ENZYMATIC SOLUTIONS TO ENHANCE BONDING, BLEACHING AND CONTAMINANT REMOVAL,

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ABSTRACT

Microbial enzymes can enhance the properties of virgin pulps and secondary fibers. Enzumes added to pulp slurries at key steps during fiber processing alter structure and composition of native substrates in specific ways. Lipases hydrolyze fatty acid esters thereby facilitating depitching and deinking. Cellulases remove fines and change surface properties, thereby enhancing drainage, deinking and contaminant removal of secondary fibers. The binding domains of cellulases exfoliate or roughen the surface of cellulose fibers. This feature may be used to enhance inter-fiber bonding. Xylanases and ligninases enhance lignin and chromophore removal thereby facilitating bleaching of draft pulps. Microbial xylanases reduce chemical demand for bleaching by 35 to 50% in softwood and hardwood pulps, respectively. Many extracellular microbial enzymes are stable at 50 to 60°C and between pH 4 and 7, and some function well at greater extremes. In some instances crude enzymes can be used, but by cloning and expressing pure enzymes at high titers more precise and economical applications can be achieved.

INTRODUCTION

enzymes increase fiber bonding and water retention, reduce beating times in virgin pulps, reduce vessel picking in tropical hardwoods, remove xylan from dissolving pulop, and enhance bleaching of kraft pulps. With recycled fibers enzymes increase freeness without affecting the mechanical properties of the fibers, and recent dtudies suggest that they can facilitate deinking.

Recycled or secondary fibers contain fines, colloids, fillers and contaminants. One of the most marked characteristics of secondary fibers is their low freeness. Freeness, or the degree of resistance to the flow of water, is an important physical property in pulp processing and papermaking. Decreased pulp freeness limits the speed at wihch the paper machine can be operated. A substantial increase in freeness enables the use of lover-grade recycled fibers and more dilute suspensions in the paper machine headbox, thereby leading to lower production costs and better paper formation. some microbial enzymes have been shown to increase freeness of secondary fibers.

Another characteristic of recycled fibers is their relatively low strength. Paper strength properties are largely a function of fiber length and interfiber bonding. Interfiber bonding increases with specific surface area (due to fibrillation) and the presence of materials that facilitate surface interactions. Refining increases

pulp strength by increasing the surface area, but it also decreases freeness. The brittleness of secondary fibers limits the amount of refining that can be used to restore bonding, because during refining, freeness decreases. The loss of freeness is attributable to the accumulation of fines. Unlike virgin fibers, secondary fibers - and particularly secondary bleached kraft fibers - create large quantities of fines during beating. Some microbial enzymes have been shown to enhance fibrillation, and hence restore fiber bonding with less beating.

The most significant contaminants are clays, inks and stickies. Whereas clays largely will wash out of the fiber, inks and stickies can present problems. When ink particles adhere to the surface of recycled fibers, they decrease paper brightness, and, if they are too large, they cause spotting. Inks and stickies can be removed either by washing or by floatation, but even the best deinking technology removes only about half of the ink present. True to their name, stickies are particularly difficult to remove. The chief problems in deinking are with non-contact inks from office copiers and laser printers and with finely dispersed inks that become enmeshed in the fiber matrix. In order to increase the amount of ink removed, refiners are used to break up and disperse the ink particles. Unfortunately, in the process, the fibers are broken and strength properties are lost. What is needed is a means to remove inks and stickies with less fiber damage. Recent evidence suggests that enzymes can alter ink or fiber surface properties, reducing the amount of mechanical action needed, and thereby facilitating contaminant removal.

Production of bleached kraft pulp is one of the most important segments of the U.S. pulp and paper industry. Kraft pulp is commonly brown and must be bleached and extracted to make white paper. Chlorine and chlorine dioxide are usually employed for bleaching kraft pulp. Some of the chlorinated aromatic hydrocarbons produced during bleaching are mutagenic, and many are recalcitrant to biodegradation. For these reasons, the pulp and paper industry is seeking ways to reduce chlorine bleaching. One useful approach found is to enzymatically open up pulp porosity, cleave covalent linkages and facilitate chemical bleaching sequences.

In this article, we review some of the literature related to the use of microbial enzymes in recycling secondary fibers, and we offer a few examples from our own research. Because this is a rapidly-emerging field, much of the literature is found in patents and symposium proceedings rather than in peer-reviewed journals.

Enhanced freeness and bond strength restoration

A mixture of cellulases and hemicellulases at low concentrations will markedly increase the freeness of recycled fibers without substantially reducing yield. The lower the initial freeness, the greater the gain following treatment. Many different cellulases and hemicellulases can be used to improve freeness, but good results have been obtained with commercial cellulases from *Trichoderma reesei* [1]. Freeness shows rapid initial increases, with over half of the observed effect occurring in the first 30 min. A relatively small amount of enzyme is

required. While the initial effects are largely beneficial, extending the reaction time with large concentrations of enzyme is detrimental. Unfortunately, crude enzyme mixtures also reduce strength properties [2].

Mixed cellulases and xylanases peel the surfaces of fibers. If treatment is limited, the enzymes remove only elements that have a great affinity for water but which contribute little to the interfiber bonding potential. By selectively removing these surface components, pulp-water, interactions are reduced and drainage increases without noticeable changes in the final mechanical strength properties of the paper. If the treatment is extended, hhowever, fibrillation becomes pronounced, and drainage decreases. If large quantites of crude enzymes are used, the average fiber length is reduced, fines disappear, and the strength properties of the fibers are lost. An optimum level of enzyme treatment is therefore required.

A recent patent [3] suggests that the drainability of mechanical pulp can also be enhanced by the addition of heicellulases. The authors claim that xylanase imptoves the freeness of deinked recycled pulp while having no detrimental effect on fiber tensile strength properties. By comparison, the tear indices of recycled pulps treated with cellulases decreased [3]. These findings suggested that xylanases might be much more effective than cellulases or crude xylanase/cellulase mixtures. Xylanases, however, remove hemicelluloses that promote interfiber bonding. This effect can also lead to poor paper properties.

Studies in our laboratory have incicated that xylanases will enhance freeness of chemical but not mechanical fibers. We compared the effects of a commercial cellulase (Celluclast™), a commercial xylanase containing cellulase activity (Pulpzyme HA™), and a cellulase-free xylanase from *Aureobasidium pullulans.* The effects of the enzymes were significant. In the case of the *A. pullulans* xylanase acting on chemical fibers, the freeness was better than observed with Celluclast™ when both were used at the same dosage level (*cf.* Tables. 1 and 2).

IU/g OD	ml CSF	
	Pulpzyme HA™	Celluclast™
0	525	520
10	550	590
50	565	590
100	570	595
200	575	550

Table 1: Effects of cellulases and cellulase.xylanase mixtures on hte freeness of secondary fibers from office waste. Each enzyme preparation wqas incubated with the pulp for 2 h at either 30°C (Pulpzyme HA™) or 50°C (Celluclast™).

enzymes have been examined extensively for their effects on fiber properties. In 1942, a patent claimed that microbial hemicellulases from *Bacillus* and various Aspergillus species could aid refining and hydration of pulp fibers [4]. In 1959, bolaski et al. patented the use of cellulases from *Aspergillus niger* to separate and fibrillate pulp [5]. The technilogy was principally applied to cotton linters and other non-wood pulps. A process patented in 1968 used cellulases from a white-rot

fungus, *Trametes suaveolens,* to reduce refining or beating time [6]. Cellulase plus cellobiase when added to pulps in water has been reported to accelerate digestion of pulp[7].

IU/g OD pulp	ml CSF	
	Newsprint	Office waste
0	395	520
10		610
50	385	
100	390	595
200	370	

Table **2:** Effects of *Aureobasidium pullulans* xylanase on the freeness of newsprint and office waste. Pulp was incubated with the indicated amount of enzyme for two hours at 40°C then tested for Canadian Standard Freeness.

The mechanisms by which cellulases and xylanases promote fibrillation are not completely understood. Presumably the enzymes preferentially remove hemicellulose and amorphous cellulose to leave behind the cellulosic microfibrils. Initial enzyme treatment will loosen fiber bundles and reduce the amount of refining necessary to attain paper strength. If the cellulose is degraded, however, the paper strength is reduced. Optimally, therefore, the most effective treatments will be those that act on the interfiber glue. This consists primarily or hemicellulose, lignin and, in some cases, pectin. Hemicellulases and ligninases should, therefore, promote fibrillation to enhance fiber bonding. Specific enzyme fractions that increase interfiber bonding without destroying fibril strength properties need to be identified.

In most studies to date, only crude enzyme preparations have been used with secondary fibers. Although this is appropriate from an applications perspective, the results tell us little about the effects of individual enzyme classes, isoenzymes or enzyme components. Many different cellulases and hemicellulases are produced by lignocellulose-degrading microbes. Not only do they vary from one organism to another, but an individual organism will often produce multiple enzymes falling into several different classes. Isoenzymes in a specific class may possess structural differences that are difficult to detect from kinetic studies but which have significant bearing on enzyme activity. It is therefore necessary to consider individual isoenzymes when looking for specific effects.

Despite their wide structural variety, cellulases and hemicellulases possess conserved domains within the enzymes. These protein domains are similar from one enzyme to another. Recent studies have shown that when the catalytic and cellulose binding domains of cellulase are separated, they have profoundly different effects on cellulosic fibers [8]. The catalytic domain has the ability to cleave glycosidic linkages, but it shows only low affinity for its substrate. The catalytic domain when used alone leads to a smoothing of the fiber surface. The binding domain does not hydrolyze glycosides, but it has a high affinity for the substrate. When used alone, it will delaminate fibrils, thereby leading to exfoliation and surface roughening. These quite opposite actions could be used to affect fiber

surface properties. The smoothing effect should improve freeness and the roughening effect should improve inter-fiber bonding.

Cellulases can be classified into enzymes that principally attack either amorphous cellulose, crystalline cellulose or cellulooligosaccharides. In addition, cellulases can exist in cell-bound or cell-associated complexes consisting of many different catalytic subunits. Hemicellulases likewise can be classified into those attacking the main chain, those removing side chains and those degrading oligomeric products, but because these polymers consist of several different sugars, the enzymes that degrade them are also more heterogeneous. The actions of cellulases and hemicellulases are synergistic in some combinations, at least with respect to sugar release, but their individual and combined effects of fiber properties are poorly understood. One component that has not been characterized very well is the short fiber or microfibril producing factor [9, 10].

Mercuric chloride has been used to create a number of "cellulase-free" xylanase preparations [23, 24, 25, 26]. In the presence of $HgCl_2$, hydrolysis of cellulosic substrates is essentially blocked; by comparison, the rate of xylan hydrolysis decreases only slightly [28]. Similar results can be obtained using cloned xylanases. Electron microscopy shows increased fibrillation in enzyme treated as compared to control pulps [29, 30] The water retention, breaking length and apparent density all increase with treatment, but viscosity decreases by over 30%, and the wet zero-span breaking length decreases significantly. Enzyme-treated pulps show enhanced beatability and better bonding due to increased fiber flexibility, but as treatment is extended, the intrinsic fiber strength decreases due to the loss of the xylan.

Deinking and contaminant removal

Patent disclosures from Oji Paper [11] and from the Korean Institute of Chemical Technology [12] claim that treating used paper with cellulases, hemicellulases or pectinases prior to deinking results in a brighter pulp of higher quality. The Oji patent states that the bonds between each component of the pulp are "relaxed"and the adhered ink is released easily. However, the basis for the effect is not understood. It is possible that the deinking results from increased fibrillation. It is also possible that the enzyme removes the surface layer of the fiber to which the ink adheres. The Korean patent suggests that cellulases lower the amount of energy required in pulping and dispersing inks in secondary fibers, because the beating time required to attain a desired freeness is reduced by almost 50% in the presence of cellulases. The enzymatic activity debonds the fibers and dislodges the ink that is caught in the fiber bundles. Other benefits such as a reduced use of hydrogen peroxide and emulsifying agents and an improved pulp strength are also claimed. Greater brightness (10%) and an improved tear index (9%) are attained. The greatest difficulties in deinking lie in the use of non-contact inks from xerographic processes. Such ink formulations include nylon and styrene. The cellulase treatment is claimed to be particularly effective with laser beam cured inks.

Another approach to enzymatically enhanced deinking is to attack the ink itself. Two recent reports in Japanese patents claim that alkaline lipases will facilitate the removal of lipid-based offset printing inks [13, 14]. This approach could be

particularly effective if inks based on natural vegetable oils are employed. To our knowledge, no one has examined the effects of enzyme treatments on the removal of stickies.

Enzymatically enhanced bleaching

In exploring applications of both crude ligninases and hemicellulases with kraft pulps, Viikari et al. [15] showed that treating pulps with hemicellulases can reduce the subsequent chlorine bleaching requirements. The original researchers [16, 17] and others [18, 19] have confirmed these studies, and several laboratories are developing this approach. Kraft pulping degradation products inactivate some xylanases, [20] and others cannot fully penetrate the pulp matrix [21]. However, these problems have not impeded research. Companies are now beginning to market enzyme preparations suitable for bleaching kraft pulps, and we have characterized a system that is particularly effective.

Researchers have filed numerous patent applications concerning the hemicellulase-facilitatedbleaching of kraft pulps. One indicates that a mixture of cellulases, hemicellulases and esterases might be used [22]; another employs the xylanase from *Aureobasidium pullulans* [23] Several other patents claim that low molecular weight xylanases from various *Chainia* sp. facilitate bleaching [24,25,26,27]. One patent specifies xylanases from *Streptomyces lividans* [28], while another specifies the use of an alkaline active, thermostable xylanase from *Thermomonospora fusca* [29]. Several patents claim the use of xylanases in combination with conventional chlorine bleaching, [30] and some specify compatibility of xylanase treatments with oxygen bleaching [31]. One recent patent application claims that xylanases can be used in conjunction with lipases to enhance bleaching [32].

There are at least two U.S. patents [33, 34] and three European patent applications [35, 36] pertaining to the use of ligninases or lactase [sic] for bleaching pulps. One of these makes use of a chemically modified lignin peroxidase [37]. In addition, a European patent application exists for the production of recombinant ligninase [38]. Olsen et al. [30] have developed an enzymatic bleaching procedure using partially purified MnP and LiP isoenzymes from *P. chrysosporium.* At least one process uses lignin and manganese peroxidases from *Phanerochaete chrysosporium* sequentially with xylanases from a *Chainia* sp. Intervening alkali extraction steps reduce residual lignin content and results in a brighter pulp without chlorine [39].

These are rapidly emerging commercial processes, and details of the technology are not readily available, but xylanases reduce consumption of bleaching chemicals by 30 to 50% with hardwood pulps and **20** to **30%** with softwood pulps. Productivity of the organism largely determines the cost of treatment, but enzyme costs can be even less than the cost of the chemicals displaced. Industrial enzymes typically cost $5 to $10 per kg to manufacture. Loading rates are on the order of 0.1%, i.e. 1 kg/ton pulp. In the case of Cl_2 bleaching, the savings in bleaching chemicals realized by using xylanase is near a break-even, but in the case of ClO_2, H_2O_2 or other more expensive bleaching chemicals that do not create chlorinated

aromatics, the savings can be significant when enzymes are used. The technology for enzymatic treatments of pulps is advancing rapidly, and increasing competition among manufacturers will continue to improve products and reduce prices.

Viikari et al. have compared the effects of two hemicellulase and two ligninase preparations for their efficacy against hardwood and softwood pulps [40, 41]. In these studies, the ligninases were not effective, but much has been learned since that time.

In the absence of cellulase, some xylanase treatments can increase viscosity while facilitating bleaching [42]. The best published example of this is the treatment of pulps with a cloned bacillus xylanase [43, 44] Lignin removal was facilitated while pulp retained viscosity and strength properties. Many other microbial xylanases have been cloned, but the peorperties of relatively few have been tested for their action on kraft pulps. Xylanases enhance bleaching of hardwood more than softwood pulps. Enzymes that will effectively enhance the bleaching of softwood kraft pulps are still needed.

Hemicellulases having different specificities for substrate degree of polymerization (DP) and side groups can remove lignin from a pine kraft pulp when applied in a sequential enzyme-perxiide delignification. The greater the cellulase activity, the greater the loss of pulp viscosity. Only limited hemicellulose hydrolysis is necessary to enhance lignin removal. Endoxylanases are most effective in enhancing the alkaline peroxide delignification of a pine kraft pulp [45]. Only a portion of the xylan is susceptible to enzyme hydrolysis following repeated enzyme treatment and lignin extraction. Relatively short treatments with low quantities of enzyme are sufficient to remove the susceptible fraction of xylan present. These findings have led to the hypothesis that the efficacy of xylanase treatments are largely limited by substrate accessibilty or structure.

Bleaching enhancement is not proportional to the amount of glucomannan removed [46]. Mannanase treatments have little effect on handsheet properties, whereas xylanasse treatments increase the beatability of unbleached kraft pulps and substantially increase the tear strength of blended pulps when both are beaten to the same degree of freeness. The differences observed between xylan and mannan solubilization might be attributable to the locations of these polymers and the cell wall.

Xylanases enhance bleqaching of hardwod more than softwood pulps. Purity of the xylanase appears to be important. Celllulases probably should not be present, but in some instances, cellulases are thought to enhance bleaching by clipping bonds between lignin and cellulose. Mixtures of hemicellulases with different action patterns can be more effective than single enzyme fractions. Glucomannanases are more useful with softwood pulps. The only reliable method to evaluate an enzyme is to determine its effects on pulp properties and bleachability. Xylanase treatments can release lignin from kraft pulp, but more commonly, they are used to reduce chemical consumption in subsequent bleaching and extraction steps. If xylanases alone are used to reduce residual lignin content, the negative effects attributable to excess xylan removal can be significant. More specific enzymes are bneeded that will cleave the lignin and lignin-carbohydrate bonds and leave the xylan intact [47].

In the absence of cellulase, some xylanase treatments can actually increase viscosity [48]. Probably the best published example of this is the treatment of pulps with a cloned bacillus xylanase [49, 50, 51]. Lignin removal was facilitated while pulp retained viscosity and strength properties. Many other microbial xylanases have been cloned, but the properties of relatively few have been tested for their action on kraft pulps. Enzymes that will effectively enhance the bleaching of softwood kraft pulps are still needed.

Enzymatic reduction of kappa number in old corrugated containers

Old corrugated containers (OCC) comprise one of the largest fractions of secondary fibers marketed today. They consist for the most part of high-quality, readily accessible softwood kraft pulp. While a high demand exists for OCC during good economic times, prices can fall dramatically as the economy weakens, this is, therefore, a potential source of fibers for converting into printing and writing grades.

In order to determine whether xylanases from *S. rosea* can reduce kappa number in OCC, we prepared a pulp from OCC with an initial kappa number of 83. It was divided into two batches; one was soaked in alkali (10%, OD pulp basis) at room temperature for 4 h; the other was used without further treatment. The control OCC and the alkali-treated OCC were each treated with 5 IU of *S. roseiscleroticus* xylanase/g OD pulp at 60°C, pH 7, and the resulting supernatant solutions from stage 1 were recovered and assayed for materials absorbing at 237 nm. Effectiveness was determined by multiplying the volume of the solution times the absorbency at each wavelength (optical density · ml = OD units).

Enzyme treatment effectively removed materials absorbing at 237 nm. Enzyme treatment also facilitated the subsequent extraction of color bodies by 1% alkali in a stage 2 extraction; notably, enzyme treatment enhanced 1% alkali extraction of material absorbing at 237 nm even when the OCC had previously been subjected to extraction with 10% alkali. In all instances removal of color bodies was greater with enzyme treatment than without.

The S. roseiscleroticus xylanase reduced the kappa number of OCC in a single stage (stage 1) enzyme treatment either with or without a prior 10% alkali extraction. The control OCC pulp prior to treatment with 10% alkali or enzyme had a kappa number of 82.8 ± 1.3. Following enzyme treatment this non-alkali extracted (control) OCC pulp had a kappa number of 74.7 ± 1.9, so the enzyme alone reduced the kappa number by 8.1 points. Following extraction with 10% alkali, the OCC had a kappa number of 66.3, but enzyme treatment could reduce this still further to a kappa number of 61.5. Thus, a stage 1 enzyme treatment alone reduced the kappa number of OCC or 10% alkali-extracted OCC significantly. The xylanase also reduced kappa number further in the second stage (stage 2) extraction with 1% alkali. For example the control OCC had a kappa number of 66.8 following extraction with 1% alkali, i.e. only slightly higher than the kappa number of 66.3 in control pulp following extraction with 10% alkali. The control OCC pulp which was treated with enzyme in stage 1 then extracted with alkali in stage 2 had a kappa number of 63.7, a full three points lower than pulp that had not been treated with alkali. Likewise OCC that was treated with 10% alkali, then enzyme, then 1% al-

kali had a final kappa number of 53.2, more than 10 points lower than the corresponding OCC that had not been treated with enzyme. By itself, enzyme reduced the kappa number 10%; together, enzyme and alkali were able to reduce the kappa number by 36%. These data are summarized in Figure 2:

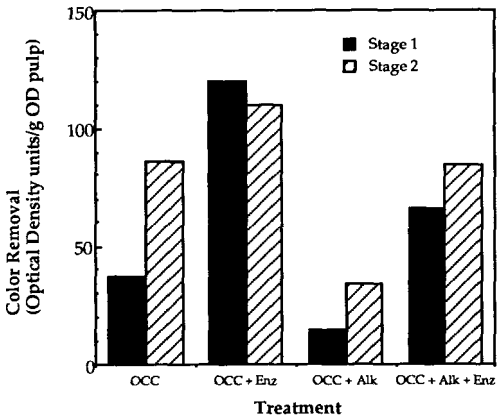

Figure 1. Color bodies released into solution from OCC by enzyme treatment at stage 1 (solid bars) or following a stage 2 extraction with 1% alkali (Hatched bars). OCC = Old corrugated containers; OCC + Enz = pulp that had been treated with enzyme prior to extraction with 1% alkali; OCC + Alk - OCC that had been treated with 10% alkali prior to a second extraction with 1% alkali; OCC + Alk + Enz = OCC that had been treated with 10% alkali, followed by enzyme treatment, followed by extraction with 1% alkali.

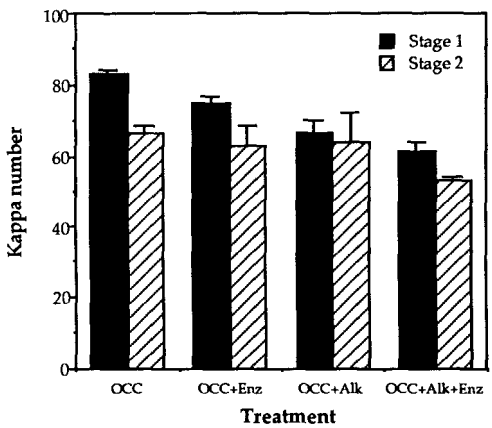

Figure 2. Reduction of kappa number of OCC following alkali and enzyme treatment. Solid bars represent OCC either with or without alkali pretreatment; hatched bars represent pulps that have been carried through a second stage alkali extraction as described in Fig. 1.

Conclusions

Microbial enzymes are sprcific tools to modify the properties of lignocellulosic materials. Many different organisms are capable of degrading cellulose, hemicellulose and lignin, and they form a correspondingly large array of enzymes. They are capable of modifying surface properties and selectively removing fiber components. Contaminant, pitch, ink, and chromophore removal are among the more important applications demonstrated thus far. Enzymes can also alter strength and freeness, and through the use of individual enzyme conponents, we may be able to bring about these changes in even more discriminating ways.

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