Chapter 1

Roles for Microbial Enzymes in Pulp and Paper Processing

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Microbial enzymes are enabling new technologies for processing pulps and fibers. Xylanases reduce the amount of chemicals required for bleaching; cellulases smooth fibers, enhance drainage, and promote ink removal; lipases reduce pitch; lignin-degrading enzymes remove lignin from pulps. Several of these processes are commercial, and others are beginning to emerge. In the future, enzyme-based processes could lead to cleaner and more efficient pulp and paper processing. The papers in this book describe fundamental and applied aspects of xylanase, cellulase, lignin-degrading enzymes, and lipase with a view toward the development of novel processes, unusual enzymatic activities and elucidation of underlying mechanisms.

Paper manufacture is one of the largest industries in the United States. In 1995, the US produced more than 82 million metric tons of paper, paperboard and secondary products with a shipped value of $166 billion (1) An industry of this magnitude supports a large, rapidly changing technical base, and because it draws heavily upon timber and water resources, it is subject to close scrutiny from environmental interests.

Several decades ago researchers realized that because paper is composed of natural polymers - cellulose, hemicelluloses, and lignin - microbial enzymes and organisms might be useful in its processing. Only in the last decade, however, have microbial enzymes been used commercially in the pulp and paper industry, and microorganisms, though long employed in waste treatment, are only now beginning to be used in other processing steps.

The main reason for the slowness in using enzymes in pulp and paper processing is that the substrates - wood and pulps - are difficult to degrade. Because it is the lignin that is removed from wood in chemical pulping, and from pulps in bleaching, the research focus historically has been on lignin-biodegrading systems. Lignin likely evolved in part as a deterrent to microbial degradation, and it continues to be an impediment to biotechnological processing of wood and pulps. Its degradation by isolated enzymes remains difficult.

The potential for environmentally benign, efficient lignin removal spurred research that led to the discovery of lignin-degrading enzymes in the early 80's, and to extensive

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investigations into their activities through the present. Even so, having ligninolytic enzymes in hand, and knowing how they function, has not shown promise for practical applications. During the past ten years the number of possible applications of enzymes in pulp and paper manufacture has grown steadily, and several have become, or are approaching, commercial use. These include enzymatic bleaching with xylanases, pitch removal with lipases, and freeness enhancement with cellulases and hemicellulases. Others such as contaminant removal and fibrillation of recycled fibers by cellulases could be commercial soon. As we gain more experience with these systems, one of the biggest barriers to their use - a reticence by industry - is diminishing. Enzymes are considered exotic, and paper makers usually cannot use them in the same manner as inorganic chemicals. Enzyme manufacturers often lack sufficient industry contacts to transfer technology to the mills, and traditional suppliers of paper chemicals face loss of some markets with increased use of enzymes. All of these factors weigh against rapid widespread application, but the industry has continued to develop. Increasingly, pulp and paper companies are employing microbiologists and biochemists to guide them in the use of biotechnology.

Our purpose in this chapter is to provide a historical framework for applications and fundamental studies in this field. Several recent in-depth reviews of individual applications (2-5) present summaries of the latest developments. Historically, development of enzyme applications in pulp and paper began with studies of the use of cellulases to facilitate fiber beating, and progressed through current efforts to apply lignin-degrading enzymes (Table I).

**Table I. Milestones in the development of enzyme technology for pulp and paper processing**

<table>
<thead>
<tr>
<th>Year</th>
<th>Development</th>
<th>Researchers and reference</th>
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<tbody>
<tr>
<td>1959</td>
<td>Pulp fibrillation by cellulases</td>
<td>Bolaski and Gallatin (6)</td>
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<tr>
<td>1984</td>
<td>Enzymatic beating with xylanases</td>
<td>Comtat, Mora and Nöe (7)</td>
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<td>1984</td>
<td>Hemicellulose removal from dissolving pulps by xylanases</td>
<td>Paice and Jurasek (8)</td>
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<td>1986</td>
<td>Prebleaching with xylanases</td>
<td>Viikari, Rannua, Kantelinen, Sundquist and Linko (9)</td>
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<tr>
<td>1988</td>
<td>Enhanced drainage with cellulases</td>
<td>Uchimoto, Endo and Yamagishi (11)</td>
</tr>
<tr>
<td>1988</td>
<td>Decreased vessel picking with cellulases</td>
<td>Irie, Matsukura and Hata (12)</td>
</tr>
<tr>
<td>1989</td>
<td>Depitching pulp with lipase</td>
<td>Kim, Ow and Eom (13)</td>
</tr>
<tr>
<td>1991</td>
<td>Deinking with cellulases and xylanases</td>
<td>Call and Mülke (14)</td>
</tr>
<tr>
<td>1993</td>
<td>Pulp delignification with laccase</td>
<td>Harazono, Kondo and Sakai (15)</td>
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**Fibrillation and Strength Enhancement**

Beating and refining are mechanical processes that enhance fibrillation and inter-fiber bonding. Properly applied, microbial enzymes can enhance or restore fiber strength, reduce beating times, and increase inter-fiber bonding through fibrillation.

Pulp fibrillation by cellulases was recognized as a means to enhance strength properties as early as 1959 (6). Cellulases from the fungus *Aspergillus niger* were used to enhance fibrillation, thereby improving the strength of paper by increasing fiber-fiber contact. It was principally applied to cotton linters and other non-wood pulps. A process patented in 1968 used cellulases from the white-rot fungus *Trametes suaveolens* to reduce refining or beating time (16). Nomura reported that cellulase plus cellobiase added to pulps facilitated fibrillation without strength loss (17) Jokinen et al have also described the use of cellulases to improve fibrillation of pulps (18).
The principal challenge in using enzymes to enhance fiber bonding is to increase fibrillation without reducing pulp viscosity. Viscosity decreases when cellulases cleave cellulose chains, lowering the degree of cellulose polymerization (number of glucose residues per chain) and destroying fiber integrity. In one attempt to get around this problem, researchers fiberized pulp using “cellulase-free” xylanase from mutants of *Sporotricum pulverulentum* and *Sporotricum dimorphosphorum*. Relatively mild xylanase treatments removed less than 2% of the total fiber weight while improving fibrillation and fiber bonding and decreasing beating times. This decreased the drainage rate (increased the Schopper-Riegler (SR) value), and the water retention value. However, at the same time it decreased viscosity, and decreased breaking length drastically. Both of these negative effects are attributable to residual cellulase activity, which these workers had attempted to inhibit. Today, many commercial, cellulase-free xylanase preparations are available through cloning and over expression, so their application in pulp fibrillation might be practical. In fact, some of the side benefits of enzymatic bleaching and deinking are increased pulp viscosity and drainage.

The exact mechanism by which enzymatic pulp fibrillation occurs is still not understood, and this area deserves more basic research.

**Drainage**

The drainage rate of a pulp - that is, the rate of water loss during paper formation - determines the speed of paper machine operation. Drainage rates of secondary or recycled fibers are particularly important because they tend to be much lower than drainage rates of primary (virgin) fibers, so that using large quantities of secondary (recycled) fiber slows the papermaking process. In the United States, where manufacturers mainly use primary fibers, paper machines tend to be designed for such stocks. Increasing the recycled fiber content requires operating the machines at lower rates.

Fuentes and Robert discovered that cellulases can improve the drainage rates of recycled fibers (23). In one study, treating recycled fibers with cellulases and xylanases reduced the SR value (i.e., it increased drainage) by 18 to 20%. Commercial enzymes were used to treat batches of recycled fibers on both laboratory (24) and pilot scales (25). Pulp strength was reduced, but it could be improved by refining the pulp before enzyme treatment. Starch sizing likewise improved mechanical properties. Cellulases are presently being used commercially to enhance drainage rates of recycled fibers in France, and one might expect to see wider application.

Current research on cellulases is refining the understanding of how they function (e.g., 26, 27). Considerable progress has been made recently in understanding cellulase structures and functions. Research is showing that cellulases vary in mode of action, pointing to the opportunity to optimize cellulase selection for specific uses. In applied research, we need to better understand the relationships between fibrillation and drainage.

**Modification of Other Pulp Properties**

Enzymes can improve paper properties in specific ways. For example, some hardwoods contain large vessels that make a rough paper surface. During printing, these vessels lead to “picking”, which prevents complete contact of the ink with the paper surface, and an imperfect image. This can be particularly important in image copy, and it is of increasing significance as vessel-rich eucalyptus pulps are used in larger quantities. Cellulase treatment can reduce “picking.” (11).

Xylanase from the fungus *Schizophyllum commune* reduced xylan 22% in a delignified mechanical aspen pulp (8). Purified xylanase from the fungus *Trichoderma harzianum* reduced the xylan content of unbleached kraft pulp by 25% (28), and a
cloned xylanase from the bacterium *Bacillus subtilis* reduced the xylan content of bleached hardwood kraft pulp 20% (29). The use of xylanase, however, has not become commercial, in part because xylan removal is incomplete. More recently obtained xylanases might prove more effective, and this application should be reexamined.

**Retting**

To date, pulping wood with isolated enzymes has not been accomplished, and is not to be expected, because enzymes cannot penetrate the lignified cell walls. Enzymes can, however, pulp herbaceous fibers. Microbial retting is an ancient process dating to the beginnings of civilization. Traditional retting uses mixed microbial populations—mainly soft-rot bacteria—introduced with crude inocula. Fibers that are retted include flax, jute, and coconut hulls (30). In this process microbial pectinases (pectin-depolymerizing enzymes) release cellulosic fibers from fiber bundles.

Contemporary practice uses selected microbial strains or isolated enzymes. The chief disadvantages of the traditional method is the bad odor that develops in the retting tanks, during handling, and in the discharge of the effluent; its uncontrolled nature; and its slowness. Enzymatic retting is faster than traditional retting, readily controlled, and produces fewer odors, but further development is required to make it competitive with the traditional methods. Commercial enzymes such as cellulases, hemicellulases, pectinases and other polysaccharidases have been applied to flax at various levels and compared to traditional retting methods (31).

Pectinolytic enzymes secreted by soft-rot bacteria (32) also cause maceration of woody bast fibers derived from the phloem of plants. These fibers, used to make cordage, matting and various fabrics, are long, strong, and commonly stiff. Pectinolytic and xylanolytic enzymes can help soften them. Alkaline presoaking enhances enzymatic activity. A combined alkali–enzyme process increased fibrillation, decreased the Canadian standard freeness value, decreased the shives content, and improved sheet formation (33). Enzymatic pulps prepared with pectinolytic enzymes produce bulkier paper with higher opacity and better printability than pulps prepared from the same stock solely by an alkaline process. Chemical and enzyme retting have both been carried out on a semi-industrial scale, and the characteristics of fibers produced by these two methods are not significantly different (34).

Combinations of cellulases, xylanases and pectinases have been used to soften and smooth the surfaces of jute-cotton blended fabrics (35). By obtaining an optimum balance of enzymes, it is possible to lower the dosing rate and improve efficiency.

In recent years, a few fundamental studies have been initiated on the enzymatic retting process. These employ purified enzymes on defined substrates, and characterization of the resulting products. A purified pectinase from an Aspergillus released three size classes of polysaccharides from flax. To ensure maximum strength of the thread manufactured from retted flax, only a small fraction of the pectins belonging to the fiber bundles need to be hydrolyzed. Some advantage might be gained, therefore, in using enzyme preparations with better specificity (36).

In developing nations, and particularly in countries where forest stands are endangered from over exploitation, better use might be made of herbaceous fibers for paper production. Such feedstocks should be amenable to enzymatic pulping, and the resulting processes should give higher yields with fewer environmental problems. Clearly, however, much more work needs to be done in this area before enzymatic pulping of herbaceous fibers will see wide application.

**Pitch Control**

Certain types of wood pulps, including sulfite pulps and various mechanical pulps—especially from pines—have high pitch contents. Pitch is a term used collectively for hy-
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Droplophobic components of wood resin and resin acids, triglycerides, waxes, etc. Pitch causes numerous problems in pulp and paper manufacture, including deposits in and on equipment, adverse effects on water absorption by the pulps, holes and tearing of the paper due to sticky deposits on dryer rolls, discoloration and hydrophobic spots in the paper. Current methods for controlling pitch include "seasoning" the wood before pulping (which allows the pitch to deteriorate by the action of indigenous microbes), and adding talc or other chemicals to the pulp to coat the resin and deactivate its surface. Seasoning takes a long time, and often leads to discoloration, and the chemicals cost money. The most troublesome components of resins are triglycerides, fatty acid esters of glycerol. These can be removed by solvent extraction or by strong alkali, but the former process is not practical, and the latter results in yield losses and discoloration. Neither is used.

Hata and coworkers at Jujo Paper Company reported in 1990 that lipases can reduce pitch problems by lowering the triglyceride content of groundwood pulp (37). A lipase obtained from *Candida cylindrica*, when added to the groundwood stock chest reduced pitch problems and talc consumption considerably. Mill trials showed that the number of defects in the paper decreased along with the frequency of machine cleaning. The dosage of chemicals was reduced, and the time used for the traditional "seasoning" of the chips (to control pitch) was greatly shortened, resulting in cleaner chips and lowered bleach consumption. Perhaps most importantly, lipase improved pulp properties. Use of lipases went on-line in Jujo mills shortly after 1990, and reportedly is now being used by other companies in Japan. Other work showed that incubating CTMP in the presence of lipase speeds water absorption while it increases the strength and the specific volume of the resulting paper (38).

In Austria, Messner and coworkers (39) showed that lipases are also effective in reducing pitch problems associated with sulfite pulps. Pitch in sulfite pulps can also present problems during paper manufacture. Deposits on exposed parts of the paper machine such as air foils or machine wire can degrade the product and impair production. Chlorinated pitch triglycerides formed during bleaching are particularly troublesome. Fischer and Messner found that treating unbleached sulfite pulps with lipase followed by alkaline extraction removed most of the triglycerides (44). Because the enzyme absorbs rapidly to pulp fibers, it is not possible to recycle it for reuse. On the other hand, its absorptive properties enable application at low pulp consistencies, and the enzymes remain active and attached during various treatments and washing stages (40). The lipase process has been scaled-up in a 12-ton per day pulp trial, and has been shown to remove 90% of the triglycerides in three h with stirring at 37°C (41).

**Bleaching Chemical Pulps**

The kraft process accounts for 85% of the total pulp production in the United States, and is the single major process world-wide. Bleached kraft pulp is a major, relatively high-value component of the total production of kraft paper. Kraft pulping removes most of the lignin, and dissolves and degrades hemicelluloses without severely damaging cellulose. The kraft process results in excellent pulp from a wide variety of wood species. Unfortunately, kraft pulping also generates large quantities of chromophores. Chromophores are composed of residual lignin and carbohydrate degradation products. They are hard to extract because they are physically entrapped in and covalently bound to the carbohydrate moieties in the pulp matrix (42-44). Manufacturers use elemental chlorine (Cl₂) and chlorine dioxide (ClO₂) to bleach the chromophores, and then they extract them, along with residual lignin, to make white ("bright") pulp. Because of consumer resistance and environmental regulation of chlorine in bleaching, pulp makers are turning to oxygen, ozone and peroxide bleaching, even though they may be more expensive and less effective than Cl₂.
The biggest success story in the use of enzymes in the pulp and paper industry is hemicellulases (mainly xylanases) as aids in pulp bleaching ("enzymatic pre-bleaching"). Viikari et al. (9) discovered that treating kraft pulps with fungal xylanases decreases the amount of bleach chemical required to attain a given brightness. Mill trials followed, confirming the laboratory results, and since the early 1990's xylanases have been used commercially in Scandinavia and Canada - and more recently in Chile. Following the initial discovery of the effectiveness of xylanase, reports confirmed that xylanases can reduce chemical demand in bleaching (45, 46). At least 15 patents or patent disclosures dealing with enzymatic treatments to enhance bleaching of kraft pulps were submitted between 1988 and 1993. In addition, there have been numerous research publications and presentations at conferences. The reason for this high interest lies in the economic importance of kraft pulping and the regulatory pressure against chlorine. Another motive for pulp makers to use xylanase for bleaching is to save chemical costs. Hemicellulase treatments are effective on both hardwoods and softwoods, but they affect hardwood kraft pulps more (47). Although promising results were obtained initially (48, 49), mannanases are not as effective as xylanases, even with softwood pulps, apparently because of limited accessibility of the substrate (49). Mannanase treatments have very little effect on handsheet properties. The mannanase from Trichoderma reesei has, however, been shown to act synergistically with xylanases to enhance pulp bleaching (50). Most proceedings' reports and many of the patent disclosures have been reviewed in earlier publications (3, 51-54). We concentrate here on recent findings on mechanisms of efficacy and enzyme characteristics.

**Mechanism.** The mechanism of hemicellulase prebleaching is not completely understood. One hypothesis suggests that precipitated xylan blocks or occludes extraction and that xylanase increases accessibility (50, 55, 56). This model is based on reports that xylan reprecipitates on the fiber surfaces (57-59). More recently, Suurnäkki et al. (60) found that no extensive relocation of xylan to the outer surface occurs during pulping, so the occlusion model might not be a sound premise. The second model suggests that lignin or chromophores generated during the kraft cook react with carbohydrate moieties (43). Hemicellulases liberate residual lignin by releasing xylan-chromophore fragments, thereby increasing their extractability.

Skjold-Jørgensen et al. (61) found that xylanase treatment decreased the demand for active chlorine (aCl) for a batch kraft pulp by 15%, but decreased aCl of pulp from a continuous process by only 6 to 7%. They also showed that DMSO extraction of residual xylan does not lead to an increase in bleachability, but that xylanase treatment does. This indicates that it is the DMSO-insoluble xylan fraction that is chemically bound to the chromophores. Paice et al. (62) have shown that the prebleaching effect on black spruce pulp is associated with a drop in the degree of polymerization, even though the xylan content decreases only slightly. Prebleaching thus appears to be associated with xylan depolymerization, even though not necessarily with solubilization of the 'xylan-derived hemicellulose components. Senior and Hamilton (22) have shown that xylanase treatment and extraction change the reactivity of the pulp by enabling a higher chlorine dioxide substitution to achieve a target brightness and that they raise the brightness ceiling of fully bleached pulps.

**Enzyme Characteristics.** Effective xylanases should have several properties. First, they should be stable on kraft pulps. Some xylanase preparations non-specifically absorb to pulp fibers and are inactivated by degradation products from kraft pulping (63). Second, they should have a neutral to alkaline pH optimum. Residual alkali leaks out of the pulp during enzyme treatment, and the pH of even well washed pulp stocks can shift upwards dramatically. Third, they should have good thermal stability (64). The pulp is hot (75°C) when it first comes out of the stock washers, and heat-tolerant enzymes generally have higher turnover numbers. Fourth, factors affecting the
interaction of the enzymes with the pulps are important. These include the effective molecular weight, net ionic properties, and specific action pattern (65). Finally, they should not be contaminated with cellulases. If cellulases are present, pulp viscosities decrease. Without cellulase, xylanase treatment increases viscosity, because some lower molecular weight xylans are removed (66). Even so, excess xylan removal can reduce burst strength and long span tensile strength by reducing inter-fiber bonding even though it does not weaken the fibers themselves (67).

Differences in kinetic properties, substrate specificities, and effects on pulp bleachability have been observed with various pure xylanase isozymes. Certain xylanases release chromophores more than others when used at the same activity levels. Four xylanases from Streptomyces roseiscleroticus released chromophores and reduced the kappa number of hardwood and softwood kraft pulps. Some resulted in greater kappa reduction (a measure of residual lignin) and others released more chromophores. Characterization of the chromophoric materials by reverse-phase HPLC indicated that compounds absorbing strongly in the visible region were relatively hydrophobic (68). More recently, the release of chromophoric groups has been reported with other Streptomyces xylanases (69), and the release of chromophores has been shown to correlate linearly with increased brightness (70). With improved knowledge of substrate specificity and interaction, it may be possible to identify enzymes that have more specific effects in releasing chromophores without substantially releasing xylan.

Xylanases can be classified structurally into two major groups: Family F or 10, and Family G or 11 (71). Family F xylanases are relatively high molecular weight and Family G are relatively low molecular weight. The Family G enzymes can be further divided into those with high and low isoelectric points (pl). Liberation of reducing sugars from purified xylan by the pl 5.5 xylanase from T. reesei correlates well with its bleaching effect on fibers, but the behavior of the pl 9.0 xylanase is more complex. It appears to be affected not only by its catalytic activity, but also by electrostatic interactions with its substrate (72). The effect of the pl 9.0 xylanase on bleachability of pulp increased more at pH 7.0 than would be expected from its activity on purified xylan.

Binding of Trichoderma xylanases to polysaccharides is affected by the pH and the ionic strength (73). Fibers carry a net negative charge at neutral pH due to the presence of carboxylic acid groups, so interaction of the enzyme with the fiber is affected by charge on the protein. Enzymes are totally bound to fibers when the pH is below their pl, but are mainly unbound at pH values above the pl. A protein with a pl of 9.0 would absorb to the fibers at pH 7, but a protein with a pl of 5.5 might not absorb at all. This effect of electrostatic interaction also seems to be modulated by the effects of counter ions (74). A more fundamental explanation of these differences is found in the structure of the Trichoderma xylanases. The pl 5.5 xylanase possesses a smaller, tighter substrate pocket and a lower pH optimum than the pl 9.0 xylanase (75). It also exhibits a fifteen-fold higher turnover number (76), and a three-fold lower $K_m$. The pl 9.0 xylanase has a more open structure, and a wider pH range, and tends to produce larger oligosaccharides. The difference in pl is attributable to the presence of more lysine and arginine residues in the pl 9.0 xylanase. These are found in a particular region of the enzyme of the enzyme, and they possibly interact with the glucuronic acid side chains of xylan (77). Thus it appears that the disproportionately greater ability of the pl 9.0 enzyme to enhance bleaching could be due to its specific interaction with charged groups. Current research is focused on obtaining improved xylanases that exhibit unusual thermal stability, (78-80) alkaline activity, (81) or high specificity for chromophore release (68, 82).

**Enzymatic Enhancement of Contaminant Removal**

Printing and writing grade papers used in offices are among the most valuable papers manufactured and sold. They amount to about 25% (20 million tons per year) of U.S.
paper production, and recycling is a rapidly growing segment of the paper industry. Less than 10% of office waste papers is recycled back into printing and writing grades, but this could increase several fold if technical problems can be resolved (83). About 88% of un-segregated office waste paper (OWP) is composed of chemical fibers (84).

Mixed wastepapers present technical and economic challenges to the paper recycler, and of the wide variety of fibers and contaminants present in the paper stock, toners and other non-contact polymeric inks from laser-printing processes are the most difficult to deal with (85). Toners and laser printing inks are synthetic polymers with embedded carbon black; they do not disperse readily during conventional repulping processes. Moreover, they are not readily removed during flotation or washing. Because of these problems, recycled papers contaminated with toners have a relatively low value. Conventional deinking uses surfactants to float toners away from fibers, high temperatures to make toner surfaces form aggregates, and vigorous, high intensity dispersion for size reduction. Most of the deinking chemicals and high-energy dispersion steps are expensive. The high-energy dispersion step is both capital- and energy-intensive and can also reduce fiber length.

Cellulases were reported in 1991 to be effective in removing conventional inks from newsprint, in research done in Korea (13); a Japanese patent was filed slightly earlier (86). Prasad and coworkers also reported enzymatic deinking of newsprint in 1992 (87). Microbial enzymes have also been shown to enhance the release of toners from office waste. When cellulases and xylanases were applied to xerographic-printed papers in a medium consistency mixer, they released toner particles and facilitated subsequent flotation and washing steps. In comparison to the control treatment (water only), enzymes released 95% more residual toner particles from recycled fibers. The amounts of enzymes required were highly cost effective with conventional deinking chemicals (88, 89). This approach employs relatively low doses of neutral or alkaline-active cellulases along with surfactant and mechanical action at high consistency. The toners are released from the surfaces of the fibers and removed by subsequent flotation. This process has been scaled up in pilot plant trials and has proved to be effective (90). Obviously, use of cellulases with pulps must be done carefully to avoid excessive depolymerization of the cellulose. Woodward et al. reported in 1994 that cellulases can be used to separate un-inked from inked fibers (91). In this approach, relatively low consistency pulp suspensions are used, and ink particles are separated during recirculation.

Alkaline lipases will facilitate the removal of soy lipid-based offset printing inks (92, 93). At present, soy ink-printed materials comprise only a small fraction of the total recycled paper, but this application may find increased value as soy-based inks become more widely used.

Applications of Other Polysaccharidases

Starch-hydrolyzing enzymes such as amylases have potential where starch needs to be removed. Thus, amylases were shown 12 years ago to be useful in freeing pulp fibers from the microcapsules in pressure-sensitive carbonless copying paper waste (94). Any process step in which starch hydrolysis and removal is an objective might be facilitated with amylases. Pectinases have been shown to effectively eliminate the cationic demand caused by pectin in peroxide bleach waters (95). These enzymes also show potential for reducing energy demand in debarking spruce logs before chipping (96).

Lignin-Oxidizing Enzymes

In 1974 and 1975 we showed at the Forest Products Laboratory that synthetic 14C-labeled lignins are mineralized (oxidized to CO2) by lignin-degrading (white-rot) fungi (97). In 1976 we subjected some of the radioactive lignins to kraft cooking and sulfite cooking, and further subjected the resulting kraft lignins and lignin sulfonates to
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chlorine bleaching. Interestingly, the white-rot fungi could still mineralize these lignins (98). This told us first, that the lignin-degrading machinery of the fungi is clearly quite non-specific, because the pulping and bleaching processes severely modify the already heterogeneous lignin polymer. Second, the results told us that the chemical pulping and bleaching procedures do not produce modified lignins that are inherently environmentally recalcitrant. Third, the results suggested that we might use white-rot fungi to clean up chlorine bleach plant effluents. This turned out to be true, and we worked on that application for several years (99). Finally, the results indicated that if we could ever isolate the enzymes responsible for fungal lignin oxidation, we might be able to use them to bleach chemical pulps. We reasoned that the fungi themselves should be able to delignify chemical pulps, and we showed this to be the case in 1978 (100). Canadian workers have pursued this line of work, i.e., bleaching with living fungus cells (101). In any event, for nearly 20 years scientists have been aware of the potential of lignin-degrading enzymes in bleaching chemical pulps.

During those 20 years, lignin-oxidizing enzymes were discovered and characterized extensively (see 102 for a review). As understood today, there are three components of the lignin-oxidizing enzyme system: manganese peroxidase (MnP), lignin peroxidase (LiP), and laccases. In the presence of H2O2, MnP oxidizes Mn2+ to Mn3+, which can oxidize phenolic units in lignin. A system composed of MnP, Mn2+, H2O2, and unsaturated lipids, however, also oxidizes non-phenolic units (103) and depolymerizes lignin. The physiological significance of this system is not yet known. Lignin peroxidase, in the presence of H2O2, oxidizes both phenolic and non-phenolic units in lignin, and has also been shown to depolymerize the polymer (104). Laccase oxidizes phenolic units in lignin in the presence of O2. In the presence of certain substrate ‘mediators,’ laccase also can oxidize non-phenolic units (105).

Soon after the discovery of lignin-degrading enzyme systems, various laboratories attempted to bleach kraft pulps with them; the first report was by Linko and coworkers, and was presented as part of the same paper that included the first xylanase results, at the First International Conference on Biotechnology in the Pulp and Paper Industry, in Stockholm, in 1986 (50). Until quite recently, the lignin-oxidizing enzymes have not shown much promise in bleaching trials. Now two enzymes have given encouraging results: laccase and manganese peroxidase (14, 106). To date, the third lignin-oxidizing enzyme, lignin peroxidase, has shown little prospect. However, the amount of research that has been conducted on the practical use of these lignin-degrading enzymes has been minimal, indicating that more effort is justified.

Future Directions

In order for enzymes to have significant impacts on pulp and paper processes, they will need to be effective in consistent manners under various operating conditions. Pulp substrates are more recalcitrant and variable than the grain starch or soluble pectin substrates found in food processing applications, and the environmental factors can be more severe. Cellulases, hemicellulases and lignin-degrading enzymes generally function best under slightly acidic or neutral pH, but the most common pulping reactions and recycled fiber processes are alkaline. In addition, the pulp slurries are usually hot. It seems likely, therefore, that future research will be directed toward the discovery or engineering of enzymes that are more robust with respect to pH and temperature. Much progress has already been made in understanding the basic mechanisms of xylanases and cellulases and in engineering their properties. Lignin-degrading enzymes and the mechanisms they employ should receive increased research attention, with emphasis on how they might point to new applications in pulping and bleaching. The new experimental process employing polyoxometalates to bleach pulp oxidatively (107), for example, is conceptually based on the role that transition metals play in lignin-degrading enzymes (I. Weinstock and R. Atalla, personal communication).
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