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TOPICAL PAPER

Application of Enzymes in the Pulp and Paper Industry

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The pulp and paper industry processes huge quantities of lignocellulosic biomass every year. The technology for pulp manufacture is highly diverse, and numerous opportunities exist for the application of microbial enzymes. Historically, enzymes have found some uses in the paper industry, but these have been mainly confined to areas such as modifications of raw starch. However, a wide range of applications in the pulp and paper industry have now been identified. The use of enzymes in the pulp and paper industry has grown rapidly since the mid 1980s. While many applications of enzymes in the pulp and paper industry are still in the research and development stage, several applications have found their way into the mills in an unprecedented short period of time. Currently the most important application of enzymes is in the prebleaching of kraft pulp. Xylanase enzymes have been found to be most effective for that purpose. Xylanase prebleaching technology is now in use at several mills worldwide. This technology has been successfully transferred to full industrial scale in just a few years. The enzymatic pitch control method using lipase was put into practice in a large-scale paper-making process as a routine operation in the early 1990s and was the first case in the world in which an enzyme was successfully applied in the actual paper-making process. Improvement of pulp drainage with enzymes is practiced routinely at mill scale. Enzymatic deinking has also been successfully applied during mill trials and can be expected to expand in application as increasing amounts of newsprint must be deinked and recycled. The University of Georgia has recently opened a pilot plant for deinking of recycled paper. Pulp bleaching with a laccase mediator system has reached pilot plant stage and is expected to be commercialized soon. Enzymatic debarking, enzymatic beating, and reduction of vessel picking with enzymes are still in the R&D stage but hold great promise for reducing energy. Other enzymatic applications, i.e., removal of shives and slime, retting of flax fibers, and selective removal of xylan, are also expected to have a profound impact on the future technology of the pulp and paper-making process.

1. Introduction

Until recently, in the pulp and paper industry, the use of enzymes was not cosidered technically or economically feasible. Quite simply, suitable enzymes were not readily available, except for limited use in the modiification of starch for paper coatings. However, research by scientific institutions and enzyme producers has led to the development of enzymes that offer significant benefits for the industry. Several commercial products have been launched successfully in the past few years. Currently, the most important application of enzymes is in the prebleaching of kraft pulp. Xylanase enzymes have been found to be most effective for this purpose. Xylanase prebleaching technology is now in use at several mills worldwide. This technology has been successfully transferred to full industrial scale in just a few years. The main driving factors have been the economic and environmental advantages the enzyme brings to the bleach plant. Such intense demand for the enzyme has pushed enzyme producers to develop an entirely new industry in a remarkably short time. Enzymes have also been used to increase pulp fibrillation and water retention and to reduce beating time in virgin pulps. With recycled fibers, enzymes have been used for deinking and to restore bonding and increase freeness. Specialized applications include the reduction of vessel picking in tropical hardwood pulps and the selective removal of xylan from dissolving pulp. Enzymes have also been investigated for removal of bark, shives, pitch, and slime and for retting of flax fibers. The objective of the present article is to review the application of enzymes in the pulp and paper industry. Attention is focused on the recent advances. Information has been obtained from peer-reviewed journals, patents, and proceedings of specialized meetings/conferences.

2. Bleaching

The removal of lignin from chemical pulps is called bleaching, and it is necessary for aesthetic reasons and for improvement of paper properties. Present-day bleaching of kraft pulp uses large amounts of chlorine and chlorine chemicals. Byproducts from using these chemi-

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cals are chlorinated organic substances, some of which are toxic, mutagenic, persistent, and bioaccumulating and cause numerous harmful disturbances in biological systems (Bajpai and Bajpai, 1996a,c, 1997). The options open to pulp mills considering a change to chlorine-free bleaching are oxygen delignification, extended cooking, and substitution of chlorine dioxide for chlorine, hydrogen peroxide, and ozone. Most of these involve process modifications and/or capital investments. This climate of change has provided an opportunity for the investigation of enzymes. Enzymes provide a very simple and cost-effective way to reduce the use of chlorine, chlorine compounds, and other bleaching chemicals. Enzymes also offer a simple approach that allows for a higher brightness ceiling to be reached. This can all be achieved without major capital investment (Viikari et al., 1994).

So far, two enzyme-based approaches have been investigated. One uses hemicellulase enzymes, and the other uses ligninolytic enzymes.

2.1. Hemicellulase Enzymes. These enzymes are used commercially in pulp bleaching. The main enzyme needed to enhance the delignification of kraft pulp is reported to be *endo*-β-xylanase, but enrichment of xylanase with other hemicellulolytic enzymes has been shown to improve the effect of enzymatic treatment (Clark et al., 1990; Kantelinen et al., 1988; Paice et al., 1988). Within a short period of time (1988−1991), xylanase prebleaching technology has become one of the solutions considered by the pulp and paper industry to give an innovative, environmentally and economically acceptable answer to the pressures exerted on chlorine bleaching by regulatory authorities in Western countries and by more demanding, environmentally minded consumers. New, much lower limits on adsorbable organohalogens (AOX) levels in pulp mill effluents and the fast development of totally chlorine-free (TCF) and low AOX pulp markets led to quick responses from the industry in Canada and Northern Europe. Presently, a significant number of Scandinavian and North American mills are bleaching full-time with xylanases (Capps, 1995; Lavielle, 1992; Bajpai and Bajpai, 1992, 1996a; Viikari et al., 1994). Different paper products, including magazine paper and tissue papers manufactured from enzymatically treated pulps, have been successfully introduced to the markets (Viikari et al., 1991, 1994).

Origin of Hemicellulase Enzymes in Bleaching. The concept of biological bleaching with xylanase emerged from efforts to selectively remove hemicellulose from chemical pulps to produce cellulose acetate (Paice and Jurasek, 1984). At approximately the same time, a research program jointly carried out by the Finish Pulp and Paper Research Institute and the Technical Research Centre of Finland was focusing on lignin-degrading biochemical processes. It was found that treatment of chemical pulps with xylanases leads to savings in the consumption of bleaching chemicals, decreased environmental loadings, or increased final brightness of pulp (Viikari et al., 1986, 1987; Kantelinen, 1988). Since that time, various papers in the literature have described the benefits of xylanase treatments in pulp bleaching. Mill trials began as early as 1989 in Finland, and trials continue to be run with remarkable frequency. Since 1991, commercial use of xylanase has become a reality (Jurasek and Paice, 1992). The factors explaining this rapid development are many but can be summarized as follows:

(i) Xylanase prebleaching belongs to the soft technologies that require no or very little capital investment to operate.

(ii) Process changes are minimal in most cases (neutralization of brown stock). Mill trials are very simple and inexpensive, and they involve minimal risk.

(iii) Xylanase helps reduce pollution from bleaching.

(iv) Savings on chemicals can pay for the process.

(v) Xylanase may help to increase mill capacity where there are chlorine dioxide limitations.

(vi) The process is easily combined with many bleaching sequences for elemental chlorine-free (ECF) and TCF pulps.

In the enzymatic pretreatment for bleaching, the hydrolysis of hemicellulose is restricted to a minimum by using only small amounts of enzymes in order to maintain a high pulp yield and advantageous properties of hemicellulose in pulp (Viikari et al. 1986, 1987).

Production of Xylanases for Bleaching. Several criteria are essential for choosing a microorganism to produce xylanases. In addition to giving the desired biobleaching effect, the resulting enzyme preparation must be produced in sufficiently high quantity, and the xylanase technology must be compatible with the technology of a pulp mill. Also, it is essential that the enzyme preparation be completely free of cellulase side activity. Any cellulase activity will have serious economic implications in terms of cellulose loss, degraded pulp quality, and increased effluent treatment cost. Noncellulolytic preparations have been produced by genetic engineering, selective inactivation, or bulk-scale purification (Barnoud et al., 1986; Pederson et al., 1992). To produce xylanases, the selected organism is grown for several days in fermentation vessels containing nutrients and oxygen under specific conditions of pH, temperature, and agitation. During this time, it secretes enzymes into the growth medium. The living cell mass is then removed, leaving a xylanase-rich liquid. This is then concentrated, assayed to determine its activity, and packaged for shipment to pulp mills. With the addition of bacteriostatic preservatives, the xylanase preparation remains stable for months. Excessive temperature and freezing can cause loss of activity and should be avoided. The xylanase preparation is not corrosive or reactive and does not need resistant materials for handling.

The strains reported to be used for commercial production of xylanases include *Trichoderma reesei, Thermomyces lanuginosus, Aureobasidium pullulans,* and *Streptomyces lividans* (Bajpai and Bajpai, 1996a; Jäger et al., 1992; Senior et al., 1992a).

In practical process conditions, some properties of the enzymes, such as substrate specificity and the pH and temperature optima, are of utmost importance. Enzymes with high pH and temperature optima have been isolated and tested for improving the bleachability of the pulps (Davis et al., 1992; Högman et al., 1992; Jäger et al., 1992; Pederson et al., 1991; Senior et al., 1992a,b). Several alkali-tolerant strains of *Bacillus* species have been used for production of xylanases with pH of optima around 9 (Bajpai, 1997). The most thermostable xylanases, with a half-life of 90 min at 95 °C, have been produced by *Thermotoga* strain (Simpson et al., 1991). The pH range has been increased to about 8−9 and the temperature range to 75 °C in some commercial preparations (Bajpai 1997). Table 1 presents a list of industrial suppliers of commercial xylanases used for prebleaching.

Xylanase Treatment in Mills. Typically, xylanase is added to the pulp as an aqueous solution at the final brown stock washer. Because the enzymes are extremely potent catalysts, the desired effects are produced with small amounts of enzymes. The brown stock, which (though washed) is highly alkaline (pH 9−12), must be

Table 1. Industrial Suppliers of Xylanase Enzyme

supplier	product trade name
Clariant, U.K.	Cartazyme HS 10
Clariant, U.K.	Cartazyme HT
Clariant, U.K.	Cartazyme SR 10
Clariant, U.K.	Cartazyme PS 10
Clariant, U.K.	Cartazyme 9407 E
Clariant, U.K.	Cartazyme NS 10
Clariant, U.K.	Cartazyme MP
Genencor, Finland/Ciba Geigy,[a] Switzerland	Irgazyme 40-4X/ Albazyme 40-4X
Genencor, Finland/Ciba Geigy,[a] Switzerland	Irgazyme-10A/ Albazyme 10A
Voest Alpine, Austria	VAI Xylanase
Novo Nordisk, Denmark	Pulpzyme HA
Novo Nordisk, Denmark	Pulpzyme HB
Novo Nordisk, Denmark	Pulpzyme HC
Biocon India, Bangalore	Bleachzyme F
Rohn Enzyme OY, Finland	Ecopulp X-100
Rohn Enzyme OY, Finland	Ecopulp X-200
Rohn Enzyme OY, Finland	Ecopulp X-200/4
Rohn Enzyme OY, Finland	Ecopulp TX-100
Rohn Enzyme OY, Finland	Ecopulp TX-200
Rohn Enzyme OY, Finland	Ecopulp XM
Solvay Interox, U.S.A.	Optipulp L-8000
Thomas Swan Co., U.K.	Ecozyme
Iogen Corp., Canada	GS-35
Iogen Corp., Canada	HS-70

[a] Ciba Geigy has sold its pulp and paper enzyme techonology to Nalco Chemical Co.

neutralized with acid (usually sulfuric) to be compatible with enzyme treatment. The pulp is pumped to the high-density storage tower, where the enzyme acts. From the high-density storage tower, the enzyme-treated pulp is then pumped into the first bleaching tower, where the first contact with the oxidizing chemicals destroys the enzyme (Bajpai and Bajpai, 1996a).

Factors Affecting Treatment Efficiency. The major factors affecting the xylanase treatment efficiency include pH, temperature, enzyme dosage and dispersion, consistency, and reaction time. The optimum pH for xylan treatment varies among enzymes. Generally, the xylanase derived from strains of bacterial origin are most effective between pH 6 and 9, while those derived from strains of fungal origin should be used within the pH range of 4−6. The optimum temperature ranges from 35 to 60 °C. To obtain the best results from enzyme use, enzyme dosage must be optimized in each case. In general, the optimal dosage lies within the range of 2−5 international units (IU) of enzyme per gram of dry pulp. In addition, pulp consistency must be optimized to obtain effective dispersion of the enzyme used, to improve the efficiency of enzyme treatment. Screw conveyors and static mixers are examples of efficient mixing systems (Skerker et al., 1992). Most of the beneficial effect of bleaching can be obtained after only 1−2 h of treatment.

Effect of Xylanase Treatment on Pulp Bleaching. Xylanase treatment has been shown to reduce the requirement of chlorine for bleaching while still achieving a high brightness and good pulp properties. Results from laboratory studies and mill trials show about 35−41% reduction in active chlorine at the chlorination stage for hardwoods and 10−20% for softwoods, whereas savings in total active chlorine were found to be 20−25% for hardwoods and 10−15% for softwoods (Buchert et al., 1992; Clark et al., 1991; Perrolaz et al., 1991; Sinner et al., 1991; Skerker and Farell, 1991; Viikari et al. 1986, 1987, 1994; Bajpai et al., 1993,1994; Bajpai and Bajpai, 1996b). In the elementary chlorine-free bleaching sequences, the use of enzymes increases the productivity of the bleaching plant when the production capacity of ClO_2 is a limiting factor. This is often the case when the utilization of chlorine gas has been abandoned. In totally chlorine-free bleaching sequences, the addition of enzymes increases the final brightness value, which is a key parameter in marketing of the chlorine-free pulps. In addition, savings in the TCF bleaching are important with respect to both costs and strength properties of the pulp. The results are presented in Tables 2−7.

Table 2. Effect of Xylanase Treatment on Conventional Bleaching[a] for Various κ Factors and Chlorine Dioxide Substitution

	control		treated	
chlorine dioxide substitution (%)	κ factor	final brightness (% ISO)	κ factor	final brightness (% ISO)
10	0.200	90.0	0.05	90.0
	0.233	90.2	0.10	90.8
	0.266	90.3	0.15	91.8
40	0.150	89.2	0.05	90.0
	0.173	90.0	0.10	90.8
	0.200	91.0	0.15	91.8
70	0.200	90.0	0.05	88.7
	0.233	90.6	0.10	90.0
	0.250	90.9	0.15	90.3
100	0.150	83.5	0.10	87.4
	0.173	85.3	0.15	87.4
	0.200	87.0		

[a] Bleaching sequence: C/DEDED. Based on data from Senior and Hamilton, 1992.

The production of TCF pulp has increased dramatically during the past 2 years. TCF bleaching methods are based on different oxygen chemicals such as oxygen, ozone, and peroxide. Enzymes are frequently being used on oxygen-delignified pulps to increase the brightness. Enzymes have also been combined with ozone bleaching (Yang and Eriksson, 1992). The TCF technologies applied today are usually based on bleaching of oxygen-delignified pulps with enzymes and hydrogen peroxide.

Xylanase pretreatment has led to reductions in effluent AOX and dioxin concentrations due to the reduced chlorine requirement to achieve a given brightness (Senior and Hamilton, 1991, 1992a; Vaheri et al., 1989). The level of AOX in effluents was lower for xylanase-pretreated pulps than for conventionally bleached control pulps (Table 8) (Viikari et al., 1986, 1987; Senior and Hamilton, 1991, 1992; Berry et al., 1989; Bajpai et al., 1993, 1994; Bajpai and Bajpai, 1996b). The enzyme-treated pulps show unchanged or improved strength properties (Bajpai et al., 1993,1994; Bajpai and Bajpai, 1996b; Dunlop and Gronberg, 1994; Viikari et al., 1986). These pulps are also easier to refine than the reference pulps. Improved viscosity of the pulp has been noted as a result of xylanase treatment (Bajpai et al., 1993, 1994; Bajpai and Bajpai, 1996b; Senior et al., 1989, 1992a; Yang et al., 1992). This is probably caused by the selective removal of xylan, as determined by the pentosan values. Xylan, with lower DP than cellulose, can be expected to lower the average viscosity of kraft hemicellulose. However, the viscosity of the pulp was adversely affected when cellulase activity was present (Allison and Clark, 1992; Bajpai et al., 1993; Clark et al., 1990; Puls et al., 1990). Therefore, the presence of cellulase activity in the enzyme preparation is not desirable. In a few cases, lower mechanical strength has been obtained on xylanase-treated pulp, probably due to the presence of cellulase in the enzyme preparation (Chauvet et al., 1987).

Benefits from Xylanase Treatment. Xylanase pre-

Table 3. Effect of Cartazyme HS-10 Treatment on Chemical Usage in Different Bleaching Sequences[a]

pulp	bleaching sequence	chemical consumption (kg t^{-1} of pulp) aCl	NaOH	O_2	H_2O_2	final brightness (% ISO)
hardwood	$DE_{OP}DE_PD$	46.0	18.0	5.0	0.5	89.5
	$XDE_{OP}DE_PD$	37.0	16.0	5.0	0.5	91.0
softwood	$C_{85}D_{15}E_ODE_PD$	94.0	46.0	5.0	1.0	89.1
	$XC_{85}D_{15}E_ODE_PD$	84.0	44.0	5.0	1.0	88.9

[a] Based on data from Dunlop-Jones and Gronberg, 1994.

Table 4. Effect of Xylanase Treatment on the Increase in Brightness Improvement of Eucalyptus Kraft Pulp in Conventional Bleaching Sequence[a]

enzyme	bleaching sequence	brightness (% ISO) control	enzyme	increase in brightness due to enzyme treatment (points)
Cartazyme	CEHH	80.4	84.2	3.8
HS-10	CEH	78.1	83.0	4.9
Novozyme 473	CEHH	80.6	83.6	3.0
VAI xylanase	CEHH	80.4	82.5	2.1

[a] κ number of unbleached pulp = 25.5. Based on data from Bajpai et al., 1994.

Table 5. Effect of Xylanase Treatment on Pulp Properties[a]

pulp	bleaching sequence	brightness (% ISO)	viscosity (mPa·s)
hardwood	OXDP	87.7	17.5
kraft pulp	ODP	85.7	15.8
softwood	OXPDP	88.6	16.3
kraft pulp	OPDP	84.5	14.9

[a] Based on data from Yang et al., 1992.

Table 6. Effect of Xylanase Treatment on Bleachability of Kraft Pulps[a]

pulp	bleaching sequence	ozone consumed on pulp (%)	viscosity (mPa·s)	brightness (% ISO)
eucalyptus	OXZP	0.79	9.2	90.2
kraft pulp	OZP	0.76	9.3	84.7
pine kraft	$OXZ_1E_PZ_2P$	Z_1: 0.80/Z_2: 0.58	8.0	81.0
pulp	$OZ_1E_PZ_2P$	Z_1: 0.84/Z_2: 0.58	8.0	71.3
pine RDH	XE_PZP	0.96	5.4	85.7
pulp	E_PZP	0.95	5.0	76.3

[a] Based on data from Eriksson and Yang, 1993.

treatment of pulps, prior to bleaching, reduces bleach chemical requirements and permits higher brightness to be reached. The reduction in chemical charges can translate into significant cost savings when high levels of chlorine dioxide and hydrogen peroxide are used. Reduction in the use of chlorine chemicals clearly reduces the formation and release of chlorinated organic compounds in the effluents and in the pulps. The ability of xylanases to activate pulps and increase the effectiveness of bleaching chemicals may allow new bleaching technologies to become more effective. This means that, for expensive chlorine-free alternatives such as ozone and hydrogen peroxide, xylanase pretreatment may eventually permit them to become cost-effective (Bajpai and Bajpai, 1996a; Viikari et al., 1994).

Traditional bleaching technologies also stand to benefit from xylanase treatments. Xylanases are easily applied and require essentially no capital expenditure. Because chlorine dioxide charges can be reduced, xylanase may help eliminate the need for increased chlorine dioxide generation capacity. Similarly, installation of expensive oxygen delignification facilities may be avoided. The xylanase bleach boosting stage can also shift the degree of substitution toward higher chlorine dioxide levels while maintaining the total dosage of active chlorine. Use of high chlorine dioxide substitution dramatically reduces the formation of TOCl (Bajpai and Bajpai, 1996a; Viikari et al., 1994). Table 9 shows the various benefits obtained by the use of xylanase enzyme.

2.2. Ligninolytic Enzymes. These enzymes, unlike xylanases, attack lignin directly, and hence are more effective. Several reports in the literature suggest that these enzymes could prove useful in bleaching of kraft pulps (Arbeloa et al., 1992; Call, 1990, 1991, 1992, 1993, 1994; Call and Mucke, 1994, 1995a,b; Farell, 1987; Kondo et al., 1994, 1995; Harazono et al., 1996; Paice et al., 1995; Bourbonnais and Paice, 1995; Olsen et al., 1989). White rot fungi are the main producers of ligninolytic enzymes. The most important lignin-degrading enzymes are lignin peroxidases, manganese peroxidases, and laccases. In the absence of the living organism, the various peroxidases and laccases give only a small reduction in κ number.

It is now known that, although lignin peroxidases and laccases play an important role in degrading the lignin in vivo, in vitro the oxidation reactions catalyzed by the enzyme result in further polymerization of the lignin. These results imply that single enzymes are not able to mimic the complete biological system. Small improvements can be achieved by the addition of low-molecular-weight aromatic compounds (Olsen et al., 1989; Bourbonnais and Paice, 1990).

Despite the disillusioning experience with isolated lignin-degrading enzymes, Lignozyme GmbH Germany continued work with enzymes plus chemical mediators which create a red/ox system throughout the pulp treatment period (Call, 1987, 1990, 1992, 1993, 1994). Their idea was to find a system which is a good mimic of the natural situation. Having started in 1987 with the enzyme mediator concept, very recently Lignozyme has improved the performance of the mediator system for the laccase of *Coriolus versicolor* by changing and further fine-tuning the chemical nature of the component (Call and Mucke, 1994, 1995a,b). The mediator used is hydroxybenzotriazole. It is a rather low molecular weight, environmentally safe chemical and, with respect to cost and dosage, completely feasible compared to present consumption of chemical additives in the pulping process. The treatment of pulp with laccase alone results not in any degradation of lignin but just in a structural change or repolymerization; the laccase mediator system causes a significant κ number reduction at reasonable treatment times even if the enzyme mediator system is applied in several consecutive treatment steps to the same pulp. Laccase enzyme requires oxygen as a cosubstrate for the oxidation reaction, which has to be provided. According to the present understanding, the laccase ,while oxidizing the chemical mediator, is generating a strongly oxidizing comediator, which is the real bleaching agent. The general technical conditions for enzymatic bleaching are as follow: temperature, 40−65 °C; pH, 4−7; consistency, 1−20%; pressure, 1−14 bar; duration, 1−4 h. The results of bleaching of conventional softwood kraft pulp after extended cooking with laccase mediator system are presented in Tables 10 and 11. The system provides a broad flexibility with respect to the pulp substrate, the technical requirements for application, and the final quality of the pulp. The principal applicability has been demonstrated for softwood and hardwood pulps as well as for annual plant fibers. A repeated enzymatic treatment is possible and results in a 50−70% κ number

Table 7. Results of Mill Trials with Commercial Xylanase Enzymes[a]

mill	bleaching sequence	increase in brightness (% ISO)	reduction in chemicals	reduction in AOX (%)[b]	pulp properties
Bukoza Pulp Mill, Czechoslovakia	(CD)EDE$_H$ D	–	30% Cl_2, 30% hypochlorite	nd	unchanged
Tasman Pulp and Paper Co., New Zealand	(DC)E$_O$DED	–	20% ClO_2	20	unchanged
Canfor's International Mill, B.C., Canada	DE$_{OP}$DED	–	15.6% ClO_2	nd	unchanged
Metsa Botnia, Finland	OPPP	2–4	–	nd	unchanged
Enso Gutzeit, Finland	($D_{50}C_{50}$)$E_1D_1E_2D_1$	–	22% active chlorine	29	unchanged
Scott Paper Mill, Spain	E$_{OP}$DEPD	4–6	–	–	no attack on cellulose
Munksjo, Sweden	OQE$_1$PE$_2$P	2–4	–	–	unchanged
Ballarpur Paper Industries, India	CE$_P$H	2–3	–	nd	unchanged
Crest Brook Forest Industries, Canada	DE$_{OP}$DED	–	7–17% reduction in κ factor, increased productivity	nd	unchanged
Metsa-Sellu, Finland	–	–	12% total active chlorine	nd	unchanged
Morrum Pulp Mill, Sweden	–	–	22.9% ClO_2	22.9	unchanged
Donohue St. Felicie Mill, Quebec	–	–	24% reduction in κ factor	nd	good strength

[a]Source: Bajpai and Bajpai, 1996a. [b]nd, not determined.

Table 8. Effect of Xylanase Treatment on Effluent Quality[a]

	control	treated
pretreatment	no	yes
pH	–	4.8
xylanase charge (units/kg of pulp)	–	5000
time (h)	–	3
temp (°C)	–	37
κ number	32	31.3
viscosity (mPa·s)	25.1	30.7
($C_{70}D_{30}$) stage		
Cl_2 (kg/mt of pulp)	19.2	15.0
ClO_2 (kg/mt of pulp)	17.0	13.3
active Cl_2 (kg/mt of pulp)	64.0	49.9
(E_O) stage		
NaOH (kg/mt of pulp)	35.2	27.0
O_2 (kg/mt of pulp)	5.0	5.0
E_O κ number	4.8	4.4
effluent properties		
color (kg/mt of pulp)	155	118
AOX (kg/mt of pulp)	2.02	1.68
BOD (kg/mt of pulp)	15	11
COD (kg/mt of pulp)	74	62

[a] Unbleached κ number 32.0, viscosity 30.7 mPa·s. Based on data from Viikari et al., 1986.

Table 9. Advantages of Prebleaching with Xylanase Enzymes

bleaching	advantages
conventional	reduction of elemental chlorine and AOX; higher pulp brightness
ECF	reduction of chlorine dioxide and AOX; higher pulp brightness; increase in productivity when chlorine dioxide is limited
TCF	higher pulp brightness; reduction in chemical consumption; unchanged strength properties

reduction per treatment step. Laccase mediator system (LMS) is compatible with all other bleaching sequences. The performance of the LMS system has been proven in pilot plant trials. The summary of the results is presented in Table 12.

3. Fiber Modification

Enzymatic modification of fibers aims at decreased energy consumption in the production of thermomechanical pulps and increased beatability of chemical pulps or improvement of fiber properties (Bajpai, 1997). In high-

Table 10. Bleaching of Conventional Softwood Kraft Pulp with Laccase Mediator System[a]

treatment	κ number	viscosity (cP)	brightness (% ISO)
untreated pulp	28.7	27.5	24.7
enzyme-treated pulp	11.5	21.6	31.6
enzyme-treated pulp after TCF sequence	–	15.0	84.0

[a] Based on data from Call and Mucke, 1994.

Table 11. Bleaching of an Extended Cooked Softwood Kraft Pulp with Laccase Mediator System[a]

treatment	κ number	viscosity (mL/g)	brightness (% ISO)
untreated pulp	20	970	28.8
enzyme-treated pulp	10	950	32.8
TCF sequence	–	850	80.0
enzyme-treated pulp after TCF sequence	–	850	88.0

[a] Based on data from Call and Mucke, 1994.

yield mechanical pulps, most of the lignin and hemicellulose remains in the pulps. According to determinations of the medium pore width and immunololabelings of the untreated wood, it is evident that enzymatic modifications to the composition of mechanical pulps can be achieved only on the outer surface of the fiber. This was verified when xylan ases were applied to thermomechanical pulp (Jeffries and Lins, 1989). Even when using rather higher enzyme dosages, only about 1% of the pulp was dissolved. When combined with an alkaline pretreatment, the enzymatic treatment was substantially improved, and the amount of energy required for refining the thermomechanical pulp was decreased.

In 1942, a patent claimed that microbial hemicellulases from *Bacillus* and various *Aspergillus* species could aid refining and hydration of pulp fibers (Diehm, 1942). In 1959, Bolaski et al. patented the use of cellulases from *Aspergillus niger* to separate and fibrillate pulp. A process patented in 1968 used cellulases from a white rot fungus to reduce beating or refining time (Yerkes, 1968). The desired structural changes in the fiber which are created during beating and refining are external fibrillation and fiber swelling, which improve the flexibility and bonding ability of the fibers. The role of xylans in fiber properties was studied using xylanases from *Sporotrichium dimorphosporum* in the treatment of fully

Table 12. Pilot Plant Trial Results with Laccase Meditor System[a]

sequence	pulp	dosage of enzyme/ mediator (kg/TP)	degree of delignification (%)	max brightness (% ISO)
L-E-Q-P	A	2/13	56.6	76.5
L-E-L-E-Q-P	A	2 × 2/2 × 8	50.6/67.7	82.7
L-E-Q-(P)	B	2/8	44.2	

Conditions

parameter	L stage	E stage	Q stage	P stage
consistency (%)	10	10	5	10
temp (°C)	45	60	60	75
pH	4.5	~11.5	5	11.2
residence time (min)	120	60	30	210
pressure (bar)	2	–	–	–
dosage	enzyme: 2 kg/t; mediator: variable	NaOH	0.2% DTPA	3% peroxide

[a] Based on data from Call and Mucke, 1995.

bleached spruce sulfite and birch kraft pulps. Electron microscopic examination revealed external fibrillation and good flexibility of fibers, implying internal modification (Mora et al., 1986). The water retention value, which describes fiber swelling, was considerably increased. The enzymatically treated pulps were comparable with slightly beaten pulps. The beatability was generally enhanced, and the energy demand was reduced about 3-fold (Noe et al., 1986). Recently, the effectiveness of several commercial xylanase enzymes for energy savings in beating and refining has been examined (Bhardwaj et al., 1996). With softwood pulp, the maximum reduction in beating time was found to be 25%, while with bamboo pulp and mixed pulp (60% waste corrugated kraft cuttings and 40% unbleached softwood pulp), treatment with commercial enzymes reduced the beating time by about 18 and 15%, respectively. The strength properties of the pulp were not found to be affected.

Water removal on the paper machine has been shown to improve as a result of limited hydrolysis of the fibers in recycled paper. A mixture of xylanase and cellulase enzymes at low concentrations has been found to markedly increase the freeness of recycled fibers without substantially reducing yield (Fuentus and Robert, 1988). The lower the initial freeness, the greater the gain following treatment. Many different cellulases and hemicellulases have been found to improve freeness (Bhardwaj et al., 1995, 1997; Pommier et al., 1989, 1990). Freeness shows a rapid initial increase, with over half of the observed effect occurring in the first 30 min. A relatively small amount of the 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. Mill trials have been carried out successfully using a commercial *T. reesei* enzyme called Pergalase A40 (Pommier et al., 1990). Mixed xylanases and cellulases peel the surface of the fibers. If treatment is limited, the enzymes remove only elements that have a great affinity for water but which contribute little to interfiber binding 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 pulp. If the treatment is extended, however, fibrillation becomes pronounced and drainage decreases. If large quantities of crude enzymes are used, the average fiber length is reduced, fines disappear, and the strength properties of the fibers are lost. Therefore, an optimum level of enzyme treatment is required. It has been reported that the drainability of mechanical pulp can also be enhanced by the addition of hemicellulases (Karsila et al., 1990). The authors claim that xylanase improves 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 cellulase decreased (Karsila et al., 1990). 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.

The degree of polymerization of pulp treated with cellulase-free xylanase was found to increase, apparently due to the selective removal of xylan, which has a lower DP (Clark et al., 1989; Puls and Poutanen, 1989). Thus, treatment of pulps with xylanases has been shown to increase their viscosities (Senior et al., 1989). However, even low cellulase activities in the enzyme preparation result in decreased viscosity.

4. Production of Dissolving Pulp

Dissolving pulps are used to produce cellulosic materials such as acetates, cellophanes, and rayons (Hinki et al., 1985). Their manufacture is characterized by the derivatization and thus solubilization of highly purified cellulose. Hemicellulosic contaminants lead to color and haze in the product as well as insolubles which hamper the manufacturing process. Their extraction from pulps requires the use of high caustic loadings and appropriate pulping conditions, the latter restricted to sulfite pulping and acid-pretreated kraft pulping. The use of xylanase for purifying cellulase was first proposed by Paice and Jurasek (1984). It was found that complete enzymatic hydrolysis of hemicellulose within the pulp is difficult to achieve. Even with very high loadings of enzymes, only a relatively small amount of xylan could be removed (Christov and Prior, 1993, 1994, 1996; Christov et al., 1995; Jeffries and Lins, 1990; Roberts et al., 1990; Senior et al., 1988). The xylan content in delignified mechanical aspen pulp was reduced from approximately 20 to 10%, whereas in bleached hardwood sulfite pulp, the xylan content was decreased from 4% to only 3.5% (Paice and Jurasek, 1984). The complete removal of residual hemicellulose seems thus unattainable, probably due to the inaccessible location of the substrate. Nevertheless, xylanase treatment may reduce the chemical loading required during the caustic extraction or facilitate xylanase extraction from kraft pulps.

5. Deinking

Enzymatic deinking represents a new approach to convert secondary fibers into quality products. It has

Table 13. Patents on the Use of Enzymes in Deinking

title	enzyme used	ref
Elimination of ink from reclaimed paper	cellulase	Fukunaga and Kita, 1990
Deinking waste-printed paper using enzymes	cellulases	Eom and Ow, 1989
Enzymatic deinking process	alkaline cellulase	Baret et al., 1991
Elimination of ink from reclaimed paper	alkaline cellulase	Nomura and Shoji, 1988
Process for removing printing ink from wastepaper	cellulase or pectinase	Eom and Ow, 1990
Biological ink elimination from reclaimed paper	cellulase and/ or pectinase	Gen Y. and Go, 1991
Elimination of ink from reclaimed paper	esterase	Sugi and Nakamura, 1991
Elimination of ink from reclaimed paper	esterase	Fukuda et al., 1990
Deinking wastepaper with the incorporation of lipase	lipase	Sharyo and Sakaguchi, 1990
Removal of ink from recycled paper	lipase	Guy Vare et al., 1990
Process for wastepaper preparation with enzymatic printing ink removal	ligninolytic enzyme (laccase)	Call, 1991
Chemicals for deinking	not specified	Urushibata, 1984
Deinking chemicals	not specified	Hagiwara, 1988

proven on a laboratory and industrial scale to be an effective and economical method of deinking waste paper (Jeffries et al., 1992, 1993, 1994; Heise et al., 1995, 1996; Ow and Eom, 1990, 1991; Ow et al., 1995; Prasad, 1993; Prasad et al., 1992a,b; Prasad et al., 1993). Drainage enhancement is known to be a secondary benefit of enzymatic deinking. Many patent applications have been filed or granted conerning the use of enzymes in deinking (Table 13). Several patents specify the use of cellulases, particularly alkaline cellulases, for deinking. Few patents claim that esterases can be used, while others specify the use of lipases or pectinases. One patent application employs laccase from white rot fungi. Most of the published literature on deinking deals with cellulases and hemicelluases. The enzymatically deinked pulps possess superior physical properties, higher brightness, and lower residual ink compared to chemically deinked recycled pulps (Tables 14 and 15). More importantly, size distribution and shape of ink could be effectively controlled using the enzymatic process to maximize the efficiency of size-based flotation process. This can be accomplished by selectively varying enzyme composition, charge, and residence time as well as varying other additives and pH in the system to effectively dislodge the normally large, flat, and rigid ink particles into much fewer and non-platelet forms. The enzymatic deinking process also improves freeness compared to chemically deinked pulps (Prasad et al., 1993).

6. Removal of Pitch

Pitch is composed of fatty acids, resin acids, sterols, glycerol esters of fatty acids, other fats, and waxes and is usually defined empirically as the wood component that is soluble in methylene (Allen, 1975). It is less than 10% of the total weight of wood but causes major problems. Pitch reduction with enzymes is a very efficient biotechnological method (Fischer and Messner, 1992a,b; Fischer et al., 1993; Fujita et al., 1992a−c; Gibson, 1991; Irie et al., 1990). Different lipases have been used for removal of pitch. Few commercial preparations of lipases for pitch removal are available (Fujita et al., 1992a−c).

Enzymatic pitch control helps to reduce pitch-related problems to a satisfactory level. It reduces defects on paper web as well as the frequency of cleaning pitch deposits in the paper machine. At the same time, it also offers other advantages, such as ecofriendly and nontoxic technology, improved pulp and paper quality, reduction in bleaching chemical consumption, reduction of effluent load, and space and cost saving in a mill wood yard by using unseasoned logs. By reducing the outside storage time of logs, this method reduces wood discoloration, wood yield loss, and the natural wood degradation which occurs over longer storage time. With chemical (sulfite)

Table 14. Effect of Enzymatic Deinking on Optical Properties of Colored Offset Newsprint[a]

	enzyme dose/ g of pulp		deinked pulp		
enzyme preparation	units	mL	ISO brightness (%)	residual ink area (%)	scatter coeff. (m^2/kg)
blank[b]	–	–	41	93	34.5
control[c]	–	–	49	96	50.2
I[d]	0.2[h]	0.033	52	77	70.5
II[e]	0.2[h]	0.033	52	77	78.0
III[f]	100[i]	0.50	53	65	75.8
IV[g]	19[i]	0.033	54	79	69.9

[a] Based on data from Prasad et al., 1993b. [b] Reslushed pulp without enzyme treatment and no flotation. [c] Reslushed pulp without enzyme treatment but with flotation. [d] Enzyme preparation I contained 6 units/mL of CMCase, 10.0 units/mL of xylanase, and 42 units/mL of filter paper activity. [e] Enzyme preparation II contained 6 units/mL of CMCase, 6 units/mL of xylanase, and 1 unit/mL of filter paper activity. [f] Enzyme preparation III contained 0.20 units/mlL of CMCase, 200 units/mL of xylanase, and 0.005 Uunit/mL of filter paper activity. [g] Enzyme preparation IV contained 26 units/mL of CMCase, 580 units/mL of xylanase, and 50 units/mL of filter paper activity. [h] Dosage based on CMC. [i] Dosage based on xylan.

pulps, the applications of lipase improves the properties of resins by lowering their effectiveness. Since 1990, this method has been used commercially (Grant, 1994).

7. Removal of Slime

Like pitch, slime deposition causes significant operating problems around a paper machine. Slime is the generic name for deposits of microbial origin in a paper mill. It is impractical to run a paper mill as a sterile system. As a result, a vast array of microbes contaminate the mills, and many of the resulting slime compounds have not been characterized. When confronted with a slime, often the strategy is to try every available biocide until one is found which targets the microbe and destroys the source of slime.

In some cases, however, specific slime compounds have been characterized, and efficient strategies for their removal can be used. One such case is with levan, which is a β-2,6-linked polymer of fructose that forms a slime film. This compound is secreted by several species of *Bacillus* and *Pseudomonas* bacteria that can grow in the recirculated water around the paper machine, especially for fine paper, where the level of inhibiting compounds is low. The enzyme levan hydrolase can hydrolyze this polymer to low-molecular-weight polymers that are water soluble, thereby cleaning the slime out of the system.

Commercial levan hydrolase is supplied as the product EDC-1 by Henkel Corp. (Morristown, U.S.A.). The enzyme does not harm cellulose, so it is not harmful to the

Table 15. Effect of Enzymatic Deinking on Mechanical Properties of Colored Offset Newsprint[a]

parameters	blank[b]	control[c]	enzyme prep I[d]	enzyme prep II[e]	enzyme prep III[f]	enzyme prep IV[g]
CSF freeness (mL)	265	250	300	300	315	320
fiber length distribution						
+28	30	30	31	30	30	30
+48	25	26	28	29	29	27
+65	7	7	7	7	7	8
+150	7	5	6	6	6	7
−150	30	32	27	27	27	28
tensile index (N·m/g)	32.7	31.6	36	37	39.2	35.6
burst index (m^2/g)	1.6	1.5	1.7	1.7	1.9	1.6
tear index (m^2/g)	7.8	7.5	7.9	8.3	8.7	7.8

[a] Based on data from Prasad et al., 1993b. [b] Reslushed pulp without enzyme treatment and no flotation. [c] Reslushed pulp without enzyme treatment but with flotation. [d] Enzyme preparation I contained 6 units/mL of CMCase, 10.0 units/mL of xylanase, and 42 units/mL of filter paper activity. [e] Enzyme preparation II contained 6 units/mL of CMCase, 6 units/mL of xylanase, and 1 unit/mL of filter paper activity. [f] Enzyme preparation III contained 0.20 unit/mL of CMCase, 200 units/mL of xylanase, and 0.005 unit/mL of filter paper activity. [g] Enzyme preparation IV contained 26 units/mL of CMCase, 580 units/mL of xylanase, and 50 units/mL of filter paper activity.

paper. The enzyme is usually added at the headbox of the paper machine, although in some cases it has been added at dryer discharge. The enzyme is effective at pH 4−8 and runs best at pH 5.0.

Grace Dearborn's development work continues to pursue the control of biofilm formation in paper machine water circuits. The appropriate enzyme or combination of enzymes is determined by the extracellular polysaccharides contained in the biofilm. Initially, Dearborn had to develop both a means of simulating biofilm formation and analytical methods for identifying biofilm components. The outcome is a family of products called Darazyme. Initial trials on wood-containing and wood-free printing and writing grades allowed the accompanying microbicide to be reduced or eliminated under both acidic and alkaline conditions (Grant, 1995).

8. Removal of Shives

Shives are small bundles of fibers that have not been separated into individual fibers during the pulping process. They appear as splinters that are darker than the pulp. One of the most important quality criteria for bleached kraft pulp is the shive count. It has been found that a novel enzyme formulation, Shivex, can be used to increase the efficiency of shive removal by bleaching. By treating brownstock with Shivex, mills can increase the degree of shive removal in the subsequent bleaching by 55% (Tolan et al., 1994).

Depending upon the shive level in the incoming brownstock and the desired shive level of the bleaching pulp, this allows a mill to decrease its actual shive count or to increase its margin of safety against shives. The increase in shive removal is accompanied by an increased efficiency in the bleaching of pulp. Therefore, mills can decrease chlorine use in a bleach plant without compromising on shive counts. Shivex is a multicomponent mixture of proteins, some of which are xylanases, but the degree of shive removal by the enzyme is not directly related to the enzymes' xylanase activity or bleach-boosting effectiveness.

9. Debarking

Removal of the bark is the first step in all processing of wood. This step consumes substantial amounts of energy. Extensive debarking is needed for high-quality mechanical and chemical pulp because even small amounts of bark residues cause darkening of the product. In addition to its high energy demand, complete debarking leads to losses of raw material due to prolonged treatment in the mechanical drums. The border between wood and bark is cambium, which consists of only one layer of cells. This living cell layer produces xylem cells toward the inside of the stem and phloem cells toward the outside. In all the wood species studied, common characteristics of the cambium include a high content of pectins and the absence or low content of lignin (Dey and Brenson, 1984; Kato, 1981). The content of pectins in cambium cells varies among the wood species but may be as high as 40% of the dry weight. The content of pectic and hemicellulosic compounds is also high in the phloem (Fu and Timell, 1972). Pectinases are found to be key enzymes in the process, but xylanases may also play a role because of the high hemicellulose content in the phloem of the cambium (Viikari et al., 1989). The energy consumed in debarking was found to decrease as much as 80% after pretreatment with pectinolytic enzymes (Ratto et al., 1993). One of the major difficulties with enzymatic debarking is the poor infiltration of enzymes in the cambium of whole logs (Viikari et al., 1989; Ratto et al., 1993).

10. Retting of Flax Fibers

Enzymes have been used in processing plant fiber sources such as flax and hemp (Sharma, 1987a,b; Gillespie et al., 1990). At present, fiber liberation is affected by retting i.e., the removal of binding material present in plant tissues using enzymes produced in situ by microorganisms. Pectinases are believed to play the main role in this process, but xylanases may also be involved (Sharma, 1987a,b). Replacement of slow natural retting by treatment with artificial mixtures of enzymes could become a new fiber liberation technology.

11. Reduction of Vessel Picking

The use of tropical hardwoods such as eucalyptus for pulp production has increased in recent years. The trees grow rapidly, so the chip supply is plentiful, and the pulps are useful for many applications. The vessel elements of tropical hardwoods are, however, large and hard, and they do not fibrillate during normal beating. As a result, they stick up out of the surface of the paper. During printing, the vessels are torn out, leaving voids. This characteristic reduces the value of tropical hardwood pulps. Although increased beating can eventually increase vessel fibrillation and flexibility, it can also result in poor drainage. A patent of disclosure from Honshu Paper Co. described the use of commercial cellulases to enhance the flexibility of hardwood vessels. Enzyme treatment reduced vessel picking by 85%. At the same time, smoothness and tensile strength increased. Draining time also increased (Jeffries, 1992).

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