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## Modification of lignin for the production of new compounded materials

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**Abstract** The cell walls of woody plants are compounded materials made by in situ polymerization of a polyphenolic matrix (lignin) into a web of fibers (cellulose), a process that is catalysed by polyphenoloxidases (laccases) or peroxidases. The first attempt to transform the basic strategy of this natural process for use in human craftsmanship was the ancient lacquer method. The sap of the lacquer tree (*Rhus verniciflua*) contains large amounts of a phenol (urushiol), a polysaccharide and the enzyme laccase. This oil-in-water emulsion solidifies in the presence of oxygen. The Chinese began using this phenomenon for the production of highly creative artwork more than 6,000 years ago. It was the first example of an isolated enzyme being used as a catalyst to create an artificial plastic compound. In order to apply this process to the production of products on an industrial scale, an inexpensive phenol must be used, which is transferred by an enzyme to active radicals that react with different components to form a compounded material. At present, the following approaches have been studied: (1) In situ polymerization of lignin for the production of particle boards. Adhesive cure is based on the oxidative polymerization of lignin using phenoloxidases (laccase) as radical donors. This lignin-based bio-adhesive can be applied under conventional pressing conditions. The resulting particle boards meet German performance standards. By this process, 80% of the petrochemical binders in the wood-composite industry can be replaced by materials from renewable resources. (2) Enzymatic copolymerization of lignin and alkenes. In the presence of organic hydroperoxides, laccase catalyses the reaction between lignin and olefins. Detailed studies on the reaction between lignin and acrylate monomers showed that chemo-enzymatic

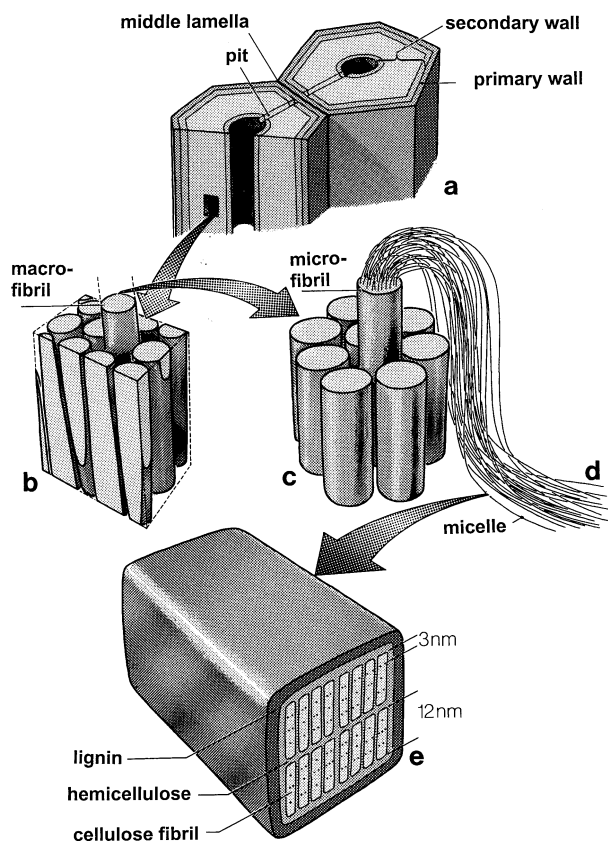
copolymerization offers the possibility to produce defined lignin-acrylate copolymers. The system allows control of the molecular weights of the products in a way that has not been possible with chemical catalysts. This is a novel attempt to enzymatically induce grafting of polymeric side chains onto the lignin backbone, and it enables the utilization of lignin as part of new engineering materials. (3) Enzymatic activation of the middle-lamella lignin of wood fibers for the production of wood composites. The incubation of wood fibers with a phenol oxidizing enzyme results in oxidative activation of the lignin crust on the fiber surface. When such fibers are pressed together, boards are obtained which meet the German standards for medium-density fiber boards (MDF). The fibers are bound together in a way that comes close to that by which wood fibers are bound together in naturally grown wood. This process will, for the first time, yield wood composites that are produced solely from naturally grown products without any addition of resins.

### Introduction

The conquest of the solid surfaces of the continents by plants was probably the most difficult venture in the development of life on earth (Gordon and Olson 1995). It took place after about 3 billion years of life in aqueous environments, first in the oceans and then in freshwater. This long lag-time is probably due to the fact that plants, which provide the basis of life for all other organisms, had to develop means to cope with the extremely low relative humidity of the atmosphere. In order to invade the continents and settle on solid soil, plants had to invent a conductive system that was able to transport rather high quantities of water at considerable speed. This meant that they had to develop pressure-stable cell walls that were able to stand the negative pressure resulting from the hydraulic laws discovered by Bernoulli. The solution to this problem was the woody cell wall, which enabled the plant not only to transport water but also

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**Fig. 1a–e** Schematic view of a woody plant cell wall at four different levels of magnification which increase from **a** to **e**. **a** Cross-section through a wood fiber showing the middle lamella and the cell wall with the primary and secondary cell walls. **b** Cross-section through a part of the secondary cell wall with macrofibrils. **c** A bundle of microfibrils. **d** The micelle strands. **e** Cross-section through a micelle, indicating the ultrastructural composition: lignin, hemicellulose and cellulose. The interspace between the wood fibers (middle lamella), the macrofibrils, microfibrils and the micelles is filled with lignin. (Drawn by G. Tambour, Forstliche Fakultät, Göttingen)

provided the structural basis for the construction of the above-ground plant body, a technical design which has yet to be surpassed by analogous human technologies (Wunderlich and Gloede 1977).

The woody cell wall is a highly structured compound material (Fengel and Wegener 1989) (Fig. 1) composed of a basic element, the lignocellulose complex, that is mechanically structured at a minimum of four consecutive levels. It is a classical compound material consisting of a fiber system embedded in a matrix. The cellulose fibers are highly ordered and mechanically strong. These fiber bundles are embedded in a film of hemicelluloses that provide a higher structural flexibility. The interspace between the carbohydrate complex is filled at all levels with a matrix compound, lignin.

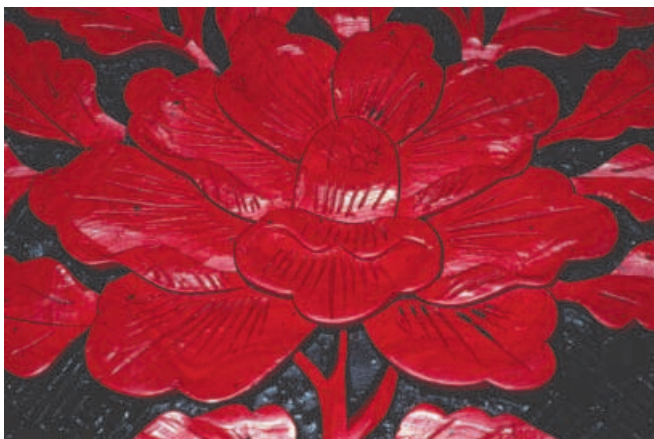
Biosynthesis of the lignin component follows a strategy that is different from the classical one by which carbohydrates are assembled (Higuchi 1997). The sequence of reactions was proposed by Freudenberg and Grion (1959)

and has been basically accepted (see Dean and Eriksson 1994; Higuchi 1997): Phenolic monomers are polymerized by the catalytic action of phenol oxidases (laccases) or peroxidases. Via a one-electron transfer reaction, the molecules are converted to radicals and polymerized to lignols, the small precursors of lignin. These react further with additional lignol radicals in a typical chain reaction to form the polymeric lignin structure. The crucial activation step, which enables lignols to react with nucleophiles, is the oxidation to quinone-methides, which is also catalysed by either phenoloxidases or peroxidases. These quinone methides then react with the oxygen of sugars to form a stable ether bond, thus linking the aromatic moiety of the lignocellulose complex to its carbohydrate part. The aromatic nucleus further reacts with additional phenol-alcohols, or lignols, to form the lignin part of the woody cell walls. These additions of more monomers to the already-existing lignin molecule, by a simple radical reaction catalyzed by either laccase or peroxidase, finally leads to the very complex structure of lignin, which forms an infinite random three-dimensional network in the middle lamella of woody plants (Goring 1989). Thus in a fully grown tree in which a continuous conductive tissue exists from the top to the roots (Zimmermann 1983), the middle lamella lignin forms a single molecule that begins a few millimeters below the top of the tree and ends a few millimeters short of the tips of its roots. In a giant redwood (*Sequoia sempervirens*), this distance can amount to about 200 m. Thus lignin is the longest single organic macromolecule on earth.

## Lacquer – an ancient biotechnology

The ingredients that form the lignocellulose complex in plant cell walls, phenols and carbohydrates together with a phenol oxidase, have been used by East Asian artists and craftsman for the creation of lacquer works for more than 6,000 years (see Watt and Ford 1991). They used the wound sap of the lacquer tree (*Rhus verniciflua*) as the material basis of their art work. When the bark of the lacquer tree is wounded, it secretes a sap, a water-in-oil emulsion composed of urushiol [60–65% (w/w)], a catechol substituted with a long unsaturated aliphatic chain, carbohydrates (gums) [6.5–10% (w/w)], the enzyme laccase [0.1–1% (w/w)] and water [20–25% (w/w)] (Kumanotani 1988). This mixture is excellently suited for plant defense against fungi, insects and phytophages. Urushiol is one of the most toxic compounds that has been discovered in the plant kingdom so far.

In the presence of oxygen, the sap eventually jells and forms a durable solid wound cover. For making lacquer, the sap is collected immediately after tapping, filtered and spread on a piece of fabric or a wooden surface (Du 1988; Burmester 1988). During the drying process, in the presence of air and high humidity, urushiol is oxidized by two agents: the phenolic part reacts with laccase and the unsaturated bonds in the side chain are oxidized directly by oxygen from the air (Kumanotani 1988). This



**Fig. 2** Contemporary Chinese lacquer artwork. The body is entirely made from solidified lacquer sap. The inner layers (*black*) are stained with graphite, the outer layers (*red*) with a plant dye

leads to a very complex reaction yielding biphenyls and nucleus-side-chain dimers (Oshima et al. 1985). Lacquer has no color of its own; therefore the artists add pigments (Fig. 2). Alternatively, the lacquer sap together with the pigments can be painted on a wooden surface. With this technique, durable paintings are created that do not lose their bright colors for millennia.

As noted above, the lacquer technique has been known for 6,000 years and is thus one of the oldest biotechnologies known. It was apparently developed either before or at least during the time when processes such as alcoholic fermentation, making bread with sour dough, and dairy techniques for milk processing were invented (Rehm and Präve 1994). It was, however, the only biotechnology in antiquity involving a cell-free system and an isolated enzyme. In addition, it was the first biotechnology to be used for making non-food products and was apparently invented about 2,000 years earlier than the making of papyri, the other important biotechnology of ancient times that also yielded a product not used for human nutrition (Hüttermann et al. 1995).

### General strategy for industrial application of the method of cell wall synthesis

A process which intends to transfer the way by which plants synthesize their cell walls or the East Asian artists produce their art on an industrial scale, needs a cheap source of phenols and an appropriate enzyme.

#### Sources of inexpensive phenols

The cheapest and most abundant source of lignin is the material derived from the pulp and paper industry, which produces technical lignins as by-products in large quantities, amounting to some 30–50 million tons per year (e.g. Glasser and Kelley 1987). For the majority of this mate-

rial, no better uses than burning have been found so far. Therefore, an enormous supply of an inexpensive raw material is available world-wide for any process that can upgrade this compound to a technically useful product.

An enzyme that is able to transfer lignin to active radicals

The obvious candidate for use in technical processes involving lignin are the enzymes involved in lignin degradation from white-rot fungi. The enzymatic activities that are able to modify native lignin (Eriksson et al. 1990; Reid 1995; Highley and Dashek 1998; Bajpai et al. 1999; Mester and Tien 2000) include:

1. Lignin peroxidase (LiP, ligninase, EC 1.11.1.7) (Tien and Kirk 1983; Glenn et al. 1983) oxidizes phenolic and also non-phenolic lignin substructures by abstracting one electron and generating cation radicals that are further decomposed non-enzymatically.
2. Manganese peroxidase (MnP, EC 1.11.1.7) (Glenn and Gold 1985) acts basically in the same way as LiP except that this enzyme uses the MnII-MnIII system as redox mediator.
3. Laccase, i.e. polyphenol oxidase (E.C.1.10.3.2) (Leontievsky et al. 1997), is widely distributed and is reported to have quite different physiological functions (Hüttermann and Haars 1987). Laccase has a lower redox potential than the two enzymes mentioned above and needs a free phenolic hydroxyl group for its action. In the presence of certain organic redox-systems, so-called mediators, the enzyme is also able to oxidize substrates, e.g. the lignin from pulp, that would not otherwise react with this enzyme (Call and Mücke 1997). Furthermore, laccase has long been known to be able to polymerize phenols (Haars and Hüttermann 1980a, b).

Although in the first decade after their discovery, research on lignin-degrading enzymes mainly focused on peroxidases, it now increasingly concentrates on laccase. The main reason for this change in paradigm is probably the fact that laccase needs only oxygen for the oxidation of its reduced stage. Thus, the difficulties associated with the use of hydrogen peroxide and its fast decay can be avoided by using laccase as the catalyst. Nowadays, laccase, which originated from white-rot fungi, can be purchased commercially from Novo Nordisk (Denmark). Several reports have demonstrated that, in the presence of laccase, lignin polymerizes both *in vivo* (Hüttermann et al. 1977) and *in vitro* (Haars and Hüttermann 1980a, b).

### Polymerization of lignin for the production of particle boards

The production of wood composites such as fiber- or particle boards always follows the same basic process. Solid wood is fragmented into small strands, chips or fi-



bers. These are then supplemented with a binder and pressed to form a wood-like structure again. Using this approach, the anisotropy of wood is reduced, and wood of small dimensions or recycled timber can be converted to a useful product. In this process, basically, only one new component is added that is not present in the original wood, i.e. the binder. At present, the most important binders for wood composites are urea-formaldehyde, phenol-formaldehyde and phenyldiisocyanate.

Nimz et al. (1972) were able to use lignin as a binder using a redox-system such as potassium ferricyanide and hydrogen peroxide as an oxidizing agent during the pressing. Unfortunately, the amount of  $H_2O_2$  required for the reaction was so high that the process was too dangerous for industrial production conditions (e.g. Roffael 1979). Nimz et al. (1972) also suggested using peroxidase and hydrogen peroxide to bind the particle boards; however, no results were presented that could serve as the basis for developing a technical process.

The approach taken by our group was to use laccase as a radical donor, because this enzyme can be rather easily produced in large quantities (Kharazipour 1996). By gluing two boards of wood together, good tensile binding strengths were achieved (Hüttermann and Haars 1981). The biggest problems were posed by the required water resistance of the particle boards. The enzyme reaction takes place in water with a water-soluble enzyme and, in principle, a hydrophilic substrate. The reaction product, however, must be water-insoluble and hydrophobic in order to meet the technical requirements. Because of the high hydrophilicity of lignosulfonate, the first boards made with this compound had good tensile strength but immediately disintegrated into pieces when they came in contact with water. The "creeping" progress that was achieved step by step during about a decade of intensive research can be followed in the relevant publications (Haars et al. 1986, 1987, 1989; Hüttermann et al. 1989, 1990; Hüttermann and Kharazipour 1996; Kharazipour 1996; Kharazipour et al. 1991; Kharazipour and Hüttermann 1992, 1998). The presently achieved solution is to work with mostly water-insoluble lignins with the addition of small amounts of petrochemical resins. In this way, good-quality particle boards can be achieved (Table 1) that are completely emission-free.

**Table 1** Technological properties of 19-mm particle boards produced with either 1% polyphenyl-methane-di-isocyanate (PMDI), 9% kraft lignin and laccase, or a combination of the two systems as a binder. The boards were pressed for 3 min at 190 °C and 1.0 Mpa (adapted from Kharazipour 1996)

	1% PMDI	Lignin/ laccase	Combination of both
Internal bond strength (N mm <sup>-2</sup> )	0.19±0.01	0.19±0.02	0.40±0.02
Swelling in water			
2 h (%)	15±1	18±2	3±1
24 h (%)	>25	>25	13±1

These results have since been confirmed by Yamaguchi et al. (1991, 1992). They used dehydrogenative polymerization of vanillic acid with laccase for the binding of thermomechanical pulp and obtained paperboard with an increased plybond strength. The copolymerization of the formed lignin with the residual lignin on the surface of the thermomechanical pulp was responsible for improving the mechanical properties (Yamaguchi et al. 1994). Jin et al. (1990) successfully used brown-rotted lignin as a binder for wood composites, together with either laccase or peroxidase.

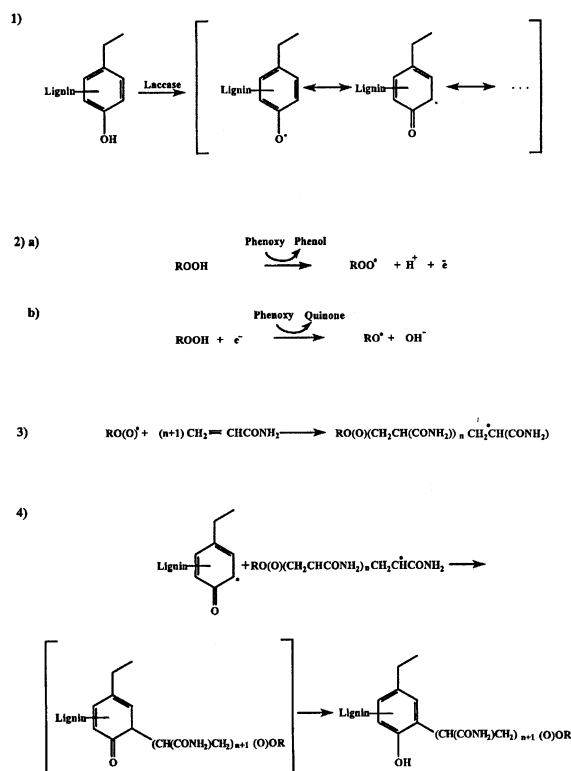
### Chemo-enzymatic copolymerization of lignin and lignin-like phenolics with acrylic compounds

Preparation of graft copolymers by introducing lignin into the main polymer backbone appears to be a suitable method to render lignin into a marketable new class of engineering plastics (Glasser 1989). Furthermore, grafting some components into the main polymer backbone may enhance the degradability by linking selected, readily degradable substituents into the polymer structure. For example, styrene graft copolymers of lignin are readily degraded by white-rot fungi, while polystyrene homopolymer was not degradable (Milstein et al. 1992).

A number of studies have been performed on the graft copolymerization of acrylic monomers onto lignin using initiation by either chemical radical starters (Meister et al. 1984, 1991; Chen et al. 1986; Huang et al. 1992) or irradiation (Koshijima and Muraki 1968; Phillips et al. 1972). All chemical initiator systems are based on peroxide compounds that form hydroxy or alkoxy radicals after homolytic or reductive cleavage of the peroxide bond. Meister et al. (1984) produced both a graft polymer and a homopolymer simultaneously in a radical process. The initiating system consisted of a chloride ion, added as  $CaCl_2$ , and a peroxide species such as hydrogen peroxide or dioxane peroxides.

Chemo-enzymatic initiation of the copolymerization of organosolv-lignin and acrylamide in aqueous dioxane (70%) was recently described. The investigators used a phenoloxidase (laccase) to generate lignin radicals and dioxane peroxides formed by auto-oxidation of the solvent (Mai 1998; Kharazipour et al. 1998; Mai et al. 1999, 2000b). These results indicate that phenoxyradicals alone, as reactive sites in lignin, possess insufficient reactivity to directly start copolymerization with acrylamide; instead, organic peroxides are also needed.

The proposed reaction mechanism (Fig. 3) summarizes the results obtained so far. It assumes that enzymatically generated phenoxy radicals can either produce alkoxy or peroxy radicals by oxidizing (Fig. 3, 2a) or reducing (Fig. 3, 2b) the peroxide. These more reactive radicals in turn are able to initiate the polymerization of acrylamide (Fig. 3, 3); The radical end of the "living" acrylamide homopolymer then combines with the phenoxy radical in a "quenching" reaction (Fig. 3, 4).



**Fig. 3** Proposed mechanism for the reaction of lignin with acrylamide catalyzed by laccase in the presence of hydroperoxides. For an explanation see the text. (From Mai 1998)

The graft efficiency of chemo-enzymatic grafting is significantly higher than in the non-enzymatic systems described previously. Whereas for the latter the acrylate homopolymer moiety was reported to be 50–60% (Chen et al. 1986) or 50% (Meister et al. 1984), only about 10% homopolymer was obtained by chemo-enzymatic grafting (Mai 1998; Mai et al. 1999, 2000a, b). The increased graft efficiency observed upon addition of laccase to the reaction medium can be explained by a higher ratio of lignin radicals than initiated acrylic chains, making the combination of these two types of radicals, i.e. grafting, more probable.

In a further study, it was attempted to incorporate lignin-like phenolic compounds into the backbone of acrylic polymers by means of chemo-enzymatic initiation (Mai et al. 2000c, in press). Various lignin-like phenolics were tested for their ability to be copolymerized with acrylamide and acrylic acid. The combined initiation by both laccase and a Fenton-like reaction generally resulted in a higher rate of incorporation than observed with the Fenton-like reaction alone.

The copolymers showed a strong variation in their average molecular weight ( $\bar{M}_w$ ) depending on the phenol applied as comonomer. It was generally observed that the initiation of laccase and *t*-butylhydroperoxide alone yielded a higher  $\bar{M}_w$  with both acrylic monomers than induction by the Fenton-like reaction or a combination of both initiator systems. This difference was espe-

cially high in the acrylic acid copolymers. The data show that chemo-enzymatic copolymerization allows control of the molecular weights of the reaction products.

Chemo-enzymatic grafting of lignin and phenols provides a method to produce a new type of water-soluble polymers with potential applications as drilling muds, cobuilders in washing detergents, or dispersion agents. The reaction conditions required are moderate and the molecular weight of the resulting copolymers can be specified with a simultaneous enhancement of the graft efficiency.

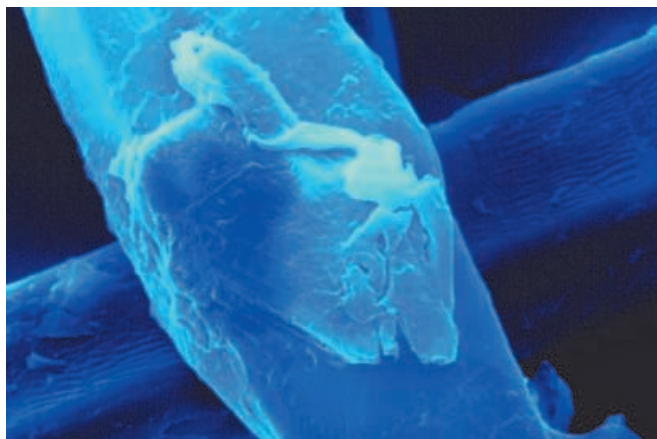
### Enzymatic activation of wood fibers for the production of wood composites

Several strategies have been developed to utilize the binding material that glues natural wood together for the binding of wood composites. The first attempts in this direction were published by Klauditz and Stegmann (1955), who demonstrated that water-resistant bonds can be obtained by pressing at high forces ( $100 \text{ g cm}^{-3}$ ) and temperatures ( $200 \text{ }^\circ\text{C}$ ) for rather long periods of time. Under these conditions, binding is obtained by pyrolytic degradation of the cell wall constituents. More recent reviews on this approach have been published by Zavarin (1984), and Ellis and Paszner (1994). Using the procedures described above, wood composites with reasonable mechanical properties can be achieved. The remaining problem to be solved is the high swelling of these boards when in contact with water. Therefore such composites have not yet been produced commercially.

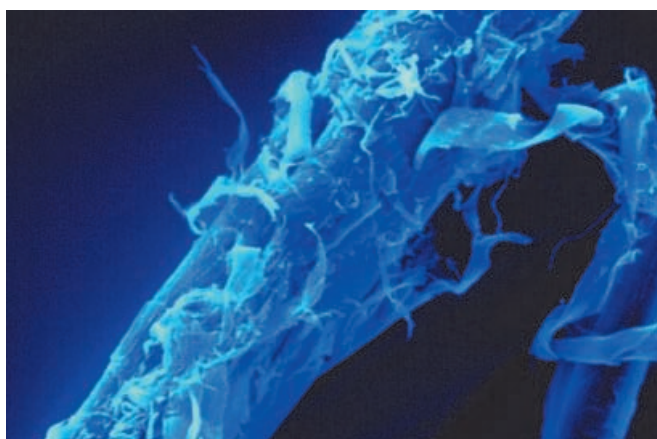
Indications that treatment with fungal enzymes alone may not only loosen the mechanical strength of wood, but may even confer intrinsic binding forces to the cell walls of fibers treated with fungi came from studies by Kühne's group (e.g. Kühne 1993). They showed that wood chips incubated via solid-state fermentation with either white- or brown-rot fungi not only have a lower demand for energy to refine them to fibers, but also a decreased demand for petrochemical resins. In addition, a definite binding of the fibers when pressed, without the addition of glues, was obtained.

During the wood fiber production process, the lignin of the middle lamella, which is the natural glue between two woody cells, is plastified at temperatures above its glass-transition point. During this process the cells separate. After cooling down to room temperature, the lignin solidifies again and forms a glassy crust on the surface of the wood fiber, which forms a barrier and reduces the binding strength of any added resin (Wagenführ 1988).

Kharazipour and Hüttermann (1993) have shown that treatment of fibers with laccase leads to a gradual increase in the  $\text{OD}_{280}$  of the supernatant. Inspection of the treated fibers in a scanning electron microscope revealed that the originally existing crust of lignin (Fig. 4) was removed completely by the enzyme treatment (Fig. 5). These data indicate that the lignin on the fiber surface is indeed susceptible to reaction with the enzyme. It there-



**Fig. 4** Surface of a wood fiber made for the production of fiber boards. The lignin from the middle lamella is plastified during the refining process and forms a crust on the surface of the fiber. (From Kharazipour and Hüttermann 1993)



**Fig. 5** Surface of a wood fiber incubated for 12 h with laccase. The crust on the surface is loosened by the enzyme treatment. (From Kharazipour and Hüttermann 1993)

fore can be expected that, during a relatively short incubation with laccase, lignin is converted into a good substrate for laccase. Thus, conditions suitable for the production of fiber boards by incubation of the fibers with laccase were tested (Kharazipour and Hüttermann 1993; Kharazipour 1996; Kharazipour et al. 1997).

Table 2 shows the results obtained following incubation of the fibers with laccase at different pH values. After 12 h, the mechanical properties of the boards pressed exactly followed the pH curve: maximal internal bond strength and minimal swelling were obtained at pH 5, which is the pH optimum of laccase. Thus, for the first time it was possible to produce wood composites without the addition of binders; instead the composites were formed solely by activation of the same intrinsic adhesive forces that act in the formation of solid wood.

Not only is laccase able to activate the lignin of the fiber surface, the same is true for peroxidases also (Kharazipour et al. 1998a, b). The required German stan-

**Table 2** Technological properties of fiber boards as a function of the pH of the laccase solution. The fibers were incubated in the laccase solution for 12 h and pressed for 5 min at 190 °C and 1.0Mpa (adapted from Kharazipour 1996)

pH	Thickness (mm)	Density (g cm <sup>-2</sup> )	Internal bond strength (N mm <sup>-2</sup> )	24 h Swelling (%)
4.0	5.1	0.78	0.60	36
5.0	5.4	0.78	0.95	23
5.5	5.3	0.77	0.80	21
6.0	5.0	0.76	0.35	26
7.0	5.0	0.78	0.38	42

dards, DIN-Norm, for fiber boards are fulfilled by both types of boards. This work has been confirmed by the studies of Felby et al. (1997a, b, 1998).

### Future perspectives

The processes described in this review have the advantage in that the manufacturing techniques are simulations of a natural process. The products do not pose emission problems either during their production or application. In addition, the compound materials based on lignin are highly compatible with the terrestrial carbon cycle and can be recycled by composting. Owing to these properties, a rather high acceptance of these products can be expected. Since laccase has recently become commercially available, the speed of product development should increase considerably.

The techniques described above are at present at different stages of technological implementation. The most advanced one in this regard is the production of fiber boards. Owing to recent developments in the wood-composite market, it is apparently the most promising development in this field. A pilot plant was established on the premises of the Forstbotanisches Institut in Göttingen. During the next 2 years, scale-up experiments that are necessary to start industrial production will be carried out.

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