Biotechnology in the pulp and paper industry: a review

Part 1: tree improvement, pulping and bleaching, and dissolving pulp applications

Patrick C. Trotter

Project leader, Biotechnology Awareness, Weyerhaeuser Co., Technology Center, Tacoma, Wash. 98477

ABSTRACT *Much research has been devoted recently to biotechnology in our industry. In Part 1, we look at the directions biotechnology research is taking in tree improvement, pulping, bleaching, and dissolving pulp production. Part 2 will cover pulp property improvement, papermaking, effluent treatment, and the use of biomass for fuels, chemicals, and other products.*

Researchers in tree improvement are concentrating on genetic manipulation of lignin content and composition in wood to make pulping easier, with less expenditure of chemicals and energy. Biopulping research is following two tracks: the use of white-rot organisms in biomechanical processes and the production of ligninase enzymes for direct use as pulping reagents. Biobleaching research focuses on hemicellulase enzymes, though xylanase enzymes also are being studied for use in preparing dissolving pulps of high quality.

KEYWORDS
Biology
Biotechnology
Bleaching
Dissolving pulps
Enzymes
Forestry
Lignin
Pulping
Trees

How to apply biotechnology in the pulp and paper industry has become a prolific area of research. Biotechnology is defined here as the use of biological organisms, systems, and processes for practical or commercial purposes. It encompasses such diverse activities as fermentation, immobilized-cell and enzyme technology, cell and tissue culture techniques, and monoclonal antibody techniques. In recent years, however, the term has been increasingly identified with techniques for gene transfer and DNA manipulation, or "genetic engineering."

In the broadest sense of this definition, biotechnology has been a part of our industry for many years for treating wastes (**Fig. 1**), fermenting sulfite liquors, preparing starch for paper sizing, and controlling the buildup of slime on paper machines. But recently, major research efforts have been aimed at pulping, bleaching, modifying fibers, and improving the trees. Kirk *et al.* (*1*) reviewed progress in the entire field in 1983, and Paice and Jurasek (*2*) followed with a review of biotechnology in pulping, bleaching, and papermaking in 1984. In 1986, I reviewed biotechnology efforts in tree improvement (*3*). Four international conferences on biotechnology in the forest products industry have been held, one in Raleigh, N.C., in May 1989 (*4*). Other symposia on the subject also have attracted interest (*5*).

Advances in biotechnology have been occurring rapidly. The purpose of this two-part report is to review what has taken place since the earlier reviews appeared and to offer a fresh look at expectations for the future. Part 1 will cover biotechnology for tree improvement plus applications in pulping, bleaching, and the production of dissolving pulp. Part 2 will deal with pulp property improvement, papermaking biotechnology, waste treatment, and conversion of biomass to fuels, food, and chemicals.

Genetic engineering in forestry: improving trees for papermaking

Classical genetic techniques have been used for years to produce superior trees for commercial forestry operations. These programs rely on the broad natural variability of gen-

otypes within populations (*6*). Selections have traditionally been made for "investment" traits such as straightness, form, and superior growth yield, although "insurance" traits such as disease resistance also are included sometimes. Recently, some commercial tree breeders have begun to include wood quality characteristics in their selection criteria as well. Wood specific gravity is the characteristic usually chosen because it is an easily measured surrogate for a number of performance parameters (*7, 8*).

Clonal propagation methods such as tissue culture and embryogenesis, which are included in the broad definition of biotechnology, could be integrated into these programs to shorten the time to get superior planting stock into the field. Nevertheless, tree breeding programs based on selection of traits require long-term investments and several generations of selects before major improvements are seen. Furthermore, in following a program of selection of traits, we presuppose that they exist in the trees' genetic makeup (*1*). Many of the possibilities being considered for improving important pulpwood species are based on traits that probably do not pre-exist in tree genomes.

Recombinant DNA techniques have been developed in recent years. These techniques make it possible to transfer specific genes into host plant genomes to create new genotypes, in effect. To apply recombinant DNA techniques to plants, it must first be possible to:

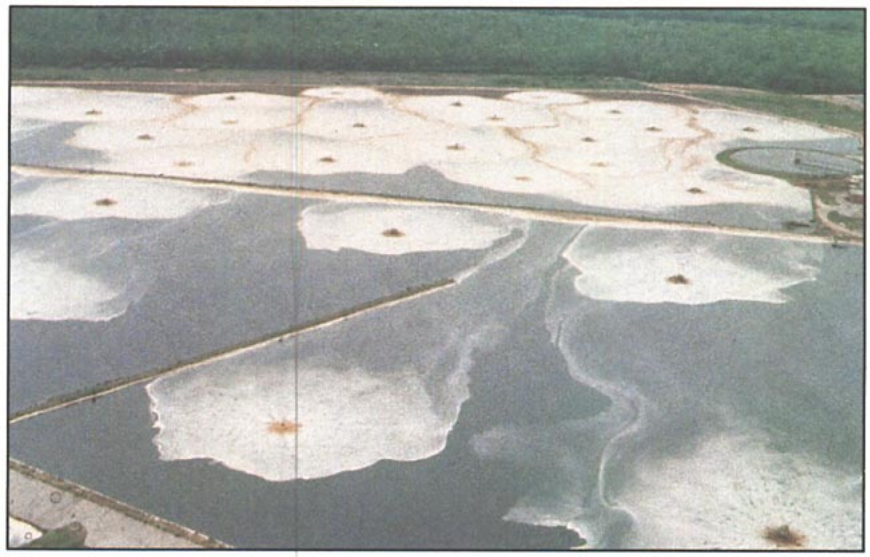
1. Identify, isolate, and clone the fragment of DNA that contains the gene of interest
2. Transfer the DNA fragment into a recipient plant cell and have it incorporated into the DNA of the cell
3. Regenerate a whole transgenic plant from that transformed cell.

I call these, collectively, "enabling technologies."

Status of enabling technologies

Strategies for carrying out Step 1—the detection, isolation, and cloning of specific genes—have become quite advanced and sophisticated in recent years. However, it still can take anywhere from $100,000 to $300,000 per year and consume up to three scientist-years to isolate and clone a recalcitrant gene (*9*). But the methods are available, and practitioners consider them quite routine.

1. Pulp mill bioponds. Effluent treatment has been a stronghold of biotechnology for many years. New research may lead to improved methods for removing color and reducing AOX.



Researchers' ability to isolate and manipulate foreign genes is outpacing our ability to know which genes to manipulate—that is, to decide which traits we want to confer or modify. There are two reasons for this. First, the length of time it takes to generate trees makes it difficult to carry out quickly enough the cause-and-effect research needed to guide gene manipulation. Second, the forest industry has yet to reach a consensus on which traits it would consider of high enough value to justify the up-front cost of this research.

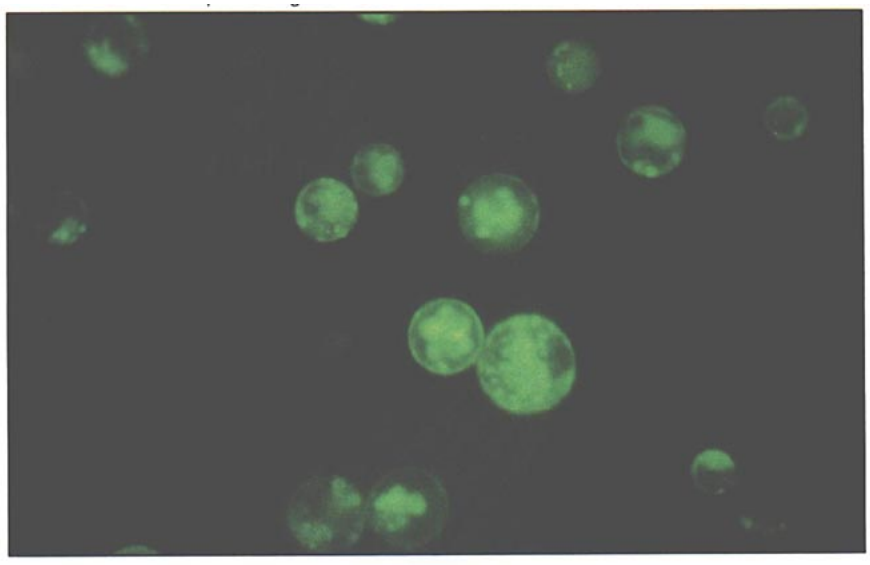
On the other hand, a spokesman for the U.S. Forest Service recently asserted that his agency does have a policy and is setting up a series of joint government-university research collaborations to tackle some of the key problems (*10*). Also, in 1988, Westvaco Corp. announced the addition of a new biotechnology research facility at its Forest Science Laboratory in Summerville, S.C. According to Edward G. Owens, director of forest research, scientists in the new facility will work on developing reliable vegetative mass-propagation systems for genetically superior pine and hardwood trees. They also will explore techniques for identifying specific genes that influence tree characteristics, fiber quality, and tree health. Perhaps these announcements signal movement toward consensus within the forest industry.

Techniques for accomplishing Step 2—the transfer and incorporation of foreign DNA into the plant cell genome—are likewise coming to hand. Five principal methods have been disclosed (*11*):

1. Agrobacterium-mediated transformation of plant cells using the Ti plasmid vector system from *Agrobacterium tumefaciens* or *A. rhizogenes*
2. Direct microinjection of protoplasts (**Fig. 2**)
3. Polyethylene-glycol-stimulated uptake of DNA by protoplasts
4. Electroporation of protoplasts
5. Ballistic injection of DNA-coated gold or tungsten particles through cell walls.

Of these methods, *Agrobacterium*-mediated transformation has become a "workhorse" system for shuttling foreign DNA into the genomes of dicotyledonous plants. Electroporation and ballistic injection also show promise, with ballistic injection receiving the lion's share of recent attention. Two types of ballistic injection devices have emerged. One was developed by scientists at Cornell University (*12, 13*) and was recently acquired by the DuPont Co. The other was developed by Agricetus, a bio-

2. Plant cell protoplasts. To successfully apply biotechnology to tree improvement, genes must be transferred into the plant cell genome.



technology company located in Middleton, Wis. (*14*). Laboratories currently working with ballistic injection to bring about the transformation of important tree species include the University of Wisconsin, Oregon State University, and North Carolina State University.

The number of plants that have been successfully transformed by one or another of these methods stands at 48. On this list are several important tree species, including poplar, walnut, apple, alder, birch, sweetgum, and eucalyptus, and the conifers Douglas-fir, loblolly pine, sugar pine, white spruce, Sitka spruce, and Englemann spruce.

Step 3—regenerating a whole transgenic plant from the transformed cell—continues to be a bottleneck. Success was reported early for poplar (*15, 16*), but transgenic plants have not been reported for any other important pulpwood species, though all of the elements needed to produce transgenic plants have seemingly been demonstrated.

Current status of genetic engineering work

Despite the bottleneck in recovering transgenic trees, at least two research groups have decided to press on with genetic manipulation studies. These scientists reason that by the time they have gene constructs in hand for manipulating important traits, somebody surely will have developed the required transformation and regeneration technology for commercially important pulpwood species. Both of these groups have chosen to work on manipulating the process of lignin formation in conifers.

Why should this be of value to the industry? Consider just the kraft pulping process. In kraft pulping, the ease of lignin removal is directly proportional to the ratio of syringyl units to guaiacyl units in the lignin (*17*). Syringyl precursors produce a less cross-linked lignin polymer than guaiacyl precursors, and it is easier for the pulping chemicals to break down and dissolve such a polymer.

Thus angiosperms, whose lignins contain both syringyl and guaiacyl units, can be pulped not only by the kraft process but also by virtually all of the other known or emerging processes, with less expenditure of chemicals and energy and in less time than gymnosperms, whose lignins consist solely of guaiacyl units (*18–20*). But pulps from gymnosperms are preferred for many applications because the fibers are generally longer and stronger than those from angiosperms (*18*). Thus, manipulating lignin composition in gymnosperms to be more like that of angiosperms would produce substantial economic gain by virtue of the savings in time, chemicals, and energy required for pulping. The saving in chemicals alone has been estimated at close to $6 billion annually for the total U.S. kraft pulping industry (*20*). Controlling the amount of lignin formed in the wood would yield economic gains of like magnitude.

Many enzymes (and therefore several genes) are involved in the total lignin biosynthesis process (*21*), but both the amount and type of lignin formed depends on just a few key steps.

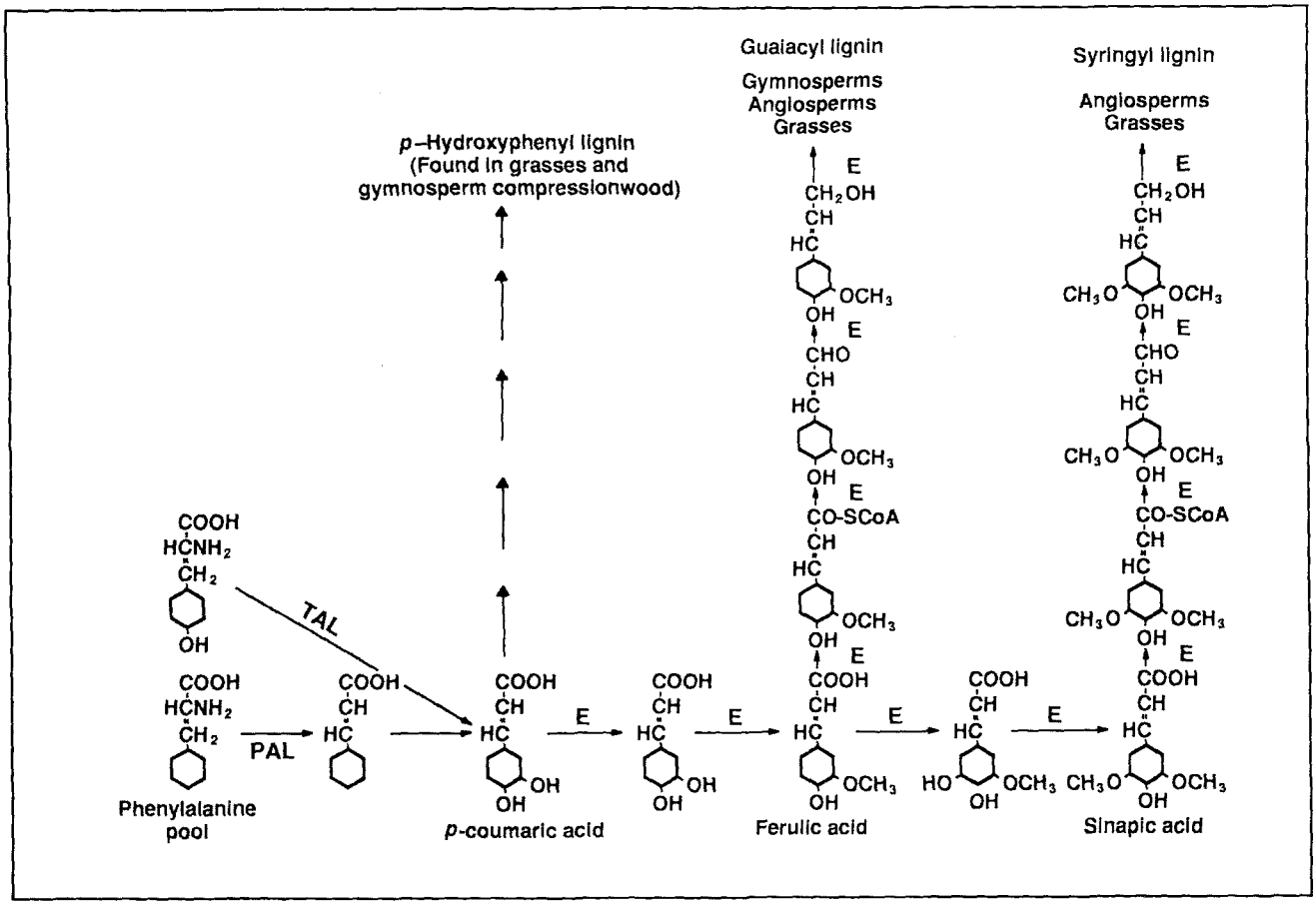
Entry into the lignin precursor pathway is governed by the enzyme phenylalanine ammonia lyase (PAL, **Fig. 3**), a well-characterized enzyme for which genes already have been isolated from several sources (*22–24*). Reducing PAL activity through gene manipulation might be one way to control entry into the pathway and reduce the amount of lignin formed (*25*).

At the other end of the pathway, cinnamyl alcohol dehydrogenase (CAD) enzymes specific to the lignin pathway (designated E3′ and E3″ in Fig. 3) catalyze the formation of coniferyl and synapyl alcohols, the precursors that actually polymerize to form lignin. Controlling the levels of these precursors, by controlling lignin-specific CAD activity, might be yet another way to reduce the amount of lignin formed (*26*).

Isolating a lignin pathway-specific CAD gene and particularly its promoter sequence would provide us, in addition, with a signal that is specific for the time and place of lignin formation. Such a promoter would be useful for genetic manipulation of other events that take place in lignifying tissue. Using as a probe the gene for another type of alcohol dehydrogenase previously isolated from pine, researchers at North Carolina State University hope to isolate the lignin-specific CAD gene and then investigate some of these possibilities (*26*). In this regard, Grand and Boudet (*27*) have isolated the lignification-specific CAD gene from mung bean and have reported that their group is setting up an experiment to alter lignin formation in that plant system.

As for the type of lignin formed, this is governed by the presence or absence of two enzymes in lignifying tissue: ferulate 5-hydroxylase (E7 in Fig. 3) and difunctional *o*-methyl transferase (E8) (*21*). Gymnosperms lack E7, and gymnosperm E8 will not catalyze the reaction that forms syringyl precursors. If genes coding for the two corresponding angiosperm enzymes

3. Pathway for biosynthesis of lignin. (Enzymes E are defined as follows. E1, E1′, E1″, hydroxycinnamate–CoA ligase. E2, E2′, E2″: hydroxycinnamoyl–CoA reductase. E3, E3′, E3″: cinnamyl alcohol dehydrogenase. E4, E4′, E4″: peroxidase. E5: *p*-coumarate 3-hydroxylase. E6 and E8: hydroxycinnamate O-methyltransferase. E7: ferulate 5-hydroxylase. PAL: phenylalanine ammonia-lyase. TAL: tyrosine ammonia-lyase.) (*21*)



could be incorporated into the gymnosperm genome, then a gymnosperm capable of producing angiosperm-type lignin ought to result. This is a line of research being pursued by a group of scientists at Michigan Technological University, who are working to isolate the genes for angiosperm E7 and angiosperm E8 from actively lignifying tissues of aspenwood. They are reported having the aspen E8 gene in hand, but they are still at the stage of sequencing to verify its identity (*28*).

Biotechnology for pulping, bleaching, and dissolving pulps

Biopulping

White-rot fungi and many other organisms attack lignin in wood, which raises the question of whether biological means may be used for pulping (*1*). One of the first attempts to exploit this opportunity was in pretreating wood for mechanical pulping. In 1971, an unpublished cooperative study between the U. S. Forest Products Laboratory and North Carolina State University showed that aspen wood pretreated with *Fomes ulmarius* gave refiner mechanical pulps with better paper strength properties than untreated aspen wood refined with the same amount of energy (*29*). In Sweden, Ander and Eriksson (*30*) found in the laboratory that birch and pine woods slightly delignified by a cellulase-negative mutant of *Phanerochaete chrysosporium* required 25–30% less energy for refining and gave pulps with better strength properties than controls. However, subsequent attempts to scale up their approach for an industrial evaluation met with only indifferent success (*31*, *32*).

One problem with fungal pretreatment is that the time required is a matter of weeks. This delay has led to suggestions that treatment might be carried out in connection with chip transport or storage or that treatment time could be reduced to days or even hours by optimizing the fungal strain and treatment conditions (*1*). Work toward the goal of optimizing treatment has continued in a number of laboratories. In Japan, Akamatsu *et al.* (*33*) compared TMPs made from aspen wood pretreated with any of 10 different white-rot fungi to TMP made from untreated aspen wood. None of the 10 fungi were lignin-specific, yet each of them reduced refining energy by about 25%, and three of them gave pulps with somewhat improved paper strength properties. At the University of Minnesota and the U. S. Forest Products Laboratory, Blanchette *et al.* (*34*) have found that even with lignin-specific organisms, the strain of white-rot fungus employed and the type of wood substrate treated may significantly influence the rate of lignin degradation and the selectivity of lignin removal. Therefore, it should be possible to select and optimize strains for lignolytic activity on particular

wood substrates.

In a recent publication from the same laboratories, Myers *et al.* (*35*) described a solid-substrate bioreactor used to treat aspen wood chips prior to refiner mechanical pulping. As in the earlier studies, the strength properties of "biopulps" prepared from chips treated in this bioreactor have been better than controls when compared at equal freeness, but treatment times are still on the order of weeks.

In the mid-1970s, researchers focused on the basidiomycete *Phanerochaete chrysosporium* for detailed biochemical study. In 1983, two laboratories reported the discovery of a lignin-degrading enzyme (ligninase or lignin peroxidase) from this organism (*36, 37*). Since then, ligninase has been studied extensively in many laboratories. Much has been learned about its mode of action, as well as about other enzymes and metabolites involved in degrading lignin (*38-40*). The key reaction is ligninase-catalyzed one-electron oxidation of aromatic rings in lignin to form cation radicals. These radicals degrade via many spontaneous reactions, some of which cleave intermolecular linkages in lignin and some of which cleave aromatic nuclei. The ligninase-catalyzed oxidation requires extracellular H_2O_2, which means that the enzyme system supplying that cofactor is also a key component of the *in vivo* lignin-degrading system. A second peroxidase (termed manganese peroxidase) also is involved, but its role is not yet clear.

Another result of this focus on *P. chrysosporium* has been to markedly increase ligninase production. Enzyme activities in early cultures were low (*40*), but improvements in strain and the optimization of culture parameters have improved activities 200-fold (*41*).

Ligninase exists as multiple isoenzymes (*42*), coded for by at least three closely related structural genes (*43, 44*). Complementary DNA (cDNA) sequences have been published for these genes (*45, 46*), and Smith *et al.* (*45*) recently cloned and sequenced a native ligninase gene whose coding region was virtually identical to one of these cDNAs.

A ligninase gene has been expressed in *Escherichia coli* (*45*), but the ligninase produced was not catalytically active. However, Farrell *et al.* (*46*) were able to reconstitute an active enzyme by inserting a protoheme moiety *in vitro*.

To sum up, biopulping research is currently following two tracks. The first is to use the white-rot organisms as pretreating agents for subsequent mechanical pulping. The second is to use the isolated ligninase enzymes. In the first case, the challenge still is to optimize the organism for the wood being treated and thus bring down the treatment time. In the second case, the thrust seems to be to produce the enzymes via recombinant DNA techniques and then to reconstitute an enzyme system that will duplicate *in vivo* lignin degradation.

In the United States, a consortium of 13 pulp, paper, and chemical manufacturers, along with the U. S. Forest Products Laboratory and the University of Wisconsin, is pursuing the white-rot organism route (*47*). Repligen Sandoz Research Corp. is a company in the United States that is active in developing recombinant DNA ligninases for pulping and other purposes.

Several "ligninase clubs" also exist worldwide. In France, the National Institute of Agronomic Research (INRA) has a heavily funded program that also involves the French company La Cellulose du Pin. In 1987, La Cellulose du Pin and the Finnish Sugar Co. announced the formation of a joint venture company called "Biopulp International" to develop and market enzyme applications for pulp and paper production. The Swiss Federal Institute of Technology also is said to have a well-funded program. The Swedish Forest Products Laboratory (STFI) is still active as well, although less active than in previous years. In Japan, Kobe Steel recently announced that it has found a way to use a strain of white-rot fungus to produce a high-quality wood pulp for about $40-50 per ton and is working with researchers at Kyushu University to overcome the long processing time required. Also in Japan, Oji Paper Co., Japan's largest paper manufacturer, is working on developing a recombinant lignin-degrading enzyme for pulping.

Biobleaching

The idea of removing lignin from kraft pulp with lignolytic organisms has been around for at least ten years. In 1979, Kirk and Yang (*48*) treated unbleached kraft pulp with intact cultures of *P. chrysosporium* and got partial delignification. No bleaching occurred, but the amount of chlorine required for subsequent bleaching was reduced by 27%. More recently, Paice *et al.* (*49*) treated unbleached hardwood kraft pulp with another white-rot fungus, *Coriolus versicolor*, and found that it bleached the pulp by up to 15 brightness points. The organism was not effective on softwood pulps, however.

The slow pace of the fungal systems presents the same obstacle for bleaching as it does for pulping, so isolated ligninases have been looked at as well. Egan (*50*) has reported that ligninases will depolymerize lignin and, in combination with an extraction stage, will partially bleach kraft pulp. Other workers have not enjoyed the same success. Viikari *et al.* (*51*) found that ligninases isolated from the white-rot fungi *Phlebia radiata* and *P. chrysosporium* had no detectable effect on either the kappa number or the brightness of a pine kraft pulp when applied to the pulp directly. If applied after a hemicellulase treatment, the ligninases did seem to have a slight positive effect on the kappa number.

The use of hemicellulase enzymes offers an alternative approach to biological bleaching. Lignin is now known to be covalently bound to hemicelluloses in wood and pulp (*52*). Viikari and coworkers (*53*) showed that cleaving this bond by treatment with hemicellulase enzymes does indeed facilitate lignin removal from both pine and birch kraft pulps. They reported a 25% reduction in the consumption of active chlorine by their pretreated pine kraft pulp or, for the same charge of active chlorine, delignification to lower kappa numbers than the reference pulp. They also reported a significant reduction in chlorine dioxide consumption by a hemicellulase-treated pine kraft pulp when it was subsequently bleached to 89-90% ISO brightness by a (D_c)EDED sequence. Papermaking properties of the hemicellulase-treated pulp were similar to those of the normal pulp. Finally, Viikari *et al.* (*51*) reported that kappa numbers of unbleached pine and birch kraft pulps were both reduced by 50% from original values by treating the pulps with hemicellulase followed by perox-

ide, with an increase in brightness concomitant with the reduction in kappa number. These pulps were comparable to chlorine-bleached pulps.

The hemicellulase systems used by Viikari and coworkers (*51, 53*) were endoxylanases and β-xylosidases produced by three organisms—*Aspergillus awamori, Streptomyces olivochoromogenes,* and *Bacillus subtilis.* Hemicellulases from the first two organisms were more effective than those from the third, and some decrease in pulp viscosity was experienced, suggesting that some cellulase activity occurred, with cellulase present as an impurity in all preparations. Bernier *et al.* (*54, 55*) have produced clones of *E. coli* that produce endoxylanase and β-xylosidase in the absence of cellulase. Paice *et al.* (*56*) and Juracek and Paice (*57*) have used these pure enzymes to increase the rate of lignin removal from pulp, reducing kappa number and increasing brightness, while fully retaining pulp viscosity and strength properties.

Bleaching with hemicellulase enzymes has shown enough promise to attract potential suppliers to work on inexpensive, rapid methods for bulk purification of these enzymes. Forintek Canada Corp. is one such company and has published information about its process (*58*), which utilizes the fungus *Trichoderma harzianum.* Enzyme-treated pulps also have been evaluated in the laboratories of pulp and paper companies (*59*).

Enzyme treatment for dissolving pulp

Biological alternatives to alkaline extraction of hemicellulases from dissolving-grade pulps has received little attention to date. Paice and Jurasek (*60*) evaluated the ability of xylanase from *Schizophyllum commune* to remove residual xylan from a bleached hardwood pulp for the industrial production of dissolving pulp. The xylan content was partially reduced, but pulp viscosity was decreased markedly. Using a cellulase-free xylanase produced by an *E. coli* clone (*54*), partial removal of xylan was again obtained, but now pulp viscosity was retained completely (*57*).

There is room for much more to be done here. Although only a small segment of the pulping industry manufactures dissolving pulp, it is nevertheless an important segment.□

Literature cited

1. Kirk, T. K., Jeffries, T. W., and Leatham, G. F., Tappi J. 66(5): 45(1983).
2. Paice, M. G. and Jurasek, L., Present and future applications of biotechnology in the pulp and paper industry, miscellaneous reports MR 61, PPRIC, Pointe Claire, Quebec, 1984, pp. 1-16.
3. Trotter, P. C., Tappi J. 69(7): 22(1986).
4. Kirk, T. K., Tappi J. 72(5): 33(1989).
5. Coombs, J., Hall, D. O., Smith, W. H., and Goodell, B. S., Biomass 15(Special Issue): 75(1988).
6. Farnum, P., Timmis, R., and Kulp, J. L., Science 219: 694(1983).
7. Megraw, R. A., Wood quality factors in loblolly pine, TAPPI PRESS, Atlanta, 1985.
8. Van Buijtenen, J. P., Proceedings of the 18th International Union of Forestry Research Organization (IUFRO) World Congress, Division 5, Forest Products, IUFRO, Vienna, Austria, 1986, p. 50.
9. Sederoff, R. R., North Carolina State University, Raleigh, personal communication.
10. Krugman, S., U.S. Forest Service, Washington, D.C., remarks made at the 1988 meeting of the North American Quantitative Forest Genetics Group, University of California-Davis, Davis, California, July 29, 1988.
11. Plant Genetic Transformation and Gene Expression: a Laboratory Manual (Draper, J., Scott, R., Armitage, P., and Walden, R., Eds.), Blackwell Scientific Publications, Boston, 1988.
12. Klein, T. M., Wolf, E. D., Wu, R., and Sanford, J. C., Nature 327: 70(1987).
13. Sanford, J. C., Trends in Biotechnology 12: 299(1988).
14. Christou, P., McCabe, D. E., and Swain, W. F., Plant Physiology 87: 671(1988).
15. Fillatti, J. J., McCown, B. H., Sellmer, J., Haisig, B., and Comai, L., Mol. Gen. Genet. 206: 192(1987).
16. Pythoud, F., Sinkar, V. P., Nester, E. W., and Gordon, M. P., Bio/Technology 5: 1323(1987).
17. Chang, H. M. and Sarkanen, K. V., Tappi 56(3): 132(1973).
18. Rydholm, S. A., Pulping Processes, Wiley-Interscience, New York, 1965.
19. Chiang, V. L. and Sarkanen, K. V., Wood Sci. & Tech. 17: 217(1983).
20. Chiang, V. L., Cho, H. J., Puumala, R. J., Eckert, R. C., and Fuller, W. S., Tappi J. 70(2): 101(1987).
21. Higuchi, T., Biosynthesis and Biodegradation of Wood Components (Higuchi, T., Ed.), Academic Press, New York, 1985, p. 141.
22. Chappell, J. and Hahlbrook, K., Nature 311: 76(1984).
23. Kuhn, D. N., Chappell, J., Boudet, A., and Hahlbrook, K., Proceedings of the National Academy of Sciences 81: 1102(1984).
24. Cramer, C. L., Ryder, C. B., Bell, J. N., and Lamb, C. L., Science 227: 1240(1985).
25. Chang, H.-M. and Sederoff, R. R., North Carolina State University, Raleigh, 1987, personal communication.
26. Sederoff, R. R., North Carolina State University, Raleigh, 1987 and 1989, personal communication.
27. Grand, C. and Boudet, A. M., Characterization and expression of lignification-specific genes, paper presented at the 3rd Chemical Congress of the North American Continent, Toronto, Canada, June 6-10, 1988, American Chemical Society, Washington, D.C.
28. Chiang, V., Michigan Technological University, 1989, personal communication.
29. Kirk, T. K. and Kringstad, K., U.S. Forest Products Laboratory, unpublished data, 1971.
30. Ander, P. and Eriksson, K. E., Svensk Papperstid. 78: 641(1975).
31. Samuelsson, L., Mjoberg, P. J., Hartler, N., Vallander, L., and Eriksson, K. E., Svensk Papperstid. 83: 221(1980).
32. Eriksson, K. E. and Vallander, L., Svensk Papperstid. 85(6): R33(1982).
33. Akamatsu, I., Yoshihara, K., Kamishima, H., and Fujii, T., Mokuzai Gakk. 30: 697(1984) (in Japanese).
34. Blanchette, R. A., Burnes, T. A., Leatham, G. F., and Effland, M. J., Biomass 8: 93(1988).
35. Myers, G. C., Leatham, G. F., Wegner, T. H., and Blanchette, R. A., Tappi J. 71(5): 105(1988).
36. Tien, M. and Kirk, T. K., Science 221: 661(1983).
37. Glenn, J. K., Morgan, M. A., Mayfield, M. B., Kuwahara, M., and Gold, M. H., Biochem. Biophys. Res. Commun. 114: 1077(1983).
38. Buswell, J. and Odier, E., CRC Crit. Rev. Biotechnology 6: 1(1987).
39. Kirk, T. K. and Farrell, R. L., Ann. Rev. Microbiology 41: 465(1987).
40. Kirk, T. K., Biochemistry 1(1): 71(1988).
41. Asther, M., Corrieu, G., Drapron, R., and Odier, E., Enzyme Microb. Technol. 9: 245(1987).
42. Leisola, M. S. A., Kozulic, B., Muesdoerffer. F., and Fiechter, A., J. Biol. Chem. 262: 419(1987).
43. Tien, M. and Tu, C. P. D., Nature 326: 520(1987).
44. de Boer, H. A., Zhang, Y. Z., Collins, C., and Reddy, C. A., Gene 60: 93(1987).
45. Smith, T. L., Schalch, H., Gaskell, J., Covert, S., and Cullen, D., Nucleic Acids Res. 16: 1219(1988).
46. Farrell, R. L., Belew, M. E., Maione, T. E., and Sadowick, B. A., FEMS Symposium: Biochemistry and Genetics of Cellulose Degradation (J. P. Aubert, P. Beguin, and J. Millet, Eds.), London, Academic Press, 1988, p. 349.
47. Biopulping consortium progress report, Tappi J. 71(7): 17(1988).
48. Kirk, T. K. and Yang, H. H., Biotech Letters 1: 347(1979).
49. Paice, M. G., Jurasek, J. J., Ho, C., Bourbonnais, R., and Archibald, F.,

4th International Conference on Biotechnology in the Pulp and Paper Industry, TAPPI, Atlanta, Ga., 1989, p. 41.
50. Egan, M., paper presented at the 2nd Annual Pulp and Paper Chemicals Outlook Conference, Corpus Information Services, Ltd., Don Mills, Ontario, 1985.
51. Viikari, L., Ranua, M., Kantelinen, A., Linko, M., and Sundquist, J., Proceedings of the 4th International Symposium on Wood and Pulping Chemistry, TAPPI, Atlanta, Ga., 1987, p. 151.
52. Yamasaki, T., Hosoya, S., Chen, C. L., Gratzl, J. S., and Chang, H.-M., Proceedings of Ekman Days International Symposium on Wood and Pulping Chemistry, Vol. II, 1981, p. 34.
53. Viikari, L., Ranua, M., Kantelinen, A., Sundquist, J., and Linko, M., Proceedings of the 3rd International Symposium on Biotechnology in the Pulp and Paper Industry, Swedish Forest Products Research Laboratory, Stockholm, Sweden, Swedish Association of Pulp and Paper Engineers, Stockholm. 1986, p. 67.
54. Bernier, R., Jr., Driguez, H., and Desrochers, M., Gene 26: 59(1983).
55. Bernier, R., Jr. and Desrochers, M., J. Gen. Appl. Microbiology 31: 513(1985).
56. Paice, M. G., Bernier, R., Jr., and Jurasek, L., Biotechnology and Bioengineering 32: 235(1988).
57. Jurasek, L., and Paice, M. G., Biomass 15: 103(1988).
58. Tan, L. U. L., Yu, E. K. C., Louis-Seize, G. W., and Saddler, J. N., Biotechnology and Bioengineering 30: 96(1987).
59. Clark, T. A., McDonald, A. G., Senior, D. J., and Mayers, P. R., 4th International Conference on Biotechnology in the Pulp and Paper Industry, TAPPI, Atlanta, Ga., 1989, p. 39.
60. Paice, M. G. and Jurasek, L., J. Wood Chem. Technol. 4: 187(1984).

Author's note: The author has just learned that Enso-Gutzeit Oy, the Finnish Co., has run tests of enzymatic bleaching at a 1000 metric ton scale. The objective—to economically reduce chlorine consumption—was achieved. A reduction of 25-30% was reported.

I thank the TAPPI Wood Chemistry Committee for the opportunity to write this review article. Thanks also to my colleagues R. C. Eckert and D. C. Johnson for their thoughtful critiques of an earlier version of the manuscript.

Received for review Oct. 5, 1989.

Accepted Oct. 16, 1989.