical group [Palmer, 1991]. This is termed absolute specificity when enzymes act only on one particular substrate. Enzyme catalyzed reactions are product specific as well as being substrate specific [Palmer, 1991]. Uncatalyzed reactions will give rise to a wide range of products. Besides being substrate and product specific, enzymes also exhibit stereochemical specificity. If a substrate can exhibit in two stereochemical forms, chemically identical but with a different arrangement of atoms in 3-D space, then only one of the isomers will undergo reaction as

a result of catalysis by a particular enzyme. The only enzymes which act on both stereoisomeric forms of a substrate are those whose function is to interconvert L and D isomers [Palmer, 1991].

There must be at least three different points of interaction between enzymes and substrate [Palmer, 1991]. These interactions have either a binding or a catalytic function. The binding sites link to specific groups in the substrate, ensuring that the enzyme and substrate molecules are held in a fixed orientation with respect to each other, with the reacting groups in the vicinity of catalytic sites. The active site is the region which contains the binding site and the catalytic sites. The binding and catalytic sites must be either amino acid residues or cofactors. The substrate binding may involve a variety of linkages, but the bonds formed are usually relatively weak (i.e. non-covalent) [Palmer, 1991]. The active site often includes both polar and non-polar amino acid residues, creating an arrangement of hydrophilic and hydrophobic microenvironments not found elsewhere on an enzyme molecule. The function of an enzyme may depend not only on the spatial arrangement of binding and catalytic sites, but also on the environment in which these sites occur [Palmer, 1991].

Binding domains improve the binding and facilitate the activity of the catalytic domain on the insoluble but not on soluble substrates. Cellulose binding domains (CBD's) have been divided into several different families based on their amino acid sequence similarities [Linder, 1997]. CBD's rely on several aromatic amino acids for binding to the cellulose. The CBD's binding to crystalline cellulose have different topologies but share similar rigid backbone structures for correct positioning of the side chains required for the substrate recognition and binding. Efficient wood decaying organisms, such as filamentous fungi, typically use batteries of secreted and synergistically acting cellulases, while anaerobic bacteria utilize large multi-enzyme complexes (cellulosomes), which operate at the cell-substrate interface [Linder, 1997]. The overall binding efficiency of the enzymes is much enhanced by the presence of the CBD and the enhanced binding clearly seems to correlate with better activity towards insoluble cellulose. Similar to cellulases, removal of the substrate binding domains of

Glucosylases decreases their activities on insoluble substrates, but not on soluble substrates [Linder, 1997].

The CBD's of cellulases have been grouped into several families based on similarities in their amino acid sequence [Azevedo, 2000]. Family I is the smallest with CBD's containing 33-36 amino acid residues. They occur only in fungal cellulases such as those created by *Trichoderma reesei* and *Humicola insolens*. Family II CBD's have about 110 amino acid residues and these include the CBD's of some cellulases of *Cellulomonas fimi* and *Thermomonospora fusca* [Azevedo, 2000]. Boraston et al. showed that family II CBD's disrupt the surface of cellulosic fibers and release fine particles from cotton or Avicel [Boraston, 1998]. The CBD's of family I have not been found to disrupt the cellulose structure. Jervis concluded that CBD's of bacterial cellulases from *C. fimi* (Cen A and Cex CBD's, family II) bind irreversibly to crystalline cellulose because desorption of these CBD's was not observed after dilution [Jervis, 1997]. Also, Bothwell reported irreversible adsorption for the EG's E3 and E5 from *T. fusca* (CBD's of family II), but found completely reversible binding for CBHI from *T. reesei* (family I CBD) [Bothwell, 1997].

The enzymatic activity of many different cellulases is affected by the shortening or lengthening of the linker region between CBD and the catalytic domain. Data suggest that two domains are in contact on the cellulose surface during catalyzation, and that relatively long linker regions with some flexibility are needed to express full cellulolytic activity [Linder, 1997]. Cellulases with CBD's are required in the early stages of cellulose degradation when most of the substrate is still insoluble. At later states, when the substrate has been largely solubilized into oligosaccharides, enzymes operating in the liquid phase may be preferred and brought about, e.g. by specific proteolysis of the CBD [Linder, 1997]. An aspect of CBD adsorption to cellulose is how 'tightly' or irreversibly the protein binds to cellulose. When considering the function of intact cellulases, irreversible binding through the CBD seems very likely. Instead, the enzymes should undergo a dynamic process of binding and desorption of both domains allowing progressive hydrolysis and/or relocation to new enzymatically accessible sites or the solid

substrate surface [Linder, 1997]. It is likely that the diversity of the substrates and the enzymes as well as difficulties in the experimental design have contributed to the observation of irreversible binding of cellulases and CBD's. First of all, a great variety of substrates that clearly present different binding surfaces for the celluloses have been used in the binding studies. Secondly, the binding can occur through either one or the other of the individual domains, or through both of the domains simultaneously, and that each way of binding also has a different affinity [Linder, 1997]. Finally, the catalytic and binding domains of different cellulases may have different preferred binding sites on the cellulose surface and the dominating mode of binding may depend on the enzyme concentration [Linder, 1997].

There are a couple of models or hypotheses to explain how enzymes work. One is the lock and key model which suggests that all substrates remain fixed throughout the binding process. This hypothesis (lock and key), by Fischer explains many features of enzyme specificity, but takes no account of the known flexibility of proteins, X-ray diffraction analysis and data from several forms of spectroscopy, including NMR, have revealed differences in structure between free and substrate bound enzymes [Palmer, 1991]. This hypothesis suggests that the binding of a substrate to an enzyme may bring about a conformational change. Another hypothesis is Kashland's induced fit hypothesis of 1958 that suggested that the structure of a substrate may be complementary to that of the active site in the enzyme-substrate complex, but not in the free enzyme [Palmer, 1991]. This suggests that a conformational change takes place in the enzyme during the binding of substrate which results in the required matching of structure. This essentially requires that the active site to be floppy and the substrate to be rigid.

There are two major groups of enzymes: monomeric enzymes and oligomeric enzymes. Monomeric proteins are those which consist of only a single polypeptide chain, so they cannot be dissociated into smaller units. These proteins catalyze hydrolytic reactions and may contain between 100 and 300 amino acid residues and have molecular weights in the range of 13,000 to 35,000. Some of these proteins are associated with a metal ion, but most act without the help of any cofactor. A large number of monomeric

enzymes are proteases, i.e. they catalyze the hydrolysis of peptide bonds in other proteins. Oligomeric proteins are different from monomeric proteins in that they consist of two or more polypeptide chains, which are usually linked to each other by non-covalent interactions and never by peptide bonds. The component polypeptide chains are termed sub-units and may be identical to or different from each other. If they are identical to each other they are sometimes called protomers. The vast majority of known enzymes are oligomeric.

Cellulolytic enzymes which are oligomeric in nature can be subdivided into cellobiohydrolases and endoglucanases. Cellobiohydrolases (CBH, exo-type) degrade crystalline regions and endoglucanases (EG, endo-type) prefer to degrade amorphous regions of cellulose. Cellobiohydrolase (CBHI) is an oligomeric enzyme and is probably the key enzyme needed for the efficient hydrolysis of native crystalline cellulose [Devine, 1994]. It is also the most abundant cellulase produced by the filamentous fungus *Trichoderma reesei* and the removal of its gene reduces overall activity on crystalline cellulose by 70%. CBHI has been classified on the basis of its amino acid sequence as a family C enzyme. This family includes both exo- and endoglucanases which cleave the $\beta(1,4)$ glycosidic bond by a double-displacement mechanism, resulting in retention of configuration of the product, cellobiose. Also CBHI is a two domain enzyme, consisting of a large catalytic core linked to a small cellulose-binding domain by a heavily glycosylated linker region [Devine, 1994]. Both CBH's (CBHI and CBHII) contains an active site tunnel. Most, if not all cellulases effective against crystalline cellulose share a modular structure composed of a catalytic domain linked to a distinct CBD. While the EG's have open active site clefts, the exoglucnases active sites are located in tunnels formed by long loops in the protein structure [Linder, 1997]. Although the tunnel of CBHI is about twice as long as that observed in CBHII, they both are built up from loops extending from the structural motif [Devine, 1994]. The sides of both tunnels are formed by side chains involved in a complex network of hydrogen bonds and salt links and are rich in amino acids that are known to interact with sugars. The sorption of CBHI is rapid and irreversible and CBHI shows a preference for the crystal edge, but CBHII acts at one tip of the crystal [Devine, 1994].

1.2 How Enzymes Affect Paper Softness

Enzyme technology is a versatile tool for modifying the fibers, since it has a tendency to degrade specific components [Buchert, J., et al., 1996]. The hypothesis underlying this study is that by selectively degrading a cellulose fiber, the bulk softness of the resulting fiber structure can be increased. The bulk softness of a paper sheet can be linked to the compressibility of the fibrous network. Fiber network compressibility models show that the structural rigidity of the fiber is a critical factor in determining the compressibility of the sheet [Pawlak, J.J., 2001]. This model suggests that if the flexural rigidity of the fiber can be reduced, while maintaining a constant coarseness, then the compressibility of the paper sheet can be increased at the same sheet density. The flexural rigidity of the fibers may be reduced by lowering the elastic modulus of the fibers, decreasing the moment of inertia of the fibers, or providing localized “hinge points” in the fiber. The enzymatic action on the fibers enhances localized defects in the fibers, which then serve as “hinge points” around which the fibers can flex. By creating these localized hinge points, the coarseness of the fiber is changed very little and this allows for the compressibility of the fiber structure to be increased without reducing the density of the structure. If the tensile strength of the fiber network is much lower than the fiber strength, then reducing the fiber strength should have little or no impact on the sheet tensile strength, as the tensile strength is dictated primarily by the bonding., which remains the same. The net result of engineering such a structure would be a softer, more compressible product, with the same tensile strength as the original product.

A previous study by Mansfield and Saddler [1997] looked at using enzymes to modify Douglas-Fir pulp characteristics so that they could be used for different applications. Douglas-Fir fibers are very coarse and stiff, inflexible and bulky, which yield paper products that are relatively rough and weak, primarily as a consequence of the coarse fibers providing poor interfiber bonding. In their study, it was hypothesized that if hydrolytic enzymes could be used to selectively act on the fiber wall, either from the S2 layer, the lumen or both simultaneously, it is possible that the stiff, inflexible nature of the coarser fibers could be modified to enhance collapsibility and interfiber bonding.

This would allow Douglas-Fir fibers to be used in markets other than traditional Kraft pulp applications [Mansfield, 1997]. In their study, both cellulase and xylanase treatments were used and pulp slurries were treated for one hour at 50°C under continuous agitation over a range of enzyme loadings. Results showed significant reductions in zero-span breaking length and burst index after cellulase treatment. The tensile index for the unfractionated kraft pulp decreased after cellulase treatment, while three longer fiber length fractions showed significant improvements [Mansfield, 1997]. This increase combined with the similar increase in handsheet density suggested that the fibers had collapsed and flattened. Flatter fibers would be expected to exhibit a greater surface area available for binding and this enhanced fiber to fiber contact could increase the tensile index. The fiber modifications from xylanase were very small compared to the ones previously obtained with the commercial cellulase on the different fiber length fractions [Mansfield, 1997].

It was found that a large percentage of the hemicellulose, which is important for fiber to fiber bond strength, within the pulp samples in the xylanase fiber modification study had been hydrolyzed [Mansfield, 1997]. Therefore, it is possible that, in addition to the removal of fines, the reduction in available hemicellulose played a substantial role in the previously observed reduction in paper strength. Also, the reduction in the degree of polymerization of the residual hemicellulose may have contributed to the reduced paper strength [Mansfield, 1997]. Mansfield and Saddler stated that the observed reduction in fiber strength must be a result of the modification of the fibers' cellulose component rather than any effect resulting from xylan removal. The work indicates that the 35.2 % reduction in intrinsic fiber strength resulting from the enzyme treatment occurred without any change in the degree of polymerization of the cellulose [Mansfield, 1997]. They also stated that previous studies showed that the enzymes preferentially degrade irregular zones such as kinks and nodes in the fiber wall and the localized degradation by cellulases may result in reduction in the intrinsic fiber strength. The cellulase enzymes act in a manner which consecutively removes the outer most layers of the fiber wall, ultimately reducing the thickness of the cell walls [Paice, 1992], [Gurnagul, 1992].

There have also been some studies on the effects of *Trichoderma* cellulases on pulp properties. The effects of *Trichoderma reesei* cellulases (EGI, EGII, CBHI and CBHII) and their core proteins on technical properties of the ECF bleached softwood kraft pulp were previously studied [Buchert, 1997]. In this study it was found that of the endoglucanases, EGII caused the most dramatic viscosity decrease in given cellulose hydrolysis level. The CBH's decreased the viscosity less than the EG's, however the differences were also observed between CBHI and CBHII [Buchert, 1997]. It was also found that the pulp strengths were reduced more by EGII than EGI treatment and the effect of EG's on the strength properties of the pulp after beating were most dependent on the dosage used in treatment. The removal of cellulose binding domain (CBD) from CBHII is reported to have no influence on the activity of the enzyme when soluble substrates are used but using crystalline substrates both its binding and activity were clearly impaired [Buchert, 1997].

In a study by Gerber, et al. [1997] the absorption behavior of purified *Trichoderma reesei* CBHI and EGII on bleach Kraft fibers was investigated. The results showed that the fiber history (never-dried or once-dried) had the largest influence on the extent of absorption of each enzyme. Ionic strength of the enzyme in the salt solution was shown to be dependent on the enzyme and fiber type. At high ionic strength, CBHI exhibited a higher affinity for both once-dried and never-dried fibers at low enzyme concentrations. Salt was shown to decrease the extent of adsorption at higher enzyme charge. In contrast, salt increased the maximum adsorption of EGII, most notably on the once-dried hardwood fibers [Gerber, et al., 1997]. The impact that salt had on enzyme adsorption, suggests that the ionic strength of the papermaking process water may influence the level of cellulases absorbed onto the pulp. Reinikainen showed that high ionic strengths increased the apparent affinity and binding capacity of CBHI onto microcrystalline cellulose [Reinikainen, 1994]. Also, Tenkanen's data indicated that the adsorption of purified *Trichoderma reesei* EGI and CBHI onto Avicel did not change with increasing NaCl concentrations. It was also found that the effects of salt varied according to enzyme type [Tenkanen, 1995].

Gerber found that CBHI had a higher affinity for softwood fibers than for hardwood fibers at low enzyme concentrations. The maximum adsorption of EGII onto once-dried softwood fiber increased by 80% compared to the once-dried hardwood fibers. This did not correlate to increased fiber hydrolysis. Both hardwood and softwood never-dried fibers had the capacity to adsorb two to three times more enzyme than the once-dried fibers; however at industrial enzyme addition levels the difference was substantially less [Gerber, 1997]. The higher cellulase adsorption onto never-dried fibers was probably due to its higher surface area. Upon drying, the microfibrils and nearby fiber fines collapse onto the fiber surface, and hydrogen bonding closes the physical discontinuities in the secondary cell wall. Also, hornification not only decreases the surface area by collapsing pores in the fiber wall, but most importantly, may decrease the accessibility of the enzyme on to the fiber surface [Gerber, 1997].

In a study by Oksanen, et al., on the effect of *Trichoderma reesei* cellulase and hemicellulases on the paper properties, it was found that the pretreatment of never-dried bleached pine kraft pulp prior to refining with CBHI and CBHII had virtually no effect on the development of pulp properties during refining, except for a slight decrease in strength properties [Oksanen, et al., 1997]. It was however found that EGI and EGII improved the beatability of the pulp as measured by Schopper-Riegler value, sheet density and Gurley air resistance. EGII was the most effective in improving the beating response, but combos of CBHI with EGI and EGII had similar effects on pulp properties as the EG's alone, although the amount of hydrolyzed cellulose was increased. The negative effect of EGII on strength properties was more pronounced compared with EGI. According to Noe, et al., improved beatability of kraft pulps has been obtained by using hemicellulases and cellulases [Noe, et al., 1986]. An explanation for why the EG's increased beating response is the increased fiber breakage and function of fines, rather than improved flexibilization [Oksanen, et al., 1997]. They also found that pulp hydrolyzing enzymes are potential tools for modification of pulp properties. Their report also stated that cellulases can be expected to act both on the outer fiber surface and inside the fiber wall, similarly to mannanases, but when low enzyme dosages are used, the

enzymes action is more pronounced on the outer surface of the fibers [Oksanen, et al., 1997].

In a study by Pere, et al., on the effects of purified *Trichoderma reesei* cellulases on the fiber properties of kraft pulp, purified cellulases from *Trichoderma reesei* were applied to unbleached pine kraft pulp at various dosages [Pere, et al., 1995]. The purpose was to study the individual effects of the four main cellulases – (CBHI and CBHII) and (EGI and EGII) – on pulp strength. In this study, only 0.20 – 2.05% of the pulp (dry weight) was solubilized by the enzyme treatments. It was found that both EG's dramatically decreased pulp viscosity, EGII in particular. No effect on the fiber length was observed and a clear correlation between viscosity and cellulose degradation by cellulases was detected. Also, there was no major difference in the strength properties for the CBHI treated and control pulps. In the sample treated with CBHI, the slight decrease of the tear index was compensated by the increase in tensile index at higher levels of beating [Pere, et al., 1995]. The study also reported that the comparable zero-span indices indicate that CBHI did not cause structural damage to the fibers. The EGII treatment severely damaged the strength properties of fibers, as could be expected from the viscosity data [Pere, et al., 1995]. The negative effects of EGII were found to be irreversible and could not be restored by beating. It is possible that EGII degrades certain accessible regions of the cellulose molecules within the microfibrils, loosening the structure, but not breaking the fibers. After handsheet manufacture and drying, the loosened cell wall might have partly collapsed, increasing fiber conformability, which was detected by changes in air resistance and higher density [Pere, et al., 1995]. The report also stated that a specific mode of degradation at critical points would suggest that enzymes penetrate and subsequently hydrolyze cellulose in the inner S2 layer, at least to some extent.

There have been a few studies that have looked into the interaction of shear force or agitation with enzyme treatment of pulp. One of these studies was by Lenting and Warmoeskerken, in which the mechanism of interaction between cellulase action and applied shear force was studied [Lenting, 2001]. This study found that a certain threshold

of applied shear force is required for optimal cellulase performance. The usage of cellulase technology in open-width equipment is blocked by lack of the required level of applied shear force and it cannot be compensated by additional enzyme dosage or extending the incubation time [Lenting, 2001]. Also, based on hypothesis and cellulose application in batch production equipment, it can be expected that the type of applied shear force is of importance also, in that forces of changeable directions are required to break the cellulose or weaken the microfibrils from the fibers [Lenting, 2001]. It is also pointed out that the cellulase activity is highest on the flexible amorphous cellulose when compared to that with more rigid crystalline cellulose.

A study by Azevedo, et al., looked at the effects of agitation level on the adsorption, desorption, and activities of cotton fibrils of full length and core domains of EGA (*Humicola insolens*) and Cen A (*cellulomonas fimi*) [Azevedo, et al., 2000]. The activities (at pH 7 and 50°C) of purified EGV and Cen A were determined on cotton fibers at high and low levels of mechanical agitation. Activity experiments suggested that the presence of cellulose binding domains (CBD's) is not essential for cellulase performance in textile processes, where high levels of mechanical agitation are applied [Azevedo, et al., 2000]. The adsorption or desorption processes of cellulases are enhanced by higher mechanical agitation levels and binding of cellulase with CBD of family I (EGV) is more reversible than that of the cellulase of family II (Cen A) [Azevedo, et al., 2000]. Results show that adsorption of EGV and EGV core was very low for both levels of agitation used (high level agitation (rotary wash) and low level agitation (shaken)). The action of EG (with or without its family I CBD) may be achieved via rapid adsorption or desorption and that its binding to cotton cellulose is highly reversible [Azevedo, et al., 2000]. The cellulolytic action of Cen A seems to be achieved via high levels of adsorption with low reversibility. Cen A core shows completely reversible adsorption on cotton cellulose at a high level of mechanical agitation, but, at a low level of mechanical agitation, low degree of desorption was verified [Azevedo, et al., 2000]. This shows that non-target proteins (cores) are just removed from the substrate with high mechanical agitation, whereas target proteins (with a family II CBD) remain adsorbed even at high levels of agitation. It can also be seen

that EGV and EGV core achieve three to four times the fabric loss produced by Cen A and Cen A core under the same high-agitation conditions [Azevedo, et al., 2000]. Cen A only has moderate activity on crystalline cellulose, suggesting that the surface diffusion of CBD's does not limit the substrate catalysis.

There have also been some studies on the use of enzymes in the recycled pulp industry. In a study by Pommier et al., the use of enzymes to improve the process and product quality in recycled pulp was looked at [Pommier et al., 1989]. At low enzyme concentrations, pulp freeness increases soon after contact with enzymes without any loss in the mechanical properties of the paper. This can improve machine speed and allow more dilution in the headbox, which leads to better sheet formation and better physical properties for the paper [Pommier et al., 1989]. Alternatively, different raw materials could be used, including more low-grade wastepaper, to achieve the same mechanical properties. A practical use of this phenomenon is in the recycled paper industry, in which the natural drainage of the pulp is generally low [Pommier et al., 1989]. As early as 1942, a patent claimed that “cytases” – or, actually, hemicellulases – could aid the refining of all kinds of pulps, increasing by a factor of two to six times the rate of hydration of the fibers. But there is a limitation in paper recycling, in that the fiber quality declines because of undesirable changes in the pulp furnish, caused by fines, colloids, fillers, etc. [Pommier et al., 1989]. Pommier used *T. Reesei* enzyme (0.2% wt./wt.) and wastepaper pulps (laboratory and industrial pulps) in his experiments. The wastepaper contained mostly OCC and mixed wastepaper in the ratio of 75:25 at a consistency of three percent. In the lab, the freeness increase of the pulp due to the enzymatic treatment caused a slight decrease in the paper mechanical properties. In the pilot paper machine experiments, it was found that when the pulp was treated with enzyme, the freeness increases without any loss of the mechanical properties in the paper [Pommier et al., 1989].

Some researchers from La Cellulose du Pin were the first to show that the reduced dewatering properties of recovered paper can be improved by enzymatic treatment. They used a culture filtrate of the fungus *Trichoderma reesei*, which contained a mixture of cellulose and hemicellulose degrading enzymes [Pommier et al., 1989, Pommier, 1990].

The improved pulp properties resulted in higher productivity in the manufacture of corrugated board. Strength values of the board remained unchanged when enzyme treatment was limited to 30 minutes and the enzyme dosage was not too high. A too-intensive treatment led to disintegration of the fibers by cellulases. Bhat verified the findings of Pommier using dried bleach, and unbleached softwood Kraft fibers [Bhat, 1991]. Neither Bhat, nor Pommier pursued a mechanism, but Pommier speculated that enzymes act on the surface of fibers producing a peeling effect. If a peeling effect is controlled, the enzymes will remove only some small components that have a great affinity for water, but do not contribute to hydrogen bonding of the fibers. Better drainage of the pulp could be allowed without affecting the mechanical properties [Pommier et al., 1989].

In a study by a European research consortium, recovered paper was incubated with culture filtrates and with isolated cellulase and hemicellulases [Stork, 1995]. They found that the action of endoglucanases was necessary for an improvement in the drainage of recovered paper. The effect did not appear to be due to a selective hydrolysis of the fines fraction but was a consequence of the hydrolysis of amorphous cellulose on the surface of the fibers. Depending on the origin and history of primary and secondary fibers, the endoglucanase treatment decreased the strength properties to different degrees. Endoglucanase treatment seems best suited for the treatment of recovered paper consisting of mechanical pulp but not for those papers containing considerable amounts of chemical pulp fibers [Stork, 1995].

It has been suggested that cellulases and hemicellulases could also be used as flotation aids during the deinking process. It has been claimed by several Korean and Japanese patent applications that the brightness of recovered paper could be improved by flotation in the presence of alkaline cellulases [Urushibata, 1984], [Fukunaga, 1990], [Nomura, 1988], [Eom, 1990]. Ow and Eom deinked newspaper with the aid of a cellulase and hemicellulose contained culture filtrate without the addition of other chemicals [Ow, 1990]. The brightness and bleachability of this pulp was superior to

those of conventionally deinked pulp. The authors observed a preferential hydrolysis of interfiber bonds, but no change in fiber length distribution was found.

Prasad, et al., found a selective hydrolysis of the fines and an increase in the long-fiber content of recovered paper, which was enzyme treated and deinked by flotation [Prasad, et al., 1992]. Some of the basic effects of the enzymatic treatment were studied by Jackson et al. [Jackson, 1993]. These authors used softwood kraft pulp as a substrate and tested using a xylanase preparation along with two different mixtures of cellulases and xylanases. The CSF (Canadian Standard Freeness) was increased by cellulase treatment but not by the xylanase preparation. Low enzyme dosages resulted in a reduction of the fines content, and which was related to a flocculation similar induced by polymers. On the other hand, higher enzyme dosages led to an increase in the fines content. This effect can be attributed to cellulase-induced disintegration of fibers, as described by Oltus et al. [Oltus, 1987].

In a study by Jackson, Heitmann and Joyce, in which once dried bleach softwood fibers were enzymatically treated for 30 minutes at 3% consistency, it was found that fiber length is not substantially affected by enzymatic treatment, but cellulose hydrolysis did occur [Jackson, 1993]. The fines, with their high specific surface area, seemed to be preferentially degraded, resulting in large freeness gains as a result. Microscopic analysis from this study indicated that fiber surface cleaning was occurring and this was attributed to the fact that the cell wall fibrillation created during beating was partially removed or fragmented. It was also noted in this study that at high enzyme dosages and long reaction times, fiber disintegration could occur and enzymatic treatments of recycled fiber must be controlled to minimize fiber damage [Jackson, 1993].

1.3 Understanding Softness and How it is Measured

Softness or surface feel is one of the most important properties for differentiating between different tissue products, particularly for grades such as facial and bathroom tissue [Carr, 1997]. It is important though that functional properties of tissue paper, such as tensile strength and liquid absorbency, should not be sacrificed in order to increase softness of the product. The goal of the Procter and Gamble project is to increase bulk softness by weakening fiber tensile strength and not disrupting the integrity of the overall sheet by keeping the breaking length tensile strength of the enzymatically treated sheet basically the same. For low density papers such as tissue, the fiber-to-fiber bond network is the principal factor determining the final strength of the sheet and the single fiber strength is not critical [Ramasubramanian and Perkins, 1988]. However, during the creping process, which is used often in the tissue business to increase surface softness, the fibers resist axial compression by the creping blade and maintain the stiffness of the sheet, while transferring the load to the fiber-to-fiber bonds and weakening the sheet [Ramasubramanian and Sun, 1999]. By enzymatic degradation of the fibers selectively at pre-determined locations, the fiber axial collapse and out of plane deformation can be augmented, resulting in a sheet with reduced stiffness, while not directing the energy into the fiber-to-fiber bonds. In this manner, the sheet is able to exhibit lower stiffness, and therefore increased softness, while maintaining or even increasing the strength. This would be revolutionary if a sheet can be engineered to retain strength, while losing stiffness and thus increasing bulk softness [Ramasubramanian and Lee, 1991, 1992, 1993].

Softness consists of bulk softness, which is the tactile sensation felt when tissue is gently crumpled between your hands, and surface softness, which is the sensation that is perceived when the fingers are pulled closely across the surface of a tissue sheet [Carr, 1997]. Bulk softness is largely determined by the flexibility and extensibility of the sheet, i.e., the stiffness and degree of movement of the fibers within the structure. Bending stiffness or flexural rigidity is an inverse function of softness [Hollmark, 1983]. Bending stiffness (S_B) can be related to more basic properties through the formula: $S_B \propto E * t^3 =$

$S_T * t^2$, where E is Young's modulus, t is the effective thickness of the material, and S_T is the tensile stiffness. Bending stiffness responds to variations in thickness through a square function. This means that an increase in thickness with no change in the tensile stiffness gives a large increase in bending stiffness [Hollmark, 1983]. However the existence of new materials with extremely high bulk as well as very high softness indicates that this relationship cannot be translated into softness [Commercial, 1978].

Surface softness is related to the smoothness and texture of the tissue sheet [Carr, 1997]. Surface smoothness is also a function of the mechanical properties of the sheet material [Kim, 1994]. When someone touches a sample to judge its softness, both characteristics – namely, the size and distribution of irregularities and the yield of the material under local compression- influence the magnitude of softness perception. Texture is qualitatively described by its coarseness or roughness, in the sense that a patch of wool cloth is coarser than that of a patch of silk cloth under the same conditions [Kim, 1994]. While low texture or coarseness is a component of greater softness in tissue or paper toweling, it also is desirable to have a large number of free fiber ends protruding up from the paper surface, somewhat like a nap of velvet fabric [Kim, 1994]. Brushing the tissue increases the number of free fiber ends. Softness was found to be directly related to the number of free fiber ends and inversely to the texture in Kim's study [Kim, 1994]. Garnsworthy, et al., showed that the sensations of prickliness or itchiness associated with wool cloth depend on how the fabric interacts with the skin to trigger the sensory receptors exposed to the fabric [Garnsworthy, et al., 1988].

The types of fibers used also have a contributing factor to how softness is perceived. Virgin fibers, particularly hardwood fibers, produce superior softness. The tissue industry is under pressure to use recycled fibers, so the high softness standards that customers desire are harder to meet when using less virgin fiber. The design of the paper machine used for tissue production, particularly multilayer forming can help. The low quality furnish can make up most of the sheet while the higher quality fibers impart softness and contribute to the outer layers of the sheet. As mentioned earlier in the introduction, papermakers have traditionally had a variety of tools at their disposal for

enhancing the papermaking characteristics of fibers, such as softness. These tools include: different methods of pulping, refining, beating, high consistency kneading, chemical addition etc. The use of enzymatic treatment to enhance the papermaking characteristics of fibers is less common, but offers a unique way of modifying the chemical composition and ultra-structure of the fiber.

Also mentioned earlier, was the creping process used in the tissue industry. Creping occurs as a result of the forces of adhesion and release (adhesion failure), which act upon the sheet [Carr, 1997]. Creping increases the sheets texture or coarseness. However, it also increases the sheets extensibility, which is an important factor in perception of bulk softness [Kim, 1994]. In producing toilet paper, the wet web is pressed against the surface of a Yankee dryer by a touch roll [Kuo, 2000]. After drying to the desired moisture content, the web is creped by a creping blade. This requires a fine balance between web adhesion to the dryer, the physical properties of the sheet, and the force applied by the creping blade [Kuo, 2000]. The controlled adhesion of the sheet to the Yankee dryer roll will help to develop creping by improving the crepe frequency [Sloan, 1994]. Creping is also affected by the physical properties of the formed sheet (such as tensile strength and formation), the sheet adhesion to the Yankee dryer surface and the geometry of the creping blade, which is the angle at which the creping blade strikes the Yankee dryer surface [Sloan, 1994]. The higher the adhesion level, the greater the crepe development, and ultimately, improved surface softness can be expected from this.

Crepe count is a numerical value that can be used to measure the effect of changing sheet adhesion [Sloan, 1994]. The number of crepe bars per unit length from a reel of paper is counted under a microscope. After crepe counts for a specific grade of paper and machine are collected, a benchmark can be established by which softness can be measured. Scratch is another helpful measurement. As scratch numbers fall off, the frequency of the crepe generally increases, because of the greater work done to the sheet at the creping blade [Sloan, 1994]. For the dry crepe machine, the coating strategy will be to develop a coating and release package that will maximize adhesion to the Yankee.

The coating-to-release balance will generally be at or below 1:1 ratio to maximize the coating development [Sloan, 1994].

The cylinder coating influences the forces of adhesion and release which act upon the sheet as it is creped [Carr, 1997]. The forces determine to what extent the structure and texture of the sheet is altered. A manageable coating gives rise to controlled adhesion that results in increased extensibility of the sheet. When a finer crepe structure is generated, this will improve the surface texture of the sheet [Carr, 1997]. The use of appropriate cylinder coating chemicals gives rise to a coating which gives uniform coating build up, maximum creping blade life, and protection of the Yankee surface and consistent tissue quality. The polymers used for effective coating are polyaminoamide, polyacrylamide, polyvinylalcohol, polyvinylacetate and polyethyleneoxide. The polymers give rise to various degrees of adhesion which attracts the sheet to the dryer surface [Carr, 1997]. The chemicals used as release agents are generally hydrophobic in nature. Chemicals referred to as softeners and debonders alter the bulk and surface softness of the sheet thereby improving perceived softness.

Debonders, as the name suggests, reduce bonding within the sheet through cationic surface active agents which interfere with the bonding process [Carr, 1997]. Debonding increases the bulk softness, improves flexibility, increases the water absorbing capacity and reduces tensile strength. There are some negative aspects of debonders. Reducing tensile strength is an advantage to tissue makers, which use predominately virgin fiber, but this is not generally a benefit to the recycled fiber user. Also, the debonder molecule tends to repel water. Softeners can be used together with a debonder to maximize the properties of the tissue [Carr, 1997]. The function of the softener is to improve the softness of the sheet by increasing the surface smoothness and flexibility. A softener can be a similar chemistry to a debonder, i.e. a cationic surfactant or a nonionic surface active chemical. Such chemicals serve to lubricate the individual fibers thereby encouraging more slip within the paper web. Those fibers on the surface of the sheet will be lubricated, resulting in a tactile softness. Applying the softener by

means of a spray bar eliminates any effect on the tensile strength, while maximizing the surface softness of the sheet [Carr, 1997].

As mentioned earlier, improving softness has long been a goal of tissue manufactures, but this goal is difficult to quantify. The problem is that softness is a highly subjective tactile property and it means different things to different people. The data for measuring softness is from complex and multidimensional human sensory system. In evaluating softness, users rub their fingers over the sheet while simultaneously varying pressure to judge texture and “sponginess”. They may also crumple the sheet (in-plane compression) to judge stiffness or sharpness of folds. The tissue industry relies on the subjective evaluation of softness through groups of experienced testers often known as the “softness panel”. These panels are usually done by 100’s of customers and are costly and take weeks to get results. They assign an arbitrary value to a particular grade of tissue [Carr, 1997]. A calculation based on the strength, stretch, basis weight, and thickness can be used to give a softness index for a particular sample also. This approach has met with limited success and acceptance, and consequently the softness panel still prevails. But, it has also been found that softness ratings based on subjective evaluations are often inconsistent [Kim, 1994]. An alternative to subjective evaluation is available in the form of a recently developed technique that measures fabric properties at low levels of mechanical stress. The measured mechanical/physical parameters are applied to mathematical formulas that predict softness perception [Kim, 1994]. Panel measurements have been related to lab measurements including some surface geometry parameters.

Only a few test methods exist for capturing the surface geometry itself; i.e., for specifying the x-y-z coordinates of a surface. They include stylus profilometry, laser triangulation, stereo imaging and interferometry. AFM and electrical inductance instruments are other types of coordinate measurement machines, but are not suited for tactile related measurements. Stylus profilometry is the only technique used for examining tactile related properties of tissue. A stylus tip traces lines or series of lines on surface of object and deformations of stylus provide data for scanned profiles.

Limitations of stylus profilometry include long measurement time and errors associated when stylus jumps over steep peaks or valleys. Rust et al. of NCSU's College of Textiles correlated stylus profilometry measurements of 20 tissue samples to panel softness values using 90 students [Rust, 1994]. Laser triangulation has been explored as a possible method for tissue characterization. This method uses a laser beam that shines on the sample at an angle and a detector determines apparent height of point of incidence using triangulation. Laser beam scans to create topographical map. Limitations to this method include limited resolution, noise for laser scattering and long scan times. Another method is Stereo imaging, which is an optical technique that uses multiple 2-D dimensional images from cameras at different positions to reconstruct 3-D surfaces. This method is noninvasive and can be highly accurate, but suffers from long image acquisition and processing times. One other method is interferometry, in which line diffraction gratings are superimposed, with their lines slightly off from parallel. Relatively wide fringes appear (alternate light and dark zones) that are normal to lines on the grating. Analysis of these "moiré fringes" provides information on the offset of one grating relative to the other. Computer calculations are required to relate the fringe pattern to surface structure.

The Kawabata Evaluation System for fabrics (KES-F) for measuring softness is viewed as a significant breakthrough in textile product quality control, engineering, and optimization of fabric finishing processes [Carr, 1997]. The KES-F instruments are designed to measure the low deformation forces encountered when manipulating a fabric for handle evaluation. By measuring the bending, shear, surface, and compression properties of the fabric under these conditions much of the subjectivity involved in fabric handle evaluation is removed. More recently the KES-F has been applied to the assessment of the mechanical properties of tissue [Carr, 1997]. Careful studies have shown a correlation between the perceived softness and the measurements made. The KES-F is a means of measuring the mechanical properties of tissue as it is encountered when the tissue is manipulated by hand. Several published and unpublished studies identified tensile, shear and surface as showing the best correlation with softness perception [Carr, 1997].

The softness of a particular fabric is ranked according to the PHV [Carr, 1997]. The mechanical measurements from the KES-F testing are used to calculate the PHV. The result is a figure on a scale 0-10 with 10 being the softest. The critical properties of fibers are related to softness, stiffness, and fullness. So the shear and bending properties of a particular fabric play a greater role in determining softness [Carr, 1997]. A human judge can detect difference greater than 0.5 units on the PHV scale. Softness is regarded as a “slip/stick” effect and reducing the variations in smoothness results in an increased perceived softness.

Calendering decreases bending stiffness by reducing the thickness, d , and increases it by increasing the E . The coupling disappears using the specific modulus of elasticity, $E/\rho = Ed/b$, where ρ is the density and b is the basis weight. E/ρ is then independent of calendaring. Bending stiffness is almost always symmetrical, because elastic modulus is the same in tension and compression [Niskanen, 1998]. Sometimes bending stiffness seems asymmetric because of curl in the specimen. At large bending deformations, the local strain within the sheet may increase into the nonlinear region of the stress-strain curve. Then the bending direction becomes significant. The nonlinearity in compression can be reversible, and so permanent change in curvature need occur even if the specimen goes into the nonlinear region. The nonlinearity in compression can be reversible, and no permanent change in curvature need occur even if the specimen goes into the nonlinear region [Niskanen, 1998].

There are a lot of factors that can affect the stiffness of paper and subsequently the bulk softness. Humid paper is less stiff than dry paper [Niskanen, 1998]. Also, when low bending stiffness causes problems, the cause is usually cross direction (CD) stiffness that is typically 2-4 times lower than the machine direction (MD) stiffness. The anisotropy arises from fiber orientation and CD drying shrinkage that affects the elastic modulus. Three possibilities exist to increase/improve bending stiffness. The first is thicker and bulkier paper, in that the main problem is to achieve a smooth printing surface with high bulk. The second is higher elastic modulus and this is not possible with measures that reduce paper thickness. The third is higher elastic modulus on the surface

than in the middle of the sheet. If each surface layer is 5% of paper thickness, then doubling their elastic modulus raises bending stiffness by 27%. This is the most effective way to obtain high bending stiffness and good surface properties with a maximum basis weight [Niskanen, 1998].

The following rules of thumb characterize the effect of pulp. The first is that flexible fibers give dense paper and low stiffness. The second is that stiff fibers give bulky paper and high bending stiffness. The third is that stiff bonds give high bending stiffness [Niskanen, 1998]. The bending ability of chemical pulps is better than that of mechanical pulps. Mechanical pulp fibers are stiffer than chemical pulp fibers and therefore give higher thickness to paper. Beating increases bending ability and fiber flexibility. Since flexible fibers also decrease paper thickness, bending stiffness may not change at all [Niskanen, 1998]. In the paper machine, altering fiber orientation, wet strains, and drying stresses change the anisotropy of bending stiffness. Increased fiber orientation anisotropy increases bending stiffness in MD, but decreases it in CD. Higher wet strain or drying tension increases MD stiffness, but have little effect on the CD value.

Compressive strength is typically one-third of tensile strength. The difference between compressive strength and tension is significant in severe bending [Niskanen, 1998]. In bending, paper or board yields on the compressed side first. This is often good, because the compression failure prevents the other side from breaking in tension. Compressive strength is by definition the largest force that a test piece can tolerate without failing. With paper or board, compressive strength is usually given as force per unit width. Compressive strength does not depend on basis weight, if furnish and network structure do not change [Niskanen, 1998]. In compressive strength measurement, the main problem is preventing the bulking of a thin sample. This is possible by an arrangement where the bulking load is higher than compressive strength. Compressive strength is approximately one-third of the tensile strength in MD and one-half in CD. Compressive breaking strain is one-fourth of the tensile strain in MD and one-fifth in CD [Niskanen, 1998].

Out-of-plane strength measures the ability of paper or board to withstand tensile stress in the thickness direction. There are several methods to measure it including Scott bond (according to measurement method), z-strength, delamination strength, internal bond strength, and ply-bond strength [Niskanen, 1998]. Out-of-plane strength is sensitive to any nonuniformity or layering in the z-direction of the sheet, since delamination occurs at the weakest place. The location of delamination depends on the z-directional distribution of fines and fillers, density through bonding degree, and distribution of size, if present [Niskanen, 1998]. In the layered sheet, fibers lie on top of one another in a well-defined sequence so the sheet can delaminate without breaking any fibers. Since fibers are much stronger than bonds, even a small number of broken fibers can significantly increase the delamination energy [Niskanen, 1998]. Out-of-plane strength increases with density if density is increased by wet pressing or beating. The effect comes simply from the increase in the bonded area [Niskanen, 1998].

The elastic modulus is an important property, because paper is seldom loaded anywhere near the ultimate failure stress [Niskanen, 1998]. Through bonding stiffness, elastic modulus also controls the performance of paper and board in sheet form. The elastic modulus or Young's modulus, E [GPa], measures the force necessary for a small elongation. If σ is the applied stress or force over the cross-sectional area of the specimen, and ϵ is the corresponding strain [%], then E is the following: $E = d\sigma/d\epsilon$ at $\sigma \rightarrow 0$. The elastic modulus of paper is different in the three principal directions: the machine direction (E_{MD} or E_x), cross-machine direction (E_{CD} or E_y), and thickness direction (E_{ZD} or E_z). Elastic modulus is usually the same in tension and compression. For paper, differences between tension and compression are possible because of the structure consists of fiber segments that can bond or buckle differently depending on whether the network stretches or compresses [Niskanen, 1998].

As mentioned earlier, the primary goal of this project is to increase bulk softness (influence by elastic modulus, stiffness and compressibility) by weakening fiber tensile strength and not disrupting the integrity of the overall sheet by keeping the breaking length of the enzymatically treated sheet basically the same. By enzymatic selective

degradation of the fibers at pre-determined locations (fiber defects), the fiber axial collapse and out of plane deformation can be augmented, resulting in a sheet with reduced stiffness, while not directing the energy into the fiber-to-fiber bonds. This is based upon a model of paper compressibility that shows the structural integrity of the fiber is a critical factor in determining the compressibility of the sheet [Pawlak, J.J., 2001]. This model suggests that if the flexural rigidity of the fiber can be reduced, while maintaining a constant coarseness, then the compressibility of the paper can be increased at the same sheet density. Flexural rigidity of the fibers may be reduced by lowering the elastic modulus of the fibers, decreasing the moment of inertia of the fibers, or by providing localized “hinge points” in the fiber. Enzymatic action on the fibers creates or enhances localized notches in the fibers which serve as “hinge points” around which the fiber can flex. By creating these localized weak points, the coarseness of the fiber is changed very little. This allows for the compressibility of the fiber structure to be increased without reducing the density of the structure. In this manner, the sheet is able to exhibit lower stiffness, and therefore increased softness, while maintaining or even increasing the strength.

As just mentioned, an increase in compressibility can be accomplished with a decrease in fiber strength, while keeping the overall tensile strength of the sheet the same. In the technology used in this work, the change in fiber strength resulting from enzyme treatment is measured by zero-span tensile strength measurements. The zero-span tensile strength test was first developed by Hoffman-Jacobsen in 1925 as a method of determining an index of fiber strength [Gurnagul, 1989]. It has been found in some studies that zero-span tensile strength changes with interfiber bonding and is thus affected by sheet structure [Boucai, 1971]. Cowan suggested using a “wet” zero-span test based on the premise that on rewetting a dry sheet, all interfiber bonds are effectively broken; thus, bonding would have little effect on the test [Cowan, 1988]. The rewetted test was originally recommended since it was observed that zero-span tensile index increased very fast in the beginning of a laboratory-refining curve and it was interpreted that zero-span tensile strength depended on the level of bonding in the sheet. To obtain a measure that was independent of bonding, rewetting the sheets was recommended [Mohlin, 2003].

Today, several studies demonstrate that fiber bonding does not influence the dry zero-span tensile strength. When bonding in the sheet is increased by increasing the wet pressing, the dry zero-span tensile index does not change as shown by Seth [Seth, 1999]. The reason why the dry zero-span tensile index increases with laboratory refining is instead explained by the presence of fiber curl in unrefined pulps. The fibers are straightened during laboratory refining, which gives a higher zero-span tensile strength for both dry and rewetted zero-span. In the case of laboratory pulps containing straight fibers, laboratory beating does not cause any decrease in dry zero-span tensile index [Mohlin, 2003]. Gurnagul and Page found that for a wide range of pulps, the decrease in zero-span tensile strength upon rewetting of a handsheet is caused by the weakening of the individual fibers that made up the sheet [Gurnagul, 1989]. This loss in strength depends on the extent of chemical and mechanical damage to the inter-fibrillar matrix of fibers during pulping, bleaching, and refining. Unbleached Kraft fibers are almost equally strong dry or wet, whereas sulfite pulp fibers or fully bleached Kraft pulp fibers can lose up to 30% in strength when rewetted [Gurnagul, 1989]. They also found that wet zero-span strength could not be used as an index of dry fiber strength, although it may be valuable when measuring the strength of wet fibers.

It was hypothesized by Gurnagul and Page that the magnitude of the loss in strength is controlled by chemical differences in the hemicellulose-lignin matrix holding the fibrils together in the cell wall of the fiber [Gurnagul, 1989]. As long as this matrix is not severely degraded, its strength in the wet state may remain high and there will be no loss in fiber strength when the fiber is rewetted. A degradation of the matrix either by mechanical or chemical means could weaken the fiber in the wet state, which would allow the fibrils to slide over one another, thus reducing wet fiber strength. The weaker the supporting matrix, the larger the reduction in zero-span tensile strength on wetting [Gurnagul, 1989]. The equal strength of wet and dry fibers of unbleached Kraft pulps may be explained by the hypothesis that the lignin-hemicellulose matrix though degraded, still provides a continuous network between the fibrils. Bleached pulps lose strength when wetted, because of the removal of residual lignin and hemicelluloses during the delignification and extraction stages [Gurnagul, 1989].

Mohlin and Puisseau tried to resolve the difference in wet and dry zero span tensile strength between laboratory pulps and industrial pulps by examining the defects in fibers with a microscope [Mohlin, 2003]. An examination of the refined fibers showed that some defects and some mechanical damage to the fibers could be observed and it was plausible that observable structural defects retained after the PFI-mill beating coincided with chemical degradation in the fiber wall. They found that there was no significant correlation between the number of defects before and after refining [Mohlin, 2003]. The results from the microscopic counting of defects in laboratory refined fibers, evaluated as the sum of points that showed a change in the direction of the fiber axis and mechanical damage, correlated well with the observed decrease in rewetted zero-span tensile strength. The dry zero-span tensile strength was affected very little by the localized defects. Also, it was found that extrapolating the rewetted zero-span tensile strength to zero defects, the level for the dry zero-span was reached [Mohlin, 2003]. It was also found that rewetted zero-span tensile strength responds to fiber kinks and fiber ends and that the rewetted zero-span tensile strength is sensitive to local defects and that dry zero-span tensile strength was not [Mohlin, 2003].

Mohlin [2003] also questioned how the severity of defects affected zero-span tensile index measurements. Treatments like acid hydrolysis [Frölander, 1969] and enzymatic degradation with cellulases [Gurnagul, 1992] are known to preferentially absorb in disordered regions in the cell wall, i.e. in the areas where it already is a local defect. Mohlin, Mohlin and Puisseau's study, the effect of increasing the severity of local degradation was looked at by studying the cellulase-degradation of bleached market softwood pulps. It was found that dry zero-span tensile strength and wet zero-span tensile strength were changed with severe local degradation from enzymatic absorption (cellulases) [Mohlin, 2003]. It was also observed that rewetted zero-span tensile strength is more severely affected than dry zero-span tensile strength. It was also found that instead of fiber straightening during refining, the fibers broke by tension in the weakened deformation zones (kinks) and the fiber length was reduced from 2.2 mm to 1.7 and 0.7 for the different enzyme treatment levels. Measurements of the length of undamaged

segments showed that their length did not decrease as a result of the enzyme treatment; a confirmation that the cellulase absorb in the already deformed zones [Mohlin, 2003].

It has been mentioned in these studies, that zero-span tensile strength is affected most notably by defects on fibers [Mohlin, 2003, Gurnagul, 1992]. There are two classes of structural modifications to pulp fibers that affect their papermaking properties: (a) modifications arising from compression failures within the fiber wall (wall dislocations and zones of dislocation) and (b) modifications affecting fiber straightness, i.e., fiber curl, which can be thought of as including curvature, kink and twist [Kibblewhite, 1977]. Fiber curl is defined by Perez and Kallmes as the combined in-plane and out-of-plane curvature of fibers in paper. Quantitatively, it is defined as the curl factor, which is equal to the ratio of the actual to the linear length between two points on a fiber in paper [Perez, 1965]. Fiber kinks are defined as distinct angular bends along the length of pulp fibers. Evidence suggests that unless kinks are set into position and made inflexible, they have negligible effects on both wet-web and paper strengths [Kibblewhite, 1975]. Wall dislocations are defined as fractures or dislocations in fiber walls that are clearly visible with a polarizing microscope and such fractures are located within the S_2 layer of the wall and represent fractured wall elements dislocated with respect to each other [Kibblewhite, 1977]. The visualization of dislocations is possible using polarized light microscopy because the cellulose crystal chains of the microfibrils in dislocations have a different orientation than those of the undisturbed cell wall [Thygesen, 2005]. Zones of dislocation are defined as concentrations of wall dislocations in fiber walls and they generally extend over the width of a fiber. Such regions have been described as nodes or weak points at which the greatest amount of fiber bending occurs [Kibblewhite, 1977].

There are many terms to describe these deformations including dislocations, microcompressions, slip planes, nodes, misaligned zones, crimps and kinks. Various types of stress can induce deformations in wood fibers, which may develop in the living tree or during processing like machining or pulping [Nyholm, 2001]. Deformations will lead to changes in the ultrastructure of the fibers. Robinson studied compression of wood samples and was one of the pioneers in describing dislocated regions in wood fibers

[Robinson, 1920]. He observed that dislocations, resulting from longitudinal compression of wood can be seen as light, linear regions in the fiber wall when viewed under polarized light. He suggested that the bright lines observed relate to structural changes initiated in the S_1 layer. Increased longitudinal compression will lead to an increased number of dislocations and even greater structural changes like separation between S_1 and S_2 and separation of wall layers within the S_2 layers [Keith, 1968].

When looking for the origin of dislocations in fibers, the dislocations can either have a natural origin or can be induced artificially. Robinson [Robinson, 1920] and Bienfait [Biefait, 1926] reported that deformations in the cell wall were not artifacts due to the cutting of sections, since fibers isolated by maceration commonly showed numerous dislocations (slip planes). According to Page et al. the majority of fibers show natural defects or weak areas such as pits and induced defects such as dislocations (nodes) or wrinkles, where failure is frequently initiated [Page, 1972]. Kibblewhite also suggested that dislocations (microcompressions) are a product of growth or wind stress of trees and that dislocations and zones of dislocations can give rise to microcompressions under drying stress [Kibblewhite, 1977]. A study into artificially inducing dislocations has been carried out by Iwasaki, in which they were introduced to fibers by ultrasonic treatment [Iwasaki, 1962]. The practices of chipping [Hartler 1963, 1969] or drying of paper sheets [Hartler, 1995] are other methods to artificially induce dislocations. During mechanical treatment, dislocations arise in pulp fibers due to compressive stresses acting on the fiber wall [Stone, 1961].

The occurrence of dislocations lowers fiber strength [Page, 1972] and unbeaten dried pulps with an increased number of deformations show an uneven strength distribution and reduction in the elastic modulus [Hartler, 1995]. As mentioned earlier, this reduction of strength is probably dependent of the number of dislocations and extent of deformations. However, local defects found in the fibers probably have no effect on the fiber strength measured as zero-span. Decreased paper strength is obtained first when the defects are large enough to bend and break the fibers. It has been confirmed that damage occurs at sites of dislocation (nodes) which were mechanically created during the

discharge of the cooked material from the digester. Reduction in strength as a result of this process is probably irreversible [Gurnagul, 1992]. From this, it can be seen that a large number of dislocations might reduce the elastic modulus of fibers and therefore lower the fiber strength.

Dislocations (microcompressions) make fibers more flexible with an accompanying improvement in binding potential [Hartler, 1995]. Dislocations in more flexible fibers (such as low yield earlywood fibers) resemble a soft pleat, but are more sharp-edged, like cracks in more rigid fibers. The appearance of dislocations in the fiber wall is concurrent with a decrease in fiber tensile strength [Alexander, 1968]. It was concluded by Nyholm et al. that dislocations are locally compressed regions which make the fiber more flexible, but are also more sensitive to chemical absorption. Depolymerisation of cellulose was suggested by Searle as an explanation for the susceptibility of dislocations to chemical absorption [Searle, 1924]. The “loosening up” of the cell wall resulting in separation of microfibrils or microfibril bundles will make the cell wall more accessible to all types of agents including enzymes. Hartler pointed out that the direction of microfibrils changed in microcompressed regions leading to breaking of hydrogen bonds and this should result in higher accessibility of the cellulose to chemical hydrolysis [Hartler, 1995]. The effect of acids, enzymes and other agents is related to the extent of structural change, with large deformations increasing the accessibility of cell wall elements and this would explain why cell wall hydrolysis has been observed at kinks or dislocations [Gurnagul, 1992]. Also, according to Gurnagul, it is likely that enzymes preferentially absorb to structurally irregular zones of the fiber wall, resulting in localized sites of degradation. Dislocations are not only a changed direction of fibrils, but may also contain cracks which facilitate the penetration of cell wall degrading chemicals and enzymes.

As was mentioned earlier, it has been established that there is a unique relationship between the number of microcompressions and the stiffness; the more microcompressions the less stiff the fiber is [Hartler, 1968]. Fibers that were enzymatically treated for the Procter and Gamble testing were tested for wet fiber

flexibility to determine the extent of how enzymes interact with fiber defects (dislocation, kinks, etc.) to contribute to less stiff fibers (increased flexibility) and hence increased softness as the enzyme treatment time increased. Over the past forty years many techniques have been devised to measure wet fiber flexibility. Steadman and Luner developed a method to measure flexibility by observing fibers pressed against thin wires [Steadman, 1985]. This method is relatively simple and can measure the wet flexibility of a large number of fibers more quickly and reproducibly compared to other techniques [Zhang, 2004]. The procedure used for measuring fiber flexibility is described in Steadman and Luner's paper [Steadman, 1985] and also in the methods section of Chapter 3c of this thesis, but the basics behind this procedure are that a thin, tangentially-oriented fiber network is pressed formed onto a filter paper in a handsheet mold and from here it is pressed onto a 5cm x 5cm microscope slide with parallel very thin stainless steel wires. The slide is then inverted and viewed in a microscope under incident light and the areas of the fiber that come in contact with the slide appear dark and the areas of the fiber that arcs over the stainless steel wire are not visible. Intuitively, it seems plausible that the no-contact length, L , is related to the flexibility of the fiber, and as the fiber becomes more flexible (from the enzyme absorption and weakening of dislocations and kinks), this distance will decrease [Steadman, 1985].

Chapter 2

The Modification of Bleached Kraft Softwood Fibers with Enzymatic Treatment

2.1 Introduction

The results that are shown in this section are the initial steps involved in finding an enzymatic fiber modification method for enhancing tissue properties. The main tissue property that Procter and Gamble desired to enhance was softness. The softness of a product can be broken down into two components, the bulk (i.e. structural) softness and surface softness. The surface softness is controlled by factors such as coefficient of friction, surface roughness, and surface compressibility [Ramasubramaniam, 2001]. The bulk softness, which is controlled primarily by the structure of the sheet, is related to the bending stiffness, compressibility and modulus of the structure as a whole.

Some of the common methods for enhancing paper fiber characteristics such as softness include different methods of pulping, refining, beating, high consistency kneading, chemical addition, etc. The use of enzymatic treatment of fibers is a less common method for enhancing its paper making characteristics, but it offers a unique way of modifying the chemical composition and ultra-structure of the fiber. Enzyme technology is a versatile tool for modifying the fibers, since it has a tendency to degrade specific components [Buchert, J., et al., 1996]. The proposal for our project focuses on developing the patented technology of Procter and Gamble to improve softness of tissue sheets by altering the ultra-structure of the papermaking fibers.

The hypothesis underlying the study is that by selectively degrading a cellulose fiber, the bulk softness of the resulting fiber structure can be increased. The bulk softness of a paper sheet can be linked to the compressibility of the fibrous network and a model developed by Pawlak shows that the structural integrity of the fiber is a critical factor in determining the compressibility of the sheet [Pawlak, J.J., 2001]. This model suggests that if the flexural rigidity of the fiber can be reduced, while maintaining a constant coarseness, then the compressibility of the paper sheet can be increased at the same sheet density. The flexural rigidity of the fibers may be reduced by lowering the elastic modulus of the fibers decreasing the moment of inertia of the fibers, or providing localized “hinge points” in the fiber. The enzymatic action on the fibers enhances

localized notches in the fibers, which serve as “hinge points” around which the fibers can flex. By creating these localized hinge points, the coarseness of the fiber is changed very little and this allows for the compressibility of the fiber structure to be increased without reducing the density of the structure. If the tensile strength of the fiber network is much lower than the fiber strength, then reducing the fiber strength should have little or no impact on the sheet tensile strength, as the bonding remains the same. The net result of engineering such a structure would be a softer, more compressible product, with the same tensile strength as the original product.

The first objective of this project is to develop methods for implementing selective enzymatic treatment on a commercial scale in the papermill. In order to do this, it was first necessary to duplicate the current art of the Procter and Gamble U.S. Patent 6,146,494 in order to start from a firm foundation, develop laboratory procedures and make certain that we understand the technology described. Several enzyme concentrations (0.05 %, 0.1 %, 0.5 % and 1.0 % based on grams of enzyme per gram of oven dried pulp) were used to determine which concentration was optimal for reproducing patent claims. The key patent claims are:

1. The fibrous structure should have a density of not more than about 0.4 g/cm^3 (400 kg/m^3).
2. Modified fibers that form a fibrous structure (handsheet) should have a dry tensile index that is at least as great (90 % similar) as the dry tensile index of a handsheet made from the corresponding unmodified fibers (No-Enzyme control).
3. Modified cellulosic fibers should have a dry zero span tensile index that is at least about 35 % less than the dry zero span tensile index of the corresponding unmodified cellulosic fibers (No-Enzyme control).
4. Modified cellulosic fibers should have a wet zero span tensile index that is at least about 70 % less than the wet zero span tensile index of the corresponding unmodified cellulosic fibers (No-Enzyme control).
5. Modified cellulosic fibers should exhibit a ratio of dry zero span tensile index to wet zero span tensile index from about 1.5 to about 3.

It was also desired to measure the compressibility of the different enzyme concentration samples to determine the extent of increased incubation time and enzyme concentration had on bulk softness. The results from these compressibility tests were verified with drainage and FQA tests. The following methods and materials were used to reproduce the Procter and Gamble claims and to measure and verify compressibility of enzyme treated samples.

2.2 Experimental Methods and Materials

Pulp

Northern softwood bleached kraft pulp from the National Institute of Standards and Technology (NIST) was used for this study. One conditioned NIST SW pulp sheet (488g) was broken up and soaked in water for four hours. The pulp sheet was then disintegrated in a British disintegrator for ten minutes at level 50 on a Dayton DC Speed Controller Model 5X485C. The pulps were then washed for about seven minutes with tap water in a false bottom container. For the experiments conducted at a 0.5 % enzyme addition rate (mass enzyme on OD mass fiber), the whole pulp was used as the pulp was not washed in the false bottom screen. Finally, the pulp was filtered on Buchner funnel and the consistency of pulp was measured.

Enzyme Used

The cellulase, Dyadic EXP cellulase, was obtained from Dyadic International (Jupiter, Florida). Enzyme activity was determined by measuring carboxymethyl cellulose (CMCase) and Filterpaper Activity (FPU) with 1 % enzyme solution (0.5 g cellulase weight / 50mL water). CMCase was 0.6 units/mL and FPU was 0.8 units/mL. The protein content of 1 % enzyme solution was 8.2 mg/mL.

Enzymatic Treatments

The pulp slurries (100 OD g at 3 % consistency in 50 mM sodium acetate buffer, pH 4.5 for Dyadic cellulase treatments) were heated and agitated (using a Fisher Scientific Sted-Fast Model 600 agitator set at level nine with a three blade impeller) in a water bath until the temperature reached 45°C. Once the temperature stabilized at 45°C and pH stabilized at 4.5, an enzyme charge (solids on fiber 0.05 %, 0.01 %, 0.5 % or 1.0 %) was added to the pulp slurry. Pulp/enzyme mixture was continuously agitated and kept at 45°C and pH of 4.5. A 600 ml sample (roughly 20 OD g pulp) was taken from the mixing tank at the times of 0, 30, 60, and 180 minutes. Once each sample was taken from the mixing tank, the sample was placed into a microwave oven for four minutes (boiling for two minutes) to inactivate the enzyme in the sample.

Control Treatments

There were two controls used to monitor and compare the results of the enzyme treated samples. The first control was a no enzyme control (NE control), which used the same procedure as the enzyme treatments, with the exception that no enzyme was used at all for this treatment (water was added to the pulp slurry to control the consistency) and also only one sample was needed (20 OD g total of pulp). Also, the agitator level was reduced to level three. The control was also “heat inactivated” in the same manner as the enzyme treated samples. The second control was an enzyme inactivated control (EI control), which used the same procedure as the enzyme treatments, with the exception that the enzyme was heat inactivated (microwave oven for four minutes) before mixing with pulp slurries. The samples (0, 30, 60, and 180 minutes) were “heat inactivated” in the same manner as the enzyme treatments. This control was used only for the 0.5 % enzyme concentration and its purpose was to determine whether the enzyme used retained activity after the four minute microwave heat shock (two minutes boiling).

Pulp Testing

Eight handsheets were prepared for each experimental condition according to TAPPI test method T 205 om-88, but instead of couching the fiber mat/double blotter paper/couch plate stack with five rolls, only three rolls were used and then the fiber mat that is attached to blotter paper was lifted up and placed on the bench with the fiber mat facing up. A round metal handsheet plate was placed on top of the round fiber mat and the couch plate was placed on top of this. The couch roll was then used to couch the assembly four additional times. The fiber mat (attached to round metal handsheet plate) was then peeled from blotter paper and the handsheet (still on round metal handsheet plate) was placed in a handsheet ring. All eight handsheet rings were stacked together with handsheets facing down and then placed a handsheet ring clamp. The sheets were then placed in a 50 % relative humidity and 23 °C environment to dry under restraint.

After the handsheets were dried, the basis weight, thickness, density, breaking length tensile strength, zero-span dry tensile and zero-span wet tensile strength were measured for the handsheets. All tests were conducted according to TAPPI test method T 220 om-88 and T 231 cm-96.

Short Span Compression Testing

This test was performed by using the protocol developed for the Lorentzen and Wettre STFI Compression Strength Tester, which is based upon TAPPI test method T 826 pm-86. The tests were conducted using two different samples. Initially, only a single paper sheet was placed in the instrument. However, it was determined that the method calls for a higher basis weight than a single handsheet could provide. Therefore, a second set of tests were conducted using two handsheets placed side by side. Little difference was found between the two samples on an indexed basis.

Drainage Testing

Enzymatically degraded pulp samples were tested according to Tappi method T 205 om-88 *The drainage rate of pulp*. This test was performed by taking pulp samples (1.2 oven dried grams for each enzyme concentration and incubation time sample) and placing them in a handsheet mold. The method calls for the mold to be filled and the fibers dispersed. Then the amount of time required for the water to drain the handsheet mold was measured. This method was used to evaluate the effect of fines generation on sheet drainage.

Fiber Morphology of Enzyme Treated Samples

An automated fiber analysis instrument [FQA (Fiber Quality Analyzer, Cybermetric Inc.)] was used to analyze the enzyme treated samples. This instrument evaluated the percent fines, kink index, curl index, and fiber length changes associated with increased enzyme concentration and incubation time. The FQA works by using a 10 ml sample of 0.02 % consistency pulp and diluting the sample to 600 ml. The machine withdraws the sample from the beaker and a camera, built into the machine, images the fibers. From these images the fiber properties are determined. The percent fines (length weighted) is calculated as: $\% \text{ Fines} = 100 \times (\sum n_i L_i) / L_T$, where n = number of fibers less than 0.2 mm, L_i = fines class midpoint length, and L_T = total fiber length. The kink index is calculated as: $\text{Kink Index} = [2N(21-45) + 3N(46-90) + 4N(91-180)] / L_{\text{total}}$, where N is the number of fibers and the numbers in parentheses are the range of fiber angles, so for example: two times the number of fiber angles that are between 21° and 45° is the $2N(21-45)$ part of the equation. And length weighted length, $L_w = (\sum n_i L_i^2) / (\sum n_i L_i)$.

2.3 Results and Discussion

Low Density Handsheets

The first experimental goal was to produce papers with a density below 0.4 g/cm^3 (400 kg/m^3) using a modification of TAPPI test method T 205 om-88. The patent which this research seeks to better understand (U.S. Patent 6,146,494) is only applicable to paper sheets with density below 400 kg/m^3 . Tables 1-5 show that the modified handsheet procedure can be used to produce low density ($<400 \text{ kg/m}^3$) handsheets.

Table 1: 0.05 % Enzyme concentration handsheet densities

Reaction Time (minutes)	Treated Density (kg/m^3)
0	274
30	265
60	278
180	281

Table 2: 0.1 % Enzyme concentration handsheet densities

Reaction Time (minutes)	Treated Density (kg/m^3)
0	288
30	291
60	266
180	285

Table 3: 0.5 % Enzyme concentration handsheet densities

Reaction Time (minutes)	Treated Density (kg/m^3)
0	292
30	274
60	301
180	341

Table 4: 1.0 % Enzyme concentration handsheet densities

Reaction Time (minutes)	Treated Density (kg/m³)
0	282
30	275
60	302
180	342

Table 5: EI control and NE control handsheet densities

Reaction Time (minutes)	EI Control Density (kg/m³)	NE Control Density (kg/m³)
0	307	287
30	293	
60	282	
180	293	

Replication of Major Patent Claims

Figure 1 shows the dry zero span tensile strength results for the low-density handsheets of the controls and enzyme treated samples as a function of incubation time. The control without the enzyme addition (NE control) showed the highest dry zero span tensile strength, while the enzyme treated samples clearly had lower the zero span tensile strength that progressively increased as the incubation time increased. The effect of enzyme dosage is also evident in the figure. Higher enzyme dosage led to a more rapid decrease in the dry zero-span tensile strength. The heat inactivated enzyme control (EI control) also exhibited a loss of dry zero span tensile strength although not as severe as the active enzyme. This indicates that the heat inactivated enzyme most likely retains a limited amount of activity after heat shock. Also, it is noted that for the EI control, the zero minute sample is lower than both the 0.05 % and 0.1 % enzyme dosages. This is due to the EI control using a 0.5 % heat shocked enzyme dosage. Thus, the zero minute incubation time for the EI control should be closer to the zero minute for the 0.5 % enzyme dosage. The zero minute for the EI control is actually well above the 0.5 %

enzyme dosage, but this may be attributed to the fact that the 0.5 % enzyme dosage did not have the fines removed. Furthermore, tracking zero span tensile strength for the zero minute incubation time indicates that the enzyme retains a limited amount of activity even after heat shock as can be seen in the decrease in the dry zero span tensile strength as enzyme dosage increases.

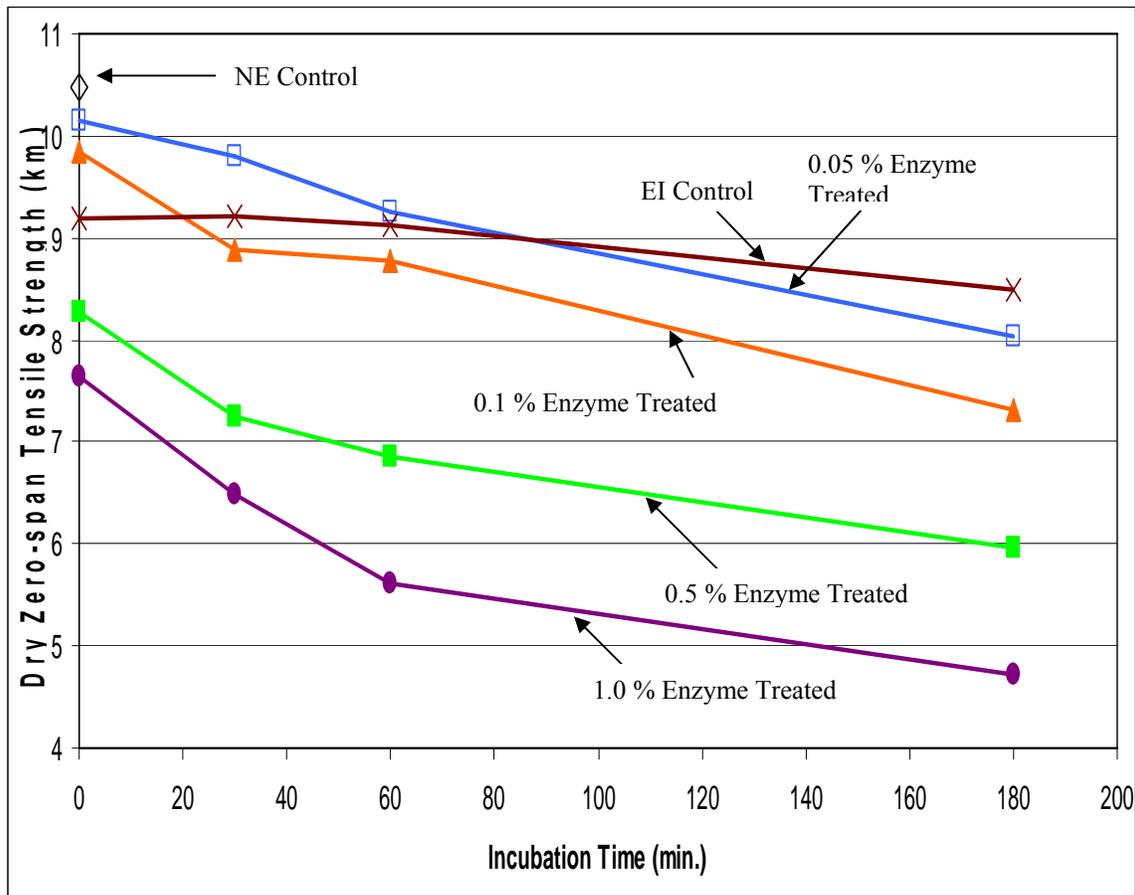


Figure 1: The dry zero span tensile strength of low density handsheets for enzyme treated samples, heat inactivated control and no enzyme control

Figure 2 shows the wet zero span tensile strength for the low-density handsheets. The trends in results are similar to the dry zero span tensile strength, cf. Figure 1. The values of the wet zero span tensile strength were considerably lower than dry zero span tensile strength. Again, the control without any enzyme addition (NE control) showed

the highest wet zero span tensile strength, while the active enzyme samples clearly lowered the wet zero span tensile strength progressively as the reaction proceeded and also as the enzyme dosage increased. The heat inactivated enzyme also exhibited a loss of wet zero span tensile strength although again, although not as severe as the active enzyme results. This is also an indication that the heat inactivated enzyme most likely retains a limited amount of activity after heat shock. Also, it can be noticed from looking at the EI control zero minute sample that its zero span tensile value is lower than that of the 0.05 % and 0.1 % enzyme concentration samples at zero minutes. This is attributed to the EI control using a 0.5 % heat shocked enzyme dosage, and because this enzyme retains a limited amount of activity, it is lower in wet zero span tensile strength.

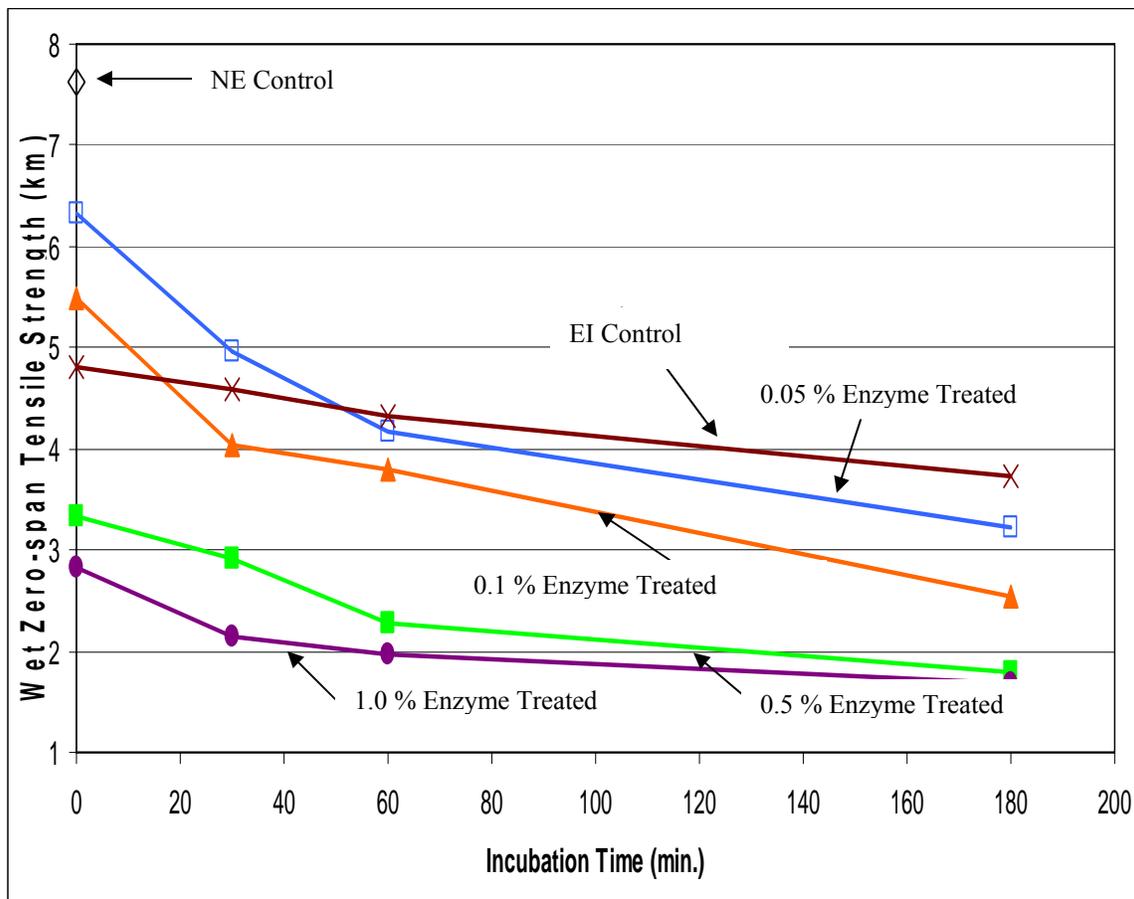


Figure 2: The wet zero span tensile strength of low density handsheets for enzyme treated samples, heat inactivated control and no enzyme control

Figure 3 shows the percentage drop in the dry zero span tensile strength as a function of incubation time when compared to the NE and IE controls. That is related to this research (U.S. Patent 6,146,494) claims that the modified cellulosic fibers should have a dry zero span tensile strength index that is at least about 35 % less than the dry zero span tensile index of the corresponding unmodified cellulosic fibers. In particular, the percentage drop should be compared to the NE control. It was found that it is possible to meet this claim at incubation times of about 60 minutes. The results for the 0.5 % enzyme concentration tests are only shown in the figure, because the EI control used the same enzyme concentration (0.5 %), and it would not be reasonable to compare the EI control results with the other enzyme concentrations. The results for the other enzyme dosages are compared to the NE control later in this work.

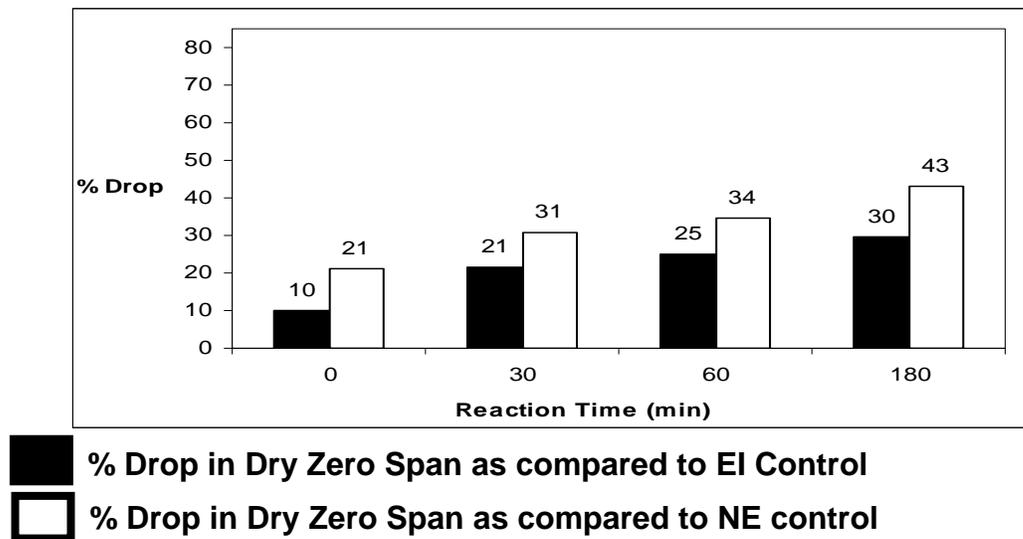


Figure 3: Patent claim #3 reproduction results (0.5 % enzyme concentration)

Figure 4 shows the percentage drop in the wet zero span tensile strength for the 0.5 % enzyme dosage when compared to the NE and EI controls. The patent claims (U.S. Patent 6,146,494) that modified cellulosic fibers should have a wet zero span tensile strength index that is at least about 70 % less than the wet zero span tensile strength index of the corresponding unmodified fibers. This decrease is based on the NE control. It was

found that it is possible to meet this claim at the incubation time of 60 minutes. The results for the 0.5 % enzyme concentration tests are only shown in this figure, because the EI control used the same enzyme dosage (0.5 %) as the enzyme treated samples, and it would not be reasonable to compare the EI control results with the other enzyme concentrations. The results for the other enzyme dosages are shown later.

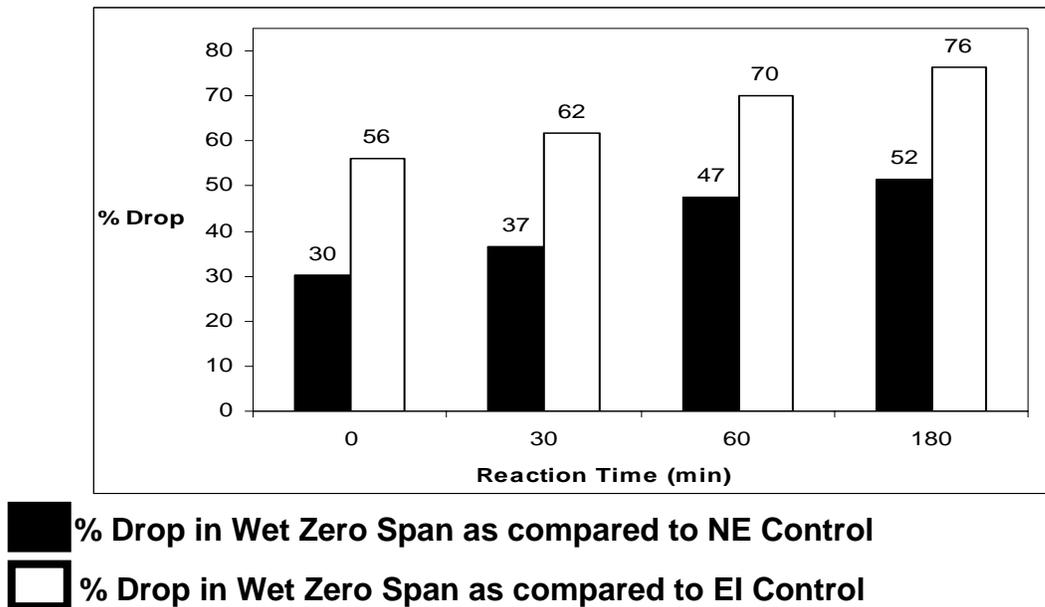


Figure 4: Patent claim #4 reproduction results (0.5 % enzyme concentration)

Table 6 and Figure 5 show the comparison in percent drop in dry zero span tensile strength between the different enzyme concentrations for extended incubation times. For completing claim number three ($\geq 35\%$ drop in dry zero span compared to NE control), it appears that this goal is achieved faster with increased enzyme concentration and that at a 0.5 % enzyme dosage one hour of incubation time is need to reach this goal.

Table 6: Patent claim #3 reproduction results (% drop in dry zero span tensile strength for treated as compared to NE control)

	0.05 % enzyme concentration	0.1 % enzyme concentration	0.5 % enzyme concentration	1.0 % enzyme concentration
Reaction Time (min)	% Drop	% Drop	% Drop	% Drop
0	3	6	21	27
30	6	15	31	38
60	12	16	34	46
180	23	30	43	55

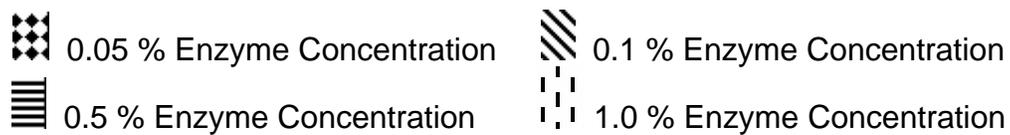
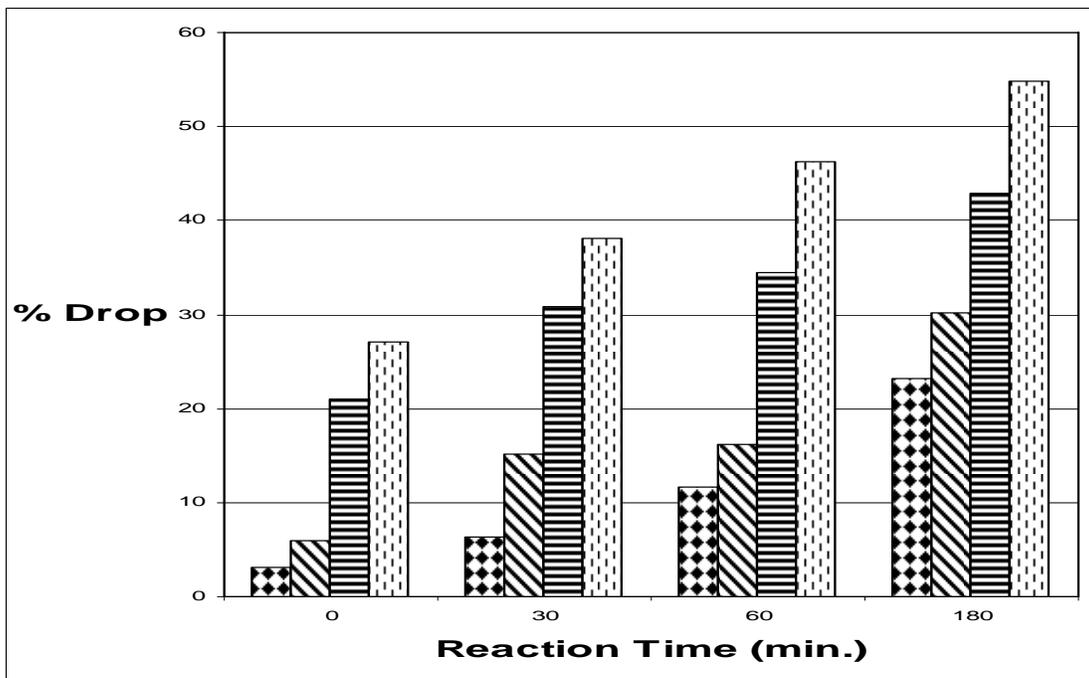


Figure 5: Patent claim #3 reproduction results (% drop in dry zero span tensile strength for treated as compared to NE control)

Table 7 and Figure 6 show the comparison in percent drop in wet zero span tensile strength between the different enzyme concentrations for extended incubation times. For completing claim number four ($\geq 70\%$ drop in wet zero span compared to NE control), it appears that this goal is reached faster with increased enzyme dosage and that at a 0.5 % enzyme concentration and one hour of incubation time the claim is satisfied.

Table 7: Patent claim #4 reproduction results (% drop in wet zero span tensile strength for treated as compared to NE control)

	0.05 % enzyme concentration	0.1 % enzyme concentration	0.5 % enzyme concentration	1.0 % enzyme concentration
Reaction Time (min)	% Drop	% Drop	% Drop	% Drop
0	17	28	56	63
30	35	47	62	72
60	45	50	70	74
180	58	67	76	78

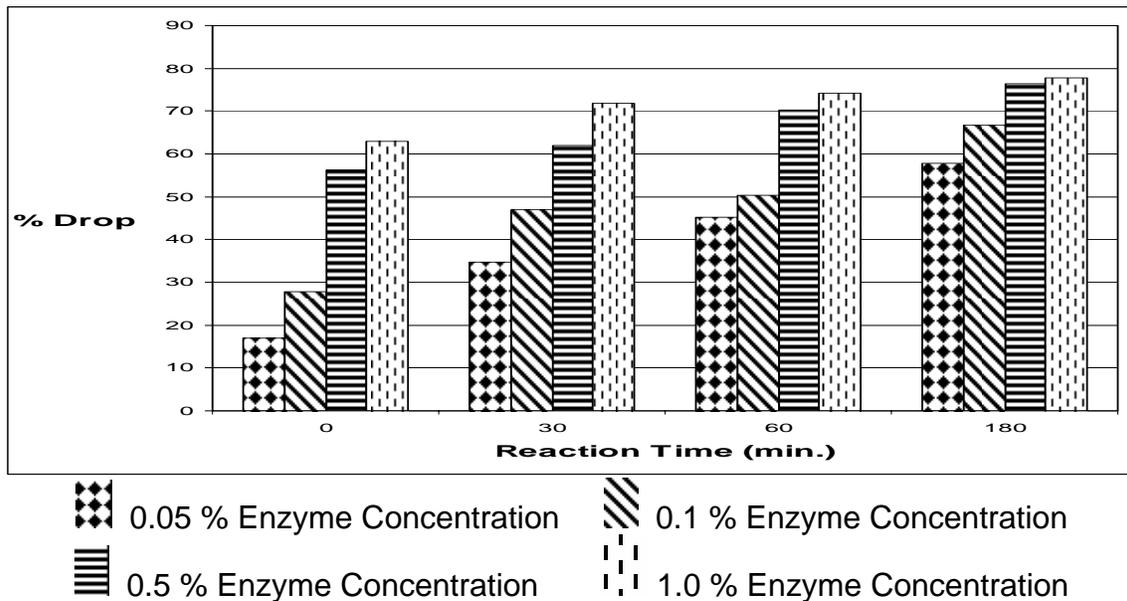
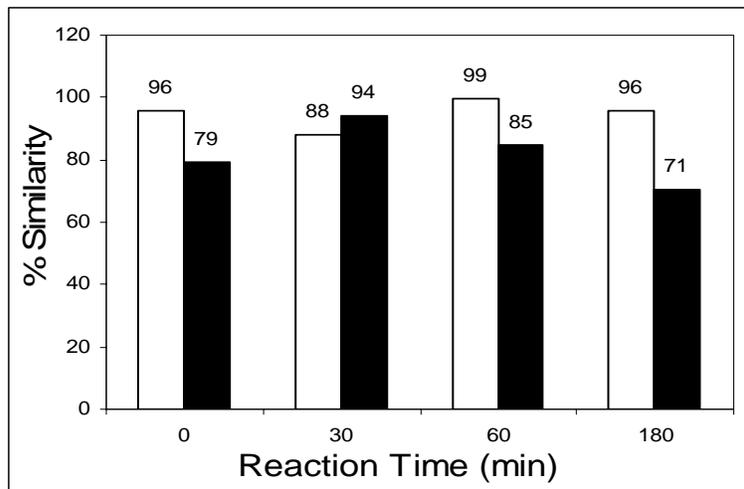


Figure 6: Patent claim #4 reproduction results (% drop in wet zero span tensile strength for treated as compared to NE control)

Figure 7 shows the results for repeating the U.S. Patent 6,146,494 claim that the modified fibers that form a fibrous structure (handsheet) should have a dry tensile index that is at least as great (90 % similar) as the dry tensile index of a handsheet made from the corresponding unmodified fibers. From the test results, it was found that we came very close to achieving this claim, more-so for the comparison between the treated samples and the EI control than the comparison between treated samples and the NE control. The results for the 0.5 % enzyme concentration tests are only shown in this table and graph, because the EI control used the same enzyme concentration as the enzyme treated samples, and it would not be reasonable to compare the EI control results with the other enzyme concentrations. The results for the other enzyme concentrations will be shown in other graphs and figures.



(Treated/EI Control) % Dry Z-span Tensile
 (Treated/NE Control) % Dry Z-span Tensile

Figure 7: Patent claim #2 reproduction results (0.5% enzyme concentration)

Table 8 and Figure 8 show the percent of dry tensile strength retained after fiber modification. The patent (U.S. Patent 6,146,494) claims that the modified fibers that form a fibrous structure (handsheet) should have a dry tensile index that is at least as great (90 % similar) as the dry tensile index of a handsheet made from the corresponding

unmodified fibers. Only the comparison between the enzyme treated samples and the NE control is shown, because the EI control used the 0.5 % enzyme dosages, which would not be relative to the other treated enzyme dosages. For this claim, it does not appear that there is a relationship between this percent similarity and the reaction time or enzyme dosage. But from looking at the results, it does appear that most of the samples come close to or reach the percent similarity claimed in the patent.

Table 8: Patent claim #2 reproduction results (% similarity between treated sample dry tensile index and NE control dry tensile index)

	0.05 % enzyme concentration	0.1 % enzyme concentration	0.5 % enzyme concentration	1.0 % enzyme concentration
Reaction Time (min)	% Similarity	% Similarity	% Similarity	% Similarity
0	88	86	79	85
30	83	91	94	95
60	85	115	85	83
180	71	67	71	84

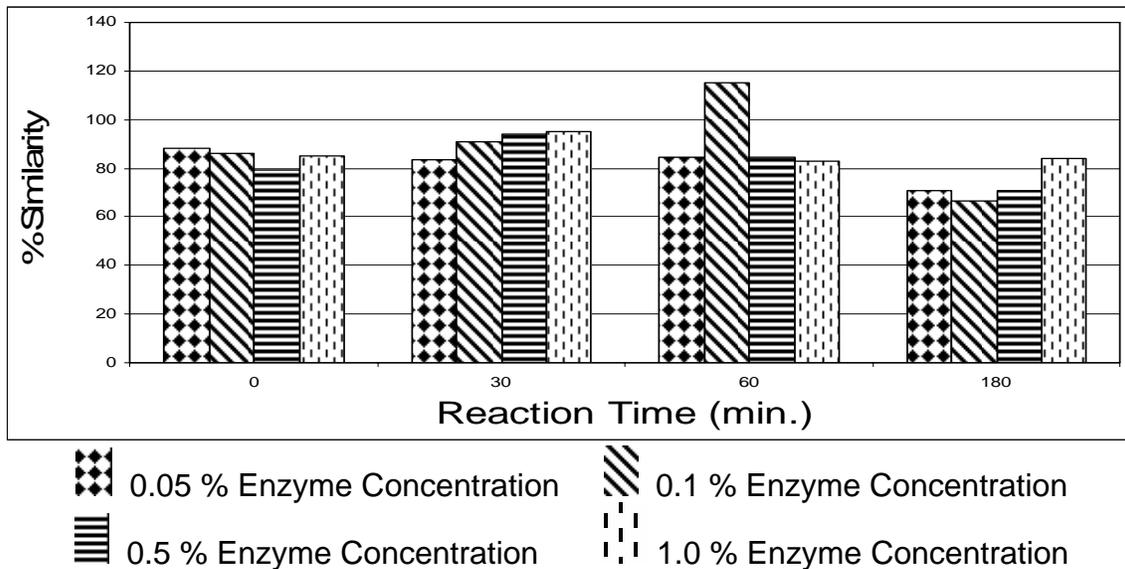


Figure 8: Patent claim #2 reproduction results (% similarity between treated sample dry tensile index and NE control dry tensile index)

Figure 9 show the results of repeating the U.S. Patent 6,146,494 claim that modified cellulosic fibers should exhibit a ratio of dry zero span tensile index to wet zero span tensile index from about 1.5 to about 3. Overall, the test results show that for the most part it was possible to repeat this claim for Trial #1 and Trial #2. From the looking at the results, it is of interest that the ratio is lower for the two controls as compared to the enzyme treated samples, especially for the NE control. The results for the 0.5 % enzyme concentration tests are only shown in this table and graph, because the EI control used the same enzyme concentration as the enzyme treated samples, and it would not be reasonable to compare the EI control results with the other enzyme concentrations. The results for the other enzyme concentrations will be shown in other graphs and figures.

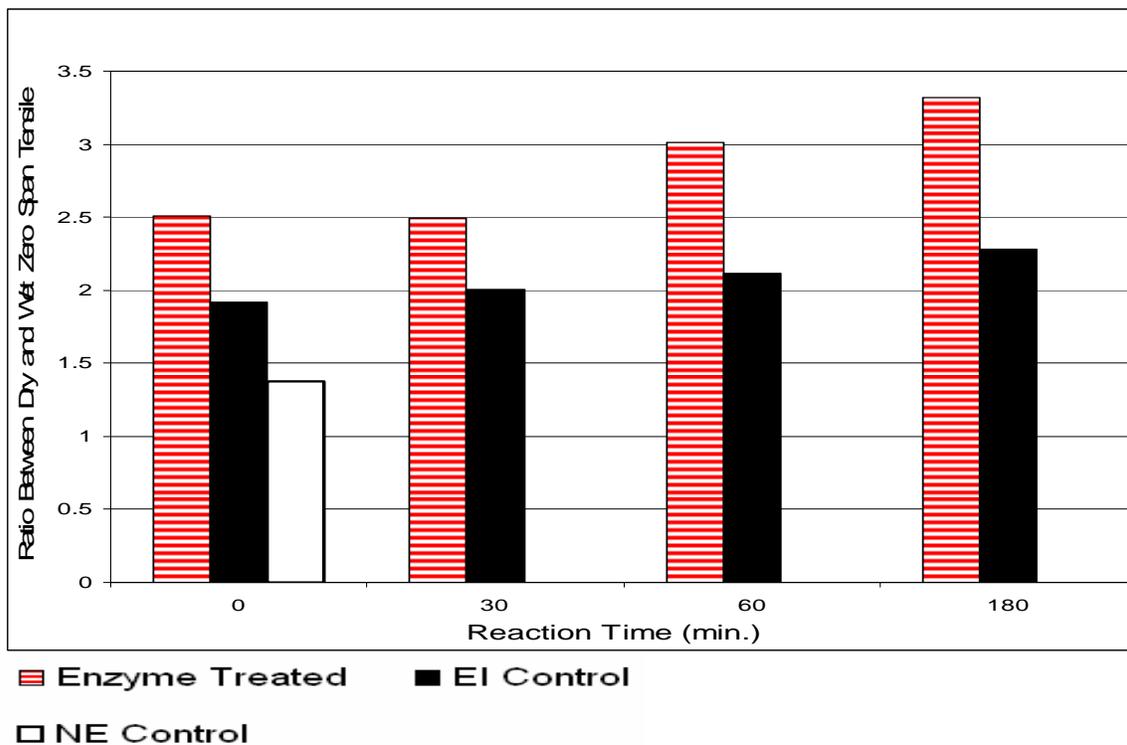


Figure 9: Patent claim #5 reproduction results (averaged for 2 trials)

Table 9 and Figure 10 show the results of repeating the U.S. Patent 6,146,494 claim that modified cellulosic fibers should exhibit a ratio of dry zero span tensile index

to wet zero span tensile index from about 1.5 to about 3 for the different enzyme concentrations. Only the comparison between the enzyme treated samples and the NE control is shown in these results, because the EI control used the 0.5 % enzyme concentration, which would not be relative to the other treated enzyme concentrations. For completing this claim, it appears that all of the samples, except for the NE control, have a ratio higher than 1.5, with only the 180 minute 1.0 % enzyme concentration sample being higher than 3. Also, the NE control ratio is very close to meeting the 1.5 ratio. It also appears that the ratio runs higher for the higher enzyme concentration samples.

Table 9: Patent claim #5 reproduction results (ratio between dry zero span and wet zero span)

	0.05 % enzyme conc.	0.1 % enzyme conc.	0.5 % enzyme conc.	1.0 % enzyme conc.	NE Control
Reaction Time (min)	Ratio	Ratio	Ratio	Ratio	Ratio
0	1.6	1.8	2.5	2.7	1.4
30	2.0	2.2	2.5	3.0	
60	2.2	2.3	3.0	2.9	
180	2.5	2.9	3.3	2.8	

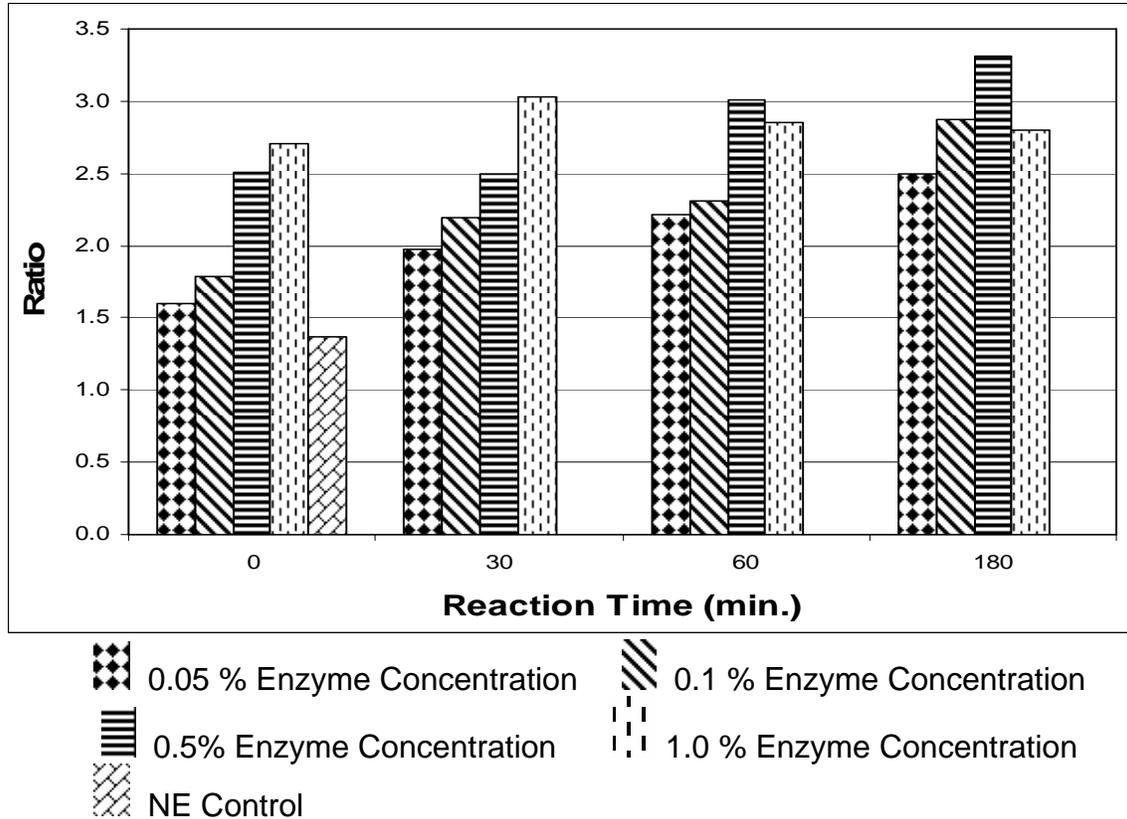


Figure 10: Patent claim #5 reproduction results (ratio between dry zero span and wet zero span)

Measurements of Short Span Compressibility

The modification of softwood fibers to enhance the softness of the tissue sheet can be related to two factors. While improving the fiber flexibility should indeed improve the out of plane compressibility, changes in the in-plane compressibility could result in significant changes in the creping performance of the paper sheet. Therefore, the short span compressive strength of the paper sheet was measured.

Tables 10-12 and Figures 11-13 show the results from the STFI compressibility testing of the handsheet samples for the different enzyme concentrations and the NE control sample. Table 14 and Figure 11 show the results obtained from using single ply handsheet test strips (60-65 g/m² range). The instructions for the STFI compressibility

tester indicated that basis weights should be between 100-400 g/m². To keep with the standards that had been used so far in the experiments, handsheets were not altered to increase the basis weight to the 100-400 g/m² range. So tests were performed using double ply test strips in the compression tester so that the basis weight would be double and fall in the acceptable basis weight range, and the results for these tests can be seen in Table 15 and Figure 12. Also the average of the results from the single and double basis weight tests can be seen in Table 16 and Figure 13. For all of the single basis weight tests, five test strips were used for each sample and each strip was tested on three different areas. For all of the double basis weight tests, three double strips were used for each sample and each double strip was tested on three different areas.

From the results of the single basis weight STFI compression tests, it appears that the compression strength is significantly higher for the higher enzyme concentrations (0.5 % and 1.0 %) than for the lower enzyme concentrations (0.05 % and 0.1 %), and also the compression strength has an overall rise from the zero minute sample to the 180 minute sample for all of the enzyme concentrations. This same effect is seen for the double basis weight and average (single and double) basis weight results also. For the single basis weight results, it can be seen that there is a significant drop in compression strength for the higher enzyme concentrations from the zero minute sample to the 30 minutes sample and that after that, the compression strength rises gradually and significantly for the remainder of the incubation time. This might be because the enzymes are degrading the dislocation and non-dislocation areas at first and creating microfibrils or fines, which would increase compressibility (lowering compression strength) and then the enzymes act to clean away the microfibrils or fines that were created at first to make a more smooth but less compressible fiber (increasing compression strength). The 0.5 % enzyme concentration 180 minute sample has a higher compression strength than that of the 1.0 % enzyme concentration 180 minute sample and this could be due to the fact that the 0.5 % enzyme concentration was not washed so there are more fines, resulting in more enzymatic degradation of the fines (higher surface for enzymes to attach to) compared to the fibers, leaving the fiber more intact and less compressible (higher compression

strength). This is seen for the single, double and average (single and double) basis weight results.

Table10: STFI compressibility strength (klbf-ft/lb) results for single basis weight testing

	0 min (average)	30 min (average)	60 min (average)	180 min (average)
NE Control	2.0			
0.05 % Enzyme Concentration	2.2	2.5	2.6	2.9
0.1 % Enzyme Concentration	2.3	2.3	2.1	3.0
0.5 % Enzyme Concentration	2.7	2.5	2.6	3.5
1 % Enzyme Concentration	2.6	2.4	2.8	3.4

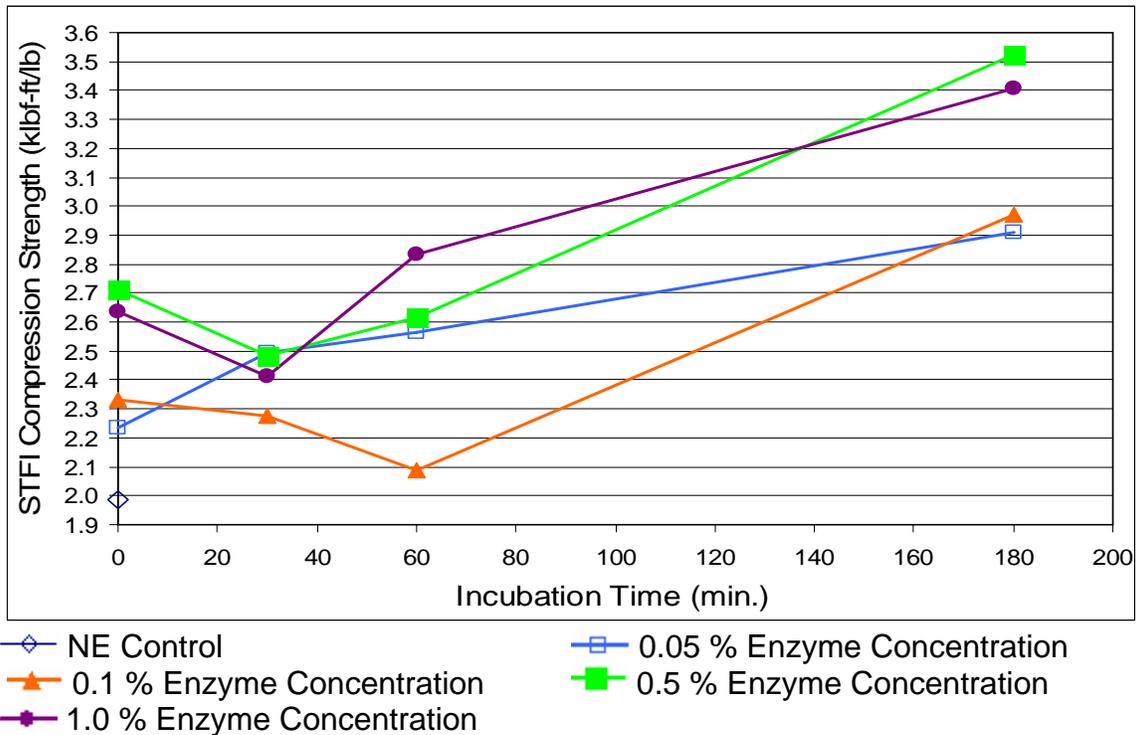


Figure 11: STFI compressibility strength (klbf-ft/lb) results for single basis weight testing

Table 11: STFI compressibility strength (klbf-ft/lb) results for double basis weight testing

	0 min (average)	30 min (average)	60 min (average)	180 min (average)
NE Control	1.7			
0.05 % Enzyme Concentration	1.9	1.9	1.9	2.1
0.1 % Enzyme Concentration	1.7	1.8	1.6	2.3
0.5 % Enzyme Concentration	2.2	1.7	2.1	2.7
1 % Enzyme Concentration	1.9	2.2	2.5	2.6

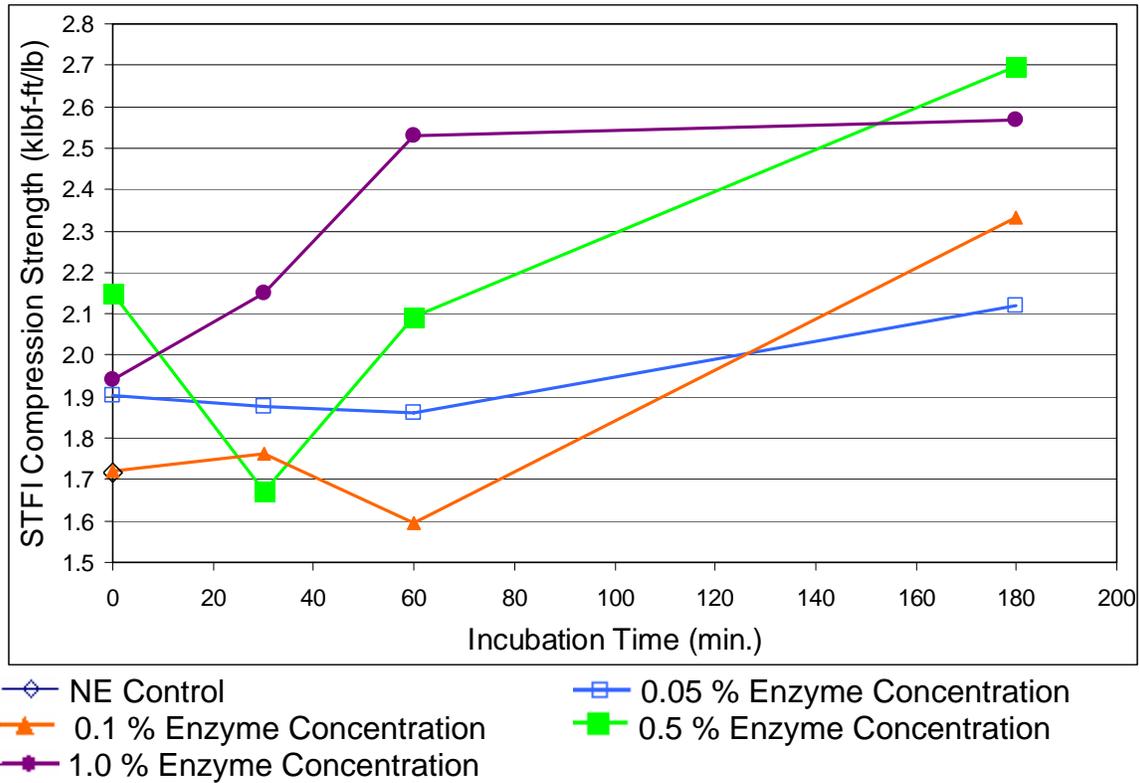


Figure 12: STFI compressibility strength (klbf-ft/lb) results for double basis weight testing

Table 12: STFI compressibility strength (klbf-ft/lb) results for averaging single and double basis weight testing

	0 min (average)	30 min (average)	60 min (average)	180 min (average)
NE Control	1.9			
0.05 % Enzyme Concentration	2.1	2.2	2.2	2.5
0.1 % Enzyme Concentration	2.0	2.0	1.8	2.7
0.5 % Enzyme Concentration	2.4	2.1	2.4	3.1
1 % Enzyme Concentration	2.3	2.3	2.7	3.0

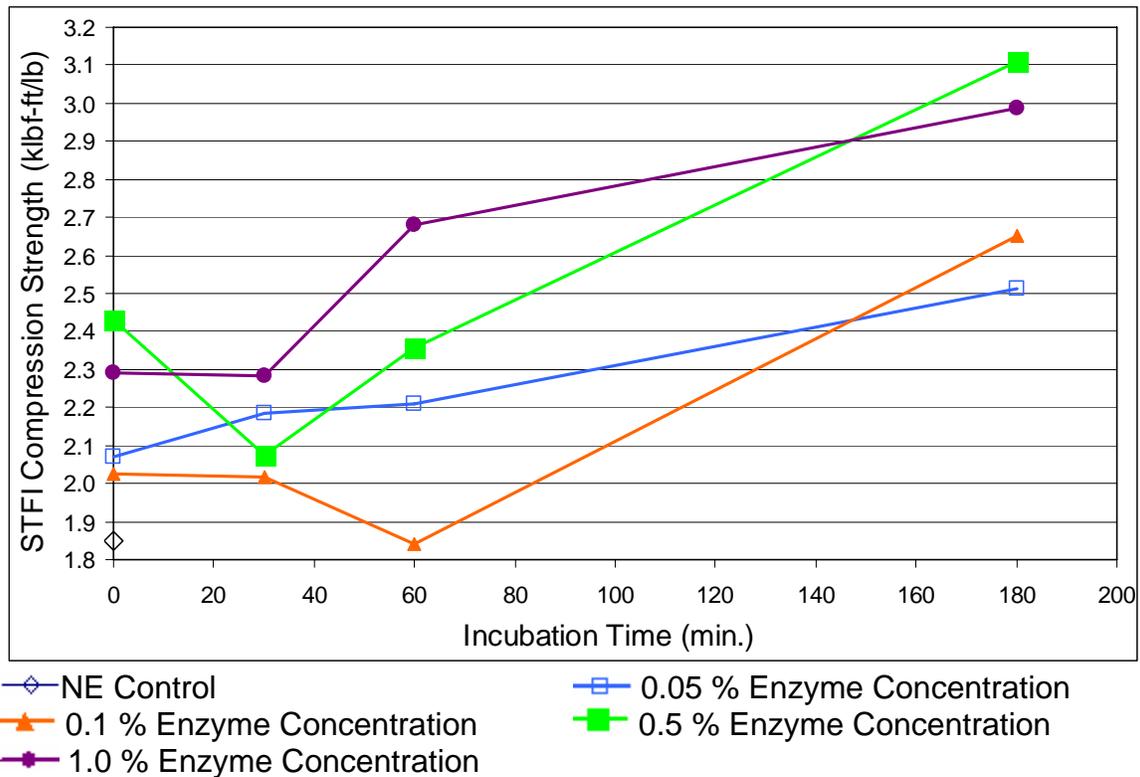


Figure 13: STFI compressibility strength (klbf-ft/lb) results for averaging single and double basis weight testing

The effect that sample handsheet density had on affecting STFI compression strength was studied and the results can be seen in Figure 14. In this graph, it appears that there seems to be a close linear correlation between STFI compression strength (average of single and double basis weight) and handsheet density for the higher enzyme concentration samples (0.5 % and 1.0 %), with the exception of the first data (zero minute samples) in each of those lines. The same is not true for the lower enzyme concentrations (0.05 % and 0.1 %), where no correlation at all could be seen. These results seem to indicate that sheet density has a close relation to STFI compression strength for higher enzyme concentrations and this could possibly be why the increase in STFI compression strength over extended incubation time is seen in Figures 11-13. Also, the handsheets for the higher concentrations are denser with the higher incubation times because of the more fines generated as seen from the results in Figure 15.

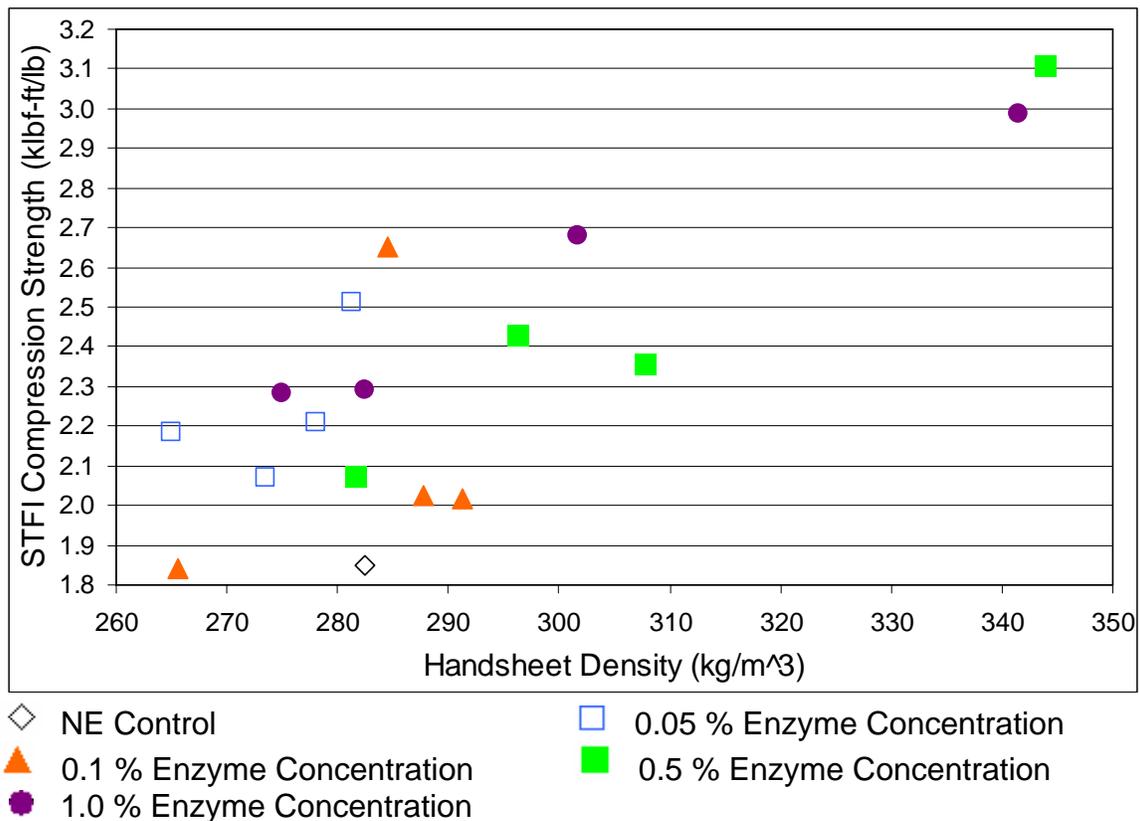


Figure 14: STFI compression strength (klbf-ft/lb) vs. handsheet density (kg/m³) for different enzyme concentrations and incubations times (min.)

Effect of Enzyme Treatment on Drainage Rate

To validate the results obtained from the STFI compression strength tests, drainage tests of pulp samples for the different enzyme concentrations and incubation times were performed. As mentioned earlier, drainage time tests can correlate to increased fines content because if the amount of fines increases, then there are fewer areas for water to drain through a pulp mass since the fines would restrict water flow. The drainage times for the 0.5 % enzyme concentration samples are significantly higher than those of the other enzyme concentrations and this could be due to the fact that these pulp samples were not washed so they contain more fines, which would mean that more time would be required for the water to drain through the pulp as described earlier. The graph in Figure 15 does not include the drainage times for the 0.5 % enzyme concentration since they would seriously skew the results for the other enzyme concentration samples, but the drainage times are included in Table 13 along with the other enzyme concentrations drainage times. From the results, it appears that the higher enzyme concentrations have significantly higher drainage times and that their drainage times increase as incubation time increases, indicating that more fines are being created. The curves seen in Figure 15 closely resemble the ones seen in Figures 11-13, indicating that fines have a close relation to the compression strength results.

Table 13: Drainage times (seconds) for different enzyme concentrations and incubation times (minutes)

	0.05 % Enzyme Conc.	0.1 % Enzyme Conc.	0.5 % Enzyme Conc.	1.0 % Enzyme Conc.	Control #2
Treated Time (min)	Drainage Times (sec)	Drainage Times (sec)	Drainage Times (sec)	Drainage Times (sec)	Drainage Times (sec)
0	3.82	3.81	7.43	3.78	3.76
30	3.80	3.77	7.44	3.76	
60	3.75	3.69	7.39	3.86	
180	3.78	3.76	7.62	3.89	

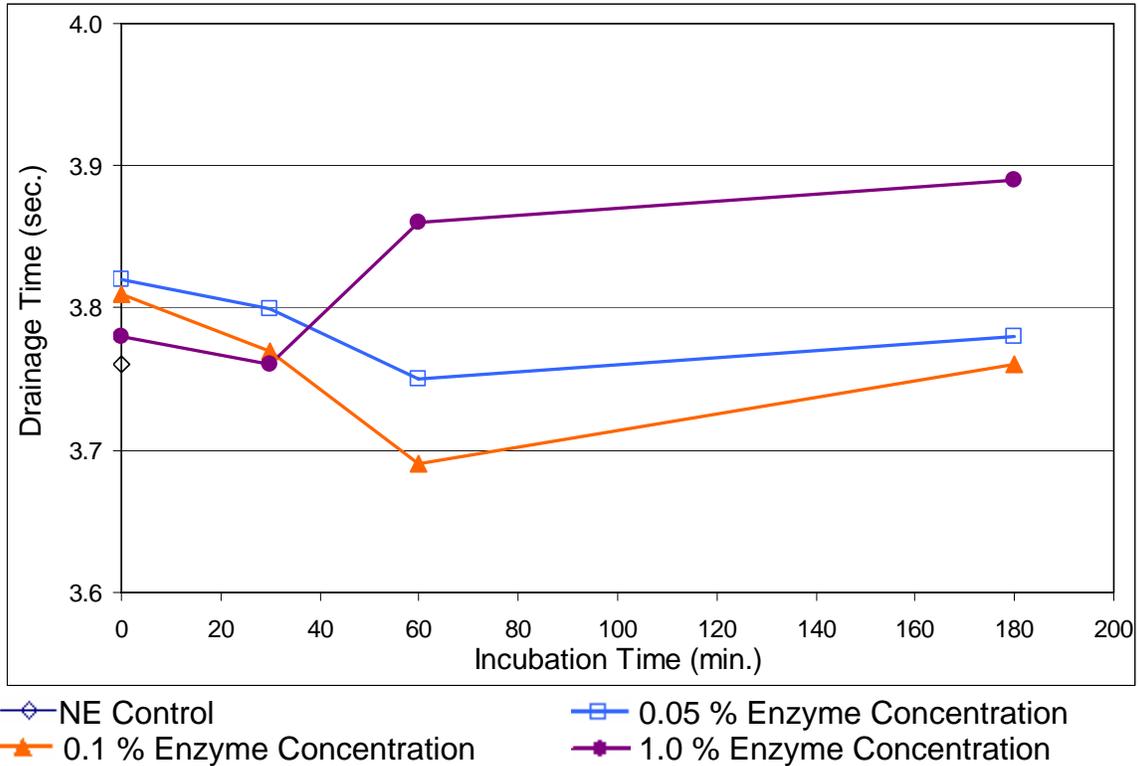


Figure 15: Drainage times (seconds) for different enzyme concentrations and incubation times (minutes)

Fiber Morphology Changes Associated with Enzyme Treatment

The Fiber Quality Analyzer (FQA) was used to characterize the changes in the fiber morphology as a function of enzyme incubation. In Figure 16 it can be noticed that the fines increase significantly over the incubation time for the higher enzyme concentrations (0.5 % and 1.0 %), but they do not continuously rise for the lower enzyme concentrations (0.05 % and 0.1 %). This data indicates that no general pattern can be detected. In the lower enzyme concentrations, it can be seen that there is a significant rise in fines initially but then there is a significant drop in fines for the three hour sample. These results follow closely with the drainage time results indicating that percent fines does increase significantly overall during the complete incubation time and that this increase in percent fines increases drainage times because of the increased restriction of

water flow from fines build up. The drop in percent fines seen for the higher enzyme concentrations at beginning of the incubation period could be due to the enzymes eating away the fiber fragments (fines) created after enzyme initially break up fibers at dislocation areas, and then the percent fines begin to rise because the enzymes are continuously eating away at fibers on the dislocation areas creating many fragments (fines). This drop is even more noticeable for the 0.5 % enzyme concentration samples because they were not washed, meaning that there were fines to be eaten up by enzyme degradation at the beginning resulting in the initial drop in percent fines, but after the initial fines were eaten up, the enzyme began to absorb more on the dislocation areas of the intact fibers, creating more fragments (fines).

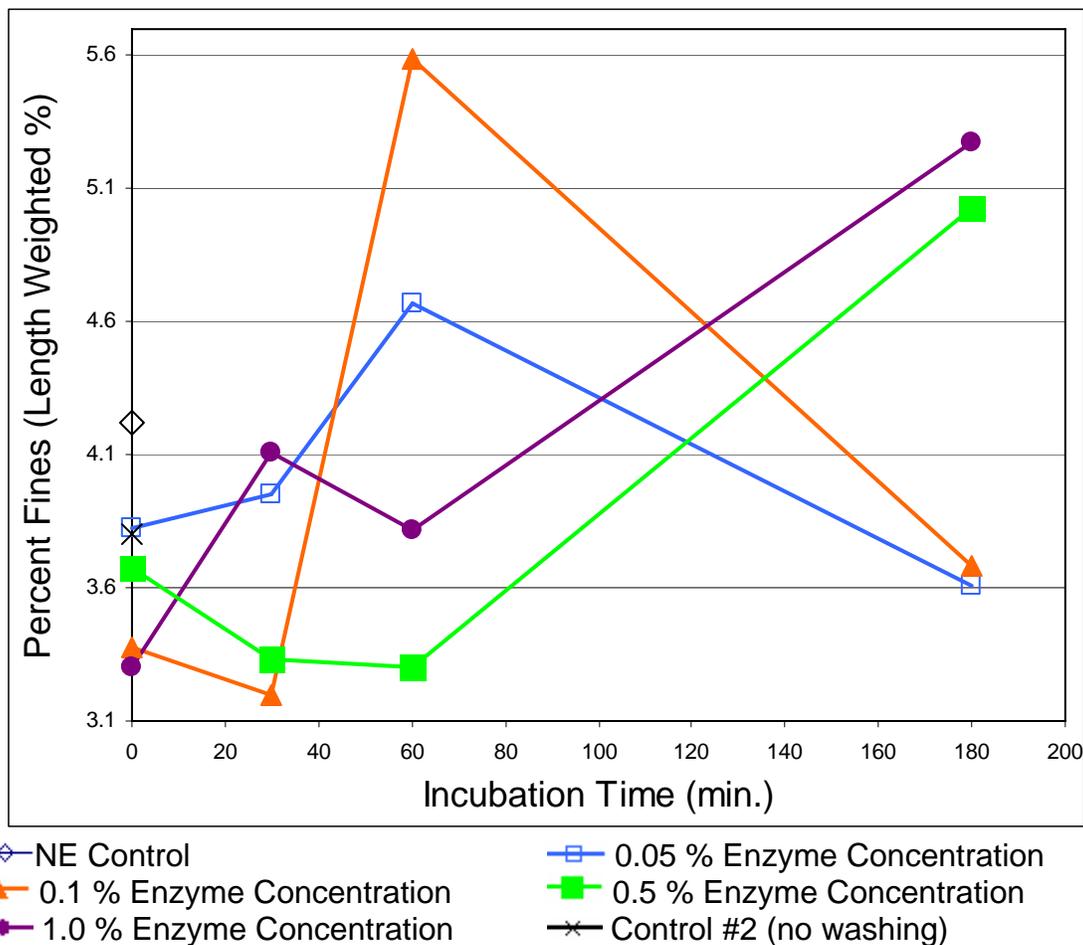


Figure 16: FQA results: percent fines (length weighted %) for different enzyme concentrations and incubation times (min.)

Figure 17 shows the FQA results for mean fiber length (length weighted mm) for the different enzyme concentrations and incubation times (minutes). From looking at the results, it appears for the higher enzyme concentration samples that the mean fiber length drops significantly over the incubation period, and this drop is more noticeable for the 1.0 percent concentration samples, which would be expected. This effect is not seen for the lower enzyme concentrations, as they remain relatively constant. These results reflect the results seen in Figure 15 for the percent fines, indicating that the intact fibers are breaking up at the dislocation areas, resulting in more fiber fragment (fines) and shorter fibers.

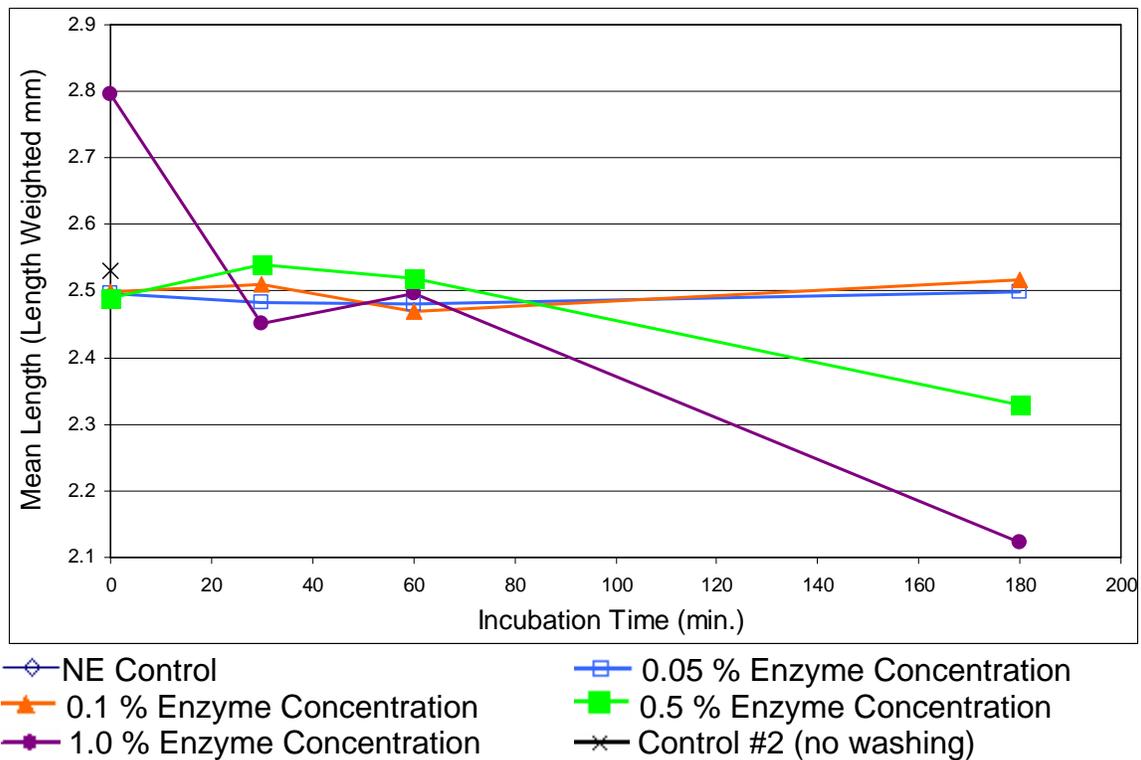


Figure 17: FQA results: mean fiber length (length weighted mm) for different enzyme concentrations and incubation times (min.)

Figure 18 shows the FQA results for mean kink index (1/mm) for different enzyme concentrations and incubation times. It can be noticed for all the enzyme concentrations, except for the lowest one (0.05 %), that there is a significant jump in kink

index in the first 30 minutes and then the indexes continuously drop over the rest of the incubation period. This is not seen in the lowest enzyme concentration (0.05 %), where the indexes drop till the 60 minute mark and then they begin to rise through the rest of the incubation period. The sudden rise seen for the other enzyme concentrations is probably due to the enzymes weakening the fibers at the dislocation areas and creating kinks, and then as the incubation period extends, the enzyme action proceeds to break up the fiber at these dislocation/kink areas and the kink that used to be on the fiber is no longer there, because the fiber is broken, resulting in a lower mean kink index as seen for the extended incubation times. The effect seen for the 0.05 % enzyme concentration is probably due to the very weak fiber kinks being eaten up by enzymes at first, resulting in the drop in mean kink index and then as the incubation period extends, the dislocations are turned into kinks and the mean kink index increases. Also, the significant difference between the 0.5 % enzyme concentration samples and the other enzyme concentration samples is probably due to the fact that the 0.5 % samples were not washed and still had many fines.

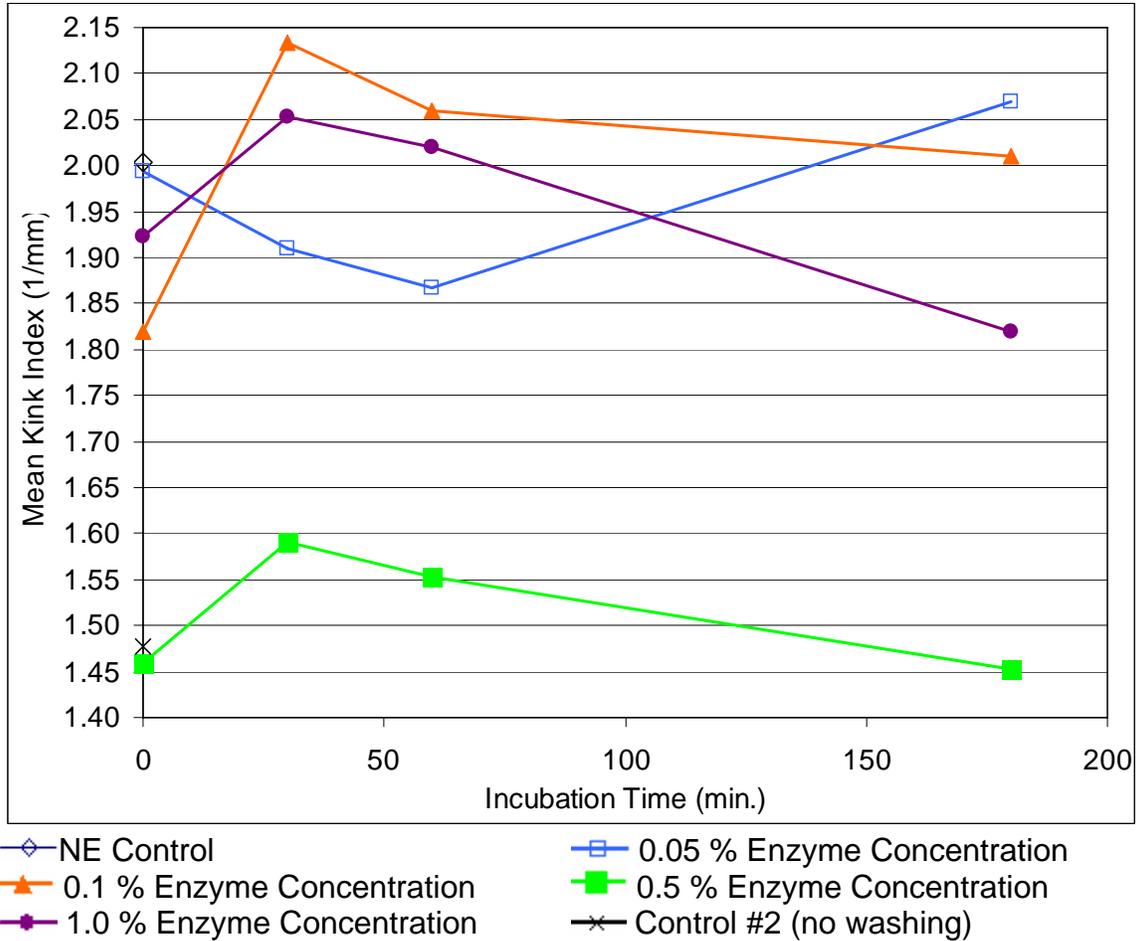


Figure 18: FQA results: mean kink index (1/mm) for different enzyme concentrations and incubation times (min.)

2.4 Conclusions

The major patent claims were repeated in the laboratory (U.S. Patent 6,146,494). Tissue grade handsheets with densities below 400 kg/m^3 were treated with enzymes and showed a gradual decrease in wet and dry zero-span tensile strengths. Enzyme concentration and incubation time affected the amount of change in the wet and dry zero span tensile strengths. Higher enzyme concentrations showed a faster change in these properties. The claims were generally satisfied when an enzyme dosage of 0.5 % was used for an incubation time of at least 60 minutes. Other combinations of enzyme dosage

and incubation could also be used to achieve the desired changes in the paper properties to satisfy the major patent claims.

The effect of enzyme degradation on a number of other fiber properties was also examined. The drainage time was found to increase for the higher enzyme concentrations as the incubation time increased. The fines content was only effect marginally and no distinct pattern was observed. The fiber kinks were in general found to first increase and then decrease as degradation of the fibers progressed. This was attributed to the initial weakening of the fiber at dislocations allowing the fiber to bend more easily resulting in an increase in the kink index. Eventually, as the fibers are degraded, the fiber length decreases. The fibers are “cut” by the enzymes at the dislocation (kinks) and thus kinks are eliminated form the fibers. Thus, a decrease in the kink index is then observed. Furthermore, this pattern of change of kink index indicates that no new kinks are formed by enzymatic degradation. The short span compression tests of the sheets showed that increased enzyme degradation results in higher short span compression strength. This was tied to the resulting increase in sheet density associated with enzyme degradation. This is an indication that fiber flexibility increases.

Chapter 3

Localized Fiber Enzyme Treatment for Fiber Modification

3A Characterization of Enzyme Absorption on Softwood Fibers: Isolation of Enzyme Activity to Fiber Dislocation Areas

3A.1 Introduction

In this section, the results from a study to characterize and assess areas of enzyme absorption on softwood fibers are discussed. As mentioned earlier in Chapter 2, enzyme technology is a versatile tool for modifying the fibers, since it has a tendency to degrade specific components [Buchert, J., et al., 1996]. It has been hypothesized that enzymes attach to and degrade on fiber defect areas, such as dislocations (notches), and kinks. If this is true, the enzymatic action on the fibers should create and/or enhance localized notches in the fibers, which serve as “hinge points” around which the fibers can flex.

A compressibility model developed by Pawlak [Pawlak, J.J., 2001] suggests that by creating these localized hinge points, the coarseness of the fiber is changed very little and this allows for the compressibility of the fiber structure to be increased without reducing the density of the structure. If the tensile strength of the fiber network is much lower than the fiber strength, then reducing the fiber strength should have little or no impact on the sheet tensile strength, as the bonding remains the same. The net result of engineering such a structure would be a softer, more compressible product, with the same tensile strength as the original product, which is the main goal of the Procter and Gamble patent.

Since the project proposal focuses on developing the patented technology of Procter and Gamble to improve softness of tissue sheets, it was decided that a study should be carried out to determine the localization of enzyme absorption on softwood fibers. As mentioned earlier, it has long been hypothesized that enzymes absorb onto fibers and create and/or enhance dislocation/notch areas by preferentially degrading these areas.

The objective of this study was to perform experiments on softwood fibers (in bulk and on single fibers) to determine whether enzymes actually absorb to already present notches and/or create notches on fibers. In this study the main goals were to:

1. Examine the effect and location of enzyme degradation of fibers in bulk and also on individual fibers using a microscope and image analysis.
2. Examine the effect of different enzyme concentrations on rate of degradation over extended treatment times using a microscope and image analysis.
3. Quantify the degree of degradation at notch areas (single fiber analysis only) using image analysis.

3A.2 Experimental Methods and Materials

Pulp

Northern softwood bleached kraft pulp from the National Institute of Standards and Technology (NIST) was used for our study. One conditioned NIST SW pulp sheet (488g) is broken up and soaked in water for four hours. The pulp sheet was then disintegrated in a British disintegrator for ten minutes at level 50 on a Dayton DC Speed Controller Model 5X485C. Next, pulp was filtered on a Buchner funnel and the consistency of pulp was measured.

Enzyme Used

The cellulase, Dyadic EXP cellulase, was obtained from Dyadic International (Jupiter, Florida). Enzyme activity was determined by measuring carboxymethyl cellulose (CMCase) and Filterpaper Activity (FPU) with 1 % enzyme solution (0.5g cellulase weight/50mL water). CMCase was 0.6 units/mL and FPU was 0.8 units/mL. The protein content of 1 % enzyme solution was 8.2 mg/mL.

Microscope Set-up

An Olympus model BH-2 microscope was used in these experiments. A Sony color video camera (Power HAD DXC-970MD) mounted on the microscope was used to image the samples on the slide glass. The optical images taken with the video camera were stored on a computer and further processed using an image analysis program (Image-Pro Version 4.5 for Windows). Defects were analyzed at 200 X magnification using cross-polarized imaging to filter out all the non crystalline and non-cellulose areas observed by the microscope. This leaves a color image of the portions of the fiber that has some degree of crystallinity with the rest of the area being black. This allows one to observe the conversion of cellulose into amorphous and soluble materials as enzyme degradation takes place.

Enzymatic Treatments (Fibers in Bulk)

Samples were prepared in 55 ml vials by using a 0.2 % consistency of NIST SW pulp and treating the different samples with both 1 % (0.4302 g enzyme/43.02 OD g pulp) and 5 % (2.151 g enzyme/43.02 OD g pulp) enzyme concentrations. The samples treated in the vials were kept at 45 °C using a water bath. After each hour of treatment, a sample from each vial was placed on a microscope slide using a graduated dropper to deliver 0.5 ml of the treated pulp. A cover glass was then placed on top of the samples and the excess liquid escaping from under the cover glass was wicked away using laboratory tissue wipes. After doing this, the slide samples were immediately examined under the microscope and images of fibers with enzyme degradation were captured digitally and saved on a computer.

Enzymatic Treatments (Single Fibers)

To prepare a microscope slide with a single fiber for analysis, some slide samples that contained many fibers had to be prepared from which a single fiber that already contained notches/dislocations could be selected and used for analysis. A bulk fiber slide

sample preparation technique was based on TAPPI method T 401 om-93 (Fiber analysis of paper and paperboard) in which 0.2 OD g of NIST SW pulp was diluted to 0.02 % consistency and then the diluted pulp was placed in a British disintegrator to break up fiber clumps. After this, 0.5 ml samples were applied to microscope slides using a 0.5 ml graduated dropper. Cover glasses were then placed on top of the samples and the samples were allowed to dry at room temperature over night. After drying the samples overnight, the cover glasses were removed and the bulk sample slide glasses were examined under the microscope at 200 X magnification till a fiber could be found that contained notches/dislocations. A pair of fine laboratory tweezers was used to extract the selected fiber from the bulk sample and then the fiber was placed on a separate clean microscope slide. On this slide, the fiber was glued down to the glass surface by using a needle to apply a very small drop of Elmer's brand Super Glue to each end of the fiber. The following figure (Figure 1) shows what a slide prepared with a single fiber would look like.

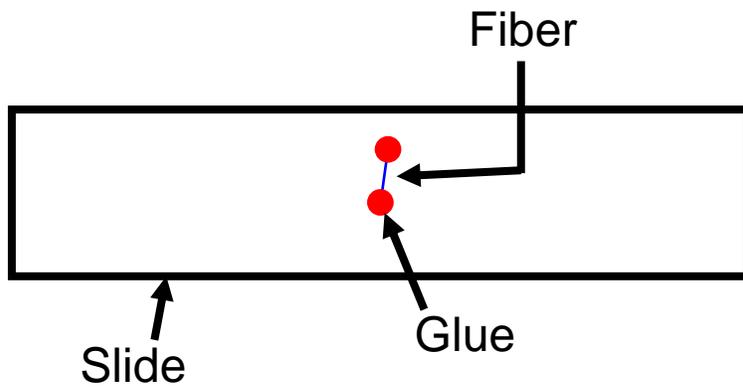


Figure 1: Single Fiber Slide Preparation

After the single fiber microscope slide was prepared, it was glued into a 40 ml beaker to keep the slide glass from moving around when the enzyme solution was added. The beaker that contained the slide glass sample was then preheated on a microscope heating element to 45°C. Enzyme solutions of varying concentrations for different experiments were prepared by adding a certain amount of enzyme to a 100 Erlenmeyer

flask and then diluting with 100 ml deionized water. Enzyme concentration was based on number of grams of enzyme used divided by 100 grams of deionized water, and then multiplied by 100. Enzyme solution was heated to 45°C in a microwave oven for 15 seconds and then it was added to the beaker by using a disposable pipette. After the image was focused in on the microscope using cross-polarized light, the images were taken every ten minutes by using the image sequencing option on the Image Pro program that automatically snapped an image set at any time interval required. The following figure (Figure 2) shows the microscope set-up used to incubate a single fiber under a microscope.

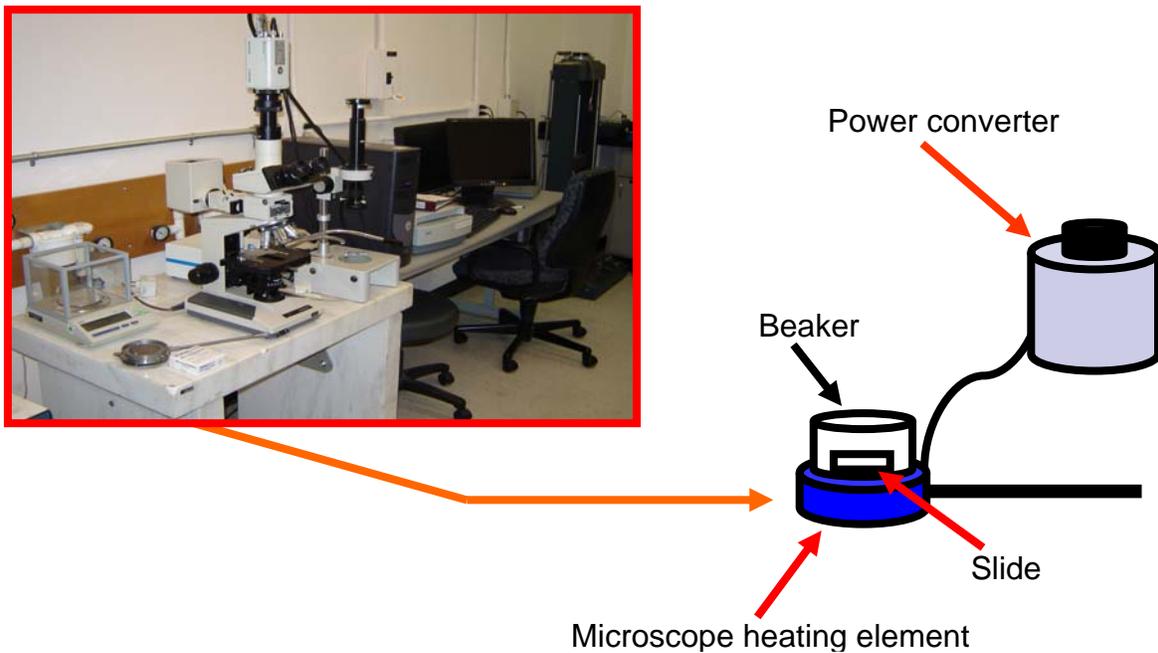


Figure 2: Microscope and Sample Incubation Set-up

Bitmap Analysis Method

To quantify the degree of degradation at notch areas for single fiber analysis, the bitmap calculating tool was used on the Image Pro program. This program calculates the optical density of a material by examining a sequence of images of a material and it

measures the amount of light that transmits through the sample. For transmitted light, it assigns a value of zero for black areas and a value of 255 for the light areas. Each pixel in the examined sequenced images was assigned a value of either zero or 255. This procedure was used because fiber areas that are left intact will appear as a light areas (bitmap 255 value) and the areas that are not fiber areas will appear black (bitmap zero value) in the cross polarized black and white images. This is useful because as the fiber is eaten away more and more by the enzyme over time, the amount of fiber (cellulosic material) will become less and less, revealing more black non-cellulosic areas in the image. When the bitmap values are averaged out later for each image, the average bitmap value for the images in the sequence should decrease more and more as the enzyme incubation time is increased and the non-enzyme area average bitmap values should remain relatively the same.

Images were first rotated with the Image Pro rotation tool so that the fiber was horizontal on the screen, and then they were converted to black and white. This is accomplished by first converting the image to a grayscale eight image and then raising the contrast to maximum level so that it is a black and white image. The areas that were to be examined (notch areas and non-notch areas) were cropped away from the original black and white sequenced images and were saved on the computer as their own file. Non-notch area image sequences were examined so that there is a control to compare the results of the notch area bitmap results to. Figure 3 shows the procedure of how an original image sequences were rotated and converted to black and white, and then notched and non-notched areas were cropped out.

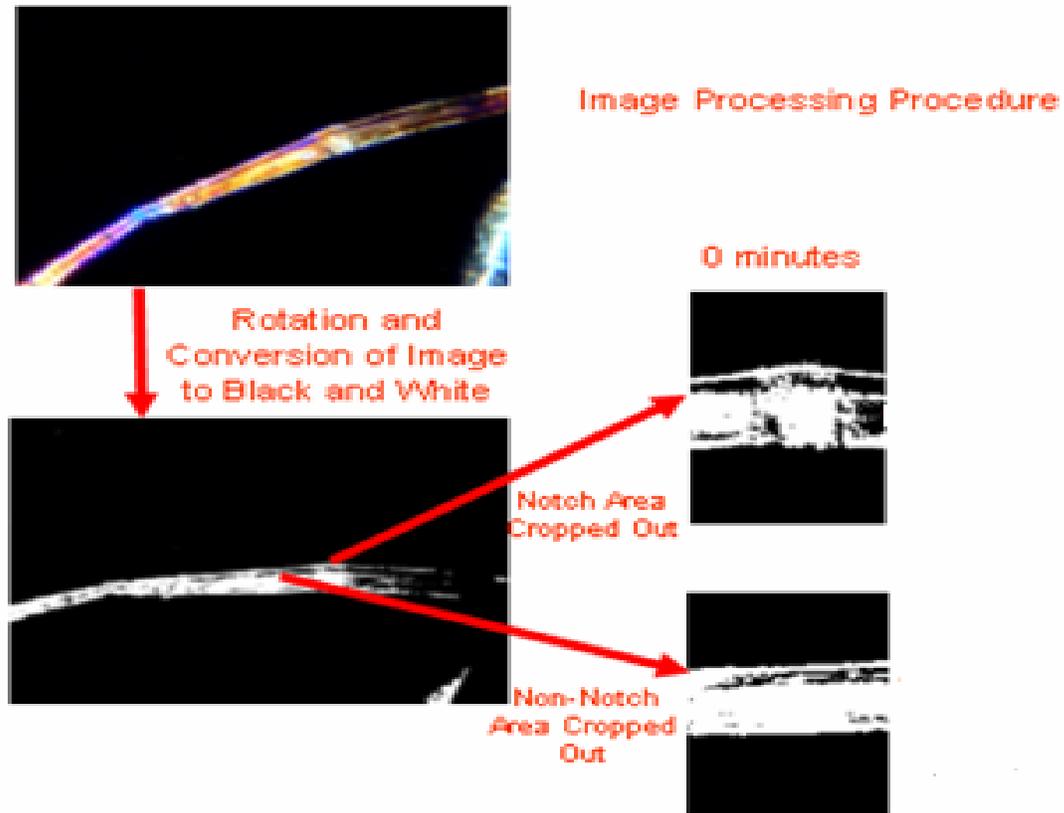


Figure 3: Procedure for Processing and Cropping Images

After the different areas of the original black and white image sequence were cropped out, they were then examined by using the bitmap analysis tool. This is done by measuring the bitmap analysis for each image in the sequence and then sending the bitmap report for each image to an excel spreadsheet. There they were stored in the same sequence as the image sequence and they were processed from the excel file later. Figure 4 demonstrates what a bitmap file looks like for an individual image.

Pixel	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	255	255	0	0	255	0	0	0	0	0	255	255	255	0	0	0
9	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
10	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
11	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
12	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
13	255	255	255	255	255	255	255	255	255	255	255	255	0	255	255	255
14	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
15	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
16	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
17	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
18	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
19	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
20	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
21	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
22	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
23	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255

Bitmap Analysis for each image is exported to excel for assessment

Each column is averaged out and then the average for all average column values is calculated

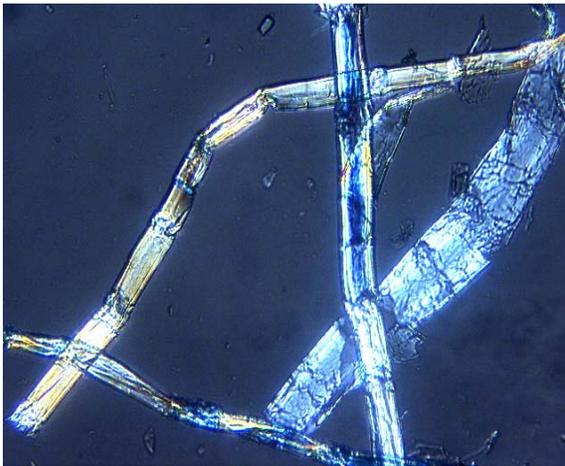
Figure 4: Bitmap Analysis for an Individual Image

As shown in figure 4, the bitmap values for each column of pixels examined in the image were averaged and then the average column values for each image were copied and pasted to another sheet in the spreadsheet where the average column values for each image were averaged out to give the average bitmap value for each image. And from here, the changes in bitmap values can be seen between the different images as the enzyme incubation time increased.

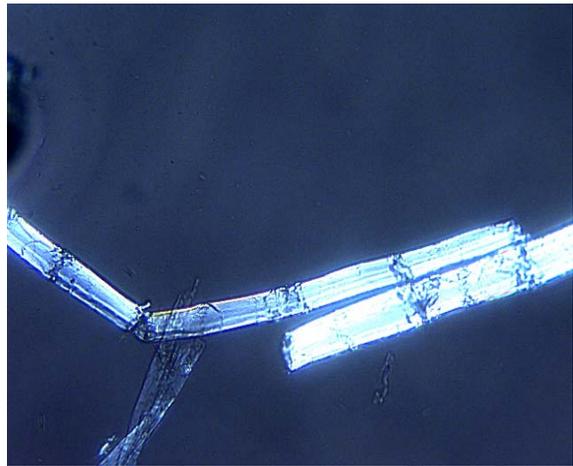
3A.3 Results and Discussion

For the first goal, which was to examine the effect and location of enzyme absorption on fibers in bulk and also on individual fibers, it was found that enzymes prefer to attach and degrade fibers at notch/dislocation areas of fiber and eat away degrade them at these locations. It was also found that enzymes do not necessarily create new notches as is seen in the following results. In time, it was found though that enzymes do convert and eat away the rest of the fiber leaving only soluble sugars. The enzyme treatment of fibers in bulk was first tested and it can be seen from the microscope

images that enzymes degraded the fibers at the notch/dislocation areas and that as incubation time increased, the fibers notch/dislocation areas degraded more and more to the point where the fibers broke up into fragments and also to the point where these fragments were totally consumed by the enzyme and converted over to soluble sugars that could not be seen. But, even after 48 hours of treatment, there still remained a small amount of insoluble fiber fragments. Tests were performed on fibers in bulk using both a 1 % (1g enzyme/100 ml DI water) and 5 % (5g enzyme/100 ml DI water) enzyme concentrations. As mentioned earlier in the method section, images of these bulk fibers were taken every hour on the optical microscope at 200 X magnification for both enzyme concentrations. The following images (Figures 5, 6, 7, 8 and 9) show the enhancement and destruction of fibers at notch/dislocation areas of fibers for different time periods (1, 3, 9, 23 and 48 hours).

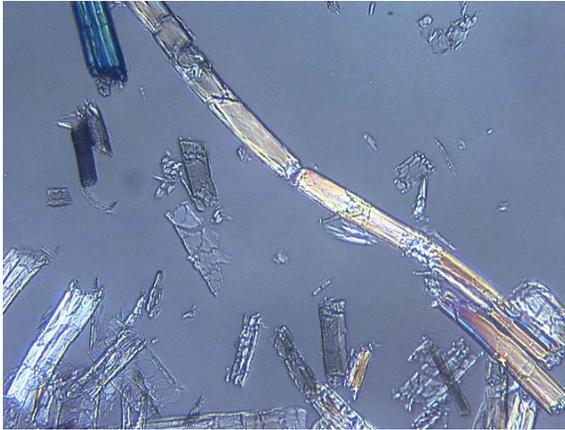


1 % enzyme for 1 hour



5 % enzyme for 1 hour

Figures 5a (left) and 5b (right): 1 % (left) and 5 % (right) enzyme concentration for 1 hour (images take using non-cross polarized light)

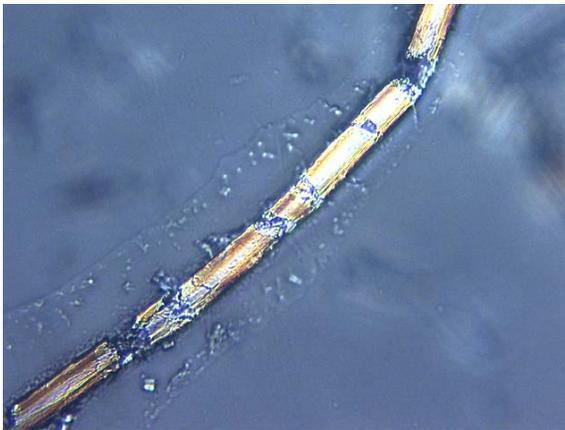


1 % enzyme for 3 hours



5 % enzyme for 3 hours

Figures 6a (left) and 6b (right): 1 % (left) and 5 % (right) enzyme concentration for 3 hours (images take using non-cross polarized light)

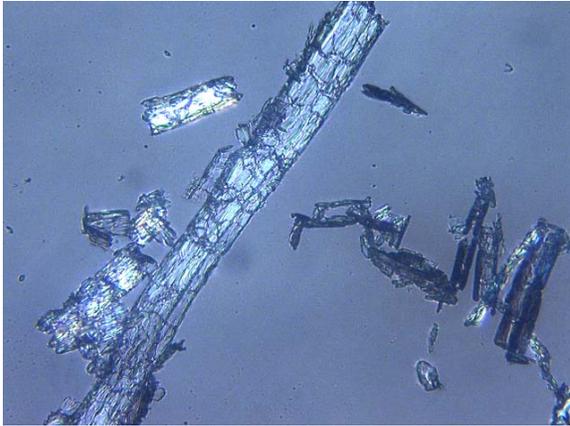


1 % enzyme for 9 hours



5 % enzyme for 9 hours

Figures 7a (left) and 7b (right): 1 % (left) and 5 % (right) enzyme concentration for 9 hours (images take using non-cross polarized light)



1 % enzyme for 23 hours



5 % enzyme for 23 hours

Figures 8a (left) and 8b (right): 1 % (left) and 5 % (right) enzyme concentration for 23 hours (images take using non-cross polarized light)



1 % enzyme for 48 hours



5 % enzyme for 48 hours

Figures 9a (left) and 9b (right): 1 % (left) and 5 % (right) enzyme concentration for 48 hours (images take using non-cross polarized light)

From looking at these images, it can be seen that with the increasing reaction time, fibers are degraded more and more by enzymes and they also begin to fragment more as time increases. It appeared that there was a slight difference between the 1 % and 5 % enzyme concentration results in that as time increased, the 5 % samples fragmented faster than the 1 % samples did. These images helped to accomplish the first goal, in that they

showed that enzymes absorbed onto the fibers at the dislocation areas and further degraded them more over time, creating fragments and eventually dissolving the fiber.

It was further shown that enzymes degrade fibers at dislocation areas when individual fibers were examined. A variety of different enzyme concentrations were used in the individual fiber study, but to show the effect of enzyme absorption on fiber dislocation areas, the results from the 1.5 % enzyme concentration (1.5 grams of enzyme/100 ml deionized water) test will be shown. These results are displayed in the following images (Figures 10 a, b, c and d) for a fiber at different enzyme incubation times (0, 70, 200 and 370 minutes). There are two dislocation areas on the fiber, and one of the dislocations is circled in red in the images.

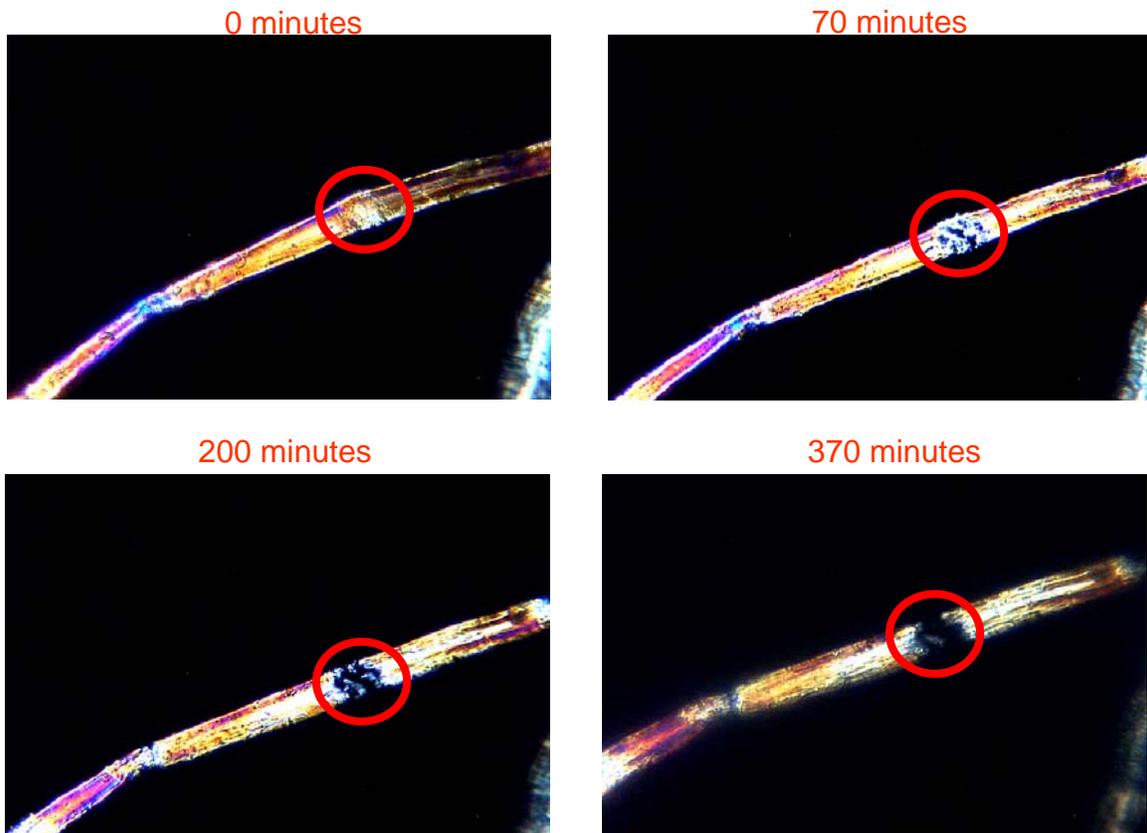


Figure 10a (upper left), 10b (upper right), 10c (lower left) and 10b (lower right): Enzyme Absorption at Dislocation Area for Increasing Incubation Time

As shown from the circled dislocation, as incubation time increases, the dislocation area on the fiber becomes more and more degraded to the point where the fiber is completely detached in the 370 minute image. It is unclear whether enzymes are degrading other areas of the fiber, but from these images it appears that the main area of absorption is at the dislocation areas. The rate of degradation is quite advanced for this high enzyme concentration, but as will be shown later, this significant degradation is not as easily observed for low enzyme concentrations (less than 0.5 %).

For the second and third goals, which were to examine the effect of different enzyme concentrations on rate of degradation over extended treatment times and to quantify the degree of degradation, single fiber tests like the 1.5 % enzyme concentration test were carried out at different enzyme concentrations and image analysis was used to quantify the rate of degradation between the different tests. Enzyme concentrations (grams of enzyme used/100 grams of deionized water) of 0 %, 0.1 %, 0.2 %, 0.4 %, 0.5 %, 1 % and 1.5 % were studied. Enzyme concentrations higher than 1.5 % resulted in images that were not clear due to the high enzyme concentration, which made the solution too cloudy for clear images to be taken. The different enzyme concentrations were examined visually and also by image analysis to quantify the differences in rates of enzyme degradation between the different enzyme concentrations. As mentioned earlier in the method section, the bitmap analysis was carried out after images were rotated and converted to black and white, from which cropped images of notch areas and non-notch areas were examined. To give an example of what the image analysis would examine over the enzyme incubation period, the 1.5 % enzyme concentration test will be shown again. In Figure 11 it can be seen how the notched area changes over time by revealing less light areas and more dark areas, and the non-notch area changes very little over enzyme incubation time.

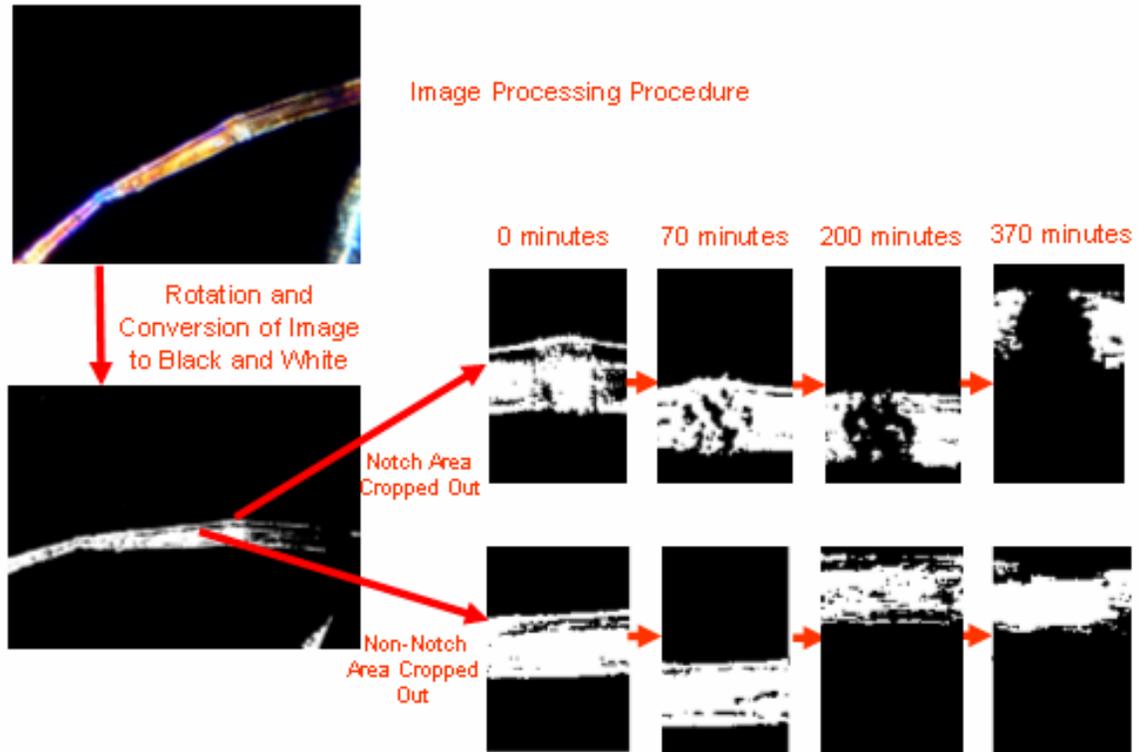
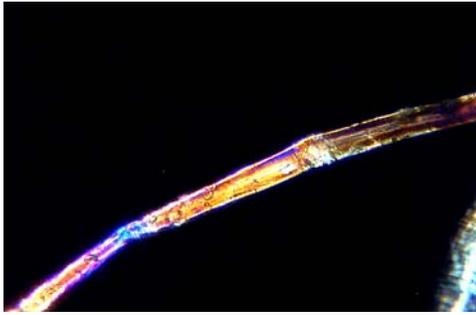


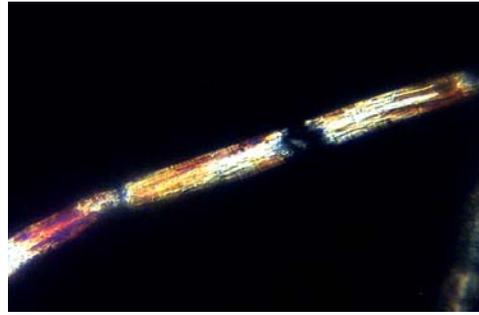
Figure 11: Example of Bitmap Procedure for 1.5% Enzyme Concentration

These images indicate that the enzymes were degrading the fiber and resulting in a lower average bitmap value as the incubation time increased. From looking at the non-notch areas it can be seen that this area does not change that much over the incubation time, except for what is seen in the first image (0 minute time) because the fiber was pushed up in the solution in the first few minutes making this area more brighter as what is seen in the other images. Because there is not much of a change to this area, there is not as much change in average bitmap value over enzyme incubation time. This indicates further that notch/dislocation areas of the fiber are more accessible areas for enzymes to attach and degrade as was seen for both bulk fiber tests and single fiber tests. Images of fibers taken for beginning and ending enzyme incubation times can be seen in the following graphs (Figures 12-19 a and b).

12a Beginning (0 minutes)

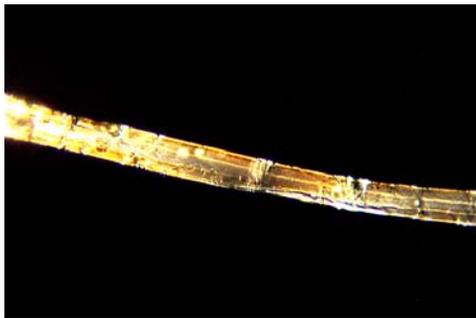


12b Ending (370 minutes)

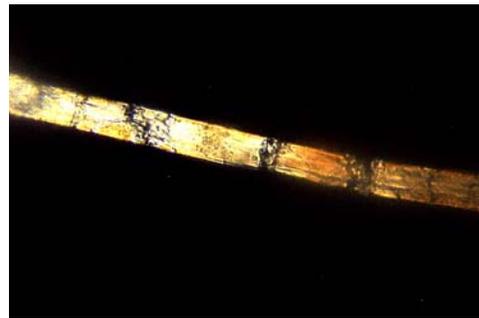


Figures 12a (upper left) and 12b (upper right): Images of Fiber for Beginning (12a) and Ending (12b) Incubation Period using 1.5 % Enzyme Concentration

13a Beginning (0 minutes)



13b Ending (370 minutes)



Figures 13a (upper left) and 13b (upper right): Images of Fiber for Beginning (13a) and Ending (13b) Incubation Period using 1.0 % Enzyme Concentration

14a Beginning (0 minutes)

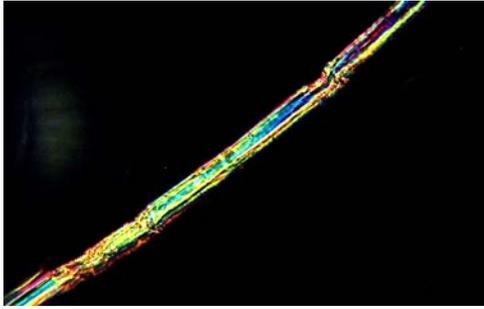


14b Ending (410 minutes)

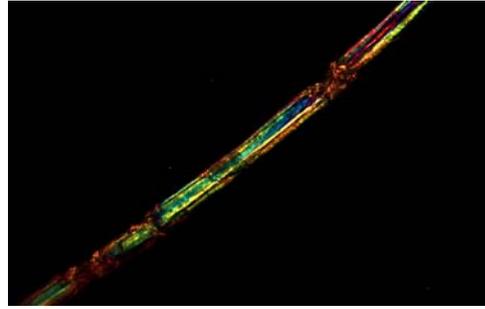


Figures 14a (upper left) and 14b (upper right): Images of Fiber for Beginning (14a) and Ending (14b) Incubation Period using 0.5 % Enzyme Concentration

15a Beginning (0 minutes)

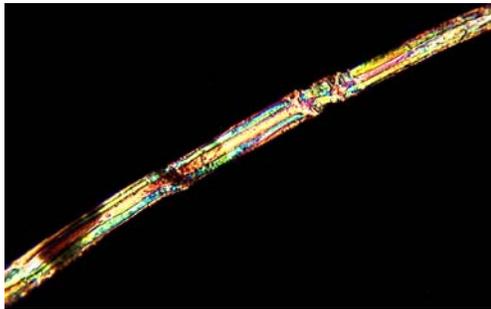


15b Ending (410 minutes)

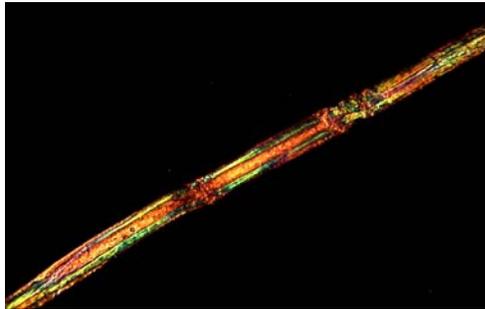


Figures 15a (upper left) and 15b (upper right): Images of Fiber for Beginning (15a) and Ending (15b) Incubation Period using 0.4 % Enzyme Concentration

16a Beginning (0 minutes)

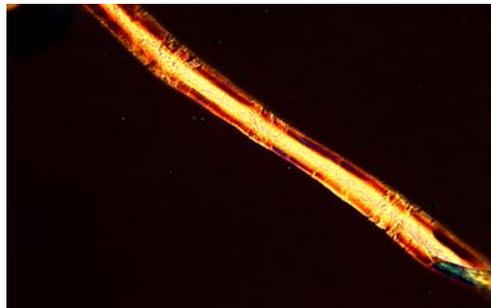


16b Ending (440 minutes)

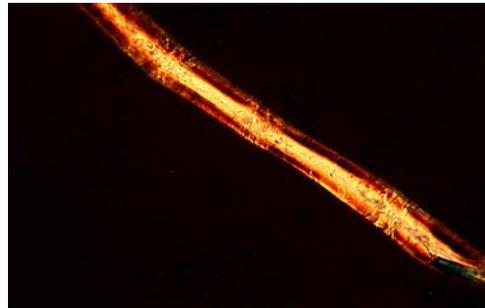


Figures 16a (upper left) and 17b (upper right): Images of Fiber for Beginning (17a) and Ending (17b) Incubation Period using 0.2 % Enzyme Concentration

17a Beginning (0 minutes)



17b Ending (410 minutes)



Figures 17a (upper left) and 18b (upper right): Images of Fiber for Beginning (18a) and Ending (18b) Incubation Period using 0.1 % Enzyme Concentration

18a Beginning (0 minutes)



18b Ending (410 minutes)



Figures 18a (upper left) and 19b (upper right): Images of Fiber for Beginning (19a) and Ending (19b) Incubation Period using 0 % Enzyme Concentration

From looking at most of the images for the different enzyme concentration tests, it can be seen that there is a considerable difference between the notch areas and non-notch areas as incubation time increased, and this is more so seen for the higher enzyme concentrations (0.5 % enzyme concentration or higher). In Figure 20, the quantitative results are shown for the above images of the different enzyme concentrations. The graph shows the number of white pixels to the number of white pixels at zero time difference between notch and non-notch areas of the fibers. This data shows that as the enzyme incubation period increases, the difference between the notch and non-notch area increases. This is because the enzymes find the notch areas to be accessible for attachment and hence degradation is more prevalent in these areas as compared to the non-notch areas causing the amount of fiber (white area 255 pixel value in black and white image) to decrease more in comparison to the non-notch area. As show by the graph, the higher enzyme concentrations had a greater effect on causing this difference as compared to the lower enzyme concentrations and this trend can be clearly seen when going from the 0 % enzyme concentration to the 1.5 % enzyme concentration. The sharp jump in the difference that is seen at the beginning of some of the enzyme concentrations could be due to the fiber swelling initially causing the fiber to appear brighter at the beginning before the degradation begins.

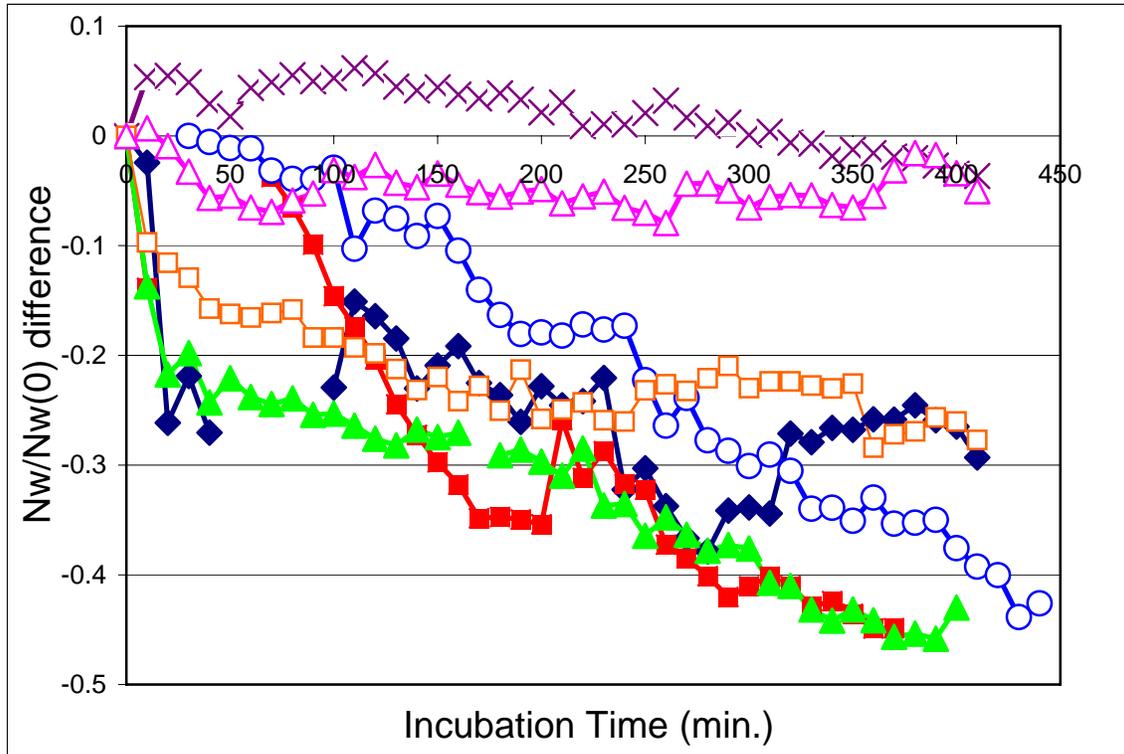


Figure 19: Notch Area to Non-notch Area White Pixel (255) Average Differential as a Function of Incubation Time

3A.4 Conclusions

From looking at the results for completing goal number one, it appears that it was proven that enzymes do prefer to degrade fibers at notch/dislocation areas and this was seen for both bulk fiber tests and single fiber tests. It was shown that as the incubation time increased, the fibers were reduced due to fiber breakage at weakened the notch/dislocation areas. This was especially evident for the higher enzyme concentrations (0.5 % or higher). From looking at the results for completing goals number two and number three, it was found that some differences can be seen between

high and low enzyme concentrations when comparing the images, in that the higher enzyme concentration showed more apparent decay of fibers at notch/dislocation areas as enzyme incubation time increased. The complete separation of the fibers at the dislocation areas is seen for the higher enzyme concentration when looking at the ending images. When looking at the bitmap quantification results for the different enzyme concentration tests, it appears that there is a considerable difference between the results for the notch areas and the results for the non-notch areas. The results show that as the enzyme incubation period increases, the difference between the notch and non-notch area increases. The higher enzyme concentrations had a greater effect on causing this difference as compared to the lower enzyme concentrations and this trend can be clearly seen when going from the 0 % enzyme concentration to the 1.5 % enzyme concentration.

3B Enzyme Interaction with Softwood Fibers

3B.1 Introduction

A study of reversibility of enzymes attachment to softwood fibers is discussed in this section. The ability to absorb enzymes onto the fraction of pulp that is to receive the enzyme treatment and then to subsequently incubate that fraction with an untreated fraction could significantly reduce capital invest. Therefore, a set of experiments were conducted to examine the effect of enzyme absorption followed by joint incubation with untreated fibers. The experiments entailed mixing a combination of enzyme treated and untreated pulp mixed after a short period (30 minutes) and conducting an incubation and comparing the zero-span tensile strengths (dry and wet) to a similar mix that is not mixed until after the incubation time has elapsed. If the enzyme permanently attached to the fibers, then the two experiments should show the same results. If the enzymes do not permanently attach, then the results will be different with the joint incubated sample being lower than the control sample.

The objective of this experiment is to determine whether the enzymes permanently absorb onto the fibers and if they fibers can be jointly incubated with other fibers that are not intended to receive the treatment. The results from these tests will be analyzed also to determine whether they meet the Procter and Gamble patent claims.

3B.2 Experimental Methods and Materials

Pulp

Northern softwood bleached kraft pulp from the National Institute of Standards and Technology (NIST) was used for this study. A single conditioned NIST SW pulp sheet (488 g) was broken up and soaked in tap water for four hours. The pulp sheet was

then disintegrated in a British disintegrator for ten minutes at level 50 on a Dayton DC Speed Controller Model 5X485C. The pulp was then washed for approximately seven minutes using a water hose in a false bottom container. Next, the pulp was filtered on Buchner funnel and consistency of pulp was measured.

Enzyme Used

The cellulase, Dyadic EXP cellulase, was obtained from Dyadic International (Jupiter, Florida). Enzyme activity was determined by measuring carboxymethyl cellulose (CMCase) and Filterpaper Activity (FPU) with 1% enzyme solution (0.5 g cellulase weight/50mL water). CMCase was 0.6 units/mL and FPU was 0.8 units/mL. The protein content of 1 % enzyme solution was 8.2 mg/mL.

Enzymatic Treatments

The pulp slurries of treated and untreated pulps (20 OD g total at 3 % consistency in 50 mM sodium acetate buffer for treated portion, pH 4.5 for Dyadic cellulase treatments) at different ratios (100 % treated/0 % untreated, 75 % treated/25 % untreated, 50 % treated/50 % untreated, 25 % treated/75 % untreated and 0 % treated/100 % untreated) were heated and agitated (using Fisher Scientific Sted-Fast Model 600 agitators set at level four with a three blade impeller) in separate stainless steel beakers in a water bath till temperature reached 45 °C. Once the temperature stabilized at 45 °C and pH 4.5, an enzyme charge (0.5 % based on OD weight of pulp) was added to the treated pulp beaker. After 30 minutes of the pulp being mixed with the enzyme, this pulp, the treated pulp, and the untreated pulp were mixed together and allowed to mix for an additional 2.5 hours. Once the incubation period of 3 hours total was complete, the enzyme in the sample was heat shocked in a microwave oven by heating the sample for four minutes (two minutes boiling). After this, the pulp slurry was drained on a Buchner Funnel and handsheets were made.

Reference Treatments

For the three hour reference experiment, a fraction of the pulp was dosed with enzymes. After three hours the fraction of treated pulp was mixed with the untreated portion for one minute. The combined pulp was then heat shocked (microwave for four minutes (two minutes boiling)) and filtered through a Buchner funnel before forming hand sheets.

For the no enzyme (NE) control experiment, the same procedure as for the treated experiments was used, with the exception that no enzyme was used at all for this treatment (so only water was added to the pulp slurry control the consistency) and also only 1 sample was needed (20 OD g total of pulp). This sample was also heat shocked in the same manner as the enzyme treated samples.

Pulp Testing

Eight handsheets were prepared for each experimental condition according to TAPPI test method T 205 om-88, but instead of couching the fiber mat/double blotter paper/couch plate stack with five rolls, only three rolls were used and then the fiber mat that is attached to blotter paper was lifted up and placed on the bench with the fiber mat facing up. A round metal handsheet plate was placed on top of the round fiber mat and the couch plate was placed on top of this. The couch roll was then used to couch the assembly four additional times. The fiber mat (attached to round metal handsheet plate) was then peeled from blotter paper and the handsheet (still on round metal handsheet plate) was placed in a handsheet ring. All eight handsheet rings were stacked together with handsheets facing down and then placed a handsheet ring clamp. The sheets were then placed in a 50 % relative humidity and 23 °C environment to dry under restraint.

After the handsheets were dried, the basis weight, thickness, density, breaking length tensile strength, zero-span dry tensile and zero-span wet tensile strength were

measured for the handsheets. All tests were conducted according to TAPPI test method T 220 om-88 and T 231 cm-96.

3B.3 Results and Discussion

In Figure 1, the results from the dry zero span tensile strength tests can be seen for the different ratios of enzyme treated to untreated pulps mixed either after 30 minutes (treated) or at the end of the total three hour incubation period (three hour control). The results show that the treated samples have lower dry zero span tensile strengths than the three hour control. It also appears that the difference between the treated samples and the three hour control decreases as the percentage of enzyme treated pulp increases. This indicates that enzymes are not completely absorbed on the fiber during the first 30 minutes, or the enzymes once absorbed can desorb and reabsorb on another fiber or a combination of these two phenomena. The higher dry zero-span tensile strengths for the three hour control indicates that these mixed pulps are not as affected by the enzymes, because the enzymes have been active only on the enzyme treated portion for an extended time (three hours). The lower dry zero span tensile strengths for the treated samples indicates that more than the portion that was initially exposed during the first 30 minutes receives the enzyme treatment. Also, the decreasing difference between treated and three hour control samples as enzyme treated ratio increases indicates that the enzyme treated and three hour control samples become more similar because the untreated portion is becoming smaller meaning that the enzymes cannot affect as many untreated fibers, making them more similar to the three hour control samples. Also, the control NE control dry zero span tensile strength is slightly lower than the treated and three hour control dry zero span tensile strengths for the 0 % enzyme ratio.

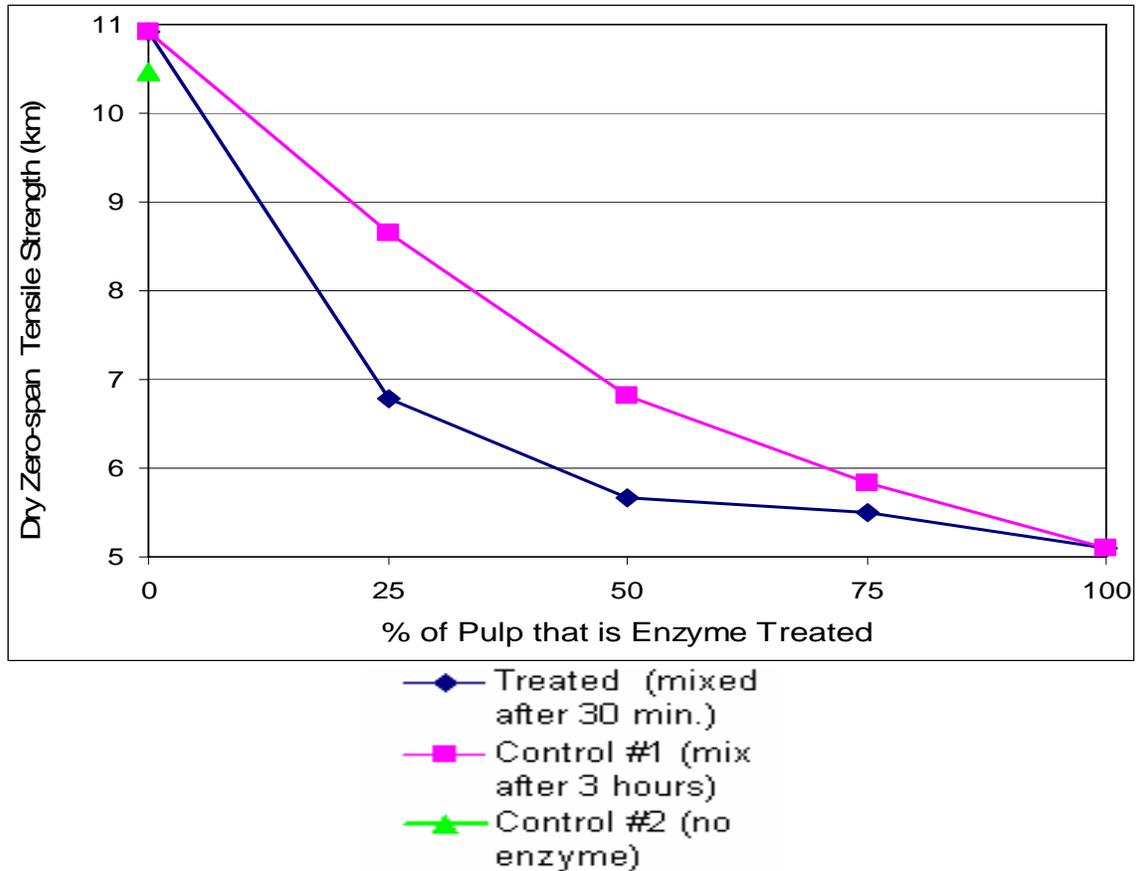


Figure 1: Dry Zero Span Tensile Strength Results for Different Enzyme Treated to Untreated Pulp Ratios

In Figure 2, the results from the wet zero span tensile strength tests can be seen for the different ratios of enzyme treated to untreated pulps mixed either after 30 minutes or at the end of the total three hour incubation period (three hour control). These results followed a very similar pattern to the dry zero span tensile strength. This further supports the notion that a portion of the enzymes remain in solution attaching to fibers after being combined, or that the enzymes are desorbed and re-deposited, or a combination of both of these phenomenon.

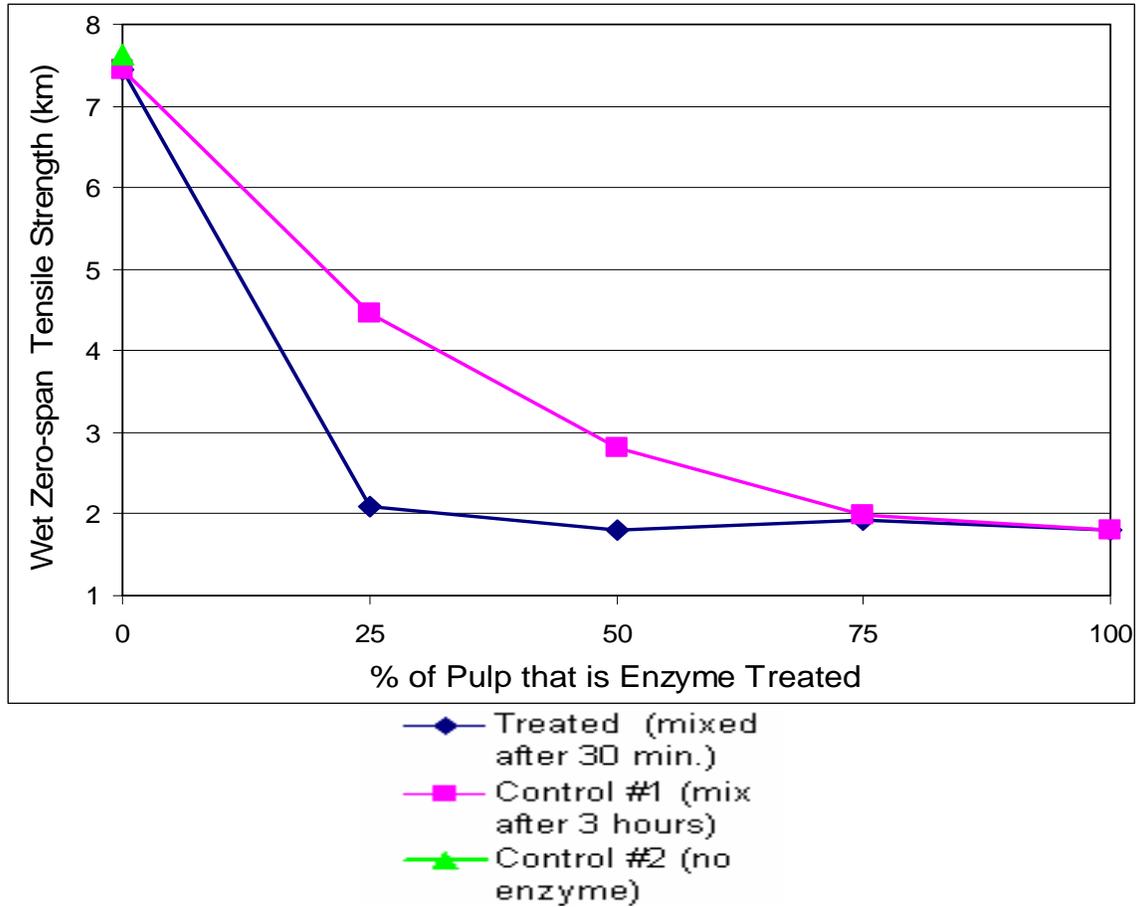


Figure 2: Wet Zero Span Tensile Strength Results for Different Enzyme Treated to Untreated Pulp Ratios

Tables 1, 2 and 3 display the densities of the handsheets produced for physical testing. These densities are within the requirements of the first claim of the Procter and Gamble Patent, which stated that the fibrous structure should have a density of not more than about 0.4 g/cm³ (400 kg/m³). The following tables display the densities of the treated handsheets (mixed after 30 minutes), the three hour control (mixed after three hours) and the NE (no enzyme) control.

Table 1: Treated (mixed after 30 min.) Handsheet Densities

% of Pulp that is Enzyme Treated	Treated Density (kg/m³)
0	314
25	303
50	344
75	353
100	355

Table 2: 3-Hour Control (mixed after 3 hours) Handsheet Densities

% of Pulp that is Enzyme Treated	3-Hour Control Density (kg/m³)
0	314
25	316
50	351
75	351
100	356

Table 3: NE Control (no enzyme) Handsheet Densities

% of Pulp that is Enzyme Treated	NE Control Density (kg/m³)
0	287

Table 4 and Figure 3 show the results for repeating Procter and Gamble's U.S. Patent 6,146,494 claim #3 that modified cellulosic fibers should have a dry zero span tensile index that is at least about 35% less than the dry zero span tensile index of the corresponding unmodified cellulosic fibers (NE control). It was found from the test results that we were able to get to the 35 % drop and higher by using either at least the 25 % enzyme treated pulp or at least the 50 % enzyme treated ratio for the three hour control (mixed after three hours). This also indicates that the treated pulps need a lower percentage of enzyme treated pulp to get closer to the 35 % in dry zero span tensile strength when comparing to the three hour control pulps. This indicates one way to

dramatically improve the efficiency of the enzyme treatment. Using a much smaller enzyme dosage (when compared to the entire sample), there is a larger decrease in the total strength when compared to a sample that is more or less incubated jointly.

Table 4: Patent Claim #3 Reproduction Results

% of Pulp that is Enzyme Treated	% Drop in Zero Span Dry Tensile Strength for Treated as compared to NE Control
0	-4
25	35
50	46
75	48
100	51
% of Pulp that is Enzyme Treated	% Drop in Zero Span Dry Tensile Strength for 3-Hour Control as compared to NE Control
0	-4
25	17
50	35
75	44
100	51

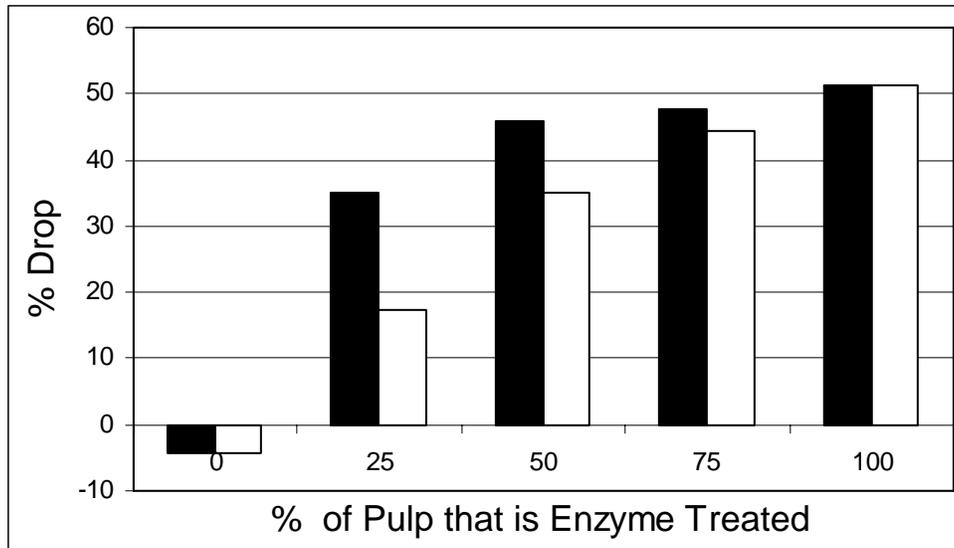


Figure 3: Patent Claim #3 Reproduction Results

Table 5 and Figure 4 show the results for repeating Procter and Gamble's U.S. Patent 6,146,494 claim #2 that the modified fibers that form a fibrous structure (handsheet) should have a dry tensile index that is at least as great (90 % similar) as the dry tensile index of a handsheet made from the corresponding unmodified fibers (NE control). It was found that for the zero enzyme control a 77 % similarity was found, indicating that this patent claim was not achieved. The other ratios are a little lower in percent similarity and there does not seem to be any correlation when looking at percent enzyme ratio or at whether the pulps were treated or three-hour control.

Table 5: Patent Claim #4 Reproduction Results

% of Pulp that is Enzyme Treated	% Drop in Zero Span Wet Tensile Strength for Treated as compared to NE Control
0	3
25	73
50	76
75	75
100	76
% of Pulp that is Enzyme Treated	% Drop in Zero Span Wet Tensile Strength for 3-Hour Control as compared to NE Control
0	3
25	41
50	63
75	74
100	76

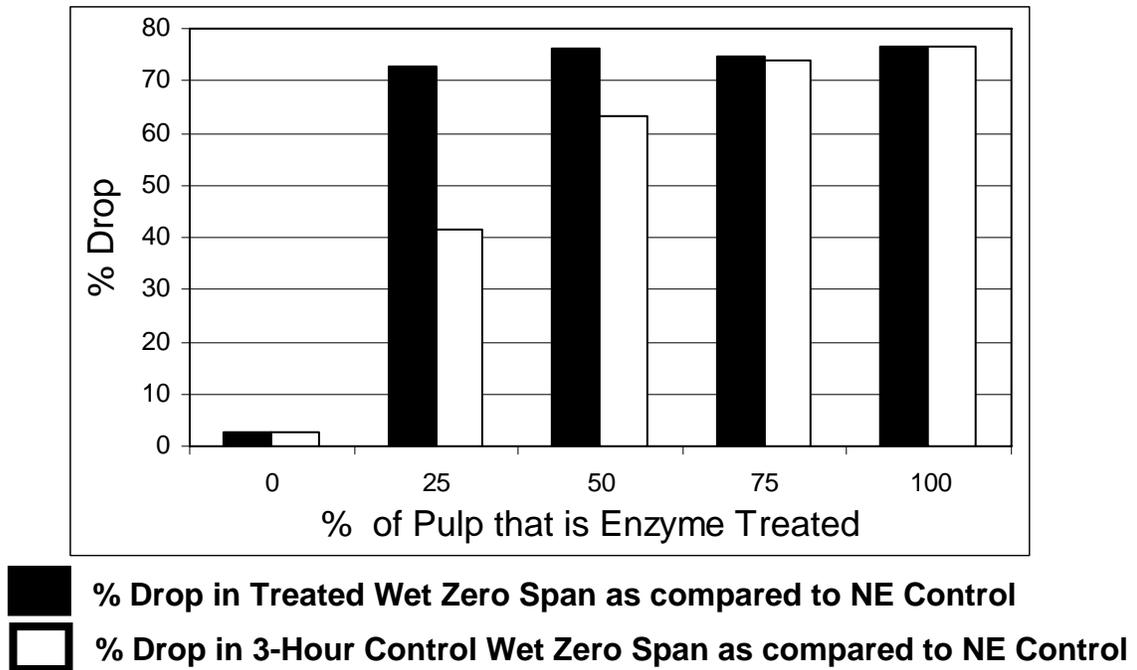


Figure 4: Patent Claim #4 Reproduction Results

Table 6 and Figure 5 show the results for repeating Procter and Gamble's U.S. Patent 6,146,494 claim #2 that the modified fibers that form a fibrous structure (handsheet) should have a dry tensile index that is at least as great (90 % similar) as the dry tensile index of a handsheet made from the corresponding unmodified fibers (NE control). From the test results, it was found that we only came somewhat close (77 %) to meeting this 90 % similarity for the 0 % enzyme treated ratio for both treated and 3-hour control pulps. The other ratios are a little lower in percent similarity and there does not seem to be any correlation when looking at percent enzyme ratio or at whether the pulps were treated or 3-hour control.

Table 6: Patent Claim #2 Reproduction Results (0.5% enzyme concentration)

% of Pulp that is Enzyme Treated	% Similarity b/w Treated Dry Tensile and NE Control Dry Tensile
0	77
25	63
50	62
75	63
100	70
% of Pulp that is Enzyme Treated	% Similarity b/w 3-Hour Control Dry Tensile and NE Control Dry Tensile
0	77
25	70
50	52
75	68
100	70

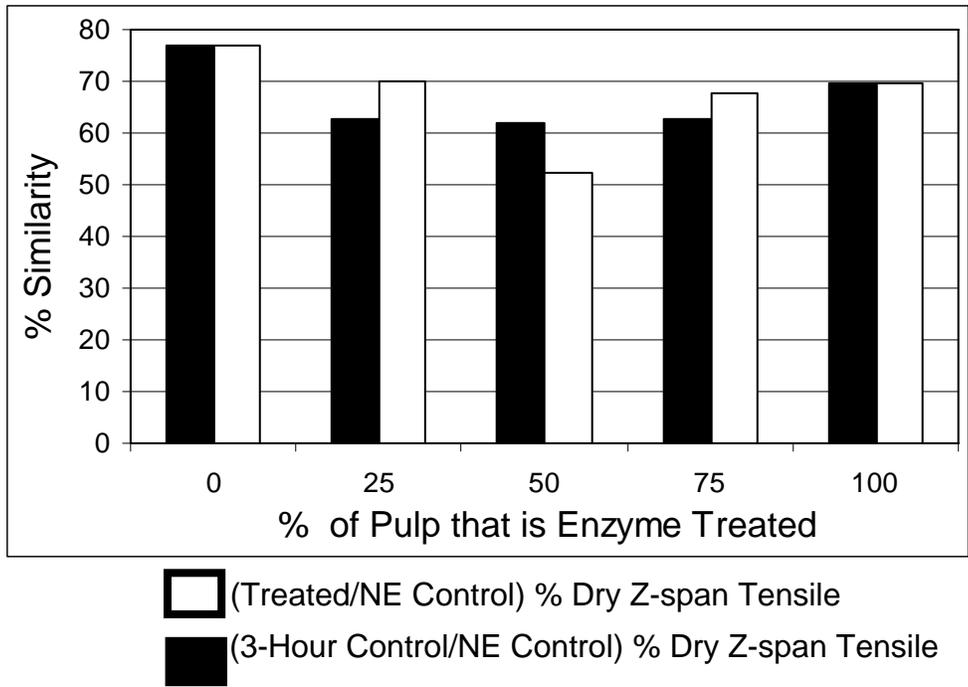


Figure 5: Patent Claim #2 Reproduction Results (0.5 % enzyme concentration)

Table 7 and Figure 6 show the results of repeating Procter and Gamble’s U.S. Patent 6,146,494 claim #5 that modified cellulosic fibers should exhibit a ratio of dry zero span tensile index to wet zero span tensile index from about 1.5 to about 3. Overall, the test results show that it is possible for the most part to repeat this claim for the treated, three-hour control and NE control pulps. From looking at the results, it is of interest that the ratio is pretty much the same for the treated, three-hour control and NE control for the 0 % enzyme used ratio, and this ratio is significantly lower than the other percent enzyme used ratios. There does not seem to be any correlation when looking at percent enzyme ratio or at whether the pulps were treated or control #1.

Table 7: Patent Claim #5 Reproduction Results

Treated % Enzyme Treated	Ratio between dry zero span and wet zero span
0	1.5
25	3.2
50	3.1
75	2.8
100	2.8
3-Hour Control	
% Enzyme Treated	Ratio between dry zero span and wet zero span
0	1.5
25	1.9
50	2.4
75	2.9
100	2.8
NE Control %	
Enzyme Treated	Ratio between dry zero span and wet zero span
0	1.4

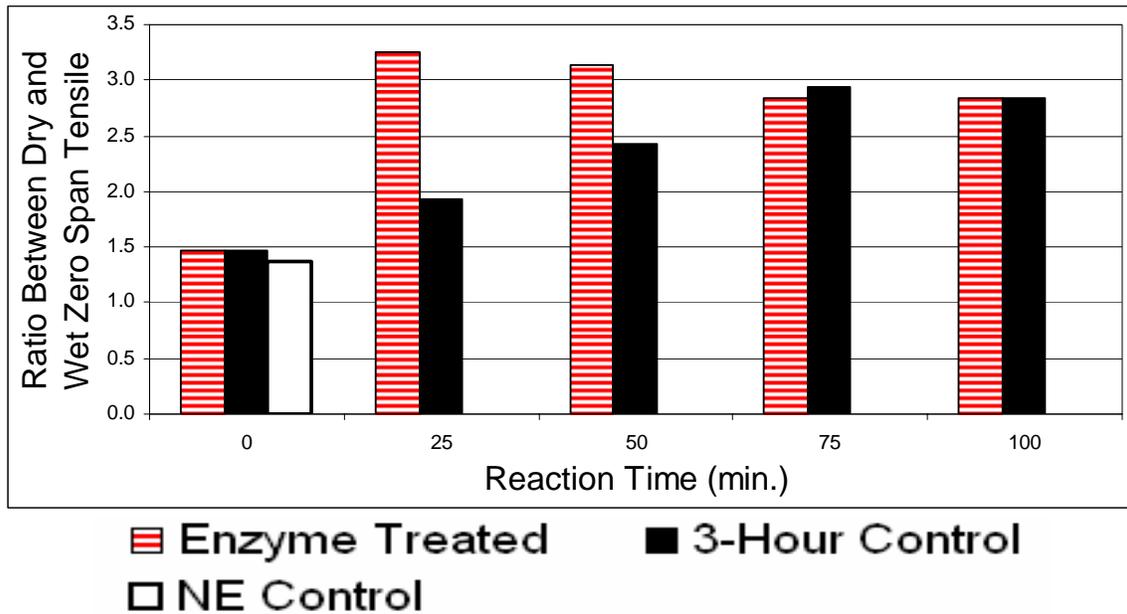


Figure 6: Patent Claim #5 Reproduction Results

3B.4 Conclusions

From the results, it can be concluded that not all enzymes absorb to fiber dislocations or other areas at the beginning of the incubation period. Some enzymes may attach to fibers, but some move around and attach later. These results indicate that if a certain ratio of softwood pulp is going to be enzyme treated and you want to achieve a certain amount of fiber strength loss for enhancing softness, then the mixing of enzyme treated pulp with untreated pulp should occur right before the enzyme is denatured (heat shocked). The results for completing the Procter and Gamble patent claims indicate that these claims can be reproduced with either treated or NE control pulps, with the exception of repeating claim #2 (90 % similarity in tensile strength).

3C Effect of Enzyme Treatment on Wet Fiber Flexibility for Refined Softwood Fibers

3C.1 Introduction

A study of the effect of how enzymatically treating refined softwood fibers has an affect on wet fiber flexibility is discussed in this section. In section 3A it was shown that enzymes prefer to absorb at notch (dislocation) and/or kink areas of fibers and that over extended enzyme incubation time, the enzymes cause the fibers to lose dry and wet zero-span tensile strength, increase the amount of kink and curl in the fibers, and decrease the mean fiber length. From these results, it was hypothesized that enzymatic degradation on the softwood fibers would cause the fibers to become more flexible since the enzymes degrade the notches and kinks, creating more kinks and weakening the kinks already present. This effect would cause the fibers to bend more at these kink (joint) areas of the fiber to allow the fiber to be more flexible. As mentioned earlier in section 2, the main tissue property that Procter and Gamble desired to be enhanced was softness and the softness of a product can be broken down into two components, the bulk (i.e. structural) softness and surface softness. The bulk softness, which is controlled primarily by the structure of the sheet, is related to the bending stiffness, compressibility and modulus of the structure as a whole.

As mentioned earlier in section 2, a recently developed model shows that the structural integrity of the fiber is a critical factor in determining the compressibility of the sheet [Pawlak, J.J., 2001]. This model suggests that if the flexural rigidity of the fiber can be reduced, while maintaining a constant coarseness, then the compressibility of the paper sheet can be increased at the same sheet density. The flexural rigidity of the fibers may be reduced by lowering the elastic modulus of the fibers decreasing the moment of inertia of the fibers, or providing localized “hinge points” in the fiber. The enzymatic action on the fibers creates localized notches in the fibers, which serve as “hinge points”

around which the fibers can flex. By creating these localized hinge points, the coarseness of the fiber is changed very little and this allows for the compressibility of the fiber structure to be increased without reducing the density of the structure. If the tensile strength of the fiber network is much lower than the fiber strength, then reducing the fiber strength should have little or no impact on the sheet tensile strength, as the bonding remains the same. The net result of engineering such a structure would be a softer, more compressible product, with the same tensile strength as the original product.

To determine the flexibility of the softwood fibers, a method developed by Steadman and Luner [Steadman and Luner, 1985] was used. The method involves pressing softwood fibers to slides that have a parallel grid of evenly spaced thin stainless steel wires affixed to them and measuring the no-contact length of the fiber over the wire under incident light conditions on a microscope. The methods and materials needed for this experiment were developed from the Steadman and Luner report and a study that was done recently, the Pulp and Paper Department at North Carolina State University by Zhang [Zhang, 2004]. Some preliminary experiments were done first to see if these methods and materials would work to measure flexibility in the manner reported by Steadman and Zhang. These preliminary tests will be discussed in the methods and materials section.

Along with trying to determine if the flexibility of the fibers would change with the increased enzyme treatment time, it was also desired to see how these changes would affect the dry and wet zero spans of the different samples and to make certain that the results would still comply with the Procter and Gamble patent claims since the pulp had to be refined as will be discussed in the methods and materials section. As mentioned earlier, the key patent claims are:

1. The fibrous structure should have a density of not more than about 0.4 g/cm^3 (400 kg/m^3).

2. Modified fibers that form a fibrous structure (handsheet) should have a dry tensile index that is at least as great (90 % similar) as the dry tensile index of a handsheet made from the corresponding unmodified fibers.
3. Modified cellulosic fibers should have a dry zero span tensile index that is at least about 35 % less than the dry zero span tensile index of the corresponding unmodified cellulosic fibers.
4. Modified cellulosic fibers should have a wet zero span tensile index that is at least about 70 % less than the wet zero span tensile index of the corresponding unmodified cellulosic fibers.
5. Modified cellulosic fibers should exhibit a ratio of dry zero span tensile index to wet zero span tensile index from about 1.5 to about 3.

Also, to make certain that the enzymes were affecting the fiber length, percent fines, kink and curl, FQA tests were performed to determine the effect of enzyme degradation at the different enzyme treatment times.

3C.2 Experimental Methods and Materials

Flexibility Measurement and Preliminary Tests

For this procedure, a thin, tangentially oriented fiber network is formed on a filter paper, which has been placed on the wire of a standard handsheet mold. The network is then pressed in contact with a 5 cm x 5 cm glass slide, which has several thin stainless steel wires (25.4 μm diameter) attached across its surface in the form of a parallel grid (spaced every 3 mm). After pressing, the slide is air-dried, and is then inverted and viewed in a microscope under transmitted and incident light according to the Steadman method [Steadman and Luner, 1985]. The exact procedure for preparing the slides, measuring flexibility and the theoretical calculations are contained in Steadman's report. When the fiber is viewed under incident light, the areas of intimate contact between the fibers and the glass appear dark, and the area which is not in contact, i.e. as the fiber arcs over the wire, is not visible. The no-contact length, L , is related to the flexibility of the

fiber, and as the fiber becomes more flexible, this distance will decrease. Theoretically, the load on the fiber can be calculated from the pressing pressure and the deflection is equal to the diameter of the stainless steel wire. The flexibility of a fiber can be expressed as:

$$\text{Flexibility} = 1/(E I) = (72 d)/(P*W*S^4), \text{ in units of } N^{-1}m^{-2}$$

Where E = Modulus of Elasticity, $N*m^{-2}$

I = Moment of Inertia, m^4

d = wire diameter, m

P = pressing pressure, $N*m^{-2}$

W = projected fiber width, m

S = a mathematical estimate of the loaded fiber span, m

From the report by Zhang [Zhang, 2004], the correct form to calculate the loaded fiber span S, based on the Pythagorean Rule, is given by Cresson [Cresson, 2002]:

$$S = \sqrt{(d^2 + (L/2)^2)}$$

Where L = less-visible length, m

In Figure 1, an example of how fibers cross over the wires and what the less visible length (L) and width (W) are for the NIST SW fibers examined. In Figure 2, there is a diagram of a slide with a parallel grid of stainless steel wires.

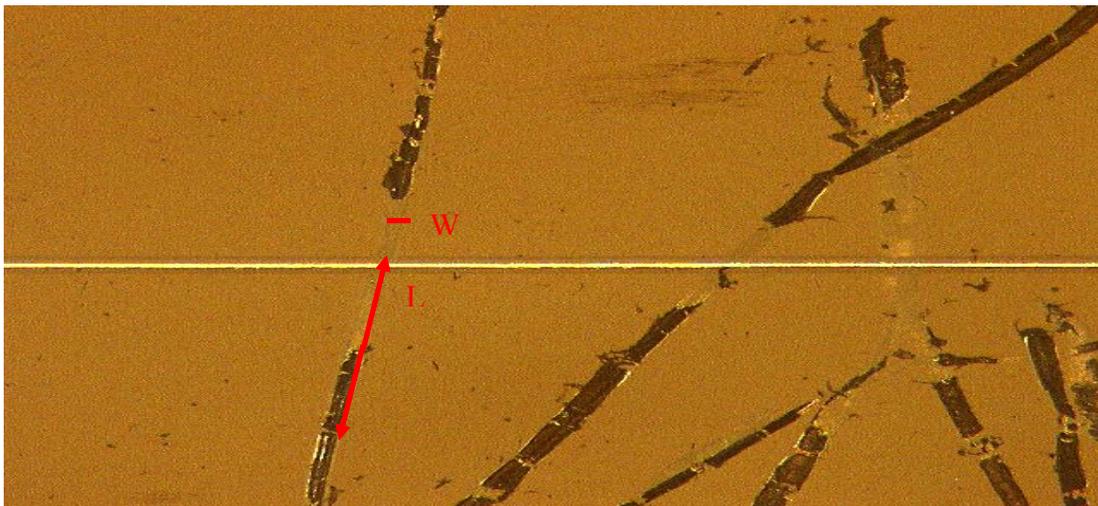


Figure 1: Fiber Examined for Flexibility

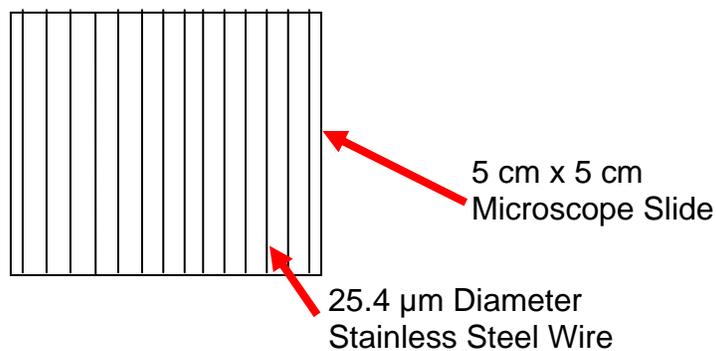


Figure 2: Slide with Parallel Grid of Stainless Steel Wires

Measuring the less visible length and width of the fibers for flexibility measurement involves using an Olympus model BH-2 microscope with a Sony color video camera (Power HAD DXC-970MD) mounted on top of the microscope, used to record and take images of the analyzed samples on the slide glass. These images taken from the video camera were transferred to an image analysis program (Image-Pro Plus Version 4.5 for Windows) on a computer where the images could be seen and analyzed. The less visible length and width could be measured using a measurement tool in the Image Pro program that gives distances in millimeters. Random sampling is assured by measuring the individual fibers in sequence as the moving stage of the microscope tracks along the wire. Around 75 images were taken for each enzyme treated sample and the images had between 1 and 4 fibers that could be evaluated for flexibility measurement.

To first see if this procedure would work for the NIST SW pulp (unrefined), several slides were prepared using the Steadman procedure. From examining the images of the fibers over the steel wires, it was determined that the fibers were too stiff for them to lie down and adhere to the microscope slide. So, it was determined that refined pulp was needed for the experiments. Some bleached kraft market pulp (softwood) that had been refined was examined on the flexibility slides, and it was found that these fibers did adhere to the slide and the less visible length of the fiber over the wire could be measured. To see how much refining was required for the pulp to drop to a low enough freeness where flexibility could be measured (450 freeness estimated), 360 oven dry grams of

NIST SW pulp were discentegrated and refined according to TAPPI procedure T 200 om-89. The pulp was refined to different times and freeness tests (TAPPI procedure T 227 om-92) and samples were taken for different refining times (0, 5, 10, 15, 20, 30, 45 and 60 minutes). The freeness measurement for the different refining times can be seen in Figure 3 and the flexibility measurements for these pulps can be seen in Figure 4.

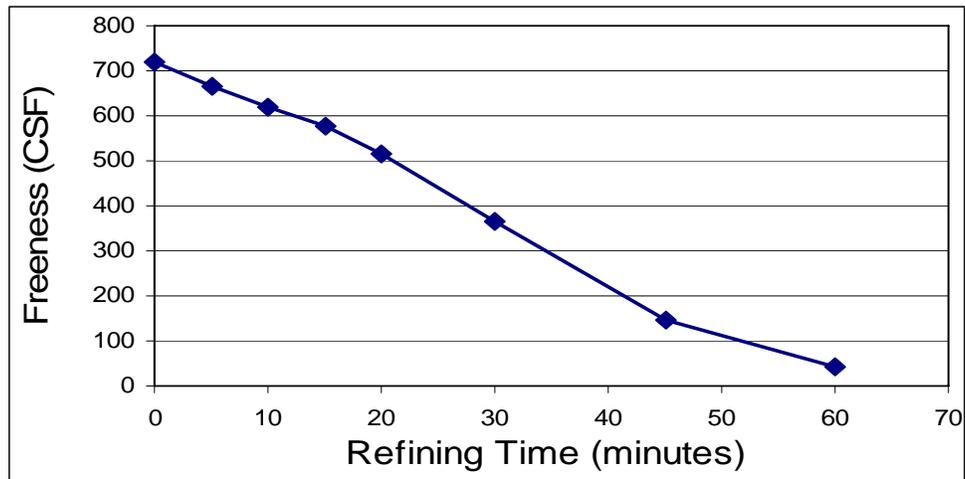


Figure 3: Freeness of NIST SW Pulp at Different Refining Times

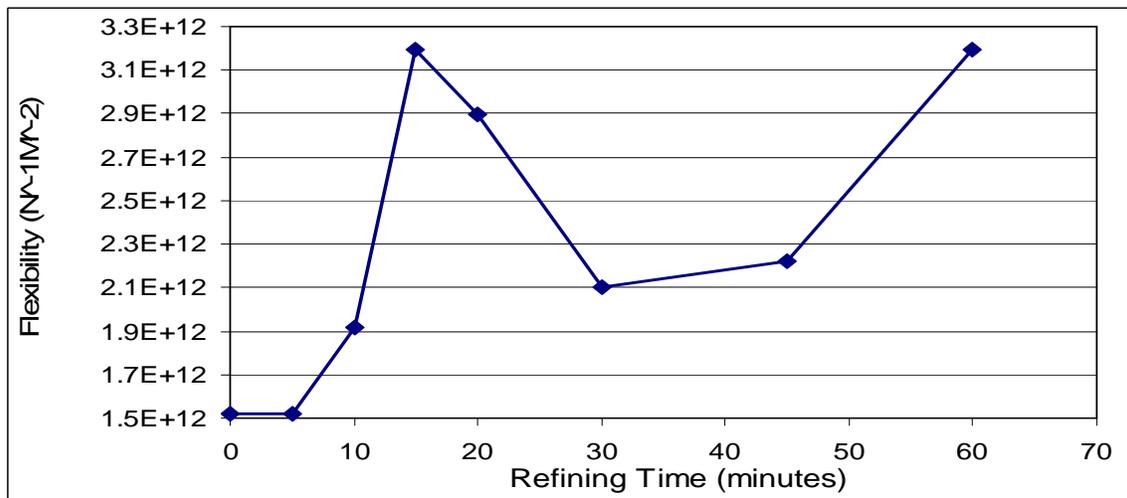


Figure 4: Flexibility of NIST SW Pulp at Different Refining Times

It was determined that 20 minutes of refining was needed to get the freeness down to ~ 450 ml CSF. So, for the preparation of the pulp needed for the first enzyme treatment trial, 360 oven-dried grams of pulp was refined for 20 minutes. The freeness was measured and it was too high (around 690 CSF), and this was probably due to the fact that last time, pulp was being pulled out every five minutes at the beginning of the trial, so there was less pulp being refined when the 20 minute sample was taken, meaning that it could have been refined more. So, the pulp for enzyme trial was refined for 15 extra minutes (samples taken out for freeness test every five minutes) till the freeness got down to 434 ml CSF. For the second enzyme treatment trial, the pulp was refined for 30 minutes and the freeness was measured as 441 ml CSF.

Pulp

Northern softwood bleached kraft pulp from the National Institute of Standards and Technology (NIST) was used for our study. 360 grams of a conditioned NIST SW pulp sheet was broken up and soaked in water for 4 hours. After this, the pulp was disintegrated for five minutes in the Valley Beater at 1.57 % consistency (23 L of total pulp slurry) according to TAPPI procedure T 200 om-89. After the pulp was discentegrated, it was refined by adding the 5500 gram weight to the Valley Beater and the pulp was refined for 30 minutes. After this stage, the pulp was drained through a false bottom screen and washed for seven minutes to wash away fines. After this, the pulp was drained to about 20 % consistency on a Buchner funnel and placed into a labeled plastic bag.

Enzyme Used

The cellulase, Dyadic EXP cellulase, was obtained from Dyadic International (Jupiter, Florida). Enzyme activity was determined by measuring carboxymethyl cellulose (CMCase) and Filterpaper Activity (FPU) with 1 % enzyme solution (0.5 g cellulase weight/50mL water). CMCase was 0.6 units/mL and FPU was 0.8 units/mL. The protein content of 1 % enzyme solution was 8.2 mg/mL.

Enzymatic Treatments

The pulp slurries of treated and untreated pulps (160 OD g total at 3 % consistency in 50 mM sodium acetate buffer for treated portion, pH 4.5 for Dyadic cellulase treatments) at different treatment times (0, 1, 2, 3, 4.5 and 6 hours) were heated and agitated (using Fisher Scientific Sted-Fast Model 600 agitators set at level ten with a three blade impeller) in separate stainless steel beakers in a water bath until temperature reached 45°C. Once temperature stabilized at 45°C and pH was 4.5, an enzyme charge (0.5% based on OD g weight to pulp) was added to the treated pulp beaker. Once each sample was taken out of the mixing tank at the respected time period, the enzyme in the sample was heat shocked in a microwave oven by heating the sample for four minutes (two minutes boiling). After this, the pulp slurry was drained on a Buchner Funnel and handsheets were made.

Control Treatment

For the NE (no enzyme) control experiment, the same procedure as for the treated experiments was used, with the exception that no enzyme was used at all for this treatment (so only water was added to the pulp slurry to control the consistency) and also only one sample was needed (20 OD g total of pulp). Again like the enzyme treated samples, the time sample (zero-minute sample) was heat shocked in the same manner as the enzyme treated samples.

Pulp Testing

Eight handsheets were prepared for each experimental condition according to TAPPI test method T 205-om88, but instead of couching the fiber mat/double blotter paper/couch plate stack with five rolls, only three rolls were used and then the fiber mat that is attached to blotter paper was lifted up and placed on the bench with the fiber mat facing up. A round metal handsheet plate was placed on top of the round fiber mat and the couch plate was placed on top of this. The couch roll was then used to couch the

assembly four additional times. The fiber mat (attached to round metal handsheet plate) was then peeled from blotter paper and the handsheet (still on round metal handsheet plate) was placed in a handsheet ring. All eight handsheet rings were stacked together with handsheets facing down and then placed a handsheet ring clamp. The sheets were then placed in a 50 % relative humidity and 23 °C environment to dry under restraint.

After the handsheets were dried, the basis weight, thickness, density, breaking length tensile strength, zero-span dry tensile and zero-span wet tensile strength were measured for the handsheets. All tests were conducted according to TAPPI test method T 220 om-88 and T 231 cm-96.

FQA Testing of Enzyme Treated Samples

An FQA (fiber quality analyzer) machine was used to analyze the enzyme treated samples to check enzyme treatment effect (percent fines, kink index, fiber length, curl index, etc) associated with increased enzyme concentration and incubation time. The FQA works by using a 10 ml sample at 0.02% consistency and diluting the sample to 600 ml and then the machine sucks up the sample and a camera built into the machine scans for and calculates kink index, fiber length, etc. The percent fines (length weighted) is calculated as: % Fines = $100 \times (\sum n_i L_i) / L_T$, where n = number of fibers less than 0.2 mm, L_i = fines class midpoint length, and L_T = total fiber length. The kink index is calculated as: Kink Index = $[2N(21-45) + 3N(46-90) + 4N(91-180)] / L_{total}$, where N is the number of fibers and the numbers in parentheses are the range of fiber angles, so for example: two times the number of fiber angles that are between 21° and 45° is the $2N(21-45)$ part of the equation. And length weighted length, $L_w = (\sum n_i L_i^2) / (\sum n_i L_i)$. Curl Index = $(L/l) - 1$, where L = contour length and l = projected length.

3C.3 Results and Discussion

The main objective of this experiment was to see whether enzyme treatment would increase the amount of flexibility to softwood fiber over an extended enzyme

incubation time. Figure 5 shows the average flexibility results from two trials obtained over the extended enzyme treatment time of 6 hours (360 minutes).

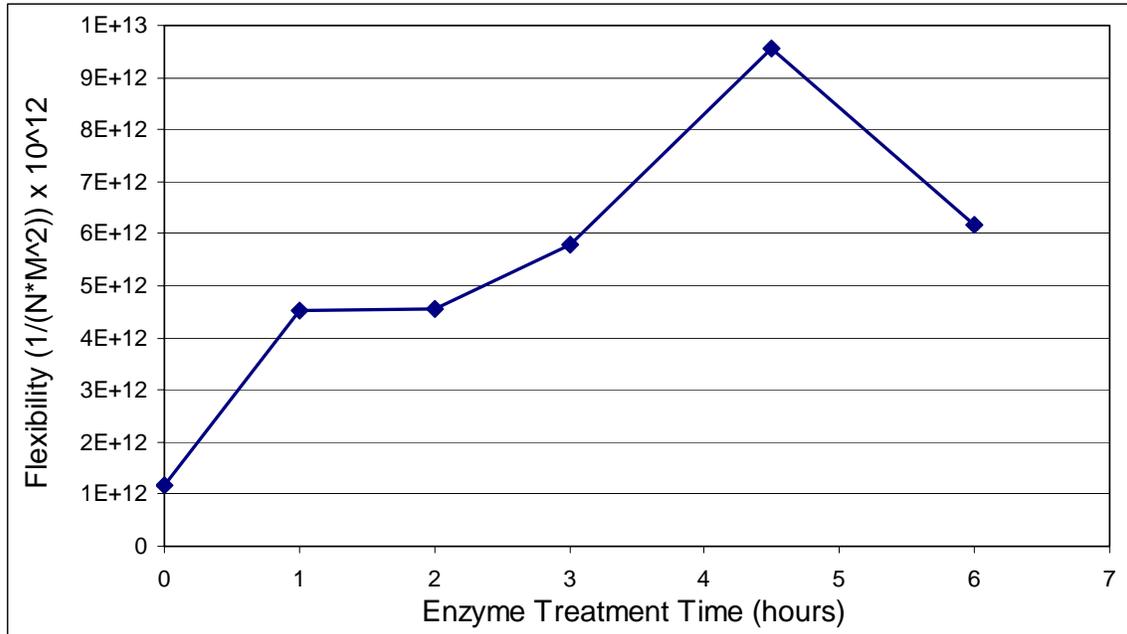


Figure 5: Average Fiber Flexibility (2 trials) vs. Enzyme Treatment Time

As can be seen from the Figure 5, the average fiber flexibility increases over the enzyme treatment time till a certain point (4.5 hours), in which the flexibility drops at the six-hour sample. The drop for the six-hour sample is due to the fiber shortening affect over extended treatment time that was explained and shown in section 3A. This shortening affect is due to the fibers being degraded at dislocation (notch) and/or kink areas and the fibers eventually breaking up at these weakened areas. This drop in fiber length results in the fibers being stiffer and causing the drop in fiber flexibility that is seen for the six-hour sample. But overall, the flexibility increases dramatically between the zero hour and 4.5 hour samples due to the fibers becoming more flexible at the kink and notch areas that have become weakened and resulting in more hinge-like action at those areas.

As mentioned in the introduction, it was also desired that the results from this trial would comply with the Procter and Gamble patent claims, even though this pulp has been refined, which is unlike what was done in the patent and different from what was done in the previous experiments explained in the earlier sections. In the production of the handsheets made for physical testing, the first goal was to produce tissue grade paper with a density below 0.4 g/cm^3 or 400 kg/m^3 using the modified TAPPI test method T 205-om88. This was a requirement in Procter and Gamble's U.S. Patent 6,146,494 for producing tissue grade paper and as can be seen from Table 1, it was not possible to reproduce handsheets with densities below 400 kg/m^3 for the refined treated and refined NE control samples. Only for the unrefined NE control, could this claim be reproduced. Table 1 also shows the average basis weights and calipers (thickness) for the basis sheets used in the two trials.

Table 1: Densities, Basis Weights and Calipers of Handsheets

Reaction Time (minutes)	Density (kg/m³)	Basis Weight (g/m²)	Caliper (inches)
0 (Unrefined NE Control)	286.7	64.1	0.0088
0 (Refined NE Control)	470.9	63.0	0.0053
0	440.7	60.5	0.0054
60	441.3	67.3	0.0060
120	436.7	64.4	0.0058
180	465.8	71.5	0.0060
270	476.0	58.4	0.0048
360	470.4	67.5	0.0056

As can be seen from the table, the densities for the different enzyme treatment times and the refined control are all significantly higher than that of the unrefined NE control. This indicates that refining has an effect on increasing the density of the handsheet because all other procedures, including handsheet making procedure remain the same for the refined pulp handsheets with the exception of refining. From looking at

the basis weights, it can be seen that basis weights are pretty much in the same range for the enzyme treated samples and for both the unrefined and refined controls, indicating that basis weight is not contributing to the difference in density. But, looking at the caliper section of the table, it can be seen that the caliper for the zero minute unrefined control is significantly higher than that of the other samples (enzyme treated and refined control) that have been refined. This decrease in caliper is probably due to the improved fiber-to-fiber bonding, which means that the paper sheet is less dense since the sheet will have less dead space between the fibers, resulting in the increase in density that is seen in the results.

Figure 6 shows the dry zero span tensile strength results for the handsheets of the refined enzyme treated tests, the controls (refined and non-refined) and also the different enzyme concentration tests shown in section two. The refined control showed the highest dry zero span, while the active enzyme refined (0.5 % enzyme concentration) samples progressively lost zero-span tensile strength as the reaction time increased, with the exception of the three-hour sample that increased. This increase can not be explained, but is the result average of the two trials. The values for the 0.5 % enzyme concentration refined samples fall somewhere between the 0.5% enzyme concentration unrefined samples and the 0.1 % enzyme concentration unrefined samples, indicating that the refining had an effect on increasing the zero-span tensile strength for the 0.5 % enzyme concentration. This is also shown for the refined control sample, in that its dry zero span tensile strength is higher than the unrefined control sample.

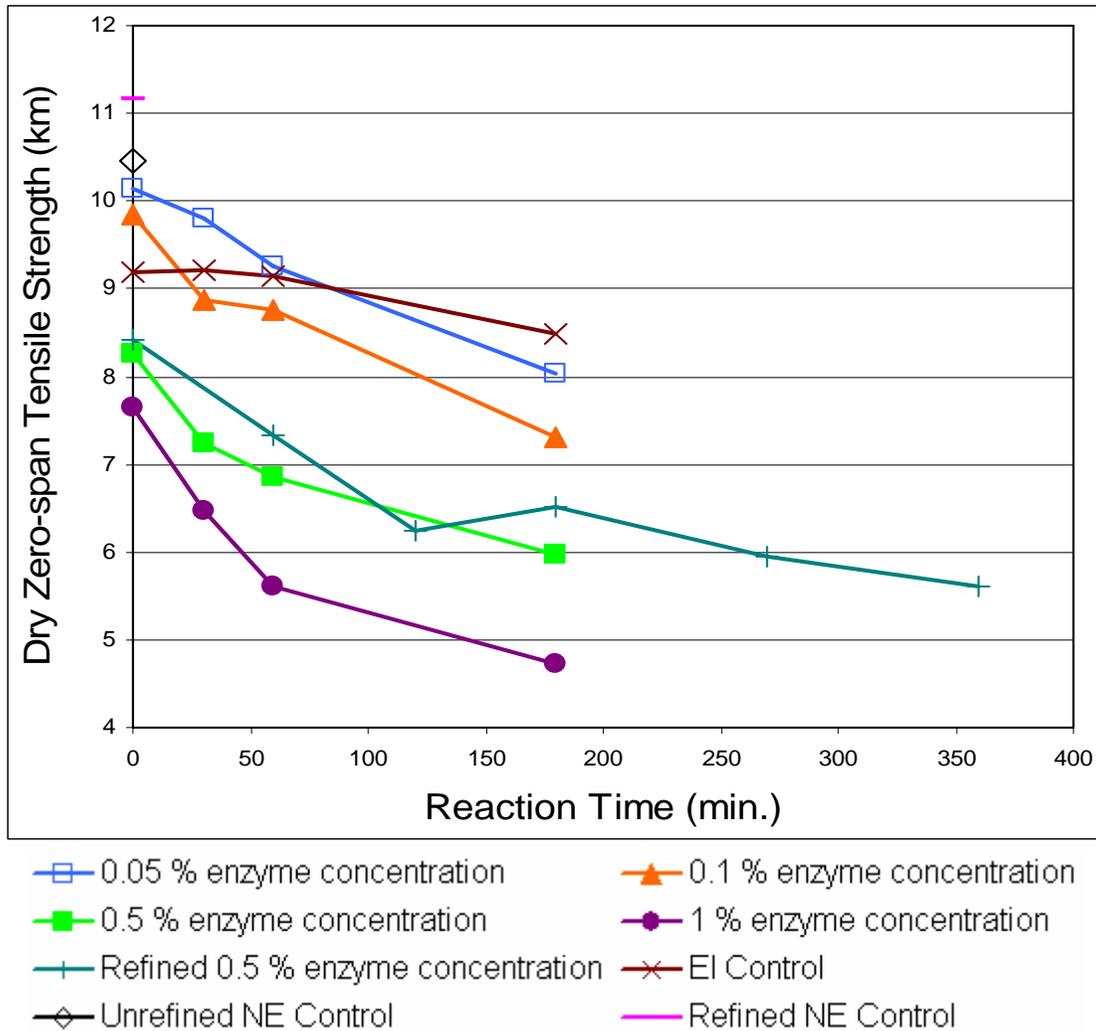


Figure 6: Dry Zero Span Tensile Strength for Enzyme Treated Samples, EI Control and NE Controls

Figure 7 shows the wet zero span tensile strength results for the handsheets of the refined enzyme treated tests, the controls (refined and non-refined) and also the different enzyme concentration tests shown in section two. The control # 2 (refined) showed the highest wet zero span, while the active enzyme refined (0.5 % enzyme concentration) samples progressively lost zero-span tensile strength as the reaction time increased, with the exception of the 4.5-hour sample that increased. This increase can not be explained, but is the result average of the two trials. The values for the 0.5 % enzyme concentration refined samples fall somewhere between the 0.5 % enzyme concentration unrefined

samples and the 1 % enzyme concentration unrefined samples for the first 1.5 hours or so, then they drop off to lower values than the 1 % enzyme concentration. This indicates that the refining might cause the fibers to lose wet zero span tensile strength as reaction time increases, which is different than the control #2 refined, which saw an increase in wet zero span tensile strength compared to the unrefined control #2 and the dry zero span tensile strength results which showed higher zero span tensile strength results for the refined samples.

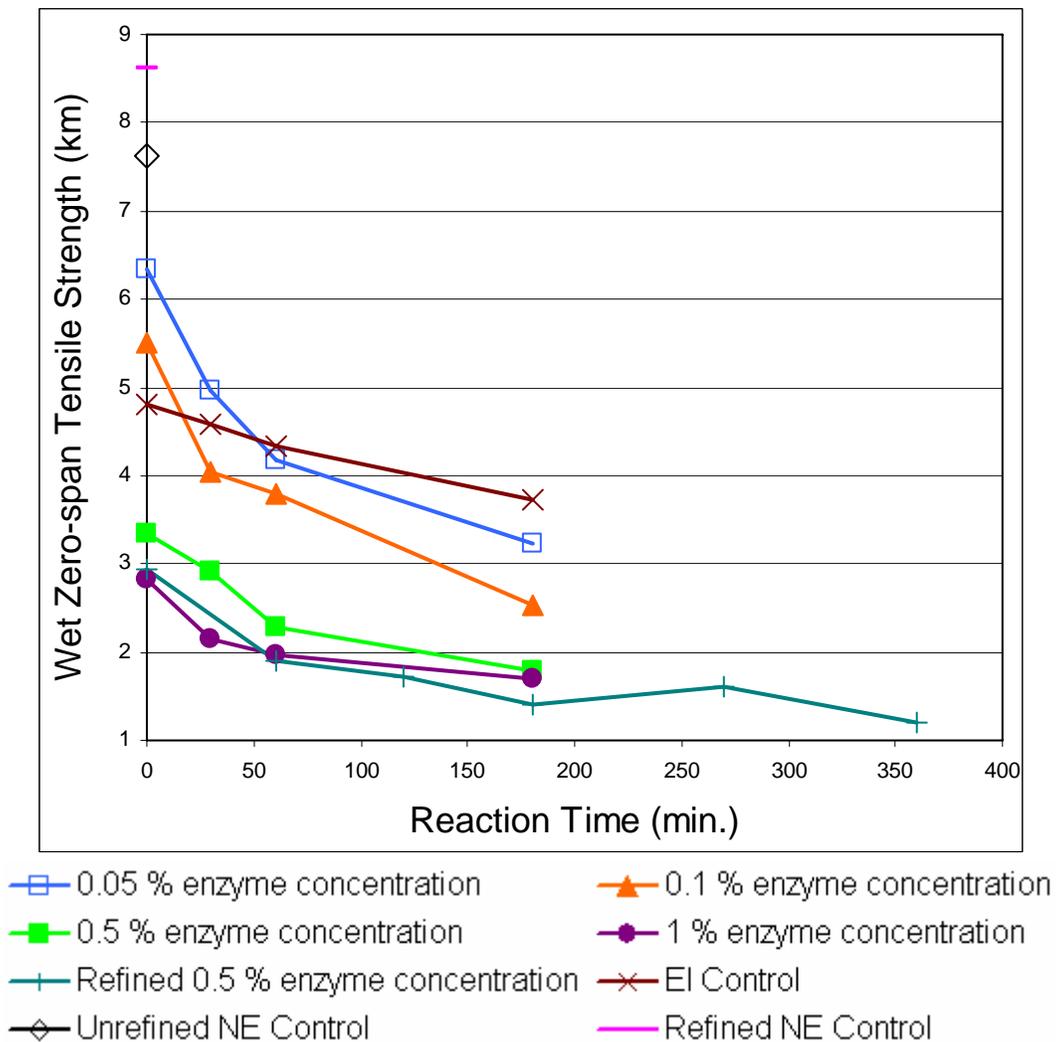


Figure 7: Wet Zero Span Tensile Strength for Enzyme Treated Samples, EI Control and NE Controls

Table 2 and Figure 8 show the results for repeating Procter and Gamble's U.S. Patent 6,146,494 claim that modified cellulosic fibers should have a dry zero span tensile index that is at least about 35 % less than the dry zero span tensile index of the corresponding unmodified cellulosic fibers (refined NE control). It was found from the test results that it was possible to almost (34 %) achieve the 35 % drop in dry zero span tensile strength after 1 hour of enzyme treatment. This percent drop progressively increased all the way to the six-hour (360 minutes) treatment time (50%), with the exception of the three-hour (180 minutes) sample that dropped slightly in the percent dry zero span tensile strength drop.

Table 2: Patent Claim #3 Reproduction Results

Reaction Time (min)	% Drop in Zero Span Dry Tensile Strength for Treated as compared to Refined NE Control
0	25
60	34
120	44
180	42
270	47
360	50

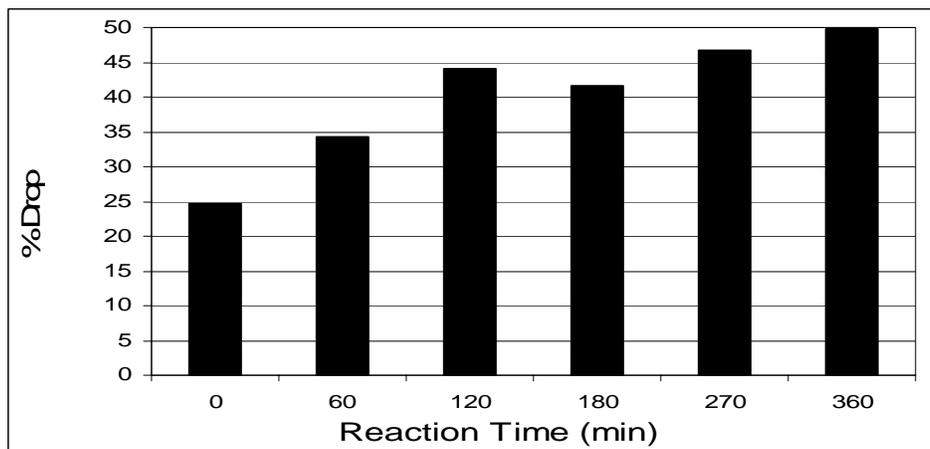


Figure 8: Patent Claim #3 Reproduction Results

Table 3 and Figure 9 show the results for repeating Procter and Gamble’s U.S. Patent 6,146,494 claim that modified cellulosic fibers should have a wet zero span tensile index that is at least about 70 % less than the wet tensile index of the corresponding unmodified fibers (refined NE control). It was found from the test results that it was possible (78 %) achieve the 70 % drop in wet zero span tensile strength after one hour of enzyme treatment. This percent drop progressively increased all the way to the six hour (360 minutes) treatment time (86 %), with the exception of the 4.5 hour (270 minutes) sample that dropped slightly in the percent wet zero span tensile strength drop.

Table 3: Patent Claim #4 Reproduction Results

Reaction Time (min)	% Drop in Zero Span Wet Tensile Strength for Treated as compared to Refined NE Control
0	66
60	78
120	80
180	84
270	81
360	86

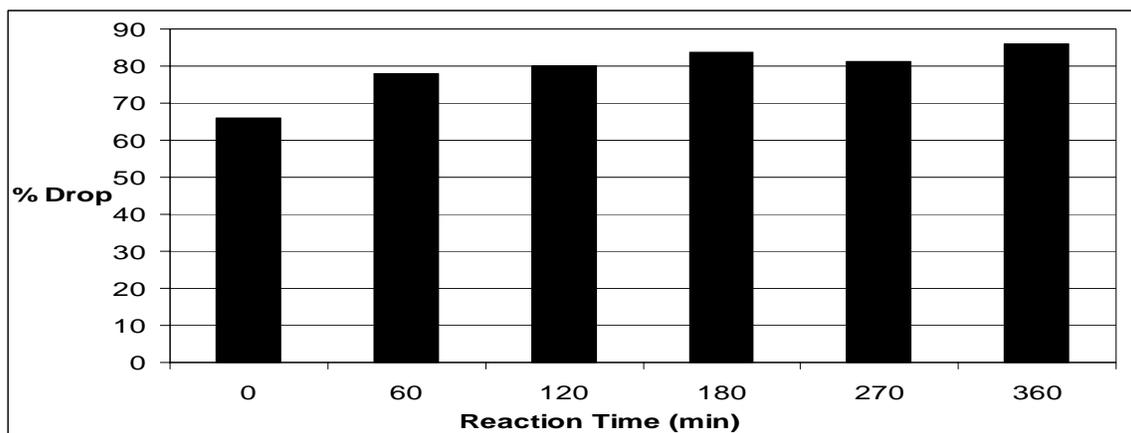


Figure 9: Patent Claim #4 Reproduction Results

Table 4 and Figure 10 show the results for repeating Procter and Gamble’s U.S. Patent 6,146,494 claim that the modified fibers that form a fibrous structure (handsheet) should have a dry tensile index that is at least as great (90 % similar) as the dry tensile index of a handsheet made from the corresponding unmodified fibers (refined NE control). From the test results, it was found that the 90 % similarity could only be achieved at the zero minute sample and that the percent similarity drops significantly as the reaction time increases with the exception of the six hour sample in which it only increased slightly. This is the result of the enzyme treated samples having lower tensile strengths than the control sample. Generally, it is a known fact that refining increases tensile strength of paper, but in this case, the fiber have been enzyme treated and this might be the reason why the tensile strength drop for the increasing enzyme treatment time.

Table 4: Patent Claim #2 Reproduction Results

Reaction Time (min)	% Similarity b/w Treated Dry Tensile and Refined NE Control Dry Tensile
0	90
60	66
120	59
180	58
270	57
360	61

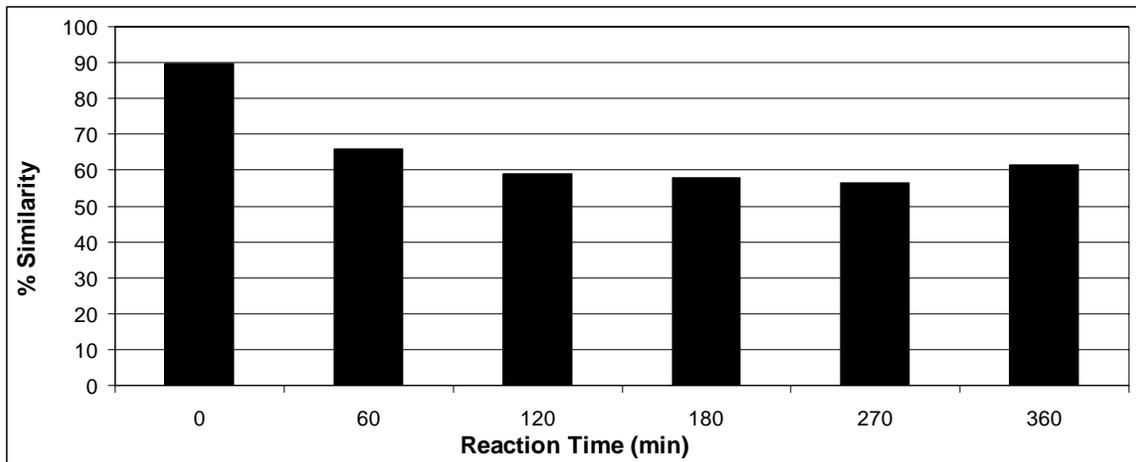
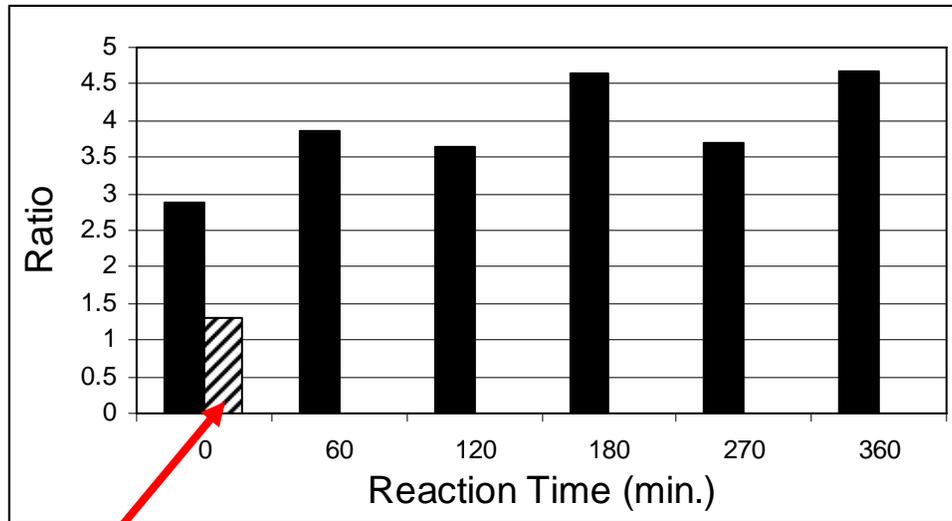


Figure 10: Patent Claim #2 Reproduction Results

Table 5 and Figure 11 show the results of repeating Procter and Gamble’s U.S. Patent 6,146,494 claim that modified cellulosic fibers should exhibit a ratio of dry zero span tensile index to wet zero span tensile index from about 1.5 to about 3. Overall, the test results show that we were able for the most part to repeat this claim for two trials. From the looking at the results, it is of interest that the ratio is lower for the control as compared to the enzyme treated samples. Overall the ratio increases over the enzyme treatment time from 2.9 for the zero minute sample to 4.7 for the 360-minute sample.

Table 5: Patent Claim #5 Reproduction Results

Treated Time (min)	Ratio between dry zero span and wet zero span
0 (Refined NE Control)	1.3
0	2.9
60	3.9
120	3.6
180	4.7
270	3.7
360	4.7



Unrefined NE Control

Figure 11: Patent Claim #5 Reproduction Results

The Fiber Quality Analyzer (FQA) was also used to validate the results of the flexibility tests. In Figure 12 it can be seen that overall the percent fines increased significantly over the incubation time, with the percent fines decreasing only slightly for the 60 minute sample. This slight drop is probably due to the enzymes absorbing to and eating away the fines that are already present in the pulp at the beginning of the enzyme treatment, causing the percent fines to decrease slightly at first. The percent fines begin to rise because the enzymes are continuously eating away at fibers on the dislocation areas creating many fragments (fines). These results will correspond with the change in the mean fiber length results, which will be shown next after the fines results.

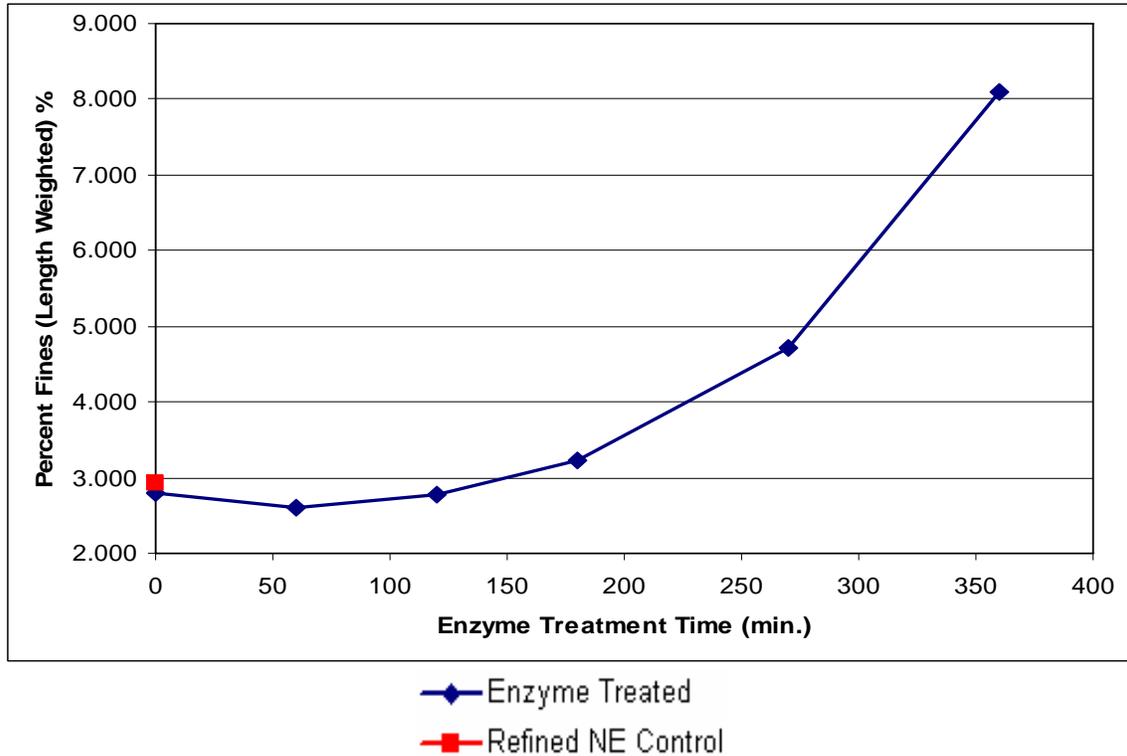


Figure 12: FQA Results: Percent Fines (Length Weighted %) for Different Enzyme Reaction Times (min.)

Figure 13 shows the FQA results for mean fiber length (length weighted mm) for the different enzyme reaction times (minutes). From looking at the results, it appears that the mean fiber length drops significantly over the incubation period. These results reflect the results seen in Figure 12 for the percent fines, indicating that the intact fibers are breaking up at the dislocation areas, resulting in more fiber fragment (fines) and shorter fibers. The significant drop in mean fiber length and significant increase in percent fines in the last 1.5 hours of the treatment corresponds to the significant decrease in flexibility that is seen in Figure 5.

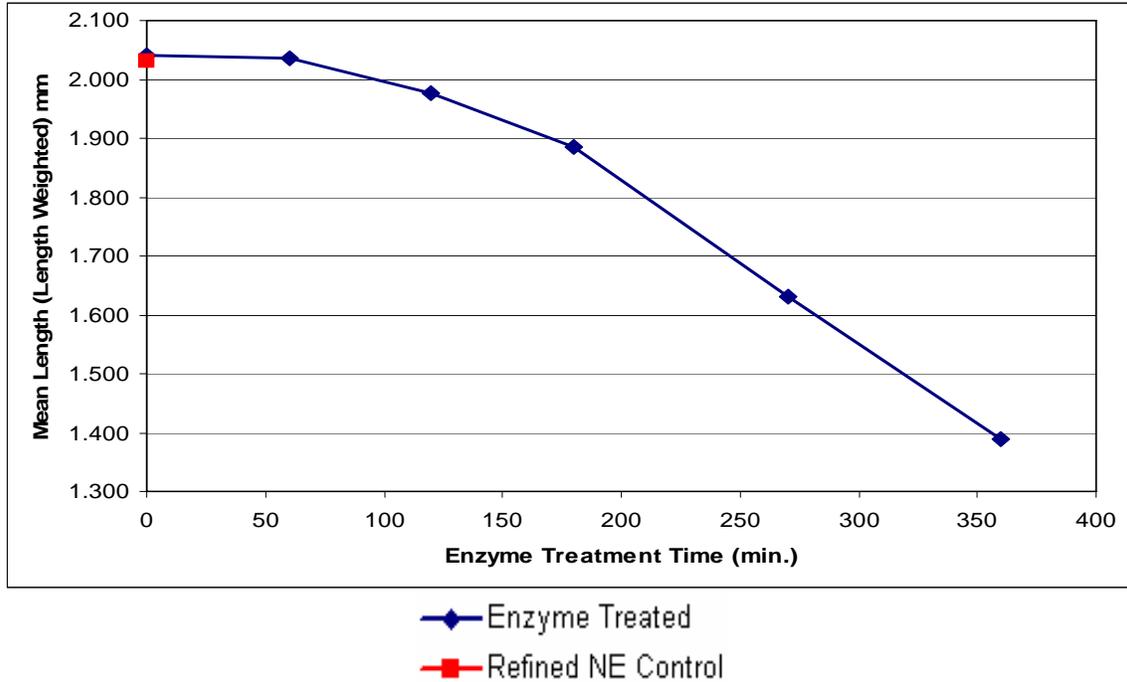


Figure 13: FQA Results: Mean Fiber Length (Length Weighted mm) for Different Enzyme Reaction Times (min.)

Figure 14 shows the FQA results for mean kink index (1/mm) for different enzyme reaction times. It can be noticed from the graph that, except for the 270-minute sample, that there is a consistent increase in mean kink index as the enzyme treatment time increases. The increase in kink index is probably due to the enzymes weakening the fibers at the dislocation areas and creating kinks, and then as the incubation period extends, the enzyme action proceeds to break up the fiber at these dislocation/kink areas and the kink that used to be on the fiber is no longer there, because the fiber is broken, resulting in a lower mean kink index as seen for 270 minute sample. Then the fibers that have been broken up after 270 minutes begin to create more kinks at dislocation areas as the enzyme continues to degrade and weaken the fibers till the end of the treatment period (360 minutes). This does not entirely reflect what is seen in the flexibility results in Figure 5, because the drop in flexibility is not seen till the 360 minute sample, but for the most part it does indicate that as more kinks are created on the fibers, this allows the fiber to be more flexible since the fiber can bend at the new hinge areas (kinks), allowing

the fibers to bend more easily over the stainless steel wires on the slide glass and hence, hence indicating greater flexibility.

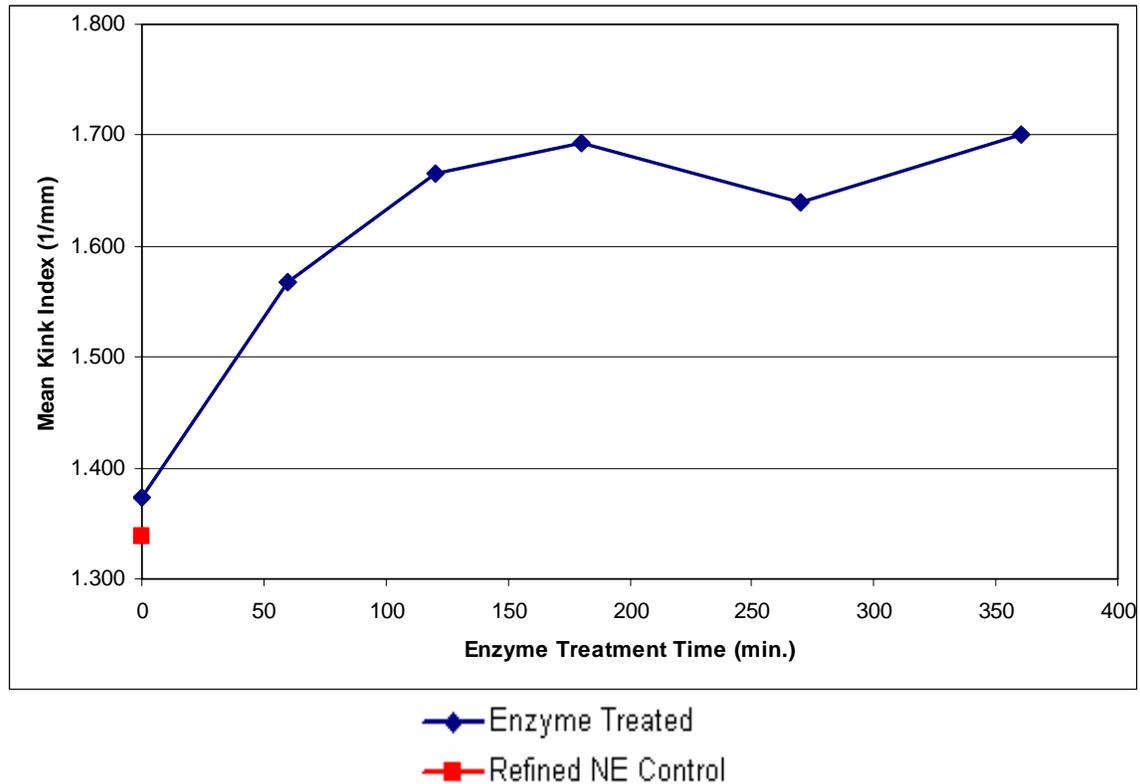


Figure 14: FQA Results: Mean Kink Index (1/mm) for Different Enzyme Reaction Times (min.)

Figure 15 shows the FQA results for mean curl index for different enzyme reaction times. From the results, it can be seen that there is a significant increase in the curl index in the first 60 minutes of the reaction, then there is a significant drop in curl index at the 120 minute sample, then the curl index remains constant to the 180 minute sample, after which, the curl index drops significantly till the end of the reaction (360 minutes). The increase seen at the beginning of the reaction is probably due to the enzymes eating away and weakening the fibers at dislocation and/or kink areas causing the fibers to be better able at bending, resulting in higher curl index. But after a certain amount of time (three hours), the fibers begin to break up at these weakened areas,

resulting in shorter, stiffer fibers, and hence lower curl index. This reflects the results seen in Figures 12 and 13 in which the fiber lengths decrease significantly during the last three hours and the percent fines increases significantly in the last three hours. This overall effect relates to what is seen in Figure 5, in which the flexibility decreases during the last one and a half hours due to the fibers shortening, causing the fibers to become stiffer with less curls.

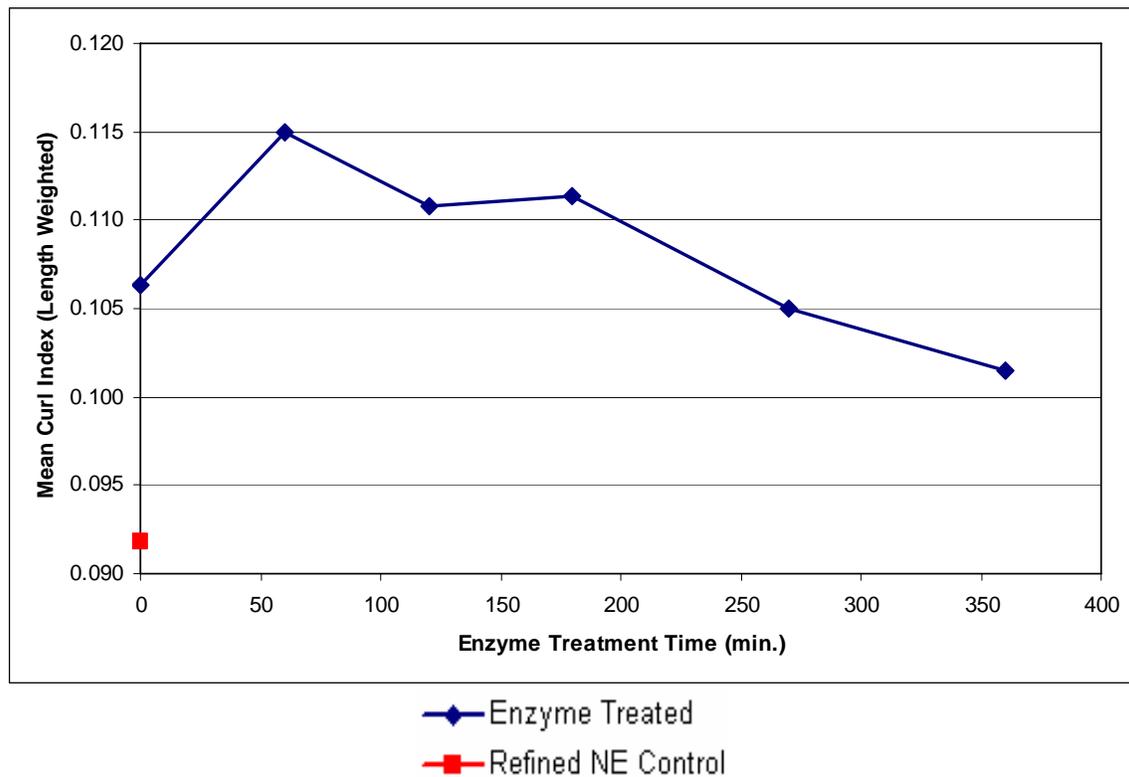


Figure 15: FQA Results: Mean Curl Index (1/mm) for Different Enzyme Reaction Times (min.)

3C.4 Conclusions

From the results, it can be concluded enzymes significantly affect fiber flexibility up to certain point (4.5 hours at 0.5 % enzyme concentration), when the flexibility begins to decrease due to fibers breaking up by enzymatic degradation, which was shown in the

FQA results. Also, it was shown that the results for the refined enzyme treated pulp samples could reproduce most of the claims stated in the Procter and Gamble patent. Only the handsheet density requirement ($\leq 400 \text{ kg/m}^3$) and the 90 % similarity in tensile strength between enzyme treated and untreated samples requirement were not entirely met, and this is more than likely due to the refining that was done to the pulp to allow for the flexibility measurements to be made. The overall goal was to show that flexibility would increase over extended enzyme treatment time. The bulk softness, which is controlled primarily by the structure of the sheet, is related to the bending stiffness, compressibility and modulus of the structure as a whole. These results prove that enzyme treatment can increase flexibility over extended treatment time, resulting in lower bending stiffness, and hence higher bulk softness.

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Appendices

Appendix A – Procedure for Preparing Pulp

1. Break up one pre-wetted (slightly dampened with distilled water to allow easier tearing) sheet of NIST softwood pulp into small pieces (roughly 2" x 2" in size).
2. Place broken-up pieces in a clean bucket and fill with distilled water to the top of the pulp. Allow pulp to disintegrate in bucket for four hours.
3. Disintegrate pulp slurry in British disintegrator by filling half of disintegrator container with pulp the other half with distilled water (fill only to the top of the bevels) and set disintegrator speed at level 50 using Dayton DC speed controller Model 5X485C for ten minutes.
4. It will take at least four runs on the disintegrator since disintegrator can not hold all of the pulp slurry that comes from one NIST SW sheet.
5. Filter disintegrated pulp on Buchner funnel.
6. Place pulp cakes from funnel in plastic bags and mix up the pulp cakes by hand in the bag.
7. Take out three samples (roughly five to six grams) from the bag and weigh each one of the samples out (wet weight).
8. Place samples in oven overnight or in speed drier for 20 minutes.
9. Weigh the three samples after drying (dry weight) and calculate the % consistency (% K) of each sample by taking the dry weight of the sample and dividing it by the wet weight and multiplying by 100. The equation for calculating consistency is:

$$\% K = ((\text{pulp weight, OD g})/(\text{pulp weigh, OD g} + \text{water weight, g})) * 100$$

10. Calculate the average consistency of the combined pulp cakes by averaging the consistencies of the three samples.

Appendix B – Procedure for Enzyme Treatment of Softwood NIST Pulp

1. At least 20 OD g of pulp will be need for all of the tests in each time sample taken (0 min, 30 min, 60 min, and 3 hours) and an additional 20 OD g is needed to help with agitation of pulp. So 100 OD g of pulp will be need from the bag of disintegrated pulp cakes.

2. Calculate the amount of pulp needed for 100 OD g by using the following ratio:

$(\% \text{ consistency}/100) / (1 \text{ g total}) = (100 \text{ OD g}) / (X \text{ g total})$, and solve for X to find the amount of pulp needed for 100 OD g.

3. Calculate the amount of 0.05 molar sodium acetate buffer needed for three liters of pulp based on buffer recipe:

$(210.14 \text{ g C}_6\text{H}_8\text{O}_7\text{-H}_2\text{O} / 1 \text{ mol C}_6\text{H}_8\text{O}_7\text{-H}_2\text{O}) * (0.05 \text{ mol C}_6\text{H}_8\text{O}_7\text{-H}_2\text{O} / \text{L}) * 3 \text{ L} * (1000 \text{ ml} / 210.14 \text{ g}) = 150.1 \text{ ml}$ of buffer needed to regulate pH at 4.5 with roughly three liters of pulp.

4. Calculate the amount of dilution water needed for making 3 % consistency pulp:

$100 \text{ OD g} / 0.03 = 3333.33 \text{ g}$ (use 3033.33 g for diluting pulp initially and use 300 g for mixing with the powder enzyme before adding enzyme to pulp)

$3033.33 \text{ g} - (X \text{ g of pulp used}) - 100 \text{ OD g} - 150.1 \text{ g buffer} = Y \text{ g}$ of dilution water (deionized water) needed for making 3 % K pulp.

5. Calculate the amount of enzyme needed for 0.5 % enzyme concentration based on OD g weight:

$$100 \text{ OD g} * 0.005 = 0.5 \text{ g enzyme}$$

6. Place 0.5 g of enzyme in 500 ml beaker and add 300 g of deionized water to beaker for mixing purposes.
7. Place pulp, dilution water (deionized water), and buffer in a one gallon mixing container.
8. Place mixing container in a water bath and place stirrer (Fisher Scientific Sted-Fast™ Stirrer Model SL 600) in the pulp.
9. Turned on water bath and set to 45°C and also turned on stirrer to level nine setting.
10. Check pH to make sure that pulp pH is leveled out to 4.5.
11. Place enzyme mix in water bath.
12. Once pulp and enzyme are up to 45°C and the pulp pH is 4.5 (both temperature and pH are checked before each time sample is taken), the enzyme mix is added to the pulp and a 600 ml sample is taken from the mixing container after 30 seconds and this is labeled as zero min treated time sample.
13. A 600 ml time sample is then taken out for each of the following times, 30-min, 60-min, and 3-hour.
14. After each 600 ml time sample is taken from the mixing container and placed in a 1000 ml beaker, the sample is directly placed in a microwave oven for four minutes (two minutes boiling) to heat shock the enzymes.

15. After each sample is microwaved, the sample is placed in a labeled plastic bag and the bag for each time sample is placed in a refrigerator.
16. Once all time samples have been treated and heat shocked, each sample is filtered on a Buchner funnel and the pulp cake samples are placed back in their respective time sample labeled bags. The samples are not washed.
17. Measure consistency of each time sample by taking roughly five to six g of pulp from each sample and weighing each sample (wet weight) and the dry each sample in the speed drier for 20 minutes and then re-weigh the samples. The percent consistency is calculated by taking the dry weight and dividing by the wet weight and multiplying by 100. The following equation is used for calculating consistency:

$$\% K = ((\text{pulp weight, OD g}) / (\text{pulp weigh, OD g} + \text{water weight, g})) * 100$$

18. Based on the consistencies for each time sample, calculate the amount of pulp needed for making handsheets (1.2 OD g) for each time sample. This is calculated by the following equation:

$$(\% \text{ consistency} / 100 \text{ g total}) = (1.2 \text{ OD g}) / (Z \text{ g total}), \text{ solve for } Z \text{ to find the amount of pulp needed for } 1.2 \text{ OD g.}$$

19. Measure and record the drainage time for each handsheet made for each time sample. Drainage time is the time period from when the water release lever is pushed down till when the water in the handsheet mold is gone.
20. Make eight handsheets for each time sample by following TAPPI test method T 205 om-88, but instead of couching the fiber matt/double blotter paper/couch plate stack with five rolls, use three rolls and then lift up fiber matt that is attached

to blotter paper and place it on bench with fiber matt facing up. Then place a round metal handsheet plate on top of round fiber matt along with placing couch plate on top of round plate and then roll couch plate four times. Then peel fiber matt (attached to round metal handsheet plate) from blotter paper and place handsheet (still on round metal handsheet plate) in handsheet ring. Stack all eight handsheet rings together with handsheets facing down and place them in a handsheet ring clamp and leave in paper testing lab overnight.

21. Measure basis weight, thickness, density, breaking length tensile strength, zero-span dry tensile and zero-span wet tensile strength for the handsheets made for each time sample by following TAPPI test method T 220 om-88.

Appendix C – Procedure for Sodium Acetate Buffer Preparation

1. Weigh out 210 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$).
2. Place the 210 g of citric acid monohydrate in a mixing container of appropriate size and add 750 ml of deionized water.
3. Mix (shake-up) solution and then place pH meter in solution.
4. Add enough NaOH (roughly 63 g) till the pH of the mixed solution is 4.5.
5. Then finish diluting the mixed solution to 1000 ml with deionized water.
6. Place the sealed container (jug) of buffer in the refrigerator till use of buffer is needed.

Appendix D – Procedure for Enzyme Inactivated Control (EI control)

1. At least 20 OD g of pulp will be need for all of the tests in each time sample taken (0 min, 30 min, 60 min, and 3 hours) and an additional 20 OD g is needed to help with agitation of pulp. So 100 OD g of pulp will be need from the bag of disintegrated pulp cakes.

2. Calculate the amount of pulp needed for 100 OD g by using the following ratio:

$(\% \text{ consistency}/100) / (1 \text{ g total}) = (100 \text{ OD g}) / (X \text{ g total})$, and solve for X to find the amount of pulp needed for 100 OD g.

3. Calculate the amount of 0.05 molar sodium acetate buffer needed for three liters of pulp based on buffer recipe:

$(210.14 \text{ g C}_6\text{H}_8\text{O}_7\text{-H}_2\text{O} / 1 \text{ mol C}_6\text{H}_8\text{O}_7\text{-H}_2\text{O}) * (0.05 \text{ mol C}_6\text{H}_8\text{O}_7\text{-H}_2\text{O} / \text{L}) * 3 \text{ L} * (1000 \text{ ml} / 210.14 \text{ g}) = 150.1 \text{ ml}$ of buffer needed to regulate pH at 4.5 with roughly three liters of pulp.

4. Calculate the amount of dilution water needed for making 3 % consistency pulp:

$100 \text{ OD g} / 0.03 = 3333.33 \text{ g}$ (use 3033.33 g for diluting pulp initially and use 300 g for mixing with the powder enzyme before adding enzyme to pulp)

$3033.33 \text{ g} - (X \text{ g of pulp used}) - 100 \text{ OD g} - 150.1 \text{ g buffer} = Y \text{ g}$ of dilution water (deionized water) needed for making 3 % K pulp.

5. Calculate the amount of enzyme need for 0.5 % enzyme concentration based on OD g weight:

$$100 \text{ OD g} * 0.005 = 0.5 \text{ g enzyme}$$

6. Place 0.5 g of enzyme in 500 ml beaker and add 300 g of deionized water to beaker for mixing purposes.
7. Place pulp, dilution water (deionized water), and buffer in a one gallon mixing container.
8. Place mixing container in a water bath and placed stirrer (Fisher Scientific Sted-FastTM Stirrer Model SL 600) in the pulp.
9. Turned on water bath and set to 45°C and also turned on stirrer to level nine setting.
10. Check pH to make sure that pulp pH is leveled out to 4.5.
11. Place enzyme mix in water bath.
12. Once pulp and enzyme are up to 45°C and the pulp pH is 4.5 (both temperature and pH are checked before each time sample is taken), the enzyme mix is taken from the water bath and placed in a 1000 ml beaker and then the beaker is placed in the microwave oven and heated for four minutes (two minutes boiling).
13. Beaker is then placed in refrigerator and allowed to cool down to 45°C.
14. Once temperature of enzyme mix has reached 45°C, the enzyme mix is added to the pulp and a 600 ml sample is taken from the mixing container after 30 seconds and this is labeled as zero minute treated time sample.

15. A 600 ml time sample is then taken out for each of the following times, 30-min, 60-min, and 3-hour.
16. After each 600 ml time sample is taken from the mixing container and placed in a 1000 ml beaker, the sample is directly placed in a microwave oven for four minutes (two minutes boiling) to heat shock the enzyme.
17. After each sample is microwaved, the sample is placed in a labeled plastic bag and the bag for each time sample is placed in a refrigerator.
18. Once all time samples have been treated and heat shocked, each sample is filtered on a Buchner funnel and the pulp cake samples are placed back in their respective time sample labeled bags. The samples are not washed.
19. Measure consistency of each time sample by taking roughly five to six grams of pulp from each sample and weighing each sample (wet weight) and the dry each sample in the speed drier for 20 minutes and then re-weigh the samples. The percent consistency is calculated by taking the dry weight and dividing by the wet weight and multiplying by 100. The following equation is used for calculating consistency:

$$\% K = ((\text{pulp weight, OD g}) / (\text{pulp weight, OD g} + \text{water weight, g})) * 100$$

20. Based on the consistencies for each time sample, calculate the amount of pulp needed for making handsheets (1.2 OD g) for each time sample. This is calculated by the following equation:

$$(\% \text{ consistency} / 100 \text{ g total}) = (1.2 \text{ OD g}) / (Z \text{ g total}), \text{ solve for } Z \text{ to find the amount of pulp needed for } 1.2 \text{ OD g.}$$

21. Measure and record the drainage time for each handsheet made for each time sample. Drainage time is the time period from when the water release lever is pushed down till when the water in the handsheet mold is gone.

22. Make eight handsheets for each time sample by following TAPPI test method T 205 om-88, but instead of couching the fiber matt/double blotter paper/couch plate stack with five rolls, use three rolls and then lift up fiber matt that is attached to blotter paper and place it on bench with fiber matt facing up. Then place a round metal handsheet plate on top of round fiber matt along with placing couch plate on top of round plate and then roll couch plate four times. Then peel fiber matt (attached to round metal handsheet plate) from blotter paper and place handsheet (still on round metal handsheet plate) in handsheet ring. Stack all eight handsheet rings together with handsheets facing down and place them in a handsheet ring clamp and leave in paper testing lab overnight.

23. Measure basis weight, thickness, density, breaking length tensile strength, zero-span dry tensile and zero-span wet tensile strength for the handsheets made for each time sample by following TAPPI test method T 220 om-88.

Appendix E – Procedure for NE Control (no enzyme)

1. At least 20 OD g of pulp will be need for all of the tests in the one time sample that will be taken (zero-minute sample). So 20 OD g of pulp will be need from the bag of disintegrated pulp cakes.

2. Calculate the amount of pulp needed for 20 OD g by using the following ratio:

$(\% \text{ consistency}/100) / (1 \text{ g total}) = (20 \text{ OD g}) / (X \text{ g total})$, and solve for X to find the amount of pulp needed for 20 OD g.

3. Calculate the amount of 0.05 molar sodium acetate buffer needed for 0.6 L of pulp based on buffer recipe:

$(210.14 \text{ g C}_6\text{H}_8\text{O}_7\text{-H}_2\text{O} / 1 \text{ mol C}_6\text{H}_8\text{O}_7\text{-H}_2\text{O}) * (0.05 \text{ mol C}_6\text{H}_8\text{O}_7\text{-H}_2\text{O} / \text{L}) * 0.6 \text{ L} * (1000 \text{ ml} / 210.14 \text{ g}) = 30.02 \text{ ml}$ of buffer needed to regulate pH at 4.5 with roughly 0.6 L of pulp.

4. Calculate the amount of dilution water needed for making 3 % consistency pulp:

$20 \text{ OD g} / 0.03 = 666.67 \text{ g}$ (use 366.67 g for diluting pulp initially and use 300 g for the non-enzyme make-up water that will be added to the pulp later)

$366.67 \text{ g} - (X \text{ g of pulp used}) - 20 \text{ OD g} - 30.02 \text{ g buffer} = Y \text{ g}$ of dilution water (deionized water) needed for making 3 % K pulp.

5. Place 300 g of deionized water to a 500 ml beaker for the non-enzyme water control.

6. Place pulp, dilution water (deionized water), and buffer in a 2000 ml beaker.
7. Place mixing container in a water bath and placed stirrer (Fisher Scientific Sted-Fast™ Stirrer Model SL 600) in the pulp.
8. Turned on water bath and set to 45°C and also turned on stirrer to level four setting.
9. Check pH to make sure that pulp pH is leveled out to 4.5.
10. Placed the 300 g of non-enzyme water control in water bath.
11. Once pulp and non-enzyme water control are up to 45°C and the pulp pH is 4.5 (both temperature and pH are checked before each time sample is taken), the enzyme mix is added to the pulp and a 600 ml sample is taken from the mixing container after 30 seconds and this is labeled as 0-min treated time sample.
12. After the 0-minute 600 ml time sample is taken from the mixing container and placed in a 1000 ml beaker, the sample is directly placed in a microwave oven for four minutes (two minutes boiling).
13. After the sample is microwaved, the sample is placed in a labeled plastic bag and it is placed in a refrigerator.
14. The 0-minute time sample is then filtered on a Buchner funnel and the pulp cake sample is placed back in a labeled sample bag. The sample is not washed.
15. Measure consistency of the time sample by taking roughly five to six grams of pulp from the sample and weighing it (wet weight) and then dry the sample in the speed drier for 20 minutes and then re-weigh the sample. The percent consistency

is calculated by taking the dry weight and dividing by the wet weight and multiplying by 100. The following equation is used for calculating consistency:

$$\% K = ((\text{pulp weight, OD g}) / (\text{pulp weight, OD g} + \text{water weight, g})) * 100$$

16. Based on the consistency for the time sample, calculate the amount of pulp needed for making handsheets (1.2 OD g) the sample. This is calculated by the following equation:

$$(\% \text{ consistency} / 100 \text{ g total}) = (1.2 \text{ OD g}) / (Z \text{ g total}), \text{ solve for } Z \text{ to find the amount of pulp needed for } 1.2 \text{ OD g.}$$

17. Measure and record the drainage time for each handsheet made. Drainage time is the time period from when the water release lever is pushed down till when the water in the handsheet mold is gone.
18. Make eight handsheets for the time sample by following TAPPI test method T 205 om-88, but instead of couching the fiber matt/double blotter paper/couch plate stack with five rolls, use three rolls and then lift up fiber matt that is attached to blotter paper and place it on bench with fiber matt facing up. Then place a round metal handsheet plate on top of round fiber matt along with placing couch plate on top of round plate and then roll couch plate four times. Then peel fiber matt (attached to round metal handsheet plate) from blotter paper and place handsheet (still on round metal handsheet plate) in handsheet ring. Stack all eight handsheet rings together with handsheets facing down and place them in a handsheet ring clamp and leave in paper testing lab overnight.
19. Measure basis weight, thickness, density, breaking length tensile strength, zero-span dry tensile and zero-span wet tensile strength for the handsheets made by following TAPPI test method T 220 om-88.

Appendix F – Softwood Fiber Defects, Quantification and Assessment

Introduction

In this study, the efforts and results to develop and use a method for quantifying and assessing fiber defects are discussed. As mentioned earlier in the patent reproduction results report, enzyme technology is a versatile tool for modifying the fibers, since it has a tendency to degrade specific components [Buchert, J., et al., 1996]. It has been hypothesized that the specific components that enzyme attach to and degrade on fibers are defect areas, such as dislocations (notches), kinks and curls. The enzymatic action on the fibers creates localized notches in the fibers, which serve as “hinge points” around which the fibers can flex.

A compressibility model [Pawlak, J.J., 2001] suggests that by creating these localized hinge points, the coarseness of the fiber is changed very little and this allows for the compressibility of the fiber structure to be increased without reducing the density of the structure. If the tensile strength of the fiber network is much lower than the fiber strength, then reducing the fiber strength should have little or no impact on the sheet tensile strength, as the bonding remains the same. The net result of engineering such a structure would be a softer, more compressible product, with the same tensile strength as the original product.

By creating a method to accurately quantify and assess fiber defects and relate their change (defects/fiber ratio) to increased kneading (Hobart treatment) times, a correlation between defect ratio (using microscope counting) and zero-span tensile strength (dry and wet) for different kneading times could possibly be developed. The use of Hobart kneading is based upon the hypothesis that high consistency kneading will result in fibers that have more defects or enhance the defects of the ones already present, which would

allow for more areas of enzyme absorption to weaken the fibers. The basis for this is based upon a series of research papers originating from Korea [Lee, J. H., Seo, Y.B., Jeon, Y., Lee, H. L., Shin, J. H, Choi, C. H., Seo, S. W.]. The results of the zero-span tensile testing (dry and wet) for different Hobart kneading times and using enzyme treatments will be discussed in another report. In this study the main goals were to:

1. Determine a method that could be used to disperse fibers so that an accurate fiber count could be made under a microscope.
2. Establish a repeatable method for classifying fiber defects (kinks, curls and dislocations).
3. Quantify changes in defect ratios (defects/fiber) for increased kneading time.

The following methods and materials were used to carry out these two kneading treatment goals.

Experimental Methods and Materials

Pulp

Northern softwood bleached kraft pulp from the National Institute of Standards and Technology (NIST) was used for our study. One dry NIST SW pulp sheet (484g) is broken up and soaked in water for 20 hours. The pulp sheet was then disintegrated in a British disintegrator for 10 minutes at level 50 on a Dayton DC Speed Controller Model 5X485C. From here, pulp is filtered on Buchner funnel and consistency of pulp is measured.

Fiber Counting Methods

TAPPI method T 401 om-93 (Fiber analysis of paper and paperboard) was used as a beginning procedure for how to disperse and count fibers. This test method involves:

- A microscope of at least 100 magnification
- Slides and cover glasses
- A graduated dropper to deliver 0.5 ml
- Warm plate for drying slide samples at 50-60°C
- Using a 0.05 % consistency sample of 0.2 g OD pulp
- A fiber count of between 200 and 300

Microscope Set-up

An Olympus model BH-2 microscope was used in these experiments. A Sony color video camera (Power HAD DXC-97MD) mounted on top of the microscope was used to record and take images of the analyzed samples on the slide glass. These images taken from the video camera were transferred to an image analysis program (Image-Pro Plus Version 4.5 for Windows) on a computer where the images could be seen and analyzed. The fibers were counted at 100 X magnification and the defects were analyzed at 200 X magnification.

Hobart Mixer Kneading Method

The kneading of the pulp occurred in a four liter capacity Hobart mixer. Using 20 % consistency pulp, 120 OD g of NIST SW pulp was used and it was placed in the Hobart mixer where the pulp was kneaded for three hours total. Equivalent amounts of pulp sample were taken for increasing 30 minute intervals up to the three hour limit. Each sample (30, 60, 90, 120, 150, and 180 minute Hobart kneading times) was then rechecked for consistency because the samples dried out some during the kneading process. After checking the consistency of each sample, the samples were then washed in a handsheet mold ten times by filling the handsheet mold up with water and draining it ten times. After this, the consistency of the pulp was checked again.

Results and Discussion

For the first goal, which was to determine a method that could be used to disperse fibers so that an accurate fiber count could be made under a microscope, a technique based on TAPPI method T 401 om-93 (Fiber analysis of paper and paperboard) was used. From trial and error it was determined that the only way to prepare a microscope slide with between 200 and 300 fibers that were not clumped together was to use the following steps:

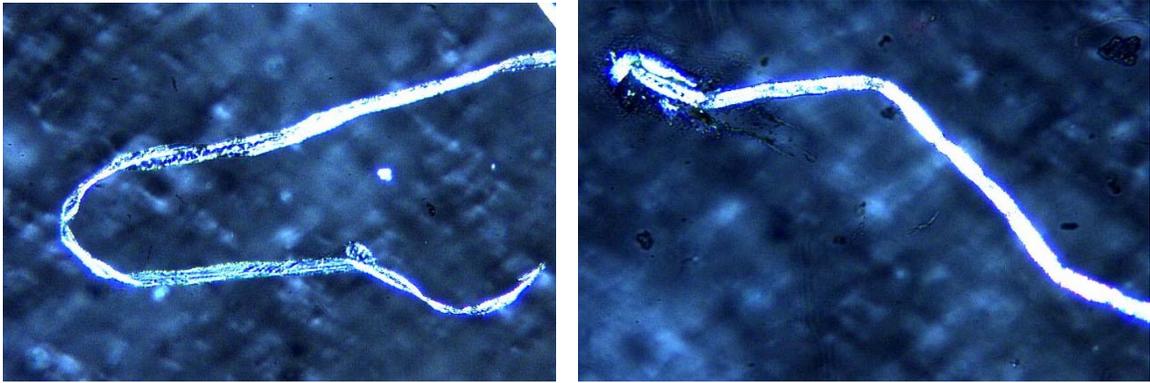
- Used 0.2 OD g pulp at 0.02 % consistency pulp
- Disintegrated in a British disintegrator for 10 minutes at level 50 on a Dayton DC Speed Controller Model 5X485C
- Allowed the sample to dry overnight on the slide with the cover glass on top

Using this technique, it was found that you could accurately count the number of fibers on slide sample and come close or to within the 200 fiber minimum mentioned in the method T 401 om-93. Table 1 shows the fiber counts from four different trials that were performed using the same slide preparation techniques.

Table 1: Fiber Count for Four Samples

Sample #1	Sample #2	Sample #3	Sample #4
179	123	163	242

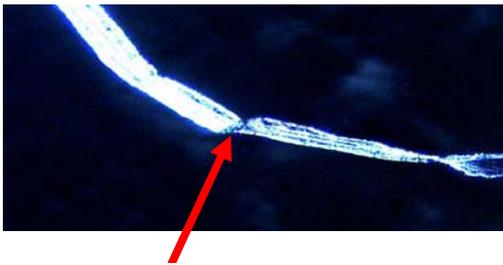
The following images in Figures 1a (left) and 1b (right) show what some of the slides prepared with fibers look like at 100 X magnification when fiber counting is being carried out.



Figures 1a (left) and 1b (right): Softwood Fibers at 100 X Magnification

As you can see from these images, fibers are not clumped together, but instead they are separated.

For the second goal, which was to establish a repeatable method for classifying fiber defects (kinks, curls and dislocations), a classification method was developed in which 200 X magnification was used to analyze the images of the slide glass fiber samples. A curl was classified as a gradual bend in a fiber; a kink was classified as a sharp angle bend in a fiber; and a dislocation (notch) was an area on a fiber in which there was damage or separation in the fiber. These dislocations could either be in the straight area of a fiber or in a kink bend also. The following images in Figure 2 and Figures 3a (left) and 3b (right) depict what is meant by kink, curl and dislocation.



Dislocation at 100 X magnification

Figure 2: Fiber with Dislocation at 100 X Magnification



Curl at 100 X magnification



Kink at 100 X magnification

Figures 3a (left) and 3b (right): Fiber with curl (3a) and Fiber with kink (3b)

A tallying table for each category (kink, curl and dislocation) was next developed, in which a sample on a slide glass would be scanned and the number of kinks, curls and dislocations would be added up for the fibers as the whole sample was scanned. Dislocations were further classified into three separate classes: Class One notches are one to three times the width of the fiber; Class Two notches are the same width as the fiber; and Class Three notches are less than the width of the fiber. The following table (Table 2) shows the tallying table used to classify the defects.

Table 2: Defect Classification and Tallying Table

Straight Defects (notches)			Bent Defects	
Class 1	Class 2	Class3	Kinks	Curls

Six different trials using this technique were performed and it was found that most notches fell into the class two category, so when notches were added up, the classes were combined to give the notches/fiber ratio. The following table (Table 3) shows the results from the six trials in which notches/fiber, kinks/fiber and curls/fiber ratios were found by calculated by dividing the number of defects in each category by the number of fibers counted for each trial.

Table 3: Defects/Fiber Count Ratios

Sample #	Notches/fiber	Kinks/fiber	Curls/fiber
Sample 1	0.61	0.49	0.55
Sample 2	0.63	0.98	0.43
Sample 3	0.66	0.97	0.41
Sample 4	0.62	1.00	0.26
Sample 5	0.69	1.12	0.70
Sample 6	0.48	0.73	0.63
Average	0.62	0.88	0.50
Standard Deviation	0.07	0.23	0.16

The following graphs (Figures 4, 5 and 6) also depict these results for each category for the different trials.

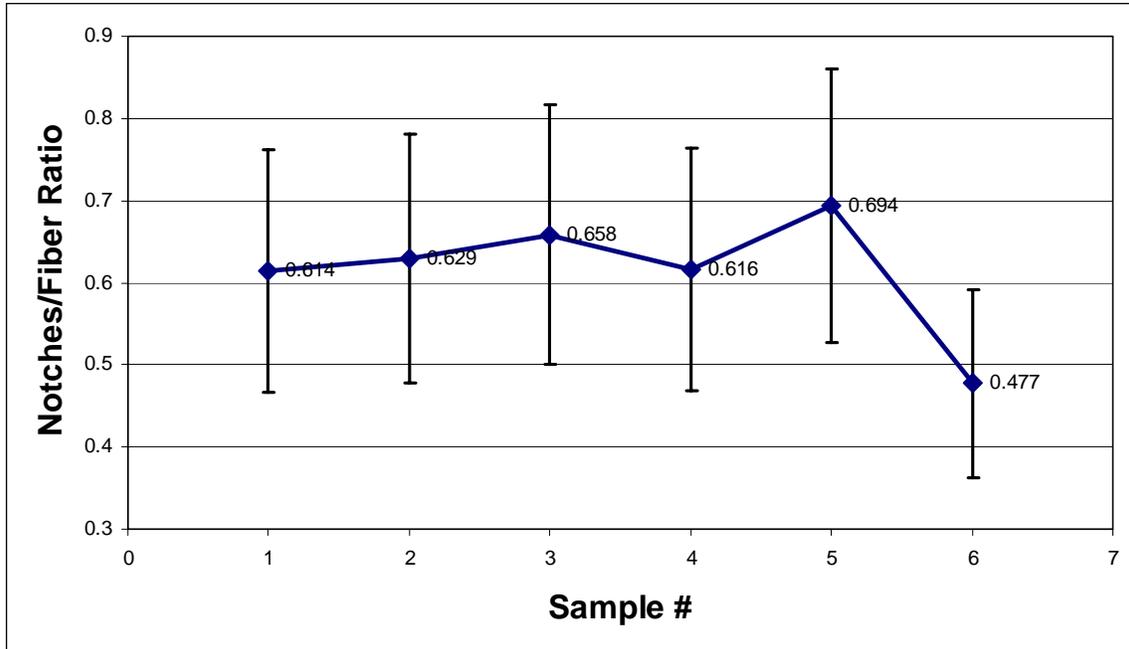


Figure 4: Notches/Fiber Ratio for Six Samples

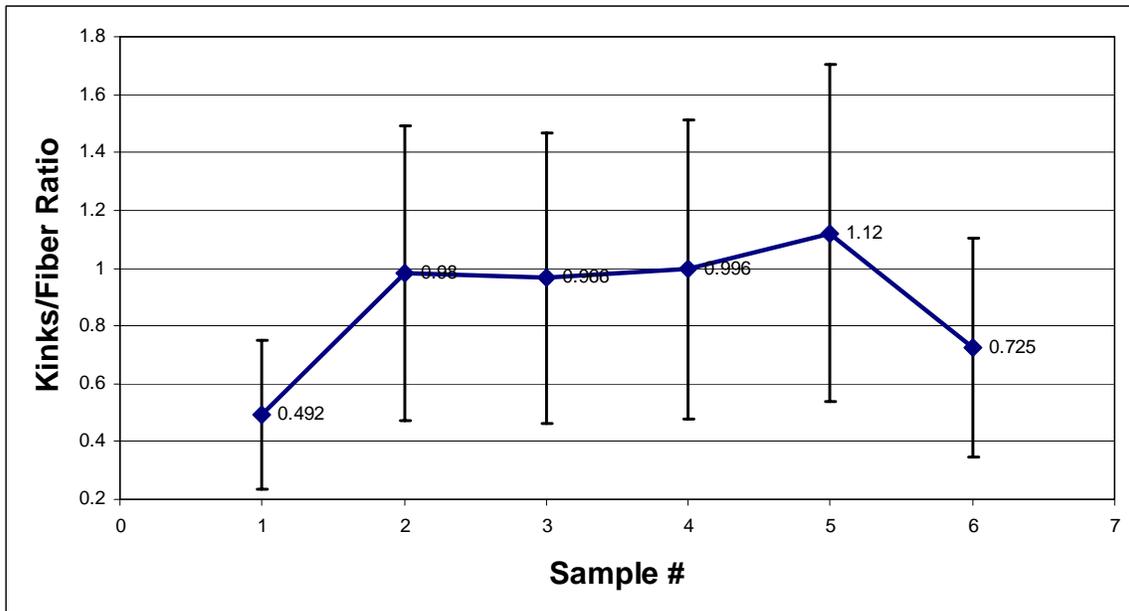


Figure 5: Kinks/Fiber Ratio for Six Samples

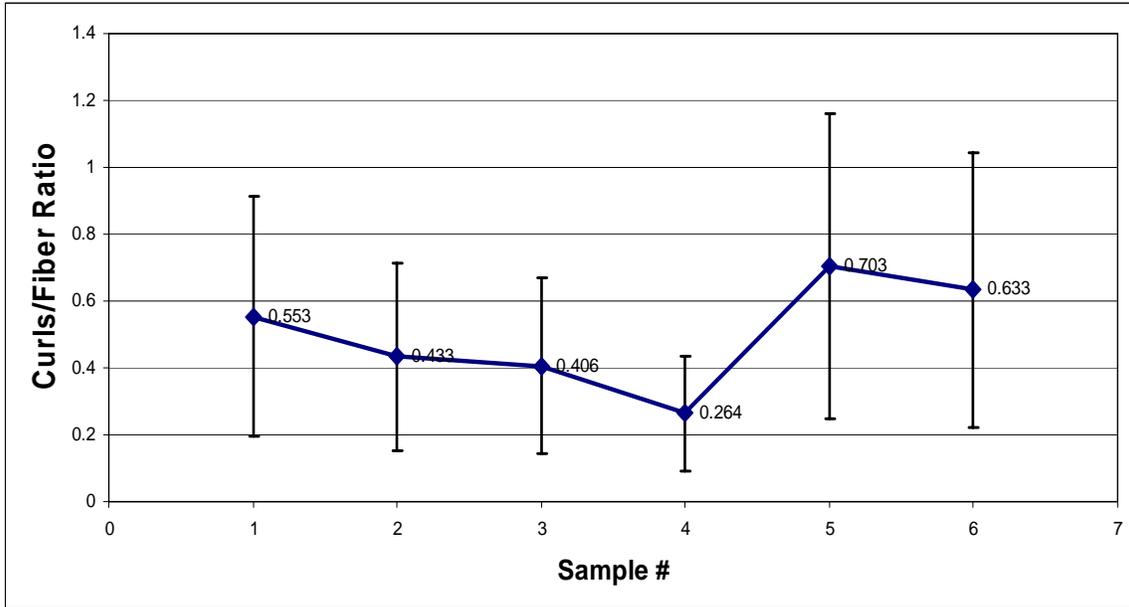


Figure 6: Curls/Fiber Ratio for Six Samples

From the table and graphs it can be seen that the notches/fiber ratio is the only category that remained somewhat constant through the six trials with the exception of the last trial. But as for the kinks/fiber and curls/fiber ratios, it can be seen that there is no noticeable repetition that can be seen in the six different trials.

It was also desired to see the effect of defect/fiber ratios for fibers in the wet state, then drying them and checking defect/fiber ratio, and then rewetting the fibers to check defect/fiber ratio again. The following table (Table 4) and graph (Figure 7) show the results.

Table 4: Defects/Fiber Count Ratios Wet, Dry and Re-Wetted States

	Notches/fiber	Kinks/fiber	Curls/fiber
Wet	0.47	0.84	0.53
Dry	0.48	0.73	0.63
Re-Wet	0.42	0.93	0.58

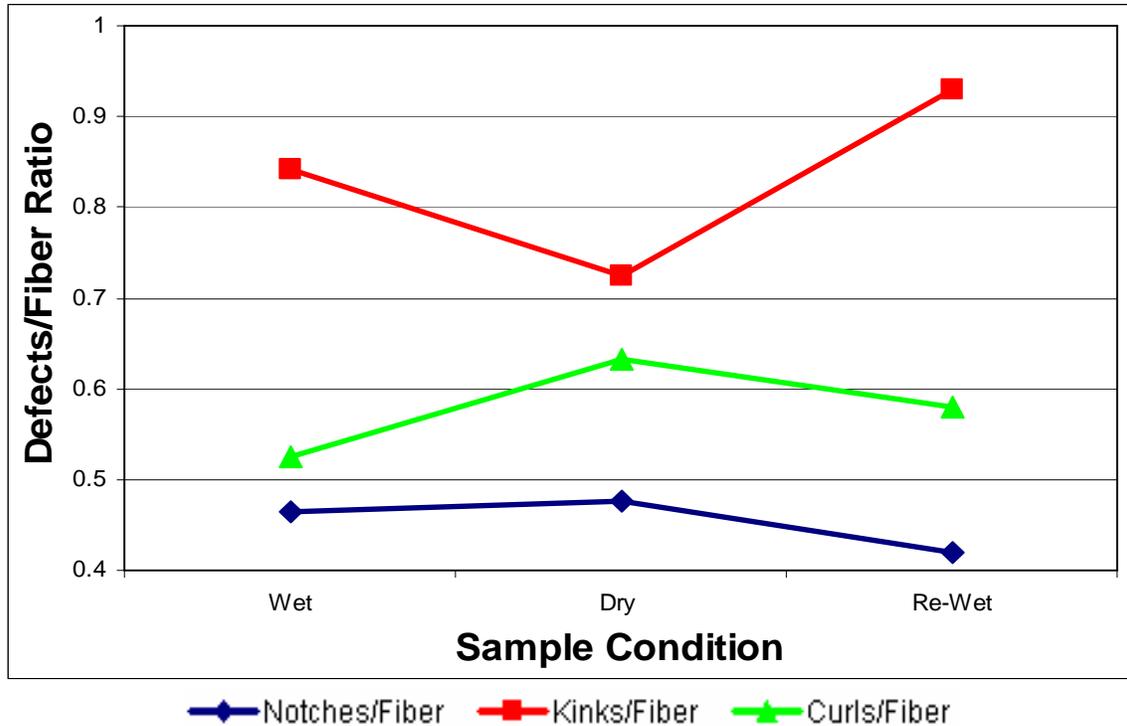


Figure 7: Defects/Fiber Count Ratios Wet, Dry and Re-Wetted States

From these results it can be seen that only notches/fiber ratio remains relatively constant for the different conditions. The curls/fiber ratio varies a little between the different states with the dry state having a higher ratio. For the kinks/fiber ratio, there seems to be a slight drop in the ratio when the fibers are dried, but there is a modest jump in the ratio when the fibers are re-wetted.

A Hobart mixer was next used to induce kneading on pulp samples to hopefully create more defects so that a difference could possibly be noticed in the defect ratios for the increased Hobart kneading times. For the increasing 30 minute Hobart kneading times, the following table (Table 5) and graph (Figure 8) show the results of these tests.

Table 5: Defects/Fiber Count Ratios for Increasing Hobart Kneading Times

Hobart Beating Time (min)	Notches/fiber	Kinks/fiber	Curls/fiber
0	0.61	0.88	0.50
30	0.37	0.46	0.66
60	0.33	0.51	0.78
90	0.32	0.64	0.55
120	0.33	0.58	0.67
150	0.49	0.53	0.76
180	0.34	0.96	0.63
180	0.38	0.64	0.74

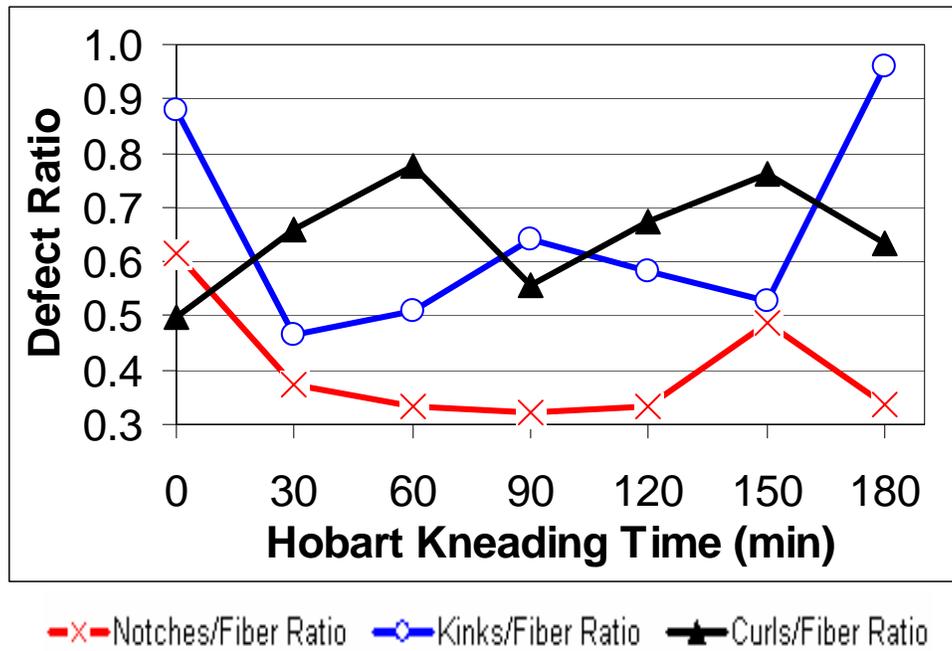


Figure 8: Defects/Fiber Count Ratios for Increasing Hobart Kneading Times

These results indicate that there is no noticeable trend in defects/fiber ratios for any category for the increasing Hobart kneading times. This is different from what was hypothesized, in which it was hoped that there would be a noticeable increase in defects/fiber ratios for increased kneading times. This indicates that the use of a microscope for counting defects does not show repeatable and reliable results.

Conclusions

From looking at the results for completing goal one, it was found that a good and repeatable method could be found for dispersing fibers onto a microscope slide to comply with TAPPI method T 401 om-93 (Fiber analysis of paper and paperboard). As for goal two, it was found that the microscope is very useful for classifying defects as kinks, curls or dislocations, but the classification and tallying method was only somewhat repeatable for the dislocation defects ratios. The kink and curls defects ratios varied too much between the different samples to prove that the method is a repeatable and useful method. The difference in the ratios between the samples could be due to counting error or it could just be due to the randomness of defects in fibers, even though they were from the same source. And for goal three, the results show that the fiber defects classification and tallying method did not show the results that were hypothesized. This is probably due to the fact that the fiber defects classification and tallying method is not accurate in the first place, most notably in the kinks and curls defects ratios. The use of an FQA (fiber quality analyzer) could show better results.

Appendix G – Pre-Enzyme Treatment, Fiber Defect Creation and Enhancement with Hobart Kneading

Introduction

As mentioned earlier, the main tissue property that Procter and Gamble desired to be enhanced was softness. The results that are shown in this study are the initial steps in developing the patented technology of Procter and Gamble for enzymatically modifying fibers to enhance tissue paper softness. This study examines the effect of pre-enzyme treating pulp by high consistency kneading. The overall goal of the study is to develop the patented technology of Procter and Gamble and to overcome commercialization obstacles of the patented fiber modification to make the enzyme degradation technology a robust commercial process.

It was mentioned earlier that some common methods for enhancing paper fiber characteristics such as softness include different methods of pulping, refining, beating, high consistency kneading, chemical addition, etc. The use of enzymatic treatment to enhance the papermaking characteristics of fibers is a less common method for enhancing paper making characteristics of fibers, but it offers a unique way of modeling the chemical composition and ultra-structure of the fiber. Enzyme technology is a versatile tool for modifying the fibers, since it has a tendency to degrade specific components [Buchert, J., et al., 1996]. It has been hypothesized that the specific components that enzymes attach to and degrade on fibers are defect areas, such as dislocations (notches), kinks and curls. The enzymatic action on the fibers creates localized notches in the fibers, which serve as “hinge points” around which the fibers can flex. As explained earlier in the patent results reproduction report, a compressibility model [Pawlak, J.J., 2001] suggests that by creating these localized hinge points, the coarseness of the fiber is changed very little and this allows for the compressibility of the fiber structure to be

increased without reducing the density of the structure. If the tensile strength of the fiber network is much lower than the fiber strength, then reducing the fiber strength should have little or no impact on the sheet tensile strength, as the bonding remains the same. The net result of engineering such a structure would be a softer, more compressible product, with the same tensile strength as the original product.

The hypothesis of this study is that by pre-treating the pulp by high consistency kneading and then enzyme treating the kneaded pulp, the net result will be fibers that have more defects or enhance the defects of the ones already present, which would allow for more areas of absorption by the enzymes to weaken the fibers. This in turn, should allow for a softer tissue sheet as hypothesized earlier. This hypothesis is based upon a series of research papers originating from Korea [Lee, J. H., Seo, Y.B., Jeon, Y., Lee, H. L., Shin, J. H, Choi, C. H., Seo, S. W.]. In these studies a Hobart Mixer was used to apply compressive impact and shear action on several pulp types before refining. After laboratory refining of the pretreated pulps, the pulps generally demonstrated higher water retention values at equivalent freeness levels compared to the same untreated pulps. The increase in water retention value supposedly reflects improved flexibility of the fibers. The fiber lengths and percent fines of the mechanically pretreated pulps were not significantly affected. The fiber curl index increased with mechanical pretreatment. It is believed that because of this increased flexibility and curl index in the Hobart treated fibers, it indicates an increase defect creation or “hinge points” which would allow the fiber to flex and result in more compressible and softer sheet. The increase in defects through Hobart mixer kneading should allow for more areas of enzyme absorption, and hence lower zero-span tensile strengths, which would reflect an increase in the sheet softness. The hypothesis is also based upon earlier research [Mohlin, Molin and de Puisseau], in which it was shown that dry zero-span tensile strength remained relatively the same as fiber defects (number/fiber) increased and wet zero-span tensile strength decreased with increasing fiber defects. The following graph (Figure 1) depicts these results:

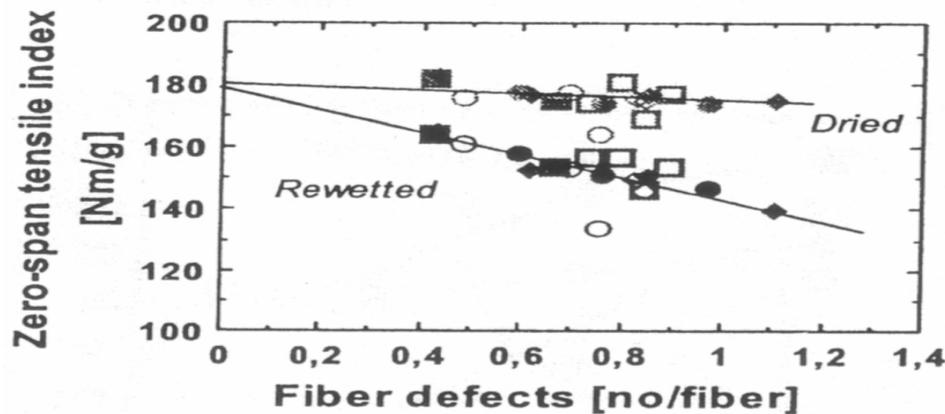


Figure 1: Zero Span Tensile Index (Dry and Wet) for Increasing Defect Ratio

The objective of this work was to examine the effect of kneading on the enzyme treatment of fibers. This objective includes two experimental goals:

1. To see if the creation and enhancement of fiber defects would be greater for kneaded pulp by measuring the effect of kneading pretreatment on kink, curl and dislocations.
2. Measure strength loss after enzyme degradation of kneaded pulp to determine whether kneading would enhance the strength loss of enzyme treated pulp.

The following methods and materials were used to carry out these two kneading treatment goals.

Experimental Methods and Materials

Pulp

Northern softwood bleached kraft pulp from the National Institute of Standards and Technology (NIST) was used for our study. One dry NIST SW pulp sheet (486 g) is broken up and soaked in water for 20 hours. The pulp sheet was then disintegrated in a British disintegrator for ten minutes at level 50 on a Dayton DC Speed Controller Model

5X485C. From here, pulp is filtered on Buchner funnel and consistency of pulp is measured.

Enzyme Used

The cellulase, Dyadic EXP cellulase, was obtained from Dyadic International (Jupiter, Florida). Enzyme activity was determined by measuring carboxymethyl cellulose (CMCase) and Filterpaper Activity (FPU) with 1 % enzyme solution (0.5 g cellulase weight/50 mL water). CMCase was 0.6 units/mL and FPU was 0.8 units/mL. The protein content of 1 % enzyme solution was 8.2 mg/mL.

Hobart Pre-Treatment

The following diagram (Figure 2) demonstrates the testing plans for the enzyme treatment used in the P&G patent results reproduction study, the Hobart pre-treatment study and also the Hobart pre-treatment plus enzyme treatment study. Using 21.5 % consistency pulp, two separate trials were performed. In trial 1, 120 OD g of NIST SW pulp was used and it was placed it in a four liter capacity Hobart mixer where the pulp was kneaded for three hours total. Equivalent amounts of pulp sample were taken for increasing 30 minute intervals up to the three hour limit. Each sample (30, 60, 90, 120, 150, and 180 minute Hobart kneading times) was then disintegrated in a British disintegrator for ten minutes at level 50 on a Dayton DC Speed Controller Model 5X485C. After disintegrating the pulp samples, they were each drained on a Buchner funnel and consistency was checked. Half of the pulp was made into handsheets, which will be discussed in a little while, and half was saved for enzymatic treatment. Also, a very small amount of pulp was saved from each sample to be analyzed with the FQA, which will also be discussed. The same procedure was used for trial two, except that 150 OD g was used and when the samples were disintegrated, the samples were disintegrated with increasing 30 second increments, starting with two minutes for the 30 minute sample, then two minutes and 30 seconds for the 60 minute sample, and so on. This was done so

that the clumps of pulp that were sticking together after the Hobart treatment would be better broken up to create more uniform handsheets.

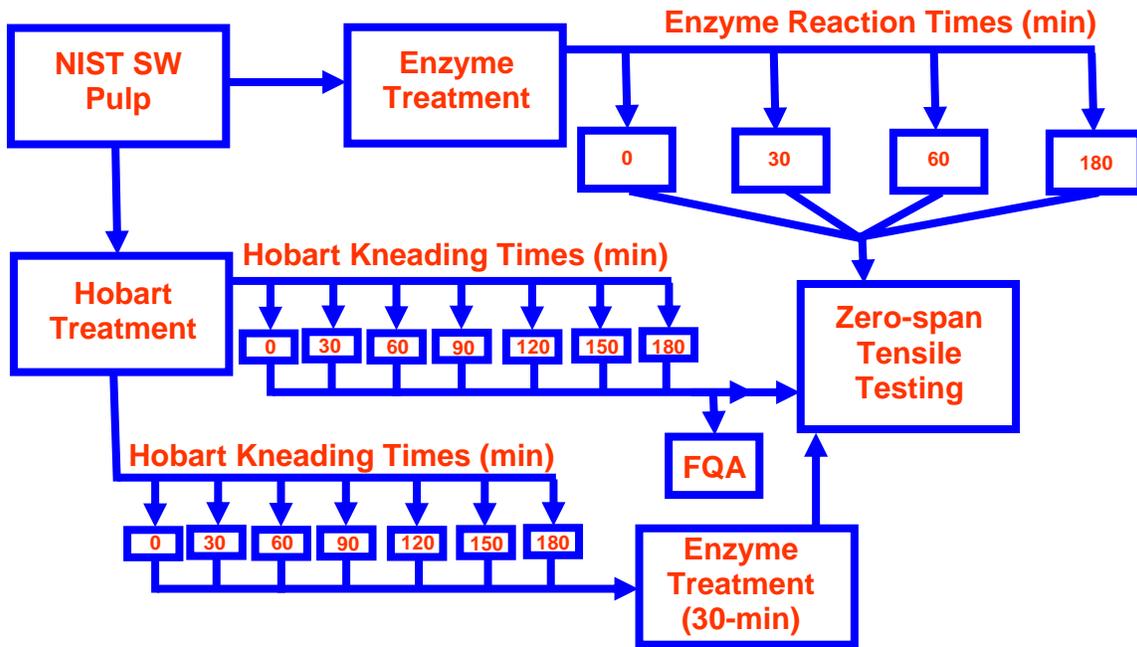


Figure 2: Experimental Plan for Three Trials

Enzymatic Treatments

The pulp slurries (at 3 % consistency in 50 mM sodium acetate buffer, pH 4.5 for Dyadic cellulase treatments) for each Hobart kneading time (30, 60, 90 minute, etc.), were heated and agitated (using a Fisher Scientific Sted-Fast Model 600 agitator set at level three with a three blade impeller) in a water bath till temperature reached 45°C. Once temperature stabilized at 45°C and pH was 4.5, a 0.5 % concentration (based on OD g of wood) charge of enzyme was added to the pulp slurry. Pulp/enzyme mix was continuously agitated and kept at 45°C and pH of 4.5. After 30 minutes of mixing, each sample was heat shocked in a microwave oven for four minutes (two minutes boiling) to deactivate the enzyme. After heat-socking the enzymes in pulp, handsheets were made by using 40 g per handsheet.

Pulp Testing

Eight handsheets were prepared for each experimental condition according to TAPPI test method T 205-om88, but instead of couching the fiber mat/double blotter paper/couch plate stack with five rolls, only three rolls were used and then the fiber mat that is attached to blotter paper was lifted up and placed on the bench with the fiber mat facing up. A round metal handsheet plate was placed on top of the round fiber mat and the couch plate was placed on top of this. The couch roll was then used to couch the assembly four additional times. The fiber mat (attached to round metal handsheet plate) was then peeled from blotter paper and the handsheet (still on round metal handsheet plate) was placed in a handsheet ring. All eight handsheet rings were stacked together with handsheets facing down and then placed a handsheet ring clamp. The sheets were then placed in a 50 % relative humidity and 23 °C environment to dry under restraint.

After the handsheets were dried, the basis weight, thickness, density, breaking length tensile strength, zero-span dry tensile and zero-span wet tensile strength were measured for the handsheets. All tests were conducted according to TAPPI test method T 220 om-88 and T 231 cm-96.

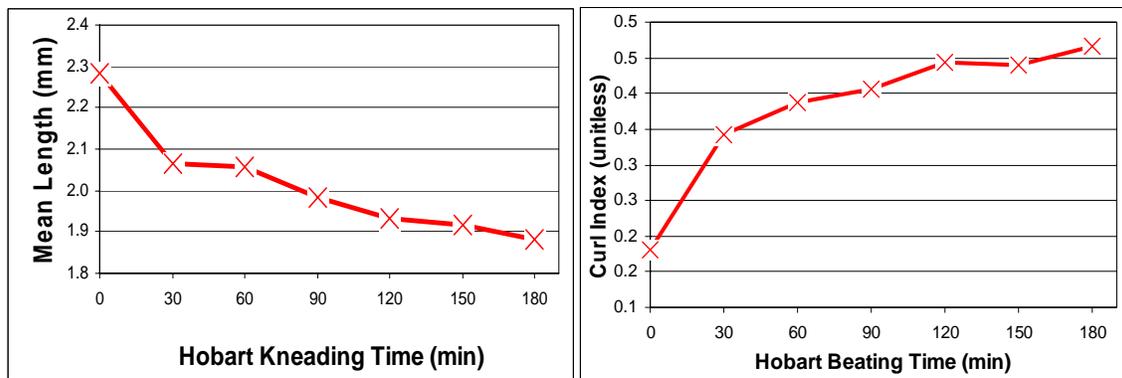
FQA Testing of Hobart Treated Samples

An FQA (fiber quality analyzer) machine was used to analyze the Hobart kneaded only samples to check for the amount of fiber defects (kink index, curl index, fiber length, etc) associated with increased Hobart kneading time. The FQA works by using a 10 ml sample at 0.02 % consistency and diluting the sample to 600 ml and then the machine sucks up the sample and a camera built into the machine scans for and calculates kink index, curl index, fiber length, etc. The kink index is calculated as: $\text{Kink Index} = [2N(21-45) + 3N(46-90) + 4N(91-180)]/L_{\text{total}}$, where N is the number of fibers and the numbers in parentheses are the range of fiber angles, so for example: two times the number of fiber angles that are between 21° and 45° is the 2N(21-45) part of the equation. Average kink angle is average of all kink angles greater than 20° divided by total number

of detected kinks. Curl Index = $(L/l)-1$, where L =contour length and l =projected length. And length weighted length, $L_w = (\sum n_i L_i^2)/(\sum n_i L_i)$.

Results and Discussion

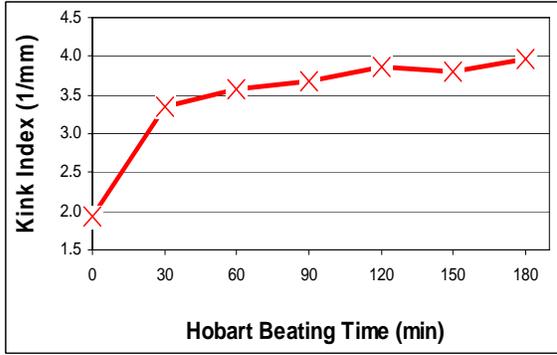
For the first goal, which was to see if the creation and enhancement of fiber defects would be greater for kneaded pulp by measuring the effect of kneading pretreatment on kink, curl and dislocations, FQA testes were performed on the different Hobart kneaded samples and also on a sample that was not kneaded. The following graphs, Figures 3, 4 and 5 indicate the results of these tests.



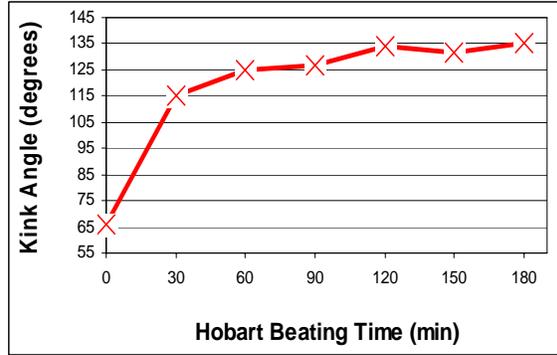
Mean Length Weighted Fiber Length vs. Hobart Kneading Time

Mean Arithmetic Curl Index vs. Hobart Kneading Time

Figures 3a (left) and 1b (right): Mean Length (left) and Curl Index (right) for Increased Hobart Kneading Time

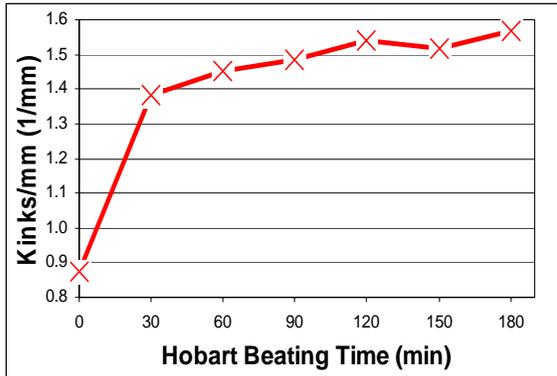


Kink Index vs. Hobart Kneading Time



Total Kink Angle vs. Hobart Kneading Time

Figures 4a (left) and 4b (right): Kink Index (left) and Kink Angle (right) for Increased Hobart Kneading Time



Kinks/mm vs. Hobart Kneading Time

Figures 5: Kinks/mm for Increased Hobart Kneading Time

From these results, it can be seen that the mean length weighted fiber length drops quite drastically in the first 30 minutes of Hobart treatment, but only moderately drops for the remaining Hobart kneading times. It can also be seen that the mean curl index, kink index, total kink angle and kinks/mm only rise drastically in the first 30 minutes of Hobart kneading, but only rise in small moderate amounts for the remaining Hobart kneading times. This seems to indicate that the Hobart mixer causes the pulp fibers to bend and/or break at the kinks and dislocations that are already present and that fiber length decreases quite a bit at first and kink index, curl index, etc. only increase drastically at first. And this seems to indicate that the instant decreases to fiber length are

because of the bending and/or breaking at the kink and dislocation areas. Afterwards it can be seen that fiber length doesn't drop that much and that is in relation to the fact that kink index, curl index, etc. do not rise that much either with the increasing Hobart kneading times. This seems to indicate that most of the kneading work occurs in the first 30 minutes in the form of fibers bending and/or breaking at already present kinks and dislocations and that no further kinks, curls or dislocations are developed because of the increased Hobart kneading time.

For the second goal, which was to measure strength loss after enzyme degradation of kneaded pulp to determine whether kneading would enhance the strength loss of enzyme treated pulp, dry zero-span tensile strength, wet zero-span tensile strength, and normal dry breaking length tensile strength were measured for both Hobart kneaded only samples and Hobart kneaded plus enzyme treated samples. These results were compared to some results from 30 minute enzyme treated samples that were not Hobart treated. These results were obtained in the reproduction of P&G patent results. The following tables (Tables 1, 2 and 3) and graphs (Figures 6, 7, 8, and 9) show the averaged results of the two trials that were performed.

Table1: Non-Enzyme Treated (Hobart only)

Hobart Beating Time (min)	Zero Span Dry Tensile (km)	Zero Span Wet Tensile (km)	Dry Tensile Strength (km)
0	10.6	7.7	1.4
30	10.4	7.3	1.6
60	8.6	6.5	1.7
90	9.3	6.9	1.7
120	8.0	5.8	1.8
150	7.3	6.0	1.8
180	6.5	5.5	1.4

Table 2: Hobart Treated + 30 min Enzyme Treatment

Hobart Beating Time (min)	Zero Span Dry Tensile (km)	Zero Span Wet Tensile (km)	Dry Tensile Strength (km)
0	7.3	3.0	1.4
30	6.9	2.7	1.5
60	6.6	2.3	1.3
90	6.8	2.5	1.7
120	5.8	2.4	1.6
150	6.2	2.6	1.6
180	5.2	2.6	1.3

Table 3: 30 min Enzyme Treatment Only

Hobart Beating Time (min)	Zero Span Dry Tensile (km)	Zero Span Wet Tensile (km)	Dry Tensile Strength (km)
0	7.3	3.0	1.4

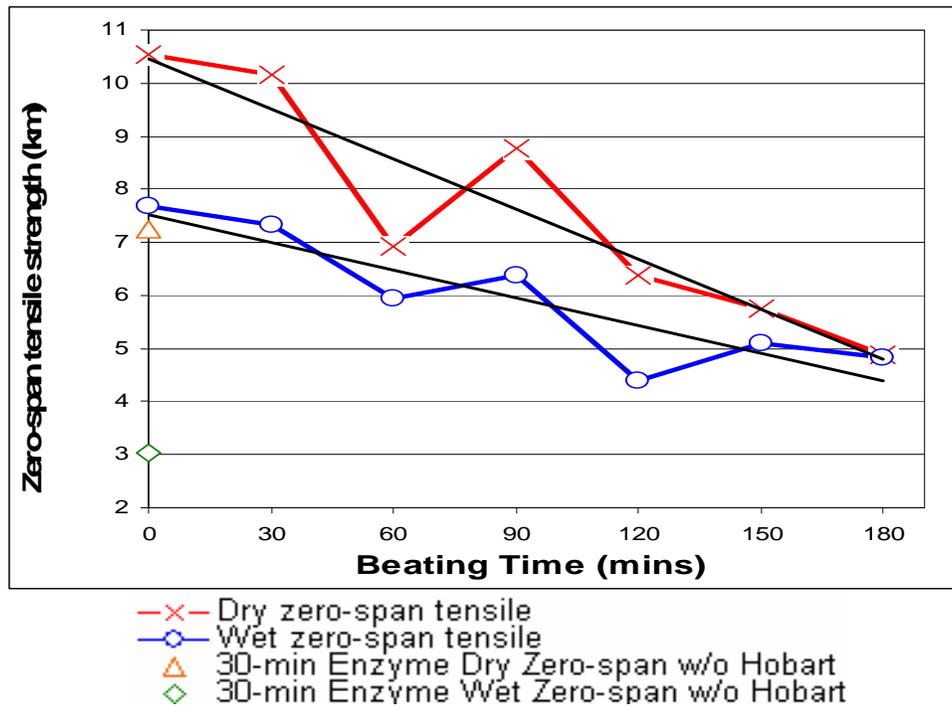


Figure 6: Hobart Pretreatment Only, Dry and Wet Zero-span Tensile Strengths for Increased Kneaded Times

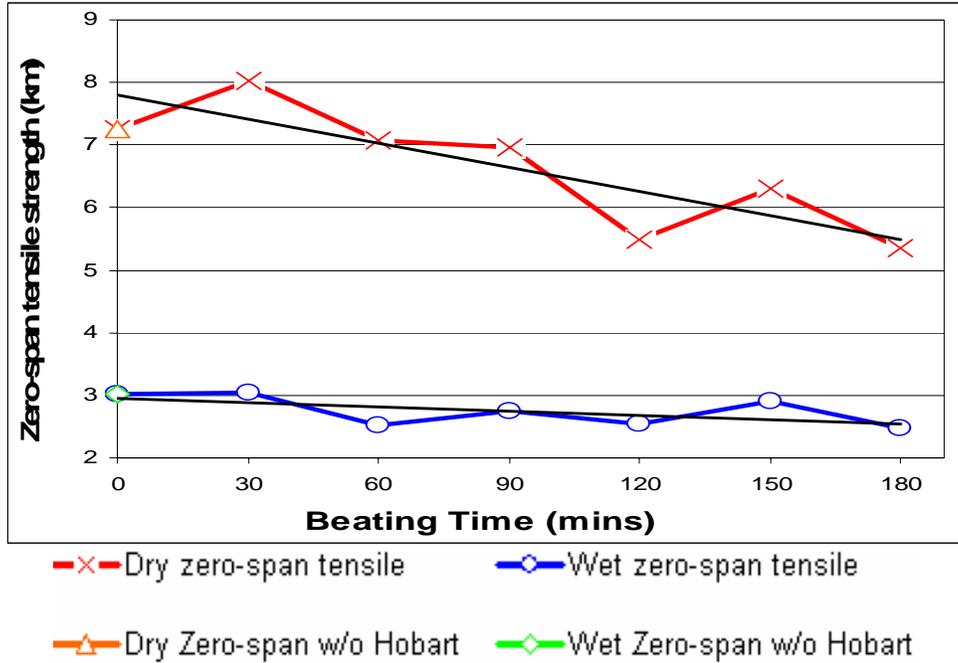


Figure 7: Hobart Pretreatment + Enzyme Treatment, Dry and Wet Zero-span Tensile Strengths for Increased Kneaded Times

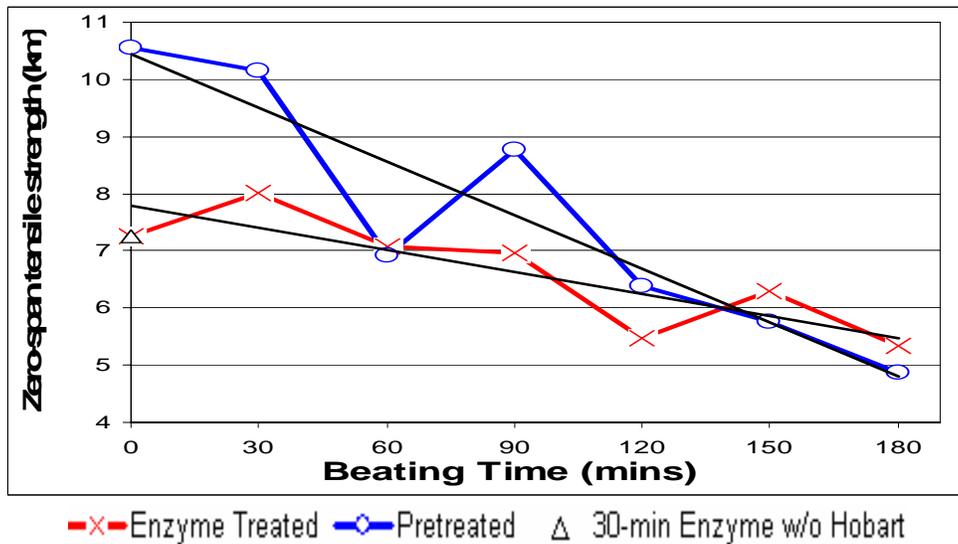


Figure 8: Dry Zero-span Tensile Strength, Enzyme Treated and Untreated at Increasing Kneading Times

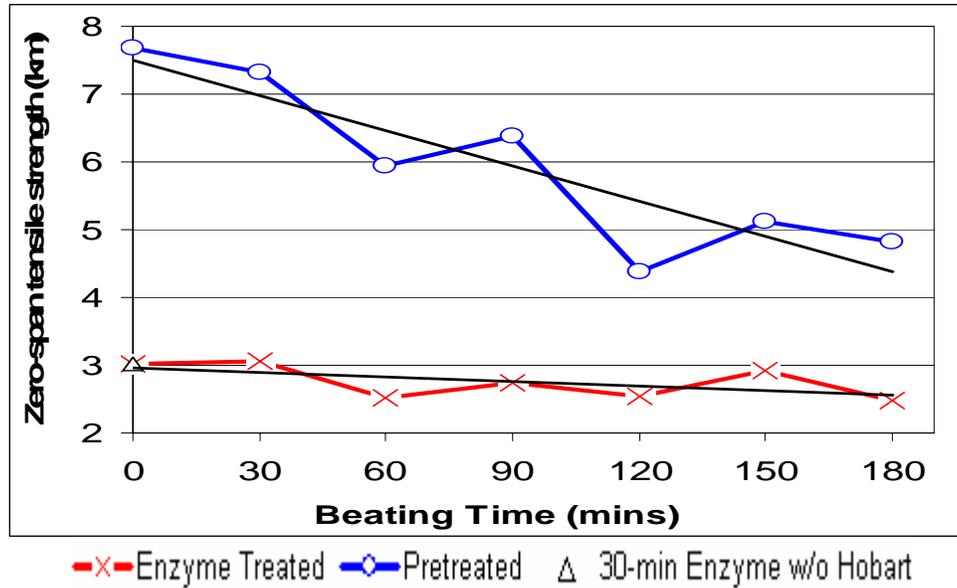


Figure 9: Wet Zero-span Tensile Strength, Enzyme Treated and Untreated at Increasing Kneading Times

From the results of the Hobart treated only samples it was found that for both dry and wet zero-span tensile strength there is a small but gradual decrease in zero-span tensile strength with increasing Hobart kneading time. The wet zero-span tensile strength is noticeably lower than the dry zero-span tensile strength, but the gap between dry and wet zero-span tensile strength become smaller as the Hobart kneading time increases. It is also shown that for the 30-minute enzyme treated samples without Hobart treatment, that for both dry and wet zero-span tensile strength, their values are quite noticeably lower than the corresponding zero-minute Hobart samples in which they were not treated in any manner. It is more so seen for the wet zero-span tensile strength. Also, it can be seen that as the Hobart kneading time approaches 180 minutes, the dry zero-span tensile strength comes close to matching the dry zero-span tensile strength of the 30-minute enzyme treatment sample without Hobart treatment. This does not happen for the wet-zero-span tensile strength, as that it does not seem to drop as much for increasing Hobart kneading time as did the dry zero-span tensile strength. Because there is a gradual drop in dry zero-span tensile strength, along with the gradual drop in wet zero-span tensile strength, these results do not match the earlier research [Mohlin, Molin and de Puiseau]

that was presented earlier in which dry zero-span tensile strength remained constant with increasing defects/fiber and the wet zero-span tensile strength dropped with increasing defects/fiber. It was thought that increased Hobart kneading time would correlate to increased defects/fiber, so that similar results would be seen.

For the results of the Hobart treated plus enzyme treatment samples, it can be seen that the dry zero-span tensile strengths are much higher than the wet zero-span tensile strengths for increasing Hobart kneading times. It is also shown that the dry zero-span tensile strength drops slightly with increased Hobart kneading time, but that the wet zero-span tensile strength remains relatively constant with increased Hobart kneading time. Again, this goes against what was seen in the earlier results. It is noticed however though that for both dry and wet zero-span tensile strength, that the Hobart treatment plus enzyme treatment samples had quite noticeable lower zero-span tensile strengths than did the ones for the Hobart treatment only samples for all Hobart kneading times. This difference is seen even more so for the wet zero-span tensile strength. For both dry and wet zero-span tensile strengths, it can be seen that the difference between the Hobart treatment only and the Hobart treatment plus enzyme treatment, zero-span tensile strengths become smaller as the Hobart kneading time increases, and this is seen even more so for the dry zero-span tensile strength, than for the wet zero-span tensile strength.

Conclusions

From looking at the FQA results to complete the first goal, it was shown that most of the kneading work occurs in the first 30 minutes in the form of fibers bending and/or breaking at already present kinks and dislocations and that no further kinks, curls or dislocations are developed because of the increased Hobart kneading time. This indicates that Hobart treatment does not seem to be an effective method for creating or inducing more defects that could be used for enzyme absorption.

Also, from analyzing the zero-span strength results for Hobart treated only and Hobart treated plus enzyme treated samples to complete the second goal, it was shown

that there is a considerable drop in zero-span tensile strengths (dry and wet) with increasing kneading times for Hobart treated only samples. For the Hobart treated plus enzyme treated samples, it is clear that there does not seem to be any effect of Hobart treatment plus enzyme treatment on the zero-span tensile strengths (dry and wet) for increased kneading time. But, there is a considerable difference between Hobart treated only and Hobart treated plus enzyme treated samples in dry and wet-zero span tensile strength with the increasing kneading times, with the Hobart treated plus enzyme treated values being lower. This seems to indicate that Hobart treatment alone can cause a drop in zero-span tensile strength, but with the addition of enzyme treatment, there does not seem to be any effect on lowering the zero-span tensile strength values with increased kneading time, except for the initial strength drop that will be seen when comparing Hobart treated only to Hobart treated plus enzyme treated only. Hobart treated plus enzyme treated zero-span tensile strength values remain relatively the same with increased Hobart kneading time.