Roles for biotechnology in manufacture

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Potential roles for biotechnology in pulp and paper manufacture are being explored increasingly in laboratories around the world. Roles that might some day be realised are in primary pulp manufacture, pulp modification, recycled fibre treatment, waste treatment, byproduct conversions and biosensor applications. Better communication between biotechnologists and industry technical staffs would speed evaluation of these possibilities.

The pulp and paper industry continually seeks to improve its processes and products. Possible changes that can be made are limited practically not only by economics, but also by the chemical and physical properties of wood, which place relatively narrow constraints on what can be done. Entirely new approaches to pulp manufacture and the various processing steps are therefore rare. Biotechnology promises new approaches. Research in this area has accelerated during the past decade, driven particularly by advances in understanding how wood components are degraded by micro-organisms and their enzymes, and spurred by very rapid advances in biotechnology in general.

Biotechnology has received increasing attention in the popular press and in scientific circles during the past 15 years because of its commercial potential in many fields. These potentials are now being realised, first with high-value products in biomedical and related areas, as in the production of human insulin and human growth hormone in bacterial cells. More recently, lower value products, such as genetically engineered enzymes in detergents, are being commercialised. The attractiveness of biotechnology lies in its potential to provide processes and products that are impractical with non-biological chemistry, to increase specificity in reactions, to provide less environmentally deleterious processes, and by virtue of the foregoing, to decrease costs.

Because wood is an ubiquitious natural material, it is the substrate for many different micro-organisms and their enzymes. In fact, one of the most natural processes is the degradation of lignocellulosic materials such as wood to carbon dioxide, water, and humic substances by micro-organisms. Consequently, there are many possibilities to apply biotechnology in wood conversion. Our purpose here is to provide a brief overview of the status of research aimed at applying biotechnology in pulp and paper manufacture, and a synopsis of the status of relevant fundamental research.[1]

The use of fungi in primary pulp manufacture is broadly referred to as **biopulping.** It is based on the ability of white-rot wood decay fungi to colonise wood rapidly and degrade lignin. Research in this area has been pioneered at the Swedish Pulp and Paper Research Institute (STFI), where cellulase-less mutants of white-rot fungi have been developed and used to partially delignify various lignocellulosic materials.[2] Producing a completely biodelignified fibre to duplicate chemical pulping is not the immediate aim; such a process would take too much time, and also, complete selectivity of lignin biodegradation does not seem possible at this time. Emphasis to date has therefore been on biomechanical pulping, in which the fungal pretreatment is used to soften wood chips befor refiner pulping. Fungal treatment followed by chemical treatements, or vice versa, have as yet received relatively little attention.

Mechanical pulping processes are a growing segment of the pulping industry because of the high yields, relatively low capital investment, low pollutant generation, and the fact that they produce pulps with properties advantageous to certain products.[3] The disadvantages of mechanical pulps are lower strength properties, high electrical energy requirements to make them, and in fact that only a few wood species are suitable. Chemical pretreatments are useful in improving the strength properties, but they generate pollutants. Research in our laboratories has shown that fungal pretreatment of chips can lead to substantial energy savings in subsequent mechanical refining, and that the resulting pulps are often stronger and have some interesting new properties. The wastes generated in biomechanical pulping would be biodegradable, and should be easy to treat.

A Biopulping Consortium in Madison, Wisconsin, is investigating the concept of biopulping in a comprehensive way.[1,4] The Consortium involves

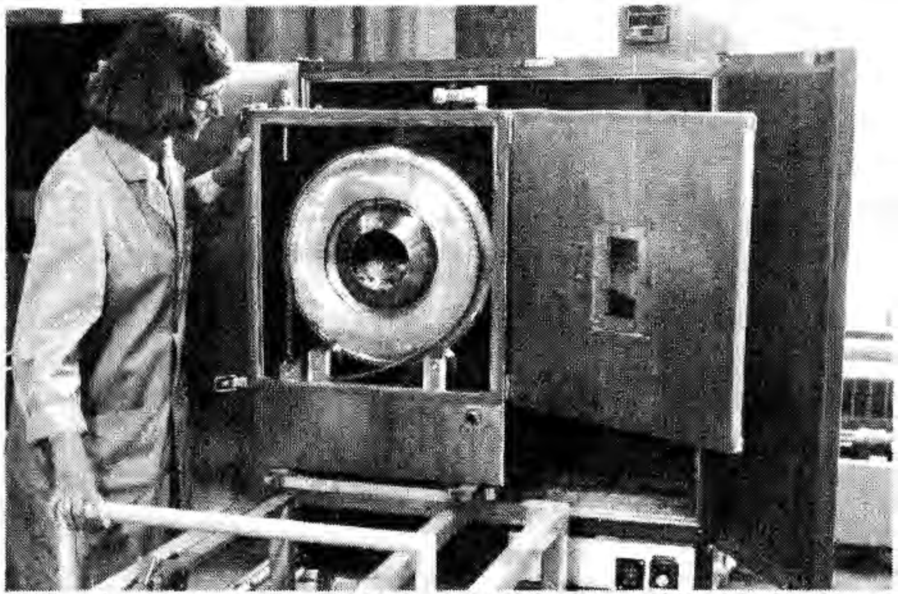

Figure 1. Apparatus to treat sterile wood chips under controlled conditions with biopulping fungi (shown is Marguerite Sykes, FPL).

17 pulp and paper and related companies, the USDA Forest Service, Forest Products Laboratory (FPL) and the University of Wisconsin, and is in cooperation with the University of Minnesota. Several species of fungi have been selected for the biopulping studies based on rapid growth and rapid lignin degradation. Bioreactors have been built (Figure 1), and approximately 60 runs of four to five kilogrammes have been completed with aspen, spruce, and loblolly pine chips. Lignin removal has ranged from three to 37 per cent in four-week fungal pretreatments. Refiner mechanical pulps prepared from the fungus-pretreated chips and from untreated control chips have been evaluated (Table 1). Energy requirements for the refining decreased up to approximately 50 per cent. Burst, tear and tensile strength of handsheets from the biopulped aspen materials increased, with burst index increasing a maximum of approximately threefold over controls. Brightness and light scattering decreased, but bleachability was not adversely affected. Properties of the handsheets overall were comparable to those of CTMPs. Surprisingly, no correlation is apparent between pretreatment efficacy and total substance loss (usually less than ten per cent), extent of lignin removal, or any of several other measured parameters. A preliminary economic analysis indicated that the decreased energy cost is worth about $250 000 of fixed asset investment for each percentage point that electrical energy requirements are reduced in a 300-ton a day model.[1,5] Current work is focused on decreasing pretreatment time, developing rapid screening procedures for pretreatment efficacy, determining the full biochemical basis for the pretreatment, and improving fungal strains.

Biopulping of sugarcane bagasse has been studied in a cooperative venture between the STFI and a Cuban laboratory. An energy saving of approximately 40 per cent achieved in a process using a cellulase-less mutant of a lignin-degrading fungus, followed by cold soda/thermomechanical pulping[6]

Patents on biopulping and related areas indicate that research is proceeding in Japan along lines similar to those in Madison.

On the subject of pulp **modification and recycled fibre treatment,** increasing concern for the environment has prompted several recent studies of enzymatic and biomimetic bleaching of kraft pulps.[1,7] Attempts to use lignin-degrading enzymes have so far been unsuccessful, although enzyme mixtures that degrade lignin extensively have not yet been described. When and if they are, bleaching with them might be more successful. More success was seen in US work with biomimetic haeme derivatives[8] and in very recent work at STFI with alkylated (palmitoylated) haemoglobin and hydrogen peroxide; the latter system caused 30 per cent or more reduction in kappa number of kraft pulps with an accompanying increase in brightness, with only slight reduction in viscosity (Table 2).[9] The haemes and haemoglobin are meant to mimic lignin peroxidases, which have haeme-active centres.

Treatments with various hemicellulose- and cellulose-degrading enzymes have also shown some promise in improving pulp properties, as studies have demonstrated in Canada, Finland, France, Japan, and the United States. These investigations showed that xylanase treatment of kraft pulps can reduce lignin content and lower the bleaching chemical consumption for subsequent chemical bleaching, with only slight loss in paper strength properties.[10] Xylanases were shown to remove up to about 25 per cent of the xylan in various pulps.[11,12] Drainage of recycled fibres was increased by about 20 per cent by treatment with a mixture of cellulases and xylanases.[13] Vessel picking in papers from hardwood pulps was reduced by 85 per cent when pulps were treated with cellulases.[14] And cellulases have been shown to reduce beating time for bleached kraft pulp.[15]

Whole living fungi have been shown to be effective in bleaching pine kraft pulp in the laboratory.[16] Promising results have recently been obtained in Canadian laboratory studies with hardwood kraft pulps. [17]

Chip treatment (freeness, CSF) (ml)	Burst index (kPa m^2/g)	Tear index (mN m^2/g)	Tensile index (Nm/g)	Density (kg/m^3)	Brightness (%)	Opacity (%)	Scattering coefficient (m^2/kg)	Fibre length index (mm)	Pulping energy (W h kg^{-1})
RMP (120)	0.66	2.75	28.1	393	64.4	93.2	61.8	0.1005	2700
BRMP (110) *Phlebia*	2.11	6.13	51.4	425	42.9	93.0	37.9	0.1060	1560
BRMP (100) *Phanerochaete*	2.04	4.64	52.5	402	40.5	94.8	39.9	0.1201	1480

Table 1. Properties of pulps and papers prepared from aspen wood.[1] *RMP, refiner mechanical pulp; BRMP, refiner mechanical pulps from fungus-pretreated wood.* Phlebia and Phanerochaete *were the fungi used.*

Pulp	Kappa number		Viscosity, dm^{3}kg^{-1}		Brightness (ISO)	
	Before bleaching	After bleaching	Before bleaching	After bleaching	Before bleaching	After bleaching
Unbleached kraft (pine)	30	22	1180	1051	23.5	35.6
Oxygen-bleached (pine)	22.5	13.5	1050	876	30.5	40.7
Unbleached kraft (birch)	18	12.3	1230	1083	40.0	52.3

Table 2. Pulp bleaching with palmitoylated hemoglobin and hydrogen peroxide.[9]

Microbes secrete polysaccharides and other polymers that are being studied for various applications in other industries. The possibility of growing such organisms directly in pulp to improve certain properties has not been reported, although a preliminary study at STFI in which a *Penicillium* species was grown in unbeaten pulp indicated that increased strength properties could be obtained.

Although enzyme treatments have potential to solve some of the problems associated with recycled fibres, this area has apparently not yet been studied. Deinking and removing size are examples of possible applications of enzymes that should be explored.

The release of **waste** bleach waters from conventional bleaching of chemical pulps into receiving waters is perhaps the most serious environmental problem created by the industry. Work at the STFI in Stockholm confirmed observations that high molecular mass chlorinated lignins are not degraded in aerated ponds, the major purification technique now in use for waste bleach waters.[18] However, that work also demonstrated that the high molecular mass chlorinated lignins are not as inert as earlier thought. They degrade in the recipient waters and generate chlorinated catechols and guaiacols.[19] This observation makes it obvious that an effective purification of bleach plant effluents must involve elimination of both low and high molecular mass chlorinated compounds. In Japan, ultrafiltration is used to remove the chlorolignins of high molecular mass. Another possibility, discussed later in this section, is to use white-rot fungi, which are the only microbes known to degrade chlorolignins efficiently.

Anaerobic systems have advantages over aerobic systems where they can be used: they generate little biomass (secondary sludge, which is a disposal problem itself), they do not require mixing, and they generate methane, which can be used for energy. Such systems are gradually being introduced into the industry,[19] but they cannot degrade chlorolignins. Nevertheless, anaerobic systems can degrade many low molecular weight compounds, including some chlorinated aromatics, and of course sugars and many other wood-derived compounds.

With these considerations in mind, STFI scientists have investigated a combination of ultrafiltration followed by anaerobic and then aerobic biotreatments.[1,20] A pilot-scale system was used to evaluate residence time and efficacy in a two-month study. Acute toxicity was completely eliminated after the combined ultrafiltration and biological treatments. Chlorate reduction was also greater than ninety-nine per cent. The predicted reductions with the optimised system are as follows: BOD (biochemical oxygen demand) 95 per cent, COD (chemical oxygen demand) 70-85 per cent, AOX (organically bound halide) 70-85 per cent, chlorate greater than 99 per cent, and acute toxicity approximately 100 per cent. This should be compared with the reductions obtained in an aerated lagoon: BOD 40-55 per cent, COD 15-30 per cent, AOX 20-30 per cent, colour about 0 per cent, and chlorinated phenols 0-30 per cent.

Work continues at North Carolina State University, in collaboration with the US Environmental Protection Agency and the FPL, on the MyCoR method, which uses *P chrysosporium* or other white-rot fungi in rotating biological contractors to decolourise, dechlorinate, and detoxify chlorolignins and low molecular weight chlorinated aromatics.[21] Recent findings show that combinations of MyCoR treatment and bacterial treatment can reduce AOX from 6.1 to 1-2 kg/ADT pulp, with 50-70 per cent colour removal. The MyCoR method is currently being evaluated on a larger scale. A modification of the MyCoR method, Mycopor, is being studied in Austria.[1,22] *P chrysosporium* is immobilised on foam squares, from which a trickling filter is constructed. Decolourisation of 80 per cent in 24 hours was achieved for a first extraction stage effluent from a chlorine bleachery using sulphite pulp. AOX was reduced at about the same rate.

These various studies indicate that combinations of physical and new biological methods should be further explored for highly efficient treatment of bleachery wastes.

Control of slime deposits in paper mill whitewater systems is another area in which biotechnological approaches have been investigated. The deposits are mainly microbial polysaccharides, which can sometimes be solubilised by enzymes,[23] fibres and resins. An improved understanding of microbial physiology, inter-reaction and ecology in different whitewater systems should make it possible to control slime problems more efficiently.

The possibility to **convert pulping and papermaking byproducts** to more valuable products by fermentation is receiving less attention now than it has in the past, when the oil crisis prompted much research into the conversion of byproduct sugars into ethanol. In the same period, various studies were made of the production of microbial protein for animal feed using various byproducts.

Fermenting glucose and other hexoses to ethanol is established technology and is readily accomplished commercially with spent sulphite liquor. Wood hydrolysates produced for the purpose of fermentation can also be converted to ethanol and other products, although such fermentations have not been performed commercially. Industrial fermentations based on conifer wood hydrolysates were developed in Germany during the Second World War, however. Such fermentations were based on the six-carbon sugars in the hydrolysates. Much of the sugar from hardwoods, however, is xylose, for which there is no commercial fermentation technology. Development of the xylose fermentation was recognised early as a key need in research aimed at bioconverting pulping and other wood manufacturing (and agricultural) wastes, Progress toward this goal is being made, but research has slowed because of lack of funding. Production of 56gL^{-1} of ethanol from xylose at rates of 1.35-2.2gL^{-1}h^{-1}, by yeasts, has been reported recently; maximum concentration and rates with glucose are commonly 85-90gL^{-1} and

11-12$gL^{-1}h^{-1}$.[24] Increased petroleum prices would of course stimulate interest in this kind of fermentation, as would a realistic view of future needs, including the need to reduce production of greenhouse gases from non-renewable carbon resources.

Microbial protein (primarily yeast) has long been produced commercially from spent sulphite liquors, in which case both five- and six-carbon sugars are converted. In the USSR approximately 30 plants hydrolyse wood with sulphuric acid and ferment the resulting sugars to protein.

At STFI a process aimed at closing the whitewater system of mechanical pulp and paper mills has been studied.[25] The principles of this system are presented in Figure 2. The water-soluble substances in the whitewaters from mechanical pulp production serve as substrates. The STFI process was tested on a pilot scale in a newsprint mill with a residence time of 17 hours in the fermenter. No build-up of organic materials was found to take place in the closed system. If part of the mycelium produced in the continuous process could be recirculated, a considerable reduction of the residence time should be realised, decreasing the necessary fermenter volume. The fungal mycelium, occurring in the form of pellets 0.2-0.4mm in diameter, is easily separated from the water by filtration. The process has also been operated on a 25m^3 scale with wastewater from a board factory. Feeding trials with the resulting fungal protein were essentially positive, particularly with ruminants.[26] Instead of being used as cattle feed, however, the fungal mycelium might be incorporated into the paper without negatively influencing the paper quality. In a closed process the fungal mycelium will correspond to approximately 1.5 percent of the paper weight. The advantages of the process are that external effluent treatment is unnecessary, water is saved, paper production is increased by incorporation of the mycelium, and possibly, energy is saved. In a cooperative project between the Austrian company Voest-Alpine, STFI and the University of Georgia, work is now in progress to develop this process.

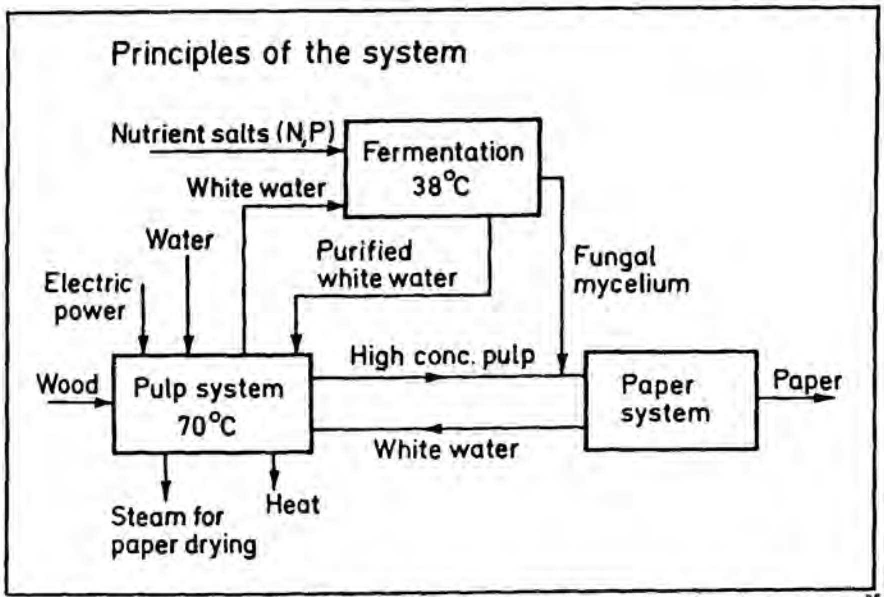

Figure 2. Flow sheet of an experimental closed whitewater system of a newsprint pulp and paper system.[25]

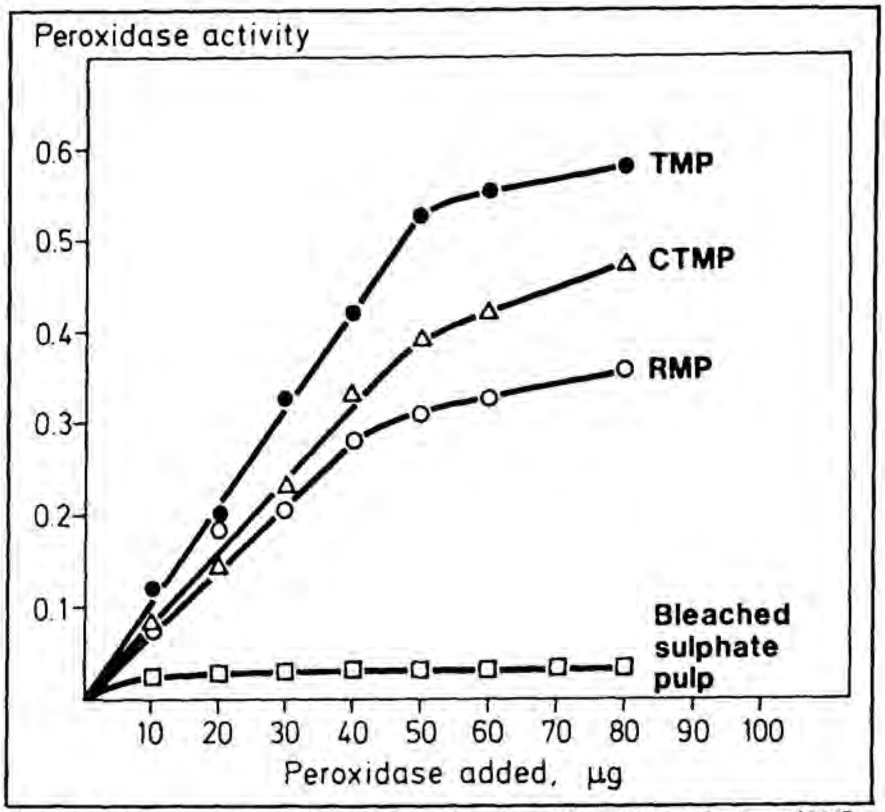

Figure 3. Relative amount of peroxidase adsorbed on the surfaces of Bauer McNettp-fractionated RMP, TMP, CTMP and kraft pulp fibres, 30-50-mesh.[28]

The possibility to convert byproduct lignins to chemicals or to more reactive polymers using microbes or enzymes has received practically no attention. White-rot fungi convert lignin to a large array of disparate degradation products.[27] Brown-rot wood-decay fungi, by contrast, have the unusual ability to demethylate lignin (convert aromatic methoxyl groups to phenolic hydroxyl groups), thereby increasing the reactivity of the polymer. Limited studies of this bidemethylation have been conducted at both the FPL and STFI. Whether a practical fermentation for this demethylation could be developed, however, remains to be determined.

Antibodies and most enzymes are highly specific, making them useful in specific assays and as components of **biosensors.** A variety of such sensors can be imagined that would be useful in the industry.

The structure and chemical composition of pulp fibre surfaces are of importance for paper strength because those properties strongly influence the bonds between fibres and fibre fragments in paper sheets. Characterisation of pulp fibre is usually achieved using physical methods including measurements of mechanical strength and hydrodymanic properties. At STFI, biosensor techniques, using enzymes and antibodies, for characterising mechanical pulp fibre surfaces have been studied.[28]

In the first study, pulps were treated with cellulases. The amount of glucose released reflected the accessible cellulose exposed on the surfaces of the pulps. Results showed that the quantity of sugar released from three types of mechanical fibres decreased in the order of RMP, CTMP, TMP.

Peroxidase, which reacts with lignin, and antibodies toward Lignin, were used in a subsequent study to investigate the exposure of lignin on the fibre surfaces (Figure 3). The more lignin exposed the more peroxidase became bound. Results showed that the peroxidase adsorbed in the order of TMP, CTMP, RMP, in exactly the reverse order in which reducing sugars were released by cellulases. Practically no peroxidase was absorbed on bleached fibres. Antibodies against lignin ranked the fibres in a somewhat different order: TMP, RMP, CTMP. Perhaps the sulphuric acid groups in the CTMP lignin prevented full recognition by the antibodies.

Knowledge gained in these studies may contribute to the development of new techniques for producing better quality mechanical pulps. Such techniques might also be used to study the influences of various modification techniques on the fibre surface structures. Monoclonal antibodies are being studied currently at the University of Georgia for developing even more specific biosensor techniques.

Figure 4. Research continues to improve the production of lignin-degrading enzymes. Such studies with Phanerochaete chrysosporium *have increased the activity of lignin peroxidase approximately 800-fold (shown examining experemental cultures is Karen Martinson, FPL).*

Fundamental studies

Chemical pulping and bleaching processes were developed commercially before a detailed understanding of the chemical reactions was obtained. Basic research followed the applied studies. Similarly, microbial waste treatment processes used in the industry were developed before much was known about the microbial processes involved, and again, fundamental investigations followed the application. However, other, more focused applications of biotechnology in pulp and paper manufacture likely will follow from fundamental studies, from an understanding of the underlying principles. Indeed, essentially all recent examples of commercial applications of biotechnology in other industries have followed basic research.

Lignin biodegradation is fundamental to many potential applications of biotechnology in the pulp and paper industry, and related research has accelerated greatly over the past 15 years. During that time the microbiology of the process was largely clarified (wood decay fungi predominate), the key features of the chemistry of biodegradation by the white-rot fungi were described, and an experimental system was developed with the efficient lignin-degrading fungus *Phanerochaete chrysosporium.* Six years ago the first lignin-degrading enzyme (a powerful peroxidase) was discovered in the *P chrysosporium* system. With that discovery the study of lignin biodegradation entered the realm of biochemistry. Subsequent investigations have described the mechanism of action of lignin peroxidase, showing that it simply oxidises aromatic nuclei in lignin by one electron to cation radicals, which undergo non-enzymatic degradative reactions of both radical and ionic nature. These reactions include aromatic ring cleavage and various other cleavages that depolymerise the polymer. The pieces of lignin thus formed are taken up by the fungus and further oxidised to CO_2. Other research seeks to improve production of lignin peroxidase for fundamental and applications research (Figure 4). A second kind of peroxidase was described in the same system five years ago: it oxidises Mn^{2+} to Mn^{3+}, which can oxidise phenolic units in lignin, leading to some degradation. The blue copper enzyme laccase, present in other lignin-degrading fungi, does the same thing, but the roles for these two latter enzymes are as yet unclear. Enzymes that generate extracellular H_2O_2 (needed by the peroxidases) were also described in the *P chrysosporium* system, including a new extracellular copper oxidase that oxidises glyoxal and related compounds with reduction of O_2 to H_2O_2. Despite all this progress, an enzyme mixture that actually depolymerises or solubilises lignin has not been described, and is prompting work toward that end. Recent literature on lignin biodegradation is reviewed briefly by Kirk and Farrell.[29]

Figure 5. Investigations have disclosed and are now characterising a large family of related genes in Phanerochaete chrysosporium *that encodes the lignin peroxidases. Such research is necessary for strain improvement (shown examining a photographic film from a DNA sequencing gel is Sarah Covert, University of Wisconsin).*

The discovery of lignin-degrading peroxidases made it possible to identify and study the encoding genes (Figure 5); over the past three years an unexpectedly large family of related genes for lignin-degrading enzymes has been described in *P chrysosporium.*[30]

Following the world oil crisis in the

1970s, considerable research effort was devoted to converting **wood and other lignocellulosics** to ethanol. The enzymatic hydrolysis of crystalline cellulose to glucose was studied extensively, providing a much clearer picture of a complex process.[31] The emphasis on cellulose-to-ethanol conversion has abated, but basic studies of cellulase systems continue.[1,32] Study continues in part because uses for cellulases have been found in food processing; also, understanding cellulases is important in biopulping and biobleaching, for which they presumably are counterprodudive.

Cellulose is degraded to glucose, cellobiose, and water-soluble cellodextrins by the synergistic action of three types of hydrolytic enzymes: endo-1,4-β-glucanases, exo-1,4-β-glucanases, and 1,4-β-glucosidases. Recent investigations have disclosed the basic similarity of these types of enzymes among different groups of fungi. Basic research has also begun to show how the enzymes bind to their substrates and how the synergy works at the molecular level. Research on cellulases has shown, too, that bacteria degrade cellulose using clusters of enzymes rather than individual endo-and exo-glucanases and that some employ phosphorylating enzymes instead of hydrolytic ones. Both bacterial and fungal cellulose genes have been cloned and sequenced, providing information on their structure and regulation.

For enzymatic hydrolysis of cellulose, only enzymes hydrolysing 1,4-β-glucosidic linkages are required. Because the structures of the xylan hemicelluloses of wood are variable, involving both linear 1,4-β-linked chains of xylose and also branched heteropolysaccharides, a more complex set of enzymes is required. Compete degradation of branched, acetylated xylans requires the concerted action of several different hydrolytic enzymes, including endo-1,4-β-xylanase, 1,4-β-xylosidase, α-glucoronidase, α-L-arabinofuranosidase, and aecetylxylan esterase. Considerable progress has been made recently in separating and characterising these enzymes.[33] Progress in characterising the enzymes that degrade the major hemicelluloses of conifer woods, galadoglucomannans, has not been as noticeable. However, research has shown that an enzyme preparation suitable for complete hydrolysis of wood mannans requires the concerted action of endomannanase, β-mannosidase, β-glucosidase, and α-galactosidase.[34]

White-rot fungi, of which *P chrysosporium* is the most studied (as it is for lignin biodegradation) employs oxidative enzymes in addition to the hydrolytic ones for cellulose and hemicellulose degradation. Cellobiose quinone oxidoreductase and cellobiose oxidase both oxidise cellobiose,[31] thereby removing cellobiose, preventing competitive inhibition of endo- and exo-glucanases.

Brown-rot fungi, the most important destroyers of wood in service, apparently are unique among microorganisms in employing an as yet undescribed oxidative system to depolymerise cellulose, after which endoglucanases and β-glucosidases apparently convert the oxidised cellulose to metabolisable sugars. The brown-rot cellulose-depolymerising system might have features that make it useful in biotechnological applications. Research is underway at the FPL and at several other laboratories to elucidate the biochemistry of this unusual system.

Perspective

Research continues to explore the potentials of biotechnology has in pulp and paper manufacture. The overall effort, however, is small. A larger effort obviously would determine more quickly how promising these new approaches are. The experience in our laboratories has shown that good communication between biotechnology researchers and the industry's technical people has many advantages for research. The potential of the applications of biotechnology to the industry is large. In order to profit from this new field, the industry must be aware of research results and be able to implement them. Our review is but one step to help achieve this goal. ☐

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Eriksson and Kirk shared the 1985 Marcus Wallenberg Prize for their fundamental research on the fungal degradation of wood.