

BIOCHEMICAL ADDITIVES FOR PAPERMAKING

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ABSTRACT

Biochemical additives encompass materials added to the paper-making operation that are derived from biological origins. Other than starch, the majority of the biochemical additives currently used in the paper industry are enzymatic. Enzymes are protein structures that speed a particular chemical reaction. The enzymes are not consumed during the reaction and can be used repeatedly. The enzymes used in the paper industry typically target one of the four major components of wood: cellulose, hemicellulose, lignin or extractives. Enzymes have been used industrially to aid in bleaching, reduce pitch, enhance strength, alter pulp freeness, and aid in paper machine cleaning. This review focuses on the use of enzymes in the papermaking operation, but also addresses the use of enzymes in other areas of the pulp and paper mill. There has also been considerable work in the use of fungus for improving both mechanical and chemical pulping operations. This is considered a separate topic and is only briefly addressed in this review. The future of biochemical additives may extend well beyond the current use of enzymes and a few notes on potential application are given.

1 INTRODUCTION

Throughout the pulping and papermaking operation, various chemicals are added to the process. These additives are used to alter the final product properties, keep the process free of deposits, and/or improve the processing of the fibers. The majority of the additives are derived from synthetic origins. However, some of these additives may be derived from biological origins. These types of additives will be referred to as biochemical additives. Starch is perhaps the most familiar additive in the papermaking process that is derived from biological origins. It is rarely used in its native form. Typically, it will undergo modification by ethylation, cationization, or degradation (enzyme or acid) to reduce its molecular weight before application. Another historical biochemical additive is rosin, which is used in rosin/alum sizing. This method of paper sizing requires an acidic pH. Rosin/alum sizing has lost favor with advent of alkaline papermaking and it is only used in select applications. While starch is currently an extremely important papermaking additive and rosin is historically an important additive, they are not the focus of this review. This review focuses on a third type of biochemical additive – enzymes. Enzymes that have been used in pulp and paper making include cellulases, hemicellulases, amylases, lipases, laccases, manganese peroxidase, lignin peroxidase, peroxidase, esterase, and protease. This review will place emphasis on the enzymes used in the papermaking operation. Also, a brief discussion about the potential future use of biotechnology and microbiology in the paper industry is given.

1.1 Basics of pulping and papermaking

Paper is a thin material consisting of randomly deposited fibers arranged in varying degrees of layering. Annually, almost 400,000,000 tons of pulp and paper products are produced worldwide and the majority of these products are based on wood fibers. Wood is processed into fibers by a number of different means including chemical pulping (ex. sulphite, kraft, and soda process) and mechanical pulping (ex. groundwood, thermo-mechanical, refiner mechanical, and chemical thermo-mechanical pulping). These processes are used to produce fibers with different properties.

Chemical pulping methods are essentially a chemical degradation of the lignin that binds the wood fibers together in the tree. The chemical pulping process results in a wood fiber that is enriched in cellulose and has had significant amounts of lignin, hemicellulose and extractives removed from it. Chemical pulping may be followed by a bleaching operation that removes near all of the lignin and hemicellulose from the wood fiber. Fully bleached

chemical wood fibers are hydrophilic and will retain 1.5–3.5 grams of water for every gram of pulp [1]. These fibers are flexible and tend to collapse during the papermaking operation.

Mechanical pulping results in fibers that are shorter and stiffer than the chemically pulped fibers. Mechanically pulped fibers are liberated from the wood by disrupting the middle lamella of the wood structure, which is the portion of the wood that interconnects the individual wood fibers. This is accomplished by a combination of mechanical force and heat. A chemical treatment may also take place to aid in the fiber liberation and reduce the amount of energy needed to separate the fibers. The approximate chemical composition of mechanically pulped fibers is the same as the original wood. Some volatiles maybe liberated during processing, and in the case of the chemically treated fibers, there may be a measurable change in the chemical composition. These fibers retain about 1.25–2.5 grams of water per gram of fiber [2]. These fibers are stiff hollow cylinders that tend to remain in a similar geometry in the paper sheet. This gives a low density and compressible paper sheet with high opacity. The lignin that remains in the paper sheet makes paper made from these types of fibers poor for archiving and, in general, lower in strength than papers made from chemically pulped fibers.

The papermaking process uses wood fibers from a pulping process and forms the fibers into a paper sheet. During this operation, the fibers are subjected to a number of different processes. There are five basic operations that take place prior to the fibers arriving at the headbox of the paper machine. These operations are screening (separation by size), cleaning (separation by density), thickening (often coincidental to screening and cleaning), dilution (consistency control), and refining (mechanical processing). Refining is critical for developing the papermaking potential of fibers, especially chemical fibers. The mechanical brushing, which occurs during refining, improves the flexibility and creates fibrillation of the fibers, cf. Figure 1. These two changes in the properties of the fibers help to create paper with more bonding and higher tensile strength.

Once the fibers are processed, they are sent to the headbox of the continuous paper machine. In the headbox, the fibers are dispersed and spread uniformly across the width of the paper machine. In the forming section, water is removed from the fibers and the initial structure of the sheet is set. The sheet then passes into a press section where the consistency is raised to about 49%. The sheet then is dried under tension using heat to about 0%–10% moisture.

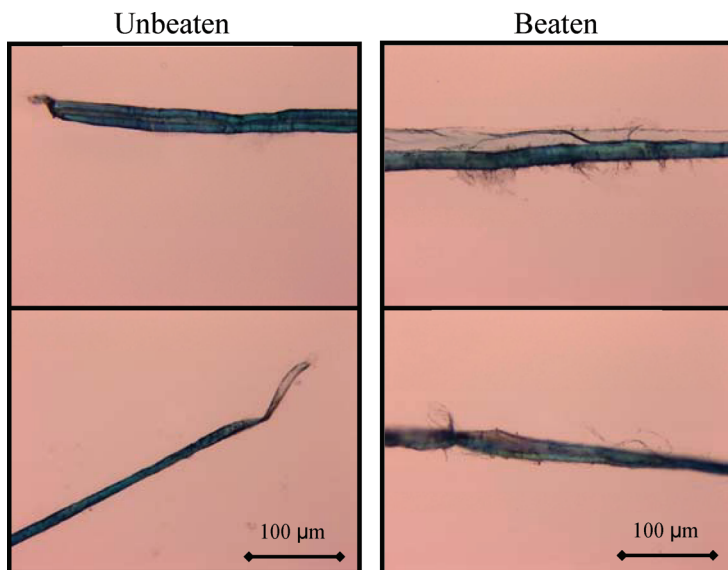


Figure 1. Optical microscope images of beaten and unbeaten softwood fibers. Note that the beaten fibers exhibit a fibrillated surface, which is thought to enhance bonding between fibers that in turn improves paper strength.

1.2 Enzyme basics

Papermakers use a variety of tools to enhance the papermaking characteristics of the fibers including: varying the pulping method, refining and 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. This section reviews the basics of enzyme technology to provide a background for further discussion of the technology.

The role of enzymes in papermaking is one of degradation. Enzymes have been targeted for use in deinking, refining, elimination of extractive and lipids, removal of stickies, debarking, degradation of hemicellulose before bleaching, enhancement of lignin degradation during bleaching and pulping. In most cases, enzymes are used to specifically degrade and remove a particular compound or classes of compounds from the pulp. The exception to this may be the use of cellulase to enhance the refining of pulp. In this case, the target is not to remove a compound from the fiber, but to enhance the ease with which the papermaking properties are developed. However, in refining

pretreatments, cellulose degrading enzymes are used to remove cellulose from the fiber. In this case, the degradation is a negative side effect of the enzyme action. The critical point is that enzymes used in the paper industry degrade fibers leaving behind a smaller fraction of material.

Enzymes are biological catalysts that increase the rate of chemical reactions. The reactants of enzymes are termed substrates [3]. 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 [3]. 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 [3].

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 [3]. 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 [3]. 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 exist 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 [3].

There must be at least three different points of interaction between enzymes and substrate [3]. 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) [3]. 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 [3]. Binding domains may also be present in the enzyme. They improve the binding and facilitate the activity of the catalytic domain on the insoluble substrates, but not on soluble substrates.

There are a number 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 [3]. This hypothesis suggests that the binding of a substrate to an enzyme may bring about a conformational change. Another hypothesis is Koshland's [4] 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 [3]. 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.

Enzymes used in industrial applications may be in a dissolved form or in a dry powdered form. Each of these preparations may contain surfactants, buffers, other stabilizing materials, and inactive protein content. Thus, the application rate of enzymes can be some what cumbersome to characterize. Enzyme application rates are typically specified in one of three ways. The simplest way to specify enzyme application rate is based on dry content of enzyme per unit of substrate. This method does not account for differences

between enzyme activity and the amount of inactive material that may be present in the enzyme preparation. The second method of dosing enzymes is based on protein content with an enzyme preparation. This technique reduces the influence of inactive material within the preparation on the perceived enzyme efficiency, but it does not account for inactive protein content within the preparation. The final means to characterize enzyme dosage is by effectiveness of the enzyme on a known substrate. For example, with cellulase, it is typical to evaluate the effectiveness of the enzyme when degrading both filter paper (insoluble substrate) and carboxymethyl cellulose (soluble substrate). The amount of enzyme that liberates a certain amount of glucose in a certain amount of time is then considered a standard unit. The deficiency in this method is that it is limited by the type of substrate used. Thus, each method of dosing an enzyme has its drawbacks and often, in industrial applications, the simplest means of dosing is used. Table I shows typical dosing rates for various types of enzymes in different applications.

2 DESCRIPTION OF CELLULASE AND ROLE OF SUBSTRATE/ ENZYME INTERACTION

In terms of the papermaking process, cellulases present one of the most interesting classes of enzymes. One of the main reasons for this is that the major component in paper is cellulose. Thus, one can speculate that cellulases have the greatest potential to impact the papermaking process. This section gives details regarding the interaction of cellulases with cellulose (the substrate) and provides a number of points to consider when enzymes interact with wood fibers.

The fact that cellulases are made of multiple types of enzymes was known as early as 1950 [5]. Reese discussed the role of two enzymes that worked synergistically to first create soluble cellulose fragments and then to degrade these fragments into sugars. Cellulolytic activity has been shown to arise for a variety of different organisms including both fungi and bacteria. Much work has focused on fungal sources of enzymes as these organisms are attributed with a more significant economic impact in terms of their degradation of wood structures and cotton fabrics. Coughlan [6] provides a detailed review of the various cellulolytic enzymes associated with different species of fungus. He identifies seven different enzymes that are responsible for the fungal degradation of cellulose and an additional three enzymes that are associated with the bacterial degradation of cellulose.

Cellulolytic enzymes are known to be oligomeric in nature and can be subdivided into cellobiohydrolases and endoglucanases. A third enzyme may

Table I. Examples of enzyme dosages for various applications

<i>Application</i>	<i>Enzyme</i>	<i>Dosage</i>	<i>Units</i>	<i>Reference</i>
Bleaching	Hemicellulase	2–10	mL of enzyme solution per od gram of pulp	Prasad <i>et al.</i> 1996
Bleaching	Laccase	5	micromoles of product per minute per gram of pulp	Paice <i>et al.</i> 1995
Bleaching	Hemicellulase	28–4000	nanokatal per gram of pulp	Viikari <i>et al.</i> 1986
Mechanical Refining Pretreatment	Hemicellulase/Cellulase Mixtures	350	grams of liquid enzyme per od metric ton of pulp	Hart <i>et al.</i> 2009
Refining Pretreatment	Cellulase	2.5	CMCase IU per od gram of pulp	Garcia 2002
Refining Pretreatment	Cellulase	0.01–2.5	mg of protein per od gram of pulp	Oksanen <i>et al.</i> 1997, Pere <i>et al.</i> 1995
Fiber Modification	Cellulase	1.12–2.92	CMCase IU per od gram of pulp	Manfield and Sadler 1999
Freeness Improvement	Cellulase	0.10%	enzyme solution (T. Reesi) on dry pulp	Pommier <i>et al.</i> 1989

also be present such as β -glucosidase, which acts to convert soluble cellobiose into glucose. β -glucosidase is not critical in degradation of cellulose, but is important for creating fermentable sugars from degraded cellulose. 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 [7]. 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 [7]. Both CBH's (CBHI and CBHII) contain an active site tunnel. Most, if not all, cellulases that are effective on 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 exoglucanases active sites are located in tunnels formed by long loops in the protein structure [8]. 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 [7]. 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 [7].

The action of cellulase on cellulose has also been shown to be inhibited by the presence of the reaction products. Berghem, Pettersson, and Fredriksson [9] showed that by continuously removing the reaction product of a cellulose degradation by enzymes, a high level of substrate degradation can be achieved. This is an important aspect to consider when one desires to degrade a large fraction of the cellulose. Berghem *et al.* [9] discussed the interaction of various enzymes within a cellulase mixture. The synergy of using endoglucanase, exoglucanase, and β -1,4-glucan cellobiohydrolase was demonstrated. An important aspect pointed out by these authors is that cellobiose can act as an inhibitor during enzymatic degradation. Cellobiose is a product of exo-glucanase and an abundance of this molecule was shown to decrease the rate of reaction. When cellobiose was removed by ultra-filtration during the reaction, the percentage of material dissolved increase from about 45% up to 80%. The addition of endo-glucanase to the mixture increased the

degree of degradation from 80% to 96%. The thermal stability of β -1,4-glucan cellobiohydrolase was also evaluated. Above 78°C the enzyme became completely denatured and exhibited no activity. However, the enzyme was shown to be capable of renaturation by dialysis with a sodium acetate buffer at pH 5.0. The enzyme regained about 40% of its activity after this process.

The CBH's within a cellulase mixture have shown a propensity to bind strongly to the cellulose substrate. The enzymes tend to bind at the corners and edges of cellulose crystals. This was observed via SEM by Chanzy *et al.* [10] and later modeled using molecular modeling [11]. This is an important aspect of the cellulose degradation mechanism as it shows that cellulases are highly specific enzymes that can be used to modify cellulose structures. It also demonstrates the importance of the cellulose binding domain of the enzyme, which greatly influences its activity on the cellulose substrate.

Cellulose binding domains (CBD's) have been divided into several different families based on their amino acid sequence similarities [8]. CBD's rely on several aromatic amino acids for binding to the cellulose. The CBD's 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 [8]. 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 glucoamylases decreases their activities on insoluble substrates, but not on soluble substrates [8].

The CBD's of cellulases have been grouped into several families based on similarities in their amino acid sequence [12]. 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* [12]. Boraston *et al.* showed that family II CBD's disrupt the surface of cellulosic fibers and release fine particles from cotton or Avicel [13]. 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 [14]. 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) [15].

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 [8]. Cellulases with CBD's are required in the early stages of cellulose degradation when most of the substrate is still insoluble. At later stages, when the substrate has been largely solubilized into oligosaccharides, enzymes operating in the liquid phase may be preferred and brought about by specific proteolysis of the CBD [8]. 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 unlikely. 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 [8]. 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 cellulases 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 [8]. 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 [8].

Much of the work related to enzymatic attack of cellulose focuses on the complex interactions of enzyme and substrate. As discussed previously, cellulase enzymes are actually a mixture of various enzymes that attack the cellulose structure in a synergistic manner. This can complicate the understanding of the cellulose degradation mechanism as it is possible for enzymes produced from the same species of organism to have varying amount of each cellulose degrading enzyme. This can become even more complex when considering differences in cellulase activity when comparing enzymes from different species of organisms, which may have different enzymes and relative concentrations of each enzyme. In this review, we will not attempt to address the differences between enzyme attack for various enzyme mixtures and enzymes originating from different organisms. This review will focus on discussing the basic concepts associated with enzyme degradation and the

influence various cellulose structures have on the activity of cellulase enzymes in general.

The rate of degradation of cellulose by cellulase enzymes is a function of a number of different factors that are related to both the enzyme and the cellulose substrate. Fan *et al.* [16] published a review of the effects of cellulose structure on enzyme degradation rates. Various forms of cellulose will have dramatically different degradation rates despite having the same chemistry. This can be explained through examination of the ultra-structure of the cellulosic materials. Fan *et al.* [16] summarizes the work of Cowling and Brown [17] and Cowling [18,19] to point out the influence of cellulose structure on enzyme degradation rates. In Cowling's 1963 [18] review (updated in 1969 by Cowling and Brown [17], and 1975 by Cowling [19]) of the influence of cellulose structure on enzyme activity, he points to the following cellulose characteristics that influence enzyme activity:

- (1) Cellulose moisture content – A minimum moisture content is needed. In some cases, it may be as little as 10% moisture. It is worth noting that a product of cellulose degradation is water and thus, once a critical level of water is reached, water is no longer a limiting factor.
- (2) The size and diffusibility of the cellulase and reagent in relationship of the pore structure within the cell wall – Much of the available surface area for enzymes to act is within the internal pore structure of the cell wall. In fact, as much as three orders of magnitude more surface area may be available in the internal portions of the cell wall [18]. Thus, the ability of the enzymes to diffuse into the cell wall is very important to effective cellulose degradation. One could speculate that smaller enzymes have more access to surface area in the internal portion of the cell wall and thus have an advantage in degrading cellulose.
- (3) The degree of crystallinity – In general, crystalline cellulose is more difficult to degrade compared to amorphous cellulose. This is a critical aspect of enzyme degradation and points to the ability of cellulase enzymes to enrich the crystalline content of cellulose.
- (4) The unit cell dimension of the cellulose – Cellulose exists in a number of different forms: Cellulose I (natural), Cellulose II, III, IV (regenerated). Certain enzymes will show enhanced effectiveness on the various types of cellulose. This implies a distinct relationship between enzyme and crystal form.
- (5) The conformation and rigidity of the cellulose chain – Coughlan [6] discusses the effect of various type of CBH on enzyme degradation. He points to the work of Fagerstam and Pettersson [20] and Wood [21] that show CBH I and CBH II work synergistically to more rapidly degrade

cellulose. It is postulated that this results from the stereochemistry of cellulose.

- (6) The degree of polymerization – This factor seems to be of limited importance to the overall degradation.
- (7) The association between cellulose and hemicellulose/lignin complex – In wood structures, the close association between lignin and cellulose inhibits the degradation of cellulose. Thus, degradation in wood by fungus typically begins at defects in the cell wall and then proceeds along the length of cellulose fibril avoiding the lignin portion of the cell wall [17].
- (8) The nature and distribution of functional groups on the cellulose chain – Substitution of the cellulose chain with chemical functional groups has varying effect on the rate of cellulose degradation. If the functional groups tend to improve the solubility of the cellulose, then, in general, the rate of degradation increases up until the degree of substitution reaches 1. Above this level, the rate of degradation tends to decrease rapidly until no cellulolytic activity occurs [5].

Of the eight factors listed above, the most significant factors seem to be the degree of crystallinity and the associated internal accessibility of cellulose to cellulase. Lee and Kim [22] examined in detail the effect of cellulose crystallinity and other structural features on enzyme degradation. They reported a number of finding that help to elucidate some critical aspects of the cellulase degradation mechanism. These researchers used a variety of different celluloses with varying degrees of crystallinity. The crystallinity index ranged from 83% (Avicel) to 2.4% (vacuum dried regenerated Solka-Floc pretreated with 85% H_3PO_4) as determined by x-ray diffraction. They determined that the rate of enzyme degradation is much faster for lower degree of crystallinity cellulose and that for the mostly amorphous cellulose the degree of crystallinity does not change significantly during degradation. The more crystalline materials did show enrichment in the crystallinity during degradation, cf. Figure 2. This is evidence indicating that the amorphous portions of the cellulose are degraded preferentially when compared the crystalline regions. Another interesting note is that the soluble protein concentration decreases during enzyme degradation, while the specific surface area (determined by solvent dried samples measure via BET isotherms) first increases and then decreases. This implies that new active sites are opened during degradation, progressively binding more enzymes to the available surface area; while at the same time having a reduction in the sugar production. The behavior shown in Figure 2 is indicative of the complex interaction of enzymes and cellulose substrate.

Lee and Kim [22] reported the effect of enzyme treatment on the degree of

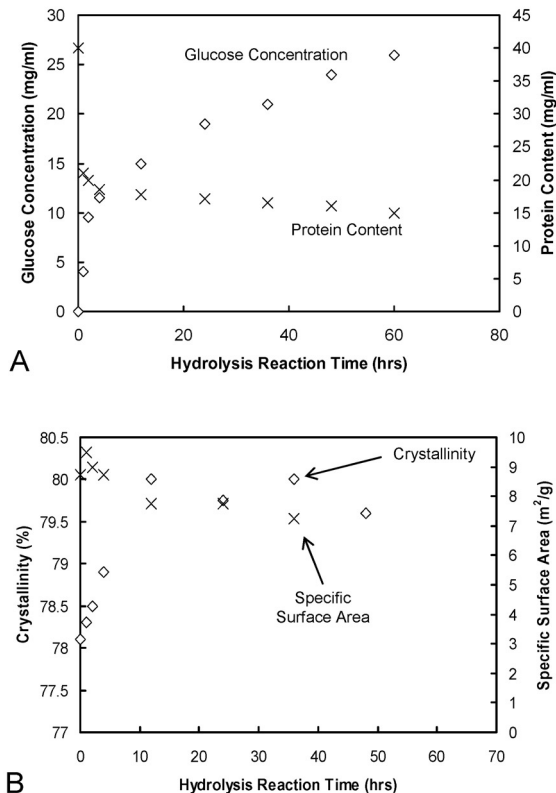


Figure 2. Effect of cellulase treatment on Solka Floc SW-40. (A) Shows increasing sugar concentration accompanied by decreasing soluble protein content. (B) Illustrates the measured increase in crystallinity that occurs along with the decrease in specific surface area. (Adapted from Lee and Kim [22])

polymerization of the insoluble fraction left after enzyme hydrolysis. For highly crystalline material the degree of polymerization changed slightly from 1210 to 1100, while the amorphous material decreased in the degree of polymerization from 1080 to 178. This would indicate that for crystalline and semi-crystalline materials, enzyme attack proceeds from the surface of the fibrillar structures, peeling away layers. In contrast, for amorphous materials degradation occurs throughout the amorphous region attacking the cellulose randomly. Wallace [23] showed a similar behavior on the microscopic scale

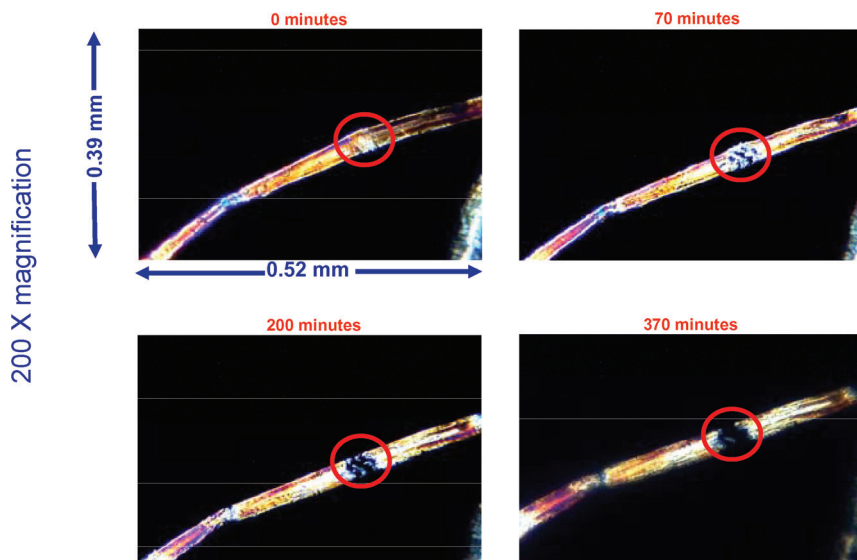


Figure 3. Enzymatic degradation of a bleach softwood kraft fiber with 1.5% enzyme concentration in the immersion liquid. Note the progressive degradation of the dislocation relative to the rest of the fiber [23].

when he examined the degradation of wood pulp fibers under polarized light. His observation indicated that defects located along the length of the fiber where more susceptible to attack than other regions of the fiber. Figure 3 show an example of a fiber degraded to different extents by a cellulase enzyme.

The initial particle size of cellulose also impacts the degradation rate. San-seethong *et al.* [24] published a work that demonstrates how influential particle size can be on degradation rate. These investigators measured the rate of degradation of microcrystalline cellulose with average particle size of 20, 50 and 100 micrometers. They observed that the smallest particles were degraded much faster than the largest particles. For instance, at a 1% (w/v) addition rate, the production of soluble sugar for the smallest particle was about 40% higher than either the 50 or 100 micrometer particle. The difference between the 50 and 100 micrometer particle was much small with the 50 micrometer particle degrading about 10% faster than the 100 micrometer particle. This preference for small, high surface area, particles is one of the main reasons why cellulase enzymes can be used to remove fine particle form the papermaking system, which can improve paper machine runnability.

3 USE OF CARBOHYDRATE DEGRADING ENZYMES IN PAPERMAKING

Enzyme technology is a versatile tool for modifying fibers, since it has a tendency to degrade specific components [25]. The main carbohydrate degrading enzymes used in papermaking fall into two broad categories: hemicellulases and cellulases. Often, cellulases will be found to have a limited amount of hemicellulase activity and the opposite is true as well. These types of enzymes have been used to reduce refining energy, alter the drainage characteristic of stock, and to modify fibers to improve a specific characteristic.

The earliest application of cellulolytic enzymes can be traced to a 1942 patent by Diehm [26]. The inventor describes a process for reducing the beating energy of straw, wood and cotton pulps using an enzymatic pretreatment. The inventor also claims that papers produced by this method exhibit enhanced strength characteristics. It is worth noting that the patent was applied for almost ten years before it was awarded (application in 1933, awarded 1942). Thus, the use of enzyme in paper applications most likely predates 1933. A second significant patent was awarded to Bolaski *et al.* in 1962 [27]. These inventors described in detail the use of cellulolytic enzymes in the refining and beating process of cotton linters for use in papermaking. They extend their claims to fall on all papermaking fibers that are treated with cellulolytic enzymes that enhance strength properties of paper.

These two patents indicate that it is relatively simple process of applying enzymes in the correct conditions to achieve improvements in the papermaking process, pulp, and paper properties. However, as we know from detailed studies of cellulase action on cellulose, there is a complex interaction between enzyme and substrate, which may allow for pulp properties to be manipulated. Pommier, Fuentes, and Goma [28] used a hemi-cellulase/cellulase mixture of enzymes to treat secondary fibers composed of old corrugated containers and mixed with undefined wastepaper. Their findings showed that an increase in freeness of pulps was evident even after a very short time from the addition of enzymes. Jackson, Heitmann and Joyce [29] continued work in this area, they investigated the short time scale effects of enzymes on pulp freeness and fines content. Their work showed that little or no hydrolysis of the pulp takes place at short time scales, and they hypothesize that the enzymes acts to bind fines to longer fibers much like a retention aid. Lee, Pawlak, and Heitmann [30] later showed that the even denatured enzymes have an effect similar to a cationic polymer in solutions of carboxymethyl cellulose. This indicates that short duration of enzyme contact with pulp slurries is very similar to the effect of a cationic retention aid. Pommier *et al.* [28] explored extended degradation of the pulp suspension up to four hours.

They show that a significant increase in the freeness of the pulp during this degradation. These researchers also showed the effect of consistency, pH and temperature during treatment. They concluded that these factors need to be optimized to create the desired effect. One significant issue facing enzyme use in an alkaline papermaking system is the fact that most cellulases prefer pH's below 7. This is evident in Pommier *et al.*'s [28] work where only a small fraction of the enzyme effect is found at a pH of 7.5.

A mill trial using a hemicellulase and cellulase enzyme mixture was published by Freiermuth, Garrett, and Jokinen [31]. Laboratory tests conducted prior to the machine trial also demonstrated the ability of carbohydrate degrading enzymes to decrease freeness. In the trial, the investigators observed the drainage rate of the pulp as a function of refining energy. They compared enzyme treated fibers to untreated fibers and show a nearly constant offset in drainage rate between the two pulps when compared at the same energy input with the enzyme treated pulps showing a lower drainage rate. This implies that a lower amount of refining energy can be used to achieve the same degree of refining. Results indicated no significant change in the controlled paper properties, but a slight decrease in the refining energy was observed. This work demonstrates that enzymes can have varying effects on pulp properties, especially when combined with other process.

In Freiermuth, Garrett and Jokinen's [31] study, enzymes were shown to both decrease drainage rate, and increase drainage rate. This apparent conflict can lead to confusion relating to the application of enzymes in the papermaking system. To understand how enzymes can have multiple effects on pulp, one needs to consider the effects of enzymes on the properties of a pulp slurry as a whole.

The attack of carbohydrate degrading enzymes on pulp slurry can be separated into three stages:

- (1) The flocculation of fine particles associated with enzyme adsorption.
- (2) Degradation of the high specific surface area fines.
- (3) Degradation of the cell wall of larger fibers.

In the following paragraphs, each of these stages of degradation will be examined more thoroughly.

(1) Adsorption of enzymes and fine particle flocculation

Jackson, Heitmann and Joyce [29] examined the effect of hemicellulase, cellulase, and a mixture of the two enzymes. As mentioned previously, they showed that at short contact times between the enzyme and pulp very little

hydrolysis takes place even though freeness is reduced. This points to the first stage in enzyme attack: binding of the enzyme to fibers and flocculation of fine particles similar to a cationic polymer. Others have observed this instantaneous increase in drainage with enzyme addition [28]. Mansfield and Wong [32] speculated that this may be a cause for the improvement in freeness of secondary pulps treated with enzyme over short durations. Lee, Pawlak and Heitmann [30] explored this mechanism using water soluble cellulose derivatives. They used soluble carboxymethyl cellulose (CMC) with varying degrees of substitution. They recognized that even highly substituted CMC showed decreases in the solution viscosity despite the lack of enzymatic activity. They showed that heat denatured enzymes continue to bind with CMC and reduce solution viscosity. The authors replicated a similar behavior using a dose of a cationic retention aid.

Lee, Shin and Ryu [33] explored the relationship between enzyme absorption and hydrolysis rate. They showed that as cellulase is adsorbed onto a substrate the rate of hydrolysis will increase in a direct manner. They also showed that there is a limited amount of enzyme that can be absorbed onto a substrate. Once that level is reached, additional enzyme will not enhance the rate of hydrolysis. Lee and Kim [22] later showed the dynamics of enzyme adsorption for substrates with varying degrees of crystallinity. Regardless of the substrate crystallinity, the enzyme concentration initially decreases very rapidly without a proportional increase in hydrolysis rate.

Jeong *et al.* [34] used a quartz crystal microbalance (QCM) and atomic force microscope (AFM) to investigate the dynamic interaction of cellulases with cellulose substrates at short times. The QCM sensor was coated with a thin regenerated cellulose layer and a cellulase solution with injected into the sample chamber. The sensitivity of the QCM allowed for the direct observation of the absorption of enzymes onto the sensor surface. The results indicated that it takes about 20 minutes before significant degradation of the cellulose takes place. However, enzymes began adsorbing almost immediately after introduction to the sample chamber. AFM images showed that the surface was significantly degraded via enzyme action. Further QCM measurements examined this process in detail [35,36]. The QCM was able to show the rapidity with which enzymes are absorbed onto regenerated cellulose as well as cellulose nano-crystal surfaces. Denatured enzymes were also absorbed onto a cellulose surface, although no cellulolytic enzyme activity was observed.

Thus, there exists a significant amount of experimental evidence that cellulases and hemicellulases can absorb onto cellulose surface without degrading them. It appears that there is a time delay between initial absorption and commencement of enzymatic activity. The reduction of fines observed by

Jackson *et al.* [29], along with soluble polymer work of Lee *et al.* [30] indicates that enzymes can act to coagulate colloidal materials and dissolved polymers. In fact, cellulases are relatively large proteins ranging in size from 2.5 nm to 8.0 nm in equivalent spherical diameter (molecular weight range 12,600 to 76,000) [17]. Cellulases also have a binding domain and a catalytic domain, which both interact with cellulose. Thus, they are reasonable candidates to act as a polymer to flocculate fine particles as the evidence indicates.

(2) Degradation of high specific surface area materials

During the second stage of enzyme action on the slurry, fine particles are hydrolyzed and degraded into soluble sugars. Enzymes attack cellulosic substrates through available surface area. While surface area is not the only factor that dictates the rate of dissolution, it is one of the most important factors when considering the degradation rate. A number of researchers have shown the effect of available surface area, both external and internal, on the overall rate of enzyme action [37,17,33] *Thus, assuming that other factors are held constant, the materials that absorb more enzymes onto their surface will degrade faster* [33]. It is well established that small particles (fines) in the papermaking system account for a disproportionately large amount of the surface area [38,39,40]. As a consequence, when enzymes are applied to fiber slurry, a large amount of enzymes will absorb onto the fines per unit weight. As described above, the initial effect will be to bind these fines to the large fibers. After this, the enzymes will begin to hydrolyze the fines at a rate faster than the larger, lower specific surface area, fibers. The net result is that the fine materials are degraded before the large fibers.

Sangseethong *et al.* [24] showed the effect of fine particle size on the rate of degradation. These researchers examined the rate of hydrolysis of the Avicel with an X-ray crystallinity ranging from 86–89% at three different particle sizes (20, 50, 100 μm). The accessible surface area was measured via solute exclusion using a 5.1 nm dextran probe. The total internal accessible surface area determined by this method accounts for 98%, 99.4%, and 99.7% of the total surface area for the 20, 50, and 100 μm particles respectively. The external surface area was calculated assuming the particle were spherical. This surface area accounts for 1.3%, 0.6%, and 0.3% of the total surface area for the 20, 50 and 100 μm particles respectively. Thus, when the total accessible surface area is determined, particle size would appear to have a minor impact on the total accessible surface area, cf. Table II. However, after one hour, the rate of enzyme degradation was almost 50% more for the 20 μm particle compared to the 100 μm particle, cf. Table II. This, in part, indicates that fines will degrade faster than the fibers.

Table II. Relationship between surface area and particle size and degradation rate

<i>Particle Size</i>	<i>External Surface Area</i>	<i>Accessible Internal Surface Area</i>	<i>Enzyme Absorption</i>	<i>Degradation Rate</i>
<i>μm</i>	<i>m²/g</i>	<i>m²/g</i>	<i>mg/g</i>	<i>μmoll/min</i>
20	0.22	17	55	0.0062
50	0.089	15	56	0.0045
100	0.044	17	54	0.004

(Adapted from Sangseethong *et al.* [24])

Jackson, Heitmann, and Joyce [29] compared the fines content, drainage rate, and soluble sugar content during the degradation of secondary fiber. Figure 4 shows the fines contents of a whole pulp after 30 minutes of reaction time with cellulase and hemicellulase mixtures. The data indicates that low enzyme addition preferentially removes the fines. Wallace [23] showed that an initial decrease in fines content can be measured even in low fine content pulp. These

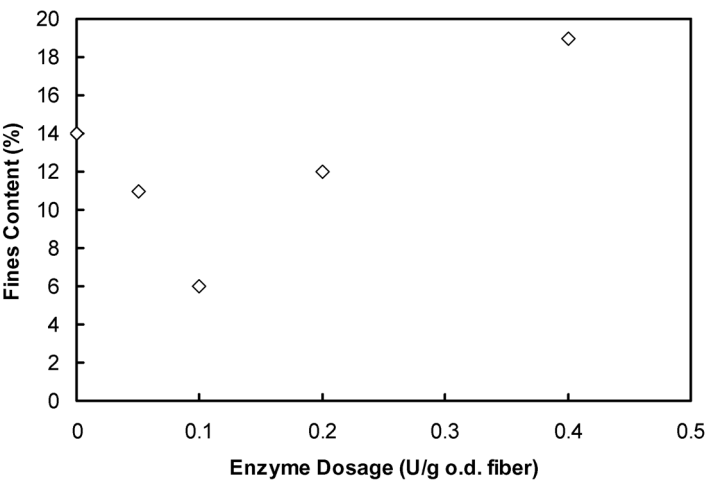


Figure 4. Effect of enzyme treatment at different dosages after 30 minutes of treatment. Note the fines decrease and subsequently increase, which represents degradation of original fines and subsequent generation of fines due to enzyme degradation of the long fiber fractions. (Adapted from Jackson, Heitmann, Joyce [29]).

works show that, after the initial addition of enzymes, the measured fines content decreases. Jackson, Heitmann and Joyce [29] also measured an increase in the soluble sugars that accompanies the reduction in fines, which indicates that the fines are being degraded by the enzymes.

Drainage improvements are found for the pulp slurry both after initial enzyme addition and after soluble sugars are detected. It is not unusual to experimentally observe an increase in mean fiber length during the early stages of treatment of a whole pulp (long fiber and fines) [23,29]. This is attributed to the initial degradation of fine materials, which leaves a greater fraction of long fibers. The elimination of fine material from the pulp slurry lowers the overall specific surface area of the pulp. This leads to an improvement in the drainage.

Improvements in drainage have been observed by a number of authors [28,31,29,41]. In the literature, it is often reported when cellulases and hemicellulases are added to secondary fiber. One of the main drawbacks to using secondary fiber is the high fines content that, in general, do not contribute to paper strength, but lower the drainage rate. Thus, applying enzymes to secondary fiber to improve drainage is rather attractive.

Pommier *et al.* [28] investigated the effects of commercial enzymes (hemicellulase and cellulase mixture) as well as preparations from *Trichoderma reesei* and *Aspergillus niger* on secondary fiber consisting of 75% old corrugated containers and 25% mixed waste papers. The whole pulp furnish was slurried prior to reacting with the enzymes. The pulps were then processed to different initial freeness ranging from about 450 ml CSF to 150 ml CSF. Enzymes were added at 0.1% based on the dry fibers for 30 minutes at 4.8 pH and 50°C. The researchers showed that as refining increases (*i.e.* freeness decreases) the gain in freeness after 30 minutes of enzyme treatment increases. Increased refining typically leads to higher levels of fines. Thus, removing fines at higher levels of refining will show more improvement in the freeness. The enzymes may also attack fibrils that have been partially liberated from surface of the fiber. This enzyme action is also likely to improve the freeness.

It is worth noting at this point the effect of cellulase concentration on the degradation rate of cellulosic materials. Due to cellulases acting on the exposed surface, there is a limit to which increasing cellulase concentration will increase degradation rate. Figure 5 shows the effect of increasing enzyme dosage on the rate of sugar production normalized to the amount of substrate present. The higher enzyme dosages correspond to a relatively small amount of cellulose being added to a fixed concentration of enzymes. As one can see, there is a limit beyond which additional cellulase does not increase the rate of degradation of the cellulose when one takes into account that

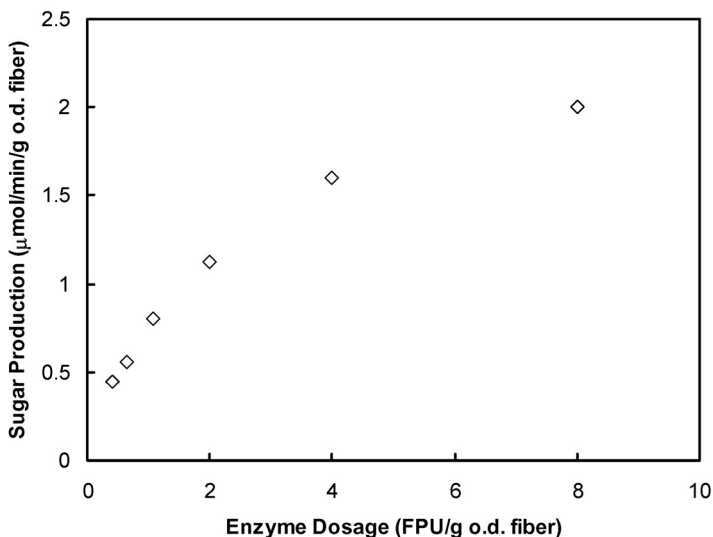


Figure 5. A plot of the sugar production rate versus the enzyme dosage. The enzyme dosage was altered by adding varying amounts of micro-crystalline cellulose to a solution of enzyme (0.008 FPU/ml). The substrate concentration varied from 0.1% to 2% (data from Sangseethong [24]).

amount of substrate available. If the enzyme dosage was the limiting factor in the reaction rate, then a straight line would be observed between the sugar production and enzyme dosage. This can be related to the limited surface the cellulase has access to at any one point in time. Once the available surface area is occupied by absorbed cellulase, no further cellulase can access the cellulose, and thus there is no further improvement in rate of degradation. The dosage of the enzyme can impact how the fiber slurry is modified. Lower dosages will tend to favor the degradation of higher specific surface area fines particles prior to significantly degrading the long fiber fraction.

During the second stage of enzyme attack, cellulases and cellulase/hemicellulase mixtures can reverse many of the effects of refining such as reducing the fines content and eliminating fibrillar structures partially liberated from the fiber surface. In fact, under certain conditions, gains of up to 250 ml CSF can be achieved with enzymatic hydrolysis [28]. Often, two major drawbacks are cited for the application of enzymes. The first is the loss of pulp yield, which in the case of drainage improvement, may not be important as the fine

particles contribute little to the paper strength. The second drawback is the degradation of the fiber strength, which typically becomes more significant at higher enzyme dosages and longer degradation times.

(3) Degradation of fiber cell wall

The loss of fiber strength during extended degradation has often been cited as one of the main draw backs to using carbohydrate degrading enzymes in papermaking. After the initial stages of enzyme degradation, cellulolytic enzymes begin to significantly degrade the long fiber fraction in pulp slurry. This fraction makes up the greatest percentage of fiber on a mass basis in the pulp slurry. These fibers are important for developing the strength and structural characteristics of paper sheets. The effective hydrolysis of the long fiber fraction is also critical when the wood fiber will ultimately be used for bio-energy production. Thus, understanding the effect of enzymatic degradation on the long fiber fraction is critical for papermaking as well as bioenergy production.

When cellulases degrade papermaking fibers three general observations can be made: (1) the fibers lose strength, (2) the fiber length is reduced, and (3) the cell wall becomes more conformable.

Fiber strength loss can be attributed to three possible phenomena. First, it is well known that cellulases degrade the fiber by hydrolysis of the cellulose chain. This results in a loss of the degree of polymerization of the cellulose. The effect of the hydrolysis on the strength loss in fibers depends greatly on the mechanism by which the enzymes attack the cellulose. Because cellulose in wood fibers is arranged in semi-crystalline structures, the cellulose within the crystalline region is not totally accessible to the enzymes. Thus, it is likely that cellulase degradation of wood fibers involve the degradation of fibril structures, which make up the cell wall. Evidence exists that the enzymes attack specific locations along the edges of fibril structures [10,11]. The enzyme degradation then takes place by successively stripping cellulose from the outer edge of the fibril structures with the enzymes working their way toward the core of the structure [10,42]. The result of this is that the viscosity (i.e. degree of polymerization) of the insoluble fraction changes by a relative small amount [22,42]. Soluble and amorphous cellulose shows large drops in viscosity and degree of polymerization, especially when endoglucanases are present [22,29]. It has been shown that there is a mild relationship between pulp viscosity and pulp strength [47]. Thus, it is not until later in the enzyme degradation process that the viscosity reduction is an important factor in pulp strength.

Although pulp viscosity may have a secondary effect on fiber strength, the

stripping of the cellulose from the fibrils will result in a decrease coarseness of the pulp [43]. The relationship between coarseness and fiber strength was described by van den Akker [44]. Thus, enzyme degradation that reduces fiber mass per unit length is likely to reduce fiber strength. It is also speculated that the removal of hemicellulose from the fiber wall decrease the inter-lamella bonding in the fibers, which reduces fiber strength [52,60]. Mansfield *et al.* [45] addressed this issue and concluded that degradation of fiber dislocations is a more important in fiber strength loss.

The degradation of fibers at dislocations is the third phenomenon that affects fiber strength. This degradation is also responsible for the loss of fiber length. Wallace [23] observed the progressive degradation of fibers at dislocations along the fiber length. In his research, he monitored the time lapsed cellulase attack on softwood fibers. By comparing the rate of degradation at the dislocations (*i.e.* notches) with the degradation of the rest of the fiber, Wallace showed that dislocations are more susceptible to enzymatic hydrolysis. This could be attributed to the disruption of the cell wall structures, which increases the accessibility of enzymes to internal cellulose fibrils. Effectively, the dislocations represent a localized increase in the internal surface area of fiber, making this part of the fiber more accessible to enzyme and thus more susceptible to enzymatic degradation. Others have observed this phenomenon as a decrease in the fiber length of the pulp slurry. Park *et al.* [46] showed images of fibers degraded by cellulases. These fibers appear to be “cut”, which is an indication that the enzymes attacked the fiber at the dislocations severing the fiber.

Fiber dislocations have been closely linked with zero span tensile strength [47]. This paper property measurement has been closely related to the strength of the fibers. Enhancing the dislocations by enzymatic degradation results in a rapid loss in the strength of the fibers, which is apparent in the reduction of zero span tensile strength.

The progressive degradation of the fibers at both the dislocations and along the edges of the fibril structure also affects the flexibility of the fiber. Wallace [23] measured the wet fiber flexibility of the fiber by measuring the conformability of the fiber dried over a fine wire [48,49]. This researcher showed that enzymatic degradation increases the flexibility of the fibers. This is an indication of the weakening of the cell wall. This increased flexibility may be attributed to weakening of dislocation, which create hinge points [23,45], loss of fiber cross sectional area (*i.e.* coarseness) [43], and/or the degradation of hemicellulases that bind together the lamella of the fiber cell wall [52,60]. The increased flexibility could also be attributed to the increase in internal pore volume that has been noted by a number of authors [45,46,50]. This additional water volume associated with the increased pore

volume would indicate that the fiber is more swollen, which is typically associated with increase conformability.

3.1 Effects on papermaking processes and paper properties

A study by Mansfield and Saddler [51] 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, then 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 [51]. 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 un-fractionated kraft pulp decreased after cellulase treatment, while three longer fiber length fractions showed significant improvements [51]. 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 bonding. This enhanced fiber to fiber contact can 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 [51].

Mansfield and Sadler [51] 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. 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 [51]. Mansfield and Sadler 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 [51]. 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 [52,53].

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 elemental chlorine free (ECF) bleached softwood kraft pulp were previously studied [54,55]. This study found that of the endoglucanases, EGII caused the most dramatic viscosity decrease at a given cellulose hydrolysis level. The CBH's decreased the viscosity less than the EG's, however, differences were also observed between CBHI and CBHII [54,55]. 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 [54,55].

In a study by Gerber *et al.* [56], 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 strengths, 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 [56]. 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 [57]. 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 with the enzyme type [58].

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 [56]. The higher cellulase adsorption onto never-dried fibers was probably due to its higher specific 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 to the internal fiber surface area [56].

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 [59]. It was however found that EGI and EGII improved the beatability of the pulp as measured by Schopper-Riegler values, sheet density and Gurley air resistance. EGII was the most effective in improving the beating response, but combinations 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 [60]. An explanation for why the EG's increased beating response is the increased fiber breakage and function of fines, rather than improved flexibility [59]. 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 [59].

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 [61]. 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 dissolved by the enzyme treatments. It was found that both EGs 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 [61]. 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 [61]. 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 [61]. 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.

In a study by Pommier *et al.*, the use of enzymes to improve the process and product quality in recycled pulp was explored [28,62]. Pommier used *T. Reesei* enzyme (0.2% w/w) and wastepaper pulps (laboratory and industrial pulps) in his experiments. The wastepaper contained mostly OCC and mixed wastepaper in the ratio of 3:1 at a consistency of three percent. At low enzyme concentrations, pulp freeness increases soon after contact with enzymes with only a slight 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 [28,62]. Alternatively, different raw materials could be used, including more low-grade wastepaper, to achieve the same 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 [28,62]. Bhat verified the findings of Pommier using dried bleached, and unbleached softwood kraft fibers [63]. Neither Bhat, nor Pommier pursued a mechanism for enzyme degradation, 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 achieved without affecting the mechanical properties [28].

In a study by a European research consortium, recovered paper was incubated with culture filtrates and with isolated cellulase and hemicellulases [64]. 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 [64].

Wallace [23] examined the effect of enzyme degradation on both the fiber strength and the wet fiber flexibility. This researcher compared the wet and dry zero span tensile strength of handsheets made from fiber treated with a commercial cellulase. Expanding on a patent by Seger *et al.* [65], Wallace showed that much of the strength lost during degradation can be recovered upon drying the fiber, as long as the hydrolysis is mild. Wallace also examined time lapsed optical microscopy of the fiber degradation, cf. Figure 3. He showed that dislocations along the length of the fiber are preferentially degraded. Thus, this can be related to the loss in zero span tensile strength. Wallace also measured the wet fiber flexibility using the method of Cresson [48,49]. A marked increase in the wet fiber flexibility was observed as cellulase hydrolysis progressed until a loss in fiber length was observed.

The importance of shear force or agitation for the effective degradation of fibers by cellulases has been noted. One of these studies was by Lenting and Warmoeskerken, in which the mechanism of interaction between cellulase action and applied shear force was studied [66]. This study found that a certain threshold of applied shear force is required for optimal cellulase performance. It was found that lower shear forces can be compensated for by higher enzyme dosages and longer retention times [66]. It can be hypothesized that based on cellulase application in batch production equipment, the type of applied shear force is of importance. Alternating the direction of the applied shear force may enhance liberation of cellulose from the fibers or loosen the microfibrils from the fibers [66]. 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.* [12], examined the effects of agitation level on the adsorption, desorption, and activities of cotton fibrils of full length and core domains of EGV (*Humicola insolens*) and Cen A (*cellulomonas fimi*) [12]. 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, where high levels of mechanical agitation are applied [12]. 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) [12]. 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. Its binding to cotton cellulose is highly reversible [12]. 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 at a high level of mechanical agitation, but, at a low level of mechanical agitation, low degree of desorption was verified [12]. 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 cellulose loss produced by Cen A and Cen A core under the same high-agitation conditions [12]. Cen A only has moderate activity on crystalline cellulose, suggesting that the surface diffusion of CBD's does not limit the substrate catalysis. Thus, the overall importance of agitation is linked to the type of enzyme used and presence or absence of a CBD.

4 PULP PROCESSING USING ENZYMES

Enzymes can be applied to fibers and wood chips prior to reaching the paper mill. The enzymes may be applied directly, or fungus may be used to generate enzymes that degrade lignin. There are three main applications of enzymes to pulp: (1) wood chip pretreatment, (2) lignin reduction, and (3) bleaching/deinking enhancement.

Akhtar *et al.* [67] have successfully demonstrated a small scale (50 tons) industrial application of fungus pretreatment before thremomechanical pulp (TMP) processing. The fungus used was a white rot fungus (*Ceriporiopsis subvermispora*), which was determined to be an effective lignin degrader. The fungus was applied to spruce wood chips that were first sterilized by the application of steam. The inoculated chips were piled and air was forced through the chips to remove metabolic heat associated with lignin degradation by the fungus. The chips were stored for two weeks prior to pulping by a TMP process. This process exhibited a 30% reduction in energy when compared to a control. The pulps had higher tensile strength, but were significantly more difficult to bleach. This work was the summation of a number of years of earlier work by Akhtar *et al.* [67,68,69]. Biopulping using fungal pretreatment has not found industrial application. A number of factors have deterred industrial application including difficulty in ensuring uniform treatment of wood chips, long storage times for chips, concerns about large scale application of fungus, and initial capital investment.

Cellulase, pectinase, and hemicellulase can be used to improve both chemical pulp quality and mechanical pulp quality. The earliest patent for using cellulases in the pulp and paper industry focuses on reducing refining energy during the mechanical processing of non-wood fibers [26]. The patent showed a significant reduction in energy required to liberate fibers during a mechanical pulping process. Pere *et al.* [70] also applied cellulolytic enzymes to a TMP processing line. In this case, cellobiohydrolase (CBH) was used to process rejects from a TMP line. The researchers found that cellulase hydrolysis of the rejects reduced the amount of energy, *i.e.* improved the efficiency, of reject refining. This was attributed to the degradation of the surface carbohydrates on the rejects. During the trial, the mill trial monitored the total carbohydrates in the white water as well as the cellulose-oligosaccharides content of the white water in order to show evidence of enzyme activity. The total white water carbohydrates showed a small increase with the application of the enzymes. However, the cellulose-oligosaccharides went from non-detectable levels to over 40 mg/l. This was direct evidence of cellobiohydrolase activity. However, this points to an environment concern when using enzymes. The products of the enzyme action may result in higher chemical and biological oxygen demand of the effluent streams. Thus, the increased expense associated with effluent treatment must be balanced with savings in other areas. In Pere *et al.*'s [70] work, the overall specific refining energy of the rejects was reduced by 10–15%, which may well justify additional water treatment costs.

Mixtures of hemicellulases and cellulases derived from *Trichoderma reesei* have been added to a chemical refiner mechanical pulping operation. Hart *et al.* [71,72] added hemicellulase and cellulase enzyme mixtures to eucalyptus wood chips in an Impressafiner[®]. This equipment was used as a pretreatment step before chemical refiner mechanical pulping. The Impressafiner[®] squeezes the chips, which can fracture the cell walls of the fiber making them more susceptible to mechanical disintegration. During this squeezing, liquid within the wood chips is pressed out. Upon release of the pressure, the enzyme solution enters into the cell wall. Thus, the Impressafiner[®] has two main purposes: (1) preprocess the chips via mechanical means, and (2) imbibe the chip with enzyme solution. Chips treated with the enzyme solutions showed a significant reduction in refining energy (24%) when transmission electron microscopy (TEM) images showed degradation of the S1–S2 interface by enzymes. The shives content, opacity and tensile index of the resulting pulps showed no statistical difference when compared to wood chips treated only with water. Hart *et al.* [71] estimate that a net savings of \$11–\$17 per ton of oven dried pulp could be realized using hemicellulase/cellulase mixtures as a mechanical pulping pretreatment.

Beyond the scope of improving the pulping process, enzymes have found use in improving the bleaching process. Both lignin degrading and hemicellulose (carbohydrate) degrading enzymes have found application in this area. The lignin degrading enzymes typically used include laccase, manganese peroxidase, and lignin peroxidase. These enzymes directly attack the lignin structure either dissolving it by breaking down the structure, or modifying the structure so that subsequent bleaching is more effective. These enzymes are typically isolated from various white rot fungi [73]. White rot fungi preferentially attack lignin in wood when deprived of nutrients [73]. Laccase reactions are mediated reactions that oxidize phenolic and non-phenolic components of lignin [74,75]. Common mediators that have been used in laccase reactions include 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) and 1-hydroxybenzotriazole (HBT). These mediators are typically faulted for their high cost and sensitivity to reaction conditions. Manganese peroxidase de-methylates the lignin producing methanol. The generation of methanol can be used to indicate the action of manganese peroxidase [76]. The effectiveness of manganese peroxidase is dependant on the presences of hydrogen peroxide. The addition of manganese ions to the bleaching operation can also improve the effectiveness of manganese peroxidase [77]. These enzymes can add significant brightness to the pulp. Manganese peroxidase has been shown to improve the brightness of pulps by up to 10 points when combined with subsequent bleaching steps. In general, the main interest of using lignin oxidative enzymes has been in conjunction with elementally chlorine free and totally chlorine free bleaching. As of yet, these enzymes have not found widespread industrial use. Prasad *et al.* [78] showed a synergistic effect of using hemicellulase along with crude enzymes derived from white rot fungus. This implies that hemicellulose degradation may be a critical part of the effectiveness of white rot enzymes to improve bleachability.

Hemicellulases are perhaps one of the most commonly used enzymes in the pulp mill. These enzymes are used to improve the bleaching of pulp by lowering chemical costs, increasing brightness, or raising pulp throughput [79,80]. Hemicellulases, especially xylanases, are typically added to the brownstock before the bleach line [81]. This requires the pH of the pulp to be lowered to a level conducive to enzyme activity (typical less than 7 pH) [79,80]. Viikari *et al.* [79] published one of the earliest papers on the use of bacterially derived hemicellulases for bleaching. In this work, enzymes were isolated from bacteria (*Streptomyces olivochromogens*) and fungi (*Aspergillus awamori*). These hemicellulases were applied to unbleached fibers and the resulting pulps were bleached by peroxide and chlorine. The pulp was treated at 2.5% consistency in citrate buffer at 5.0 pH. The enzymes were applied for 24 hours to the pulp. The enzyme treated pulps were compared to a buffer treated

reference pulp. The enzymes originating from *Aspergillus awamori* boosted the brightness of the pulp after bleaching by 2 to 3 points for the high dosage that was tested (4000 nkat/g). The lower dosage (400 nkat/g) actually reduced the brightness of the pulp. Hemicellulase from *Streptomyces olivochromogens* was shown to be more effective at raising the brightness. A dose of 28 knat/g resulted in a 2 point brightness improvement. This indicates that enzyme source can have a significant influence on its effectiveness. Overall, a 25% reduction in chlorine bleaching chemicals could be realized with the use hemicellulase enzymes.

The goal of bleaching is to remove chromophores (*i.e.* lignin) from the pulp and improve its brightness. Traditional bleaching chemistry targets the lignin that remains on the fiber after pulping, breaking it down into small pieces, and making it soluble. It is not readily understood exactly how hemicellulases improve the bleaching of pulp, but there are a number of plausible explanations. It is thought that the application of hemicellulase to the brownstock may act in three ways to improve bleaching: (1) degrading the lignin carbohydrate complex, thus weakening the bond of the lignin to the fiber [82], (2) degrading precipitated hemicellulose that covers the fibers and inhibits the action of bleaching chemicals, and/or (3) enhancing the permeability of the fibers making it easier to wash the lignin from the fiber and improving access of the bleaching chemicals to the lignin [82]. No matter the exact nature of the enzyme action, it is known that hemicellulase addition improves the bleaching. Typically, a two to five point improvement in brightness is found with same chemical loading or a 15%–25% reduction in bleaching costs is found [79,81].

Hemicellulases are degradative enzymes. In most applications, these enzymes are used for economic reasons. Thus, if these enzymes lower the pulp yield the cost savings found in reducing chemical costs might be lost. Hart and Sharp [80] presented an example showing the sensitive nature of the economics in a pulp mill. A pulp mill producing 800 tons per day was taken as an example. If enzymes lower bleaching costs by \$0.90 per oven dried ton, then a savings of \$720 per day could be realized. Assuming a wood chip cost of \$67 per OD ton, a decrease in the yield from 45% to 44.7% would completely offset the saving gained by using enzymes. The difficulty arises in measuring this small decrease in yield. Hart and Sharp used biological oxygen demand (BOD) data as an indicator of yield loss in the bleach plant associated with hemicellulase application. They showed, using a statistical method, that when enzymes are used for reducing bleaching costs, pulp mill BOD does not significantly increase. This implies that the enzymes have a negligible effect on yield. However, one of the mills that they examined showed an increase in BOD indicating a lower yield. This mill was applying

the enzymes as a de-bottlenecking measure to increase throughput. It is critical to be aware of the potential to offset economic benefits of enzymes use in the bleach plant with decreased pulp yield. Mills actively using hemicellulases should monitor BOD levels as a control parameter to guard against yield losses.

5 OTHER APPLICATIONS FOR ENZYMES IN THE PAPER INDUSTRY

Ink is adhered to the surface of paper making fibers. Disruption of the fiber surface is a means to aid in the liberation of ink from the fiber. Thus, enzyme degradation of both the fiber surface and the ink itself has been examined as aids for pulp deinking. Bajpai and Bajpai [83] provide a review of the enzymatic deinking technology that has been applied to the paper industry. They cite works showing a four point brightness improvement in the deinking process coupled with a very large decrease in the dirt count of mixed office waste (MOW) recycled pulp. Several Korean and Japanese patent applications claim that the brightness of recovered paper could be improved by flotation in the presence of alkaline cellulases [84,85,86,87]. Ow and Eom deinked newspaper with the aid of a cellulase and hemicellulase culture filtrate without the addition of other chemicals [88]. The brightness and bleachability of this pulp was superior to those of conventionally deinked pulp. The authors observed a preferential hydrolysis of intrafiber bonds, but no change in fiber length distribution was found. Li and Xu [89] applied purified cellulose binding (CBD) domains to mixed office waste pulp. The treated pulps were subjected to flotation deinking, where they showed a negative relationship between CBD and dirt count. In other words, the addition of CBD decreased the deinking effectiveness. Results that contradict earlier work showing improvements in the deinking operation with enzyme addition have also been shown by Xia, Beaudry, and Bourbonnais [90]. They used cellulase with different type of recycled pulps in a laboratory deinking operation. Their results showed that for old magazines improvements in deinking were found with cellulase treatment, while for old newsprint the deinking was worse with cellulase treatment. This conflicts the work of Ow and Eom [88], and Prasad *et al.* [91]. This was attributed to difference in processing conditions and addition point of the enzymes. Thus, when using enzymes in deinking operations, it is necessary to consider the exact nature of the enzyme and the application method.

Enzymes have also found other applications in recycled paper mills. A series of esterase enzymes have been used to reduce the impact of stickies on

a recycled paper mills. Ester bonds are very important bonds in many stickies. Thus, esterases can be effective at reducing the size and tackiness of stickies. Commercial products are available to use in the paper mill [92]. In general, they require a pH between 6.5 and 10, a temperature between 25°C and 70°C, and a contact time of at least 45 minutes with the pulp. These products have shown significant reductions in the size, tackiness, and deposition of stickies [92].

Biological treatment of wood chips has also taken place to reduce the pitch content in wood. Cartapip[®] is an albino strain of the *Ophiostoma piliferum* fungus that degrades wood extractives. The common type of *Ophiostoma piliferum* tends to stain wood blue, which has a negative impact on the brightness of pulp [93,94]. The albino strain of the fungus has been shown in the laboratory, as well as mill trials, to reduce the amount of pitch depositions in the paper mill. This is accomplished by inoculating the wood chips with the fungus prior to pulping. The fungus is allowed to grow on the wood chips for up to 5 weeks where a 50% decrease in extractives is found [93]. The pulp properties are also improved including a reduced specific refining energy consumption, and higher tensile to tear strength ratios [93]. The application of the fungus is somewhat limited due to the need for the fungus to have a certain ambient temperature to grow properly. Thus, other investigators have examined the effectiveness of using lipases for pitch reduction [95]. These investigators showed a significant decrease in the pitch deposits on the paper machine. The lipase enzymes were added to the stock preparation system where it acted to degrade the triglycerides in the pulp. A significant improvement in the runnability of high pitch content pulp was found, as well as, a higher coefficient of friction, which was attributed to triglyceride degradation. Other investigators used lipases from *Candida cylindracea* to reduce the triglycerides in pulp [96]. Both laboratory work and mill trials showed significant reduction in pitch deposits. Irie *et al.* identify the additional cost of using these enzymes at \$1.85 per ton of paper, which, in all but the most severe cases, would likely be cost prohibitive. Another enzymatic approach to pitch reduction is to use laccase in the white water system [97]. This approach showed that a significant reduction in the extractives (~20–25%) can be achieved by reacting laccase and mediator in an oxygen rich media with the thermomechanical pulp (TMP). Also, the addition of the laccase to the white water can result in even bigger decreases to the extractive circulating in the white water (~64%).

Enzymes have also been used to address biological (slime) deposits in the paper mill. A number of products exist that claim to replace biocides. However, one report by Schuetz and Wollenweber [98] illustrates that many of these “enzymatic biocides” have little or no effectiveness in killing microbial

entities. Most of these enzyme preparations consist of various cellulases, hemicellulases, amylases, proteases, oxido-reductases and other enzymes that degrade the food for the microbes or exopolymers of the microbes, which inhibit further growth and subsequent attachment to surfaces in the paper-making operation. Future work in this area could prove a very promising and fruitful area for reducing toxic chemicals used to control biological growth in the papermaking system.

Controlling dissolved substances within the wet end of a papermachine may be in part achieved by the use of enzymes. Reid and Ricard [99] used pectinases to reduce the cationic demand of mechanical pulp bleached with hydrogen peroxide. The cationic demand was reduced by up to 75% in supernatant of the TMP pulp (obtained after centrifugation) and by 40% in the whole TMP pulps by degradation of the pulp with pectinase for 2 hours at 50°C. First pass retention (total retention and ash retention) was also improved with the enzyme treated materials.

Pectinases have also been used to degrade the cambium region of logs prior to debarking [100]. This research showed that up an 80% reduction in the debarking energy could be achieved. Under possible industrial conditions, about a 50% reduction in debarking energy was achieved after 24 hours of exposure of the wood disks to the enzyme solution. This work used a laboratory scale bark peeler. Thus, the results may not translate directly to mills that use drum debarkers, but the results do indicate an opportunity to improve debarking efficiency, which may have positive impacts on pulp quality and pulp throughput.

6 FUTURE PERSPECTIVES ON BIOCHEMICAL ADDITIVES

Significant progress in the biotechnology area will allow for the production of enzymes that have excellent substrate specificity and robustness at a very affordable price. This may allow for the application of enzymes in areas that have already been identified, but were limited by these problems. Enzymes may be used in new applications to modify the ultrastructure of the fiber, aid in the processing of waste streams in the paper mill, modify under utilized wood components, or impart novel/improved properties to the final product. Beyond the application of enzymes, paper science may come to realize the potential of microbiological processing of materials. Currently, it is estimated that only 3% of all microbiological organisms are known. Many of these unexplored organisms may be very adapt at creating useful chemicals, polymers, and materials. This leaves a huge opportunity to harness microbes to process underutilized wood components and waste streams into chemicals,

which may be used as additives for the paper industry. This may lead to new directions for the paper industry whereby it processes waste streams into new and useful polymers and chemicals. For scientists to make this a reality, they will need to combine the disciplines of both engineering and microbiology. In other fields, scientists are already combining microbiology with other less obvious applications in inorganic chemistry. The use of microbiological agents to engineer new materials is becoming increasingly important. For example, Professor Angela Belcher of the Massachusetts Institute of Technology has used bacteriophages to construct nano-structured materials for uses in batteries, solar cells, and lights [101]. This is an example of how microbiological agents can be used to manipulate the materials on the micro and nano scale to improve the quality of life in a sustainable process. Scientists in the forest biomaterials arena will need to integrate the fields of microbiology, chemical engineering, materials science, and nanotechnology to develop new and useful materials from forest and agricultural resources, which will create new sustainable products.

One of the greatest challenges facing society is the shrinking petrochemical resources. There are certain materials for which no known good alternative is currently available such as polyethylene terephthalate (PET), which contains bisphenol-A (BPA). BPA has known endocrine effects and is currently not used for many food contact products [102]. However, this product still finds widespread use in food canning. Using microbiology, it may be possible to assemble a large sheet of crystalline cellulose which has excellent mechanical properties, is thermally stable, and provides excellent barrier properties that replace PET/BPA.

In our laboratory, we have begun exploring the use of microbes to produce useful polymers from wood resources. We have identified gamma poly glutamic acid (γ -PGA) as a possible matrix material for cellulose composites. γ -PGA is produced from bacterial sources and can be made in large quantities by well characterized bacteria using cellulose and hemicellulose as a carbon source. When combined with cellulose a new material is created that is thin, transparent and highly hygroscopic. We have combined γ -PGA with fibrillated cellulose, cf. Figure 6. The fibrillated cellulose was formed into thin films by vacuum dewatering. Before drying, the films were immersed in baths of PGA ranging from zero to one percent concentration. The films were then dried under restraint, cured at 150°C for varying times, and tested. The γ -PGA films showed distinctly different properties compared to the untreated samples. The γ -PGA improved the strength of the sheets, cf. Figure 7, and made the sheets more responsive to water, cf. Figure 8. These materials are very strong and durable (typical fold endurance values are more than 10,000 double folds) and hygroscopic, which is in contrast to untreated sheets. This

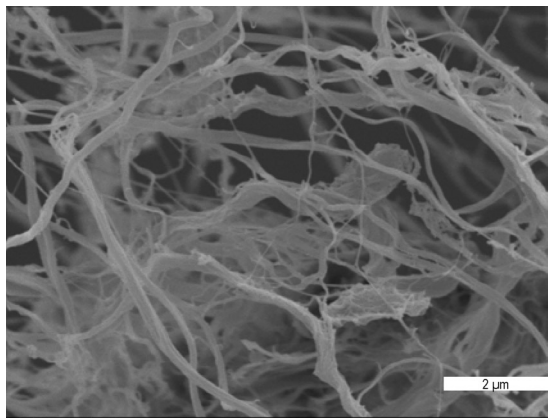


Figure 6. Fibrillated cellulose produced by extended refining of softwood pulp in a valley beater. This material will form thin strong water resistant films when dried from water.

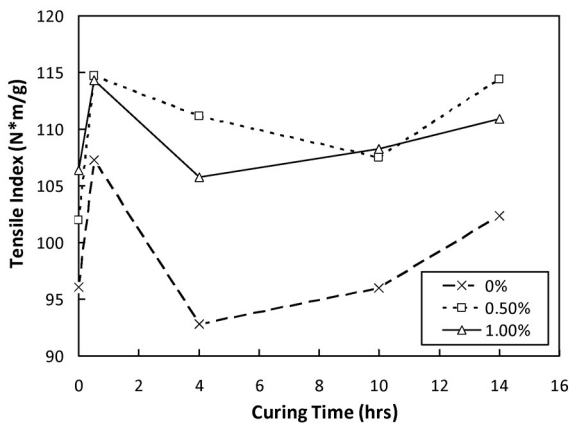


Figure 7. The effect of γ -PGA treatment and curing time on the strength of fibrillated cellulose films. The γ -PGA appears to provide an offset in strength, while the curing has a similar effect for all films. The percentages represent the concentration of the treatment bath. (Data courtesy of Rachel Ernest, North Carolina State University.)

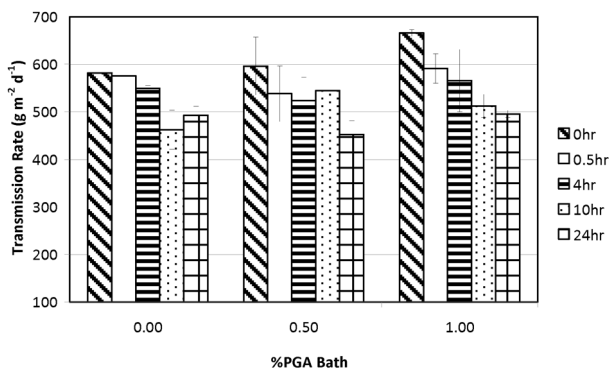


Figure 8. The water vapor transmission rate for fibrillated cellulose films treated with γ -PGA. Higher levels of treatment show more affinity for water as exhibited by an increase water vapor transmission rate. Curing of the film from 0 to 24 hours in general reduce the water vapor transmission rate. (Data courtesy of Rachel Ernest, North Carolina State University.)

material may be used in packaging applications, a drug delivery system, or in biomedical applications. This represents an initial step in the use of microbiological agents to produce polymers and chemical for application in the paper and forest biomaterials arena. There are also a wide variety of other polymers that are produced by microbiological agents, which can find useful applications and can help to fill the future demands of society for high performance materials.

7 SUMMARY AND CONCLUSIONS

Biochemical additives have not yet realized their full potential in the paper industry. A wide variety of enzymes have been applied in the paper industry including cellulases, hemicellulases, pectinases, ligninase, manganese peroxidase, lignin peroxidase, esterase, lipase, protease, and amylases. Enzymes have been applied throughout the pulp and papermill from the woodyard to the papermachine. Some of these applications have found widespread industrial use including hemicellulases to aid in bleaching and lipases to degrade extractives. There still remains a number of technical challenges that prevent the widespread use of enzymes in other applications. Most of these barriers are related to the cost/benefit analysis a mill undergoes to evaluate the enzymes and the requirements that enzymes have for retention times and

reaction conditions. In this review, the focus was placed on the use of enzymes in the papermill. Enzymes in this area tend to be carbohydrate degrading. This review examined the literature to determine a general behavior of the application of carbohydrate degrading enzymes (*i.e.* cellulase and hemicellulase mixtures) to whole fiber fractions. Three phenomena can be identified in the literature. The first is a flocculation effect caused by the binding ability of the enzyme, the second is the degradation of the fine particle fraction of the pulp, and the third is the degradation of the long fiber fraction. Thus, depending on the application rate and duration one may experience a wide variety of effects including increasing the drainage, decreasing the drainage, lowering the fines content, raising the fines content, improving the sheet strength and decreasing the sheet strength. All of these effects are possible under specific enzymatic treatment conditions. It is imperative that the papermaker understands the trade-offs and unique response of paper to enzyme treatments.

Beyond the application of carbohydrate degrading enzymes in the papermill, enzymes have been used to reduce pitch, lower TMP refining energies, improve bleaching, reduce stickies, improve deinking, increase the efficiency of shive processing, improve debarking, improve mechanical pulping, alter fiber flexibility, and improve the performance of other wet end additives. In the future, biochemical additives may extend well beyond the use of enzymes, starch, and rosin in papermaking. Biotechnology and the area of microbiology may create new opportunities to generate additives for the paper industry using current waste streams within the paper mill. Our laboratory has begun exploring the use of biotechnology and microbiology to generate novel additive for the paper industry. These technologies may also provide papermills additional revenue streams as they may produce useful and unique polymers and chemicals that can be used beyond the paper industry.

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Transcription of Discussion

BIOCHEMICAL ADDITIVES FOR PAPERMAKING

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I guess another possible explanation for the contradiction in the results is that most of the commercial cellulases would be crude extracts from fungi or bacteria and they would contain different levels of cellobiohydrolases and endoglucanases. The endoglucanases, as you say, act randomly on the glycosidic linkages of the polymer backbone and would presumably cause a lot more strength loss than the cellobiohydrolases which would act from the chain end. So it's a rather contorted way of asking a question which is: do you think it is feasible that more selective use of enzyme fractions would be economically viable, and could be used at some time in the future?

Joel Pawlak

From the point of view of economic viability, I think the previous approaches that we have taken to produce enzymes, such as the purification of a crude enzyme of some sort or some extract from fungi, will not be used in the future. We will probably have alternative ways to produce these types of enzyme. Molecular biology, for example, will be able to generate enzymes, say a cellulose degrading enzyme from an *E. coli*. That would provide a relatively pure culture for us to use. So, from an economic standpoint, the ability to get specific isozymes will probably change in the future as long as there is the push to be able to use them. Now maybe the other question is, if I can read between the lines, what role do the different cellulose isozymes play in changing the properties? And that is an important question, that's something that's been a little bit difficult to deal with because it has been difficult to get pure enzymes in a sufficient amount to be able to look at hand-sheet studies

Discussion

and actual effects on the paper making. I think using a specific strain of isozymes gives you a finer tool, but my gut instinct says that it is not going to change overall what you see happening. So if you see the microfibre edges, for example, they might have less of an effect on the degradation of strength, but still when we look at how these things interact, they tend to interact on the surface. They will interact mostly still on the surface of these notches because there is more specific surface area available there. Thus, preferential degradation of the notch will occur, and we will still see strength losses, maybe not to the same extent as when we have the full complement of the enzymes.

Lars Wågberg KTH

First of all thank you for a very good review. I enjoyed it immensely and my question is more of a comment, although I would also like to get your comments on my comment. I think you showed very clearly from one of your tables that, although many of the people that have made investigations with enzymes are good scientists, they did not know what they were doing. This is simply because they are studying such a complex mixture of fibres and fines. We have to remember that we have 5 million fibres in a gram, and we do not know how they all look.

What I missed a bit in your review was the rapid development in the work on model surfaces of cellulose, hemicellulose and lignin. The Rojas Group at NC State, we ourselves at KTH, and Janne Laine in Finland have all used the continuations of model surfaces and enzymes to establish what those materials are really doing to the different components. We have, for example, made investigations with cellulose surfaces at different crystallinity and we see things when we use cellobiohydrolases and endoglucanases, that we do not see on the macroscopic scale. What is really intriguing is if you transfer these experimental protocols to fibres in single-fibre measurements. You simply see the same mechanisms and a total new world for understanding enzymatic actions opens up. I agree with you that the enzymes are fantastic biochemical additives, but we have just seen the tip of the iceberg, and we are maybe using them in the wrong applications in the industry; we might need new, separate processes for using them more and more efficiently.

Joel Pawlak

Sure, let me just comment on your comment. I did not miss the work from the Rojas group because I am a co-author on some of the papers! This actually was brought up to me by a colleague, and the question might be more important when you look at the model surfaces used for quartz crystal

microbalance (QCM) investigations to look at enzyme degradation and absorption. Probably, one of the important things to remember, is that the model surface is very different from the fibre itself. It is maybe highly crystalline and ordered in some way, it has other things that are interacting with it. We can look at some of those things to help us understand better what is going on. I actually cite some of that work here where you can see the initial very rapid absorption of the enzymes in the first five minutes or so when the enzyme is added into the QCM and then it is not until after about 5 to 10 minutes that we actually see some sort of catalytic activity and loss of cellulose. Furthermore, if these enzymes are denatured, and then added to the QCM, we will still see absorption. That is the polymer effect that you see when these enzymes are in solution. This is interesting and it is particularly useful for understanding enzyme dynamics. Maybe not the overall activity, but the dynamics of the initial absorption of the enzyme and the delay and how enzyme absorption affects activity.