“Until now, manipulations of biological systems have been circumscribed by the set genetic makeup of the organisms.”

Biotechnology: applications and implications for the pulp and paper industry

Human progress in mastering nature has been slow mainly because of the difficulty in harnessing biological processes. Centuries of empirical testing have gone into learning how to domesticate animals, cultivate crops, preserve foods, feeds, and materials, ferment sugars, and combat diseases. Until now, manipulations of biological systems have been circumscribed by the set genetic makeup of the organisms. Research culminating in the 1970’s removed this limitation; biotechnology can now alter the heritable makeup of organisms. The tremendous potential of genetic engineering has generated scientific and industrial excitement and has ushered in the age of biotechnology.

Biotechnology, which is simply the technological use of biological agents, encompasses much more than genetic engineering. In the pulp and paper industry, it includes many aspects of the growing of trees, facets of processing wood and pulp, the utilization of by-products, and the management of wastes. Biotechnology research and development has the potential to alter many aspects of the industry. How fast these changes take place will be limited only by the imagination of scientists and engineers and by the commitment of management to R&D.

It is our purpose to survey the implications that the newly recognized potentials of biotechnology have for the industry. Included are the genetic manipulation of trees at the cellular level and various applications of enzyme and microbial technology. Many technical terms have been introduced or made more common by the advent of biotechnology; some of these are defined in a brief glossary (Table I).

The growing of trees

The tree

Pulpwood trees of the future will be superior to those of today. Classical genetic techniques, particularly with the commercially important conifers, have already been used to improve several species. Improved straightness, vigor,
disease resistance, and wood/pulp properties have resulted from selection and propagation of superior trees. Although they are effective, standard breeding and selection techniques are expensive, long-term prospects, requiring generations of trees.

Biotechnology R&D promises to decrease the time required for identifying and propagating selected trees. Presently, traditional vegetative propagation by various cuttings is used to clone trees with desirable traits. The success rate of vegetative propagation varies greatly with species or varieties and is particularly low for conifers (1). Where the rooting problems can be overcome, rapid production of select conifer clones (one-half million plants from one seedling in 5 yr) seems possible (1). The major problem with vegetative propagation is that selection of superior trees must come after the trees have reached maturity, at which time propagation by cuttings becomes problematic (1-3).

Plant tissue culture may provide an alternative means to clone superior trees (1-3). Plant cells excised from meristem tissues of many plants can be grown as cell suspensions or as callus in tissue culture. Either spontaneously or under the inducement of appropriate plant growth hormones, the cultured cells will become organized and form plantlets (p. 45). Success with woody species has been less common than with herbaceous species. The problem is not so much with establishing callus cultures as with the subsequent differentiation of the tissue and regeneration of plantlets. Nevertheless, over 200 woody species have been established in callus culture, representing over 30 genera in 20 families (1). In 25 instances, complete plants have been regenerated (1). Another potential problem is the genetic instability of rapidly grown cells (3). This whole area needs more attention both in the laboratory and in the field. With continuing research, the problems limiting cloning via tissue culture will in all likelihood be overcome (3).

Beyond cloning, plant cell culture offers the potential for rapid screening and selection of desirable genotypes in the laboratory. With *Populus* sp., the rate of tree growth under natural conditions was correlated with the rate of callus production *in vitro* (4). And with loblolly pine, good tissue culture bud production showed a correlation with rapid growth of progenies in the field (5). Traits such as growth efficiency, photosynthetic efficiency (low respiration) (2) and stress tolerance, resistance to diseases, frost, drought, salinity, herbicides, and heavy metals and other chemicals might be screened in tissue culture (1-3). Further fundamental research may disclose biochemical traits of cultured cells that correlate with cellulose, hemicellulose, or lignin content of the wood, fiber quality, specific gravity, extractives production, and other factors (6), widening the potential for rapid laboratory evaluation of new genotypes.

Selection of traits presupposes their existence in the plant's genetic makeup. Traditional methods of genetic manipulation have relied on the broad natural variability of genotypes within tree populations (2) or on the introduction of altered genes through mutagenesis. New methods include the transfer of specific genes into the host plant, which involves introducing foreign DNA into the host cells. Several methods are being developed to do this (7, 8), and very recently the first announcement was made that foreign DNA has been expressed by transformed plant cells (9). Through protoplast fusion, multiple gene transfer is also possible and is expected to become increasingly important over the next few decades in improving forage legumes, vegetables, and fruit crops (7). Even so, commercial genetic engineering of forest trees is probably a number of years in the future.

**Nitrogen nutrition**

Forest soils are often nutrient-poor. Although it is sometimes economical to use selected fertilizers such as phosphates, the high price of nitrogen fertilizer has curtailed its use even on certain food crops and is rapidly changing the way many crops are grown (10).

Biological fixation of atmospheric nitrogen (N\(_2\)) has the potential to offset the need for commercial nitrogen fertilizers. Symbiotic N\(_2\)-fixation is of special interest because it occurs in close proximity to the plant roots so that little of the fixed nitrogen is lost to competing organisms. Several examples are known in trees: alders nodulated by actinomycetes (11) (i.e., infected by the bacteria with formation of N\(_2\)-fixing nodules, Fig.1) and leguminous trees nodulated by bacteria of the genus Rhizobium (12). In New Guinea, an *Ulmaceae* species was recently found that is nodulated by Rhizobium and fixes N\(_2\) (13), offering the hope that a wider range of tree families can be nodulated. Conifers have not yet been shown to have symbiotic relationships with N\(_2\)-fixing bacteria.

It should be possible to develop N\(_2\)-fixing clones or varieties of the best pulpwood species among those that already fix N\(_2\). It may be possible to create hybrids between N\(_2\)-fixing species and other good pulpwood trees, perhaps by protoplast fusion. Better strains of symbiotic N\(_2\)-fixing bacteria can also be developed. To create a symbiotic N\(_2\)-fixing conifer may require genetic engineering of both the tree and the bacterium and is a long-term prospect that, nevertheless, should not be discounted.

It may be possible to use better methods of forest management to encourage free-living, N\(_2\)-fixing, micro-
organisms. One near-term possibility is to interplant \textsubscript{N}\textsubscript{2}-fixing alders, leguminous trees, shrubs, or ground-covers (14, 15) with conifer (or hardwood) crops. In western conifer forests, decaying wood has been shown to be a rich site of \textsubscript{N}\textsubscript{2} fixation (16). Correct management of forest litter may help ensure maximum rates of \textsubscript{N}\textsubscript{2} fixation by these organisms, thus enriching the soil (16). It might even be possible to select and improve the dominant \textsubscript{N}\textsubscript{2}-fixing microorganisms in the forest.

**Mycorrhizae**

Forest trees grow poorly unless their roots are colonized by symbiotic fungi that form root-fungus structures known as “mycorrhizae.” Mycorrhizae benefit trees in many ways, the major one being to enhance nutrient uptake, especially of phosphorus and nitrogen. They also have been shown to increase disease resistance, reduce root shock in out-planted seedlings, and increase tolerance to drought, salt, toxicants, and pH extremes (17, 18). Several-fold increases in the growth rates of hardwood and conifer seedlings in nursery and field situations—and frequently field survival itself—are observed after proper mycorrhizal fungus manipulation.

Even though the benefits of mycorrhizal associations to tree growth have been increasingly appreciated, little attention has been paid to mycorrhizae in forestry practice until very recently. In many parts of the world, natural inoculum of conifers is absent, and attempts to establish exotic pine forests failed until inoculum was provided (19). Because there are many species of mycorrhizal fungi that have varying benefits for a given tree on a given site, natural inoculation, where it does occur, may not provide the optimum association. Experiments on inoculation of southern pines with selected strains of the fungus

\textit{Pisolithus tinctorius} have dramatically increased survival and growth on adverse sites (Fig. 2). Progress toward commercial production of \textit{P. tinctorius} inoculum has been rapid (20), and this may well become the first fungus used for large-scale nursery inoculation. \textit{P. tinctorius}, however, is only one of more than 3,000 species that can be exploited worldwide. Much remains to be done in mycorrhizae research. One of the most serious bottlenecks is the current inability to culture the vesicular-arbuscular type of mycorrhizal fungi free of their host plants. These fungi are responsible for mycorrhizae formation in many tree species.

**Biological control of forest pests**

Spraying chemicals on forests to control insects or disease has met with only limited success, is environmentally questionable, and is often not cost-effective. Biotechnology can play an important role in developing pest-resistant varieties of trees.

Biotechnology is also playing a role in the development of biological control agents, particularly for insect pests. \textit{Bacillus thuringiensis} is a bacterium that infects the larvae of a wide range of insects (21) including the gypsy moth (21, 22), which is now causing dramatic defoliation of forests in the northeastern United States. Recent trials with \textit{B. thuringiensis} have given promising control of the gypsy moth in those forests. Several other microbial insecticides are commercially available (21). Many other microbial pathogens of forest insects are also known (21),
some of which hold considerable potential as new control agents for insects. It may also be possible to biologically control forest diseases, as has been demonstrated for chestnut blight in Italy, where a virus which kills the chestnut blight fungus was discovered (23). Genetic engineering can introduce new properties into biological control agents, such as enhanced virulence, broader host specificity, and longer shelf life.

The processing of wood

Biotechnology will indirectly affect the way pulp and paper are made because it will alter the raw material. Pines engineered to overproduce turpentine, or pulpwod with a lowered lignin content, for example, will have to be pulped differently from today’s wood. Problems with the juvenile wood, which dominates in young but merchantable trees genetically selected for rapid growth, have been recognized and are being addressed (24). Biotechnology might also affect pulp and paper manufacture directly.

Biopulping and biobleaching

Certain microorganisms are able to partially delignify wood, raising the possibility of biological pulping. In nature, lignin is decomposed primarily by white-rot fungi. Research has shown that their decomposition of the lignin in wood requires concomitant metabolism of carbohydrates. Certain fungi use the wood hemicelluloses to support lignin degradation, leaving much of the cellulose behind. Eriksson and coworkers in Sweden have produced mutants of other white-rot fungi that are also selective for the lignin and hemicelluloses (25, 26). Removing even a small amount of the lignin from wood or mechanical pulp with these fungi lowers the energy required for mechanical refining (Fig. 3). The time required for fungal pretreatment is on the order of days and might be reduced to hours by optimizing the fungal strain and the treatment conditions. Treatment might be done in connection with transport or storage. Pilon, Desrochers, and Jurasek (28) have demonstrated that mechanical pulp properties can be improved by treatment with white-rot fungi.

Another potential application of microbial technology to pulp manufacture is in biobleaching of pulps (25, 26). The idea stemmed from studies with a synthetic kraft lignin, which was shown to be decomposed by a white-rot fungus even more rapidly than was the original lignin. Subsequent experiments showed that the residual lignin in kraft pulp can be removed by the fungi. Only limited research has been completed on biopulping and biobleaching (25, 26). Prominent obstacles to both are that the fungal mutants which have been developed partially damage the cellulose and that we do not yet know how to increase the scale of the fungal treatment to commercial application. With biobleaching, it may not be feasible to use whole organisms because of the requirements for fungal growth such as nutrients and aeration. It may be possible in the future to use isolated enzymes for biobleaching or for otherwise improving pulps. Progress in the use of enzymes—and in the genetic improvement of lignin-degrading fungi—depends on a better understanding of the biochemistry of lignin biodegradation; fortunately, that understanding is rapidly being achieved (29).

Other possible applications

Other potential applications of biotechnology in processing wood can be envisioned: use of microbial enzymes to defibrillate fibers, use of microorganisms to improve pulp bonding or paper qualities, and perhaps even the use of microbes or enzymes to covalently cross-link fibers.

Alternative uses of wood

Microbial technology offers new approaches to utilizing trees that are marginal for pulp production, and for utilizing wood residues from harvesting and manufacturing.

Direct microbial conversion of wood to food is possible with fungi that are able to degrade lignin: white-rot fungi. The world’s second most important commercial mushroom, Lentinus edodes, is grown on small-diameter oak bolts (30, 31). This is Japan’s largest agricultural export, but is only beginning to be cultivated in the West. The high value of L. edodes and other wood-grown mushrooms can increase the value of scrub oaks and other poor pulpwood trees and of harvesting and manufacturing residues. Recent research has shown that cultivation of L. edodes on wood/bark mixtures is not only possible, but speeds mushroom production at least tenfold (32).

These and many other lignin-degrading fungi can also be used to pretreat wood to extract lignin, making the carbohydrates accessible to other microbes or enzymes (35, 34). The fungi might also be used to convert wood directly to certain chemicals (fungal metabolites). Yields of chemicals or of delignified wood, however, will be limited by the requirement for an approximately equal weight of carbohydrates to support lignin degradation (35).

Conversion of wood by non-ligninolytic microorganisms, such as those used in classical fermentations, requires either that the cellulose and hemicelluloses be hydrolyzed to simple sugars by direct acid treatment of the wood, or that cellulose and hemicelluloses be made accessible by pretreating the wood to disrupt the lignin. Simple steam explosion is an effective pretreatment for some species (36). Acid hydrolysates of wood have been converted to a variety of products (37)—including ethanol—on an industrial scale (Fig. 4), and they can probably be used as substrates for many other fermentations. Unfortunately, the yield of glucose from cellulose by such dilute acid hydrolysis is at most about 50%, and hydrolysis degradation products interfere with some fermentations. In contrast to dilute acid hydrolysis, enzymatic hydrolysis of the cellulose and hemicelluloses made accessible to the enzymes by some kind of pretreatment, can give yields approaching theoretical, and with negligible amounts of byproducts.
The resulting sugars are suitable for almost any fermentation.

In recent years, considerable advances have been made both in understanding how cellulolytic enzymes function and in their practical utilization (38). Particularly encouraging is the recent development of hyperproducing mutants of the fungus *Trichodelma reesei* (39). The economics of fermentations of wood based on enzyme hydrolysis are thus being improved through research.

An approach to using isolated cellulose and hemicelluloses that has recently received considerable attention is direct conversion in a single-stage fermentor. Certain microbes are being studied that can both hydrolyze these polymers and ferment the resulting sugars (40).

Technology is emerging rapidly for the fermentation of pentoses, which are readily obtained in good yield from hemicelluloses by mild acid hydrolysis of hardwoods. Utilizing pentoses in addition to hexoses has a profound effect on the economics of fermentation of total wood hydrolysates (41). Within the last 3 years, conditions and organisms have been described for the first fermentation of xylose to ethanol by yeasts (41). The last 10 years have seen a resurgence of research on all aspects of fermentation technology *per se*, and many improvements have been made. Further advances can be expected, including the expanded use of immobilized cells and enzymes, novel fermentations, and the wide use of genetically engineered microorganisms. Major research problems in the bioconversion of wood include development of processes for (a) economical pretreatment, (b) cost-effective cellulase (and hemicellulase) production and recovery, (c) improved yields and rates in pentose fermentation, and (d) improved product recovery. Extraction and recovery of lignin is often considered but might not be essential to achieving technical feasibility in wood bioconversion.

**Utilizing byproducts and managing wastes**

**Byproducts**

Production of microbial protein or fermentation chemicals from existing byproduct carbohydrate-rich streams can be increased. Three underutilized streams are significant: Spent sulfite liquors and hemicellulosic sugars from hardboard and fiberboard manufacture can be used for the production of both protein and chemicals; sulfite liquors already are so used. Cellulosic fines from pulp and paper mill primary sludge might be used for chemicals production, but toxic contaminants and variability preclude their use for microbial protein. Other possible fermentation substrates such as saccharinic acids from kraft black liquors may become available as improved separation processes emerge.

We have estimated the total amounts of sugar now available in the United States based on the sizes of the industries (42) and the characteristics of the waste streams (43). Roughly 1.2 million metric tons of sugar could be available annually from sulfite mills in North America. About 1.5 million metric tons of cellulosic material could be recovered from primary sludges in kraft mills (44, 45). Hardboard and insulation board plants produce about 150,000 metric tons of nonutilized sugar annually (46).

Each of these byproduct streams can potentially be used for the production of numerous fermentation chemicals (47) or protein. In general, however, they are not suitable for fermentation in their original state. They may contain toxic compounds such as inorganic salts or phenolic materials, the pH might be inappropriate, or the level of fermentable sugars (i.e., monosaccharides) may be too low. Cellulosic fines from sludges and hemicelluloses from hardboard manufacture must first be hydrolyzed to monosaccharides either by enzyme- or acid-based processes before they can be fermented by most microorganisms.

Biotecnical approaches to converting byproduct lignins to more useful products have only begun to be studied. Low-molecular-weight products of lignin biodegradation are so diverse (48) that their usefulness as chemicals is limited. On the other hand, altering the functionality while retaining the polymeric structure of lignins might be more attractive (25). Because lignins do not serve as growth substrate for microbes (26,33), their use as substrates for conversion to protein or other fermentation products is apparently not possible without extensive pretreatment.

**Wastes**

The pulp and paper industry already depends on microbial technology to treat its manufacturing wastes. Oxidation ponds, lagoons, Unox reactors, activated sludge beds, rotating biological contractors, and others are all ways of using mixed populations of microbes to destroy or detoxify wastes. Although some of these systems are sophisticated and all can be highly effective, they can be improved through research, particularly as concern for the environment grows. Microbes screened from natural populations, generated via mutation and selection, or through genetic engineering are being developed to degrade specific industrial wastes or recalcitrant products (e.g. 49).

An example of the potential of biotechnology is in decolorizing the effluent of kraft bleacheries. Color is due to polymeric lignin degradation products. These chromophoric materials are resistant to the microflora in current waste treatment facilities. The Forest Products Laboratory, in cooperation with North Carolina State University, has recently investigated the use of white-rot fungi to decolorize the highly colored first extraction-stage effluent (50). Similar research has been conducted at the University of Tokyo (51) and at the Pulp and Paper Research Institute of Canada (52). Results of these studies have been promising, indicating that scale-up and further development are warranted.

In the future, other more novel approaches may be used for the treatment of waste effluents. In initial studies, Johnson and Carlson (53) have shown that “mycelial papers” containing wood fiber with 5-10% fungal mycelia (grown on wastes) show acceptable paper characteristics. Selection or development of better fungal strains could increase the usefulness of mycelia in paper.

**Summary**

Biotechnology has the potential to benefit the pulp and paper industry in many areas, including the growing of trees, the processing of wood and pulp, the utilization of byproducts, and the management of wastes. New cell or tissue culture techniques can decrease the time required


## II. Potential impacts of biotechnology.

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<th>Area of impact</th>
<th>Near-term potentials (&lt;10 yr)</th>
<th>Long-term potentials (&lt;10 yr)</th>
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<td><strong>Growing of trees</strong></td>
<td>Cloning of superior trees via tissue culture. Improved nutrition via nitrogen-fixing bacteria, including actinomycetes. Improved nutrition and field hardiness via mycorrhizal fungus selection and management. Greater use of microbial insecticides.</td>
<td>Rapid laboratory selection from cell cultures of trees with some superior traits such as resistance to disease, frost, and drought. Trees with properties outside the species limit, such as lowered lignin content, increased fiber length, and high turpentine content. New &quot;species&quot; of trees with combined features of several current species. Symbiotic N₂-fixation in trees that do not naturally fix nitrogen. Genetically improved mycorrhizal fungi and N₂-fixing bacteria. Development of new microbial insecticides. Biopulping. Biobleaching. Biotechnical improvement of mechanical pulps. Biological pretreatment of wood for fermentation. Biological conversion of byproduct lignins.</td>
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<td><strong>Utilizing byproducts and managing wastes</strong></td>
<td>Fermentation of waste carbohydrate streams. Improvements in existing waste treatment processes. Biological decolorization of bleaching effluents.</td>
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for attaining and propagating superior trees, making it possible to screen large populations rapidly and to clone superior individuals. Genetic engineering will be used in the future to design superior trees. Microbial technology will improve tree nutrition and health through improved mycorrhizal fungi, N₂-fixing bacteria, and microbial pesticides.

In wood processing, biotechnology offers the tantalizing prospects of biopulping, biobleaching, and biologically improving mechanical pulps. Alternate uses of wood through bioprocessing can also be envisioned, particularly for residues and wood not suited for pulping. These uses include production of food and feed and conversion to fermentable sugars. Fermentation of carbohydrate-rich byproduct streams to chemicals or protein is already being practiced and can be expanded. Bioconversion of lignins to useful products may be possible. New, improved microbial waste-treatment processes are anticipated. The speed with which the many potentials of biotechnology are realized in the pulp and paper industry will depend in large part on the commitment of management to research and development. The most pressing research needs are probably in the basic biology, physiology, and biochemistry of trees and of key microorganisms.

### Concluding comments

The time-frame for some of the applications of biotechnology in the industry can only be surmised (Table II). The unexpected successes, breakthroughs, and failures that characterize research will soon make any estimate—and indeed much of the list in Table II—obsolete. For example, discovery of a new hormone in conifers that makes it possible to generate whole plants reproducibly from single cells in culture would greatly hasten the development of cellular cloning technology. Development of a medium that permits vesicular-arbuscular mycorrhizal fungi to be grown axenically would hasten their manipulation and use.

The most pressing research needs are probably in the basic biology, physiology, and biochemistry of the trees and key microorganisms. Gaps in present understanding of forest tree physiology and biochemistry limit the use of genetic engineering techniques. For example, although scientists are developing improved methods for transferring genes into plant cells, the genes responsible for desired traits are usually not known. Another example is that a critical need in biopulping and biobleaching is understanding the biochemistry of the fungal decomposition of lignin. Until the enzymes involved are known, sophisticated genetic manipulations will be impossible. Sustained basic research programs directed at eventual application are essential if we are to realize the potentials of biotechnology in the pulp and paper industry.

### Literature cited

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