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MINI-REVIEW

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Biotechnology in the wood industry

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Abstract Wood is a natural, biodegradable and renewable raw material, used in construction and as a feedstock in the paper and wood product industries and in fuel production. Traditionally, biotechnology found little attention in the wood product industries, apart from in paper manufacture. Now, due to growing environmental concern and increasing scientific knowledge, legal restrictions to conventional processes have altered the situation. Biotechnological approaches in the area of wood protection aim at enhancing the treatability of wood with preservatives and replacing chemicals with biological control agents. The substitution of conventional chemical glues in the manufacturing of board materials is achieved through the application of fungal cultures and isolated fungal enzymes. Moreover, biotechnology plays an important role in the waste remediation of preservative-treated waste wood.

Introduction

Wood is an environmentally friendly, biodegradable, nontoxic, renewable raw material which shows a neutral CO_2 balance in the case of sustainable cultivation. Against the background of global warming (the greenhouse effect), wood is an important carbon sink. About one-third of the world′s land surface is covered by forests containing a total growing stock of some 386,300 million m^3 of wood (http://www.fao.org/forestry/fo/fra/index_tables.jsp). From this stock, 3.5×10^9 m^3 are harvested yearly. This volume corresponds to about 2.1×10^9 t of roundwood (Volz 1995).

Wood is a polymeric composite whose biological and technical properties are mainly determined by the chemical composition of the cell wall. Wood cell walls are made up primarily of cellulose, hemicelluloses (polyoses) and lignin. The tensile strength of wood fibres is primarily determined by cellulose and hemicelluloses, while lignin mediates adhesion between the fibres. Cellulose, the major wood component, makes up approximately half of both softwoods and hardwoods. It is a linear polymer of high molecular weight, exclusively built up by 1–4 glycoside-linked molecules of β-D-glucose. The chains of the more complex hemicelluloses are much shorter than those of cellulose but they usually bear side-groups, such as monosaccharides and acetyl groups, and in some cases they are branched. The constituents of hemicelluloses are hexoses (glucose, mannose, galactose) and pentoses (xylose, arabinose; Fengel and Wegener 1984). Lignin is a three-dimensional polymer of phenylpropanoid units which are oxidatively polymerised by peroxidases or phenoloxidases during lignin biosynthesis (Freudenberg and Neish 1968; Nimz 1974; Boudet et al. 1995). In addition to the cell wall polymers, the properties of wood are strongly influenced by extractives, accessory compounds extractable by solvents of different polarity. These compounds encompass phenolics, terpenes, carbohydrates, fats, waxes and others (Fengel and Wegener 1984).

Besides solid wood, wood-based products such as paper products (various types of paper, cardboard) and board materials (particle, fibre, strand boards) are of industrial importance. Whilst board materials are traditionally and to date most economically produced by chemical gluing (Maloney 1993), the enzymatic production of wood-based board materials is also possible. Paper mainly consists of wood fibres or cellulose fibres which are conventionally obtained by mechanical or chemical pulping. Biotechnological methods in the pulp and paper

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industry include bio-pulping, enzymatic bleaching, pitch control and purification of wastewater. These processes were reviewed by Jaeger and Reetz (1998), Messner (1998), Bajpai (1999), Spiridon and Popa (2000) and Gutierrez et al. (2001). Small-sized timber and industrial wood residues, such as saw dust, chips, slab wood and edgings, possess additional industrial potentials. They may serve as growth substrates for edible mushrooms (reviewed by Kües and Liu 2000) or can be converted by (enzymatic) hydrolysis of cellulose and hemicellulose into ethanol as a potential substitute of, or complement to, gasoline (reviewed by Galbe and Zacchi 2002). This review on biotechnology in the wood industry focuses on biotechnological aspects in the production, handling, preservation and disposal of solid wood and wood-based materials, in particular particle and fibre boards.

Improvement of preservative penetration in wood

A limited natural durability of wood is typical for most of the European species. This necessitates the chemical protection of wood in service against fungal and insect decay. Conventional preservatives are generally based on biocides, such as copper, chromium and arsenic (CCA), creosotes, boron salts or organic compounds (Crawford et al. 2000; Peylo and Willeitner 2001; Humphrey 2002). These preservatives are either liquid (e.g. creosote) or are formulated in aqueous solutions or emulsions. To achieve sufficient protection, a deep and even penetration of these biocides is required. However, many wood species resist the uptake of such liquids (Morrell and Morris 2002).

Anatomical features are known to be the main reason for the strongly reduced ability to transport liquids into dried wood. In the sapwood of a living tree, water is transported from the roots to the leaves through specialised cell elements (tracheids in softwoods, vessels in hardwoods) which are connected to each other by cell openings, so-called "pits" (Fig. 1). After the felling of the tree and during the maturing process of heartwood formation, the wood dries out and the pit openings close down, often being incrusted by resin acids, monoterpenes, fatty acids and other extractives. These extractives are not limited to pit structures but are additionally deposited in the cell walls and partially in the cell lumen, hindering liquid transport (Bauch et al. 1974).

Various physical and mechanical methods have been studied to overcome the refractory behaviour of the heartwood and sapwood of many commercially interesting wood species. Some technical approaches are state-of-the-art, such as vacuum and pressure treatment, adapted drying schemes, the use of steam or compression forces, boring small holes or incising the wood with needles or lasers, but a sufficient impregnation depth to guarantee full protection is not reached (for a review, see Militz 1993a; Morrell and Morris 2002). Strong acids, alkalis or chelating agents have also been tested as a means of dissolving the extractives blocking the pit structures during the preservative treatment. Although partially successful, in most cases the effect is accompanied by undesirable side-reactions, such as strong swelling, cell wall destruction and strength losses (Yoshimoto et al. 1972; Tschernitz 1973; Kumar and Morell 1993).

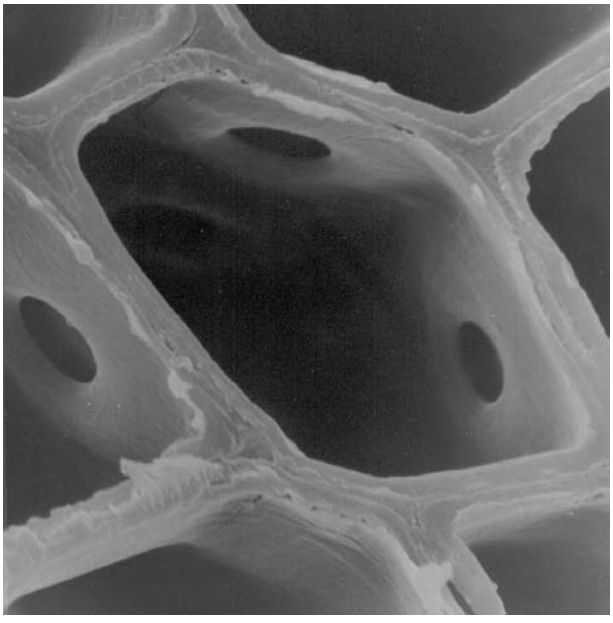

Fig. 1 Scanning electron micrograph of the cell openings, so-called "pits", which connect the water-carrying cell elements (tracheids in softwoods, vessels in hardwoods)

Pits are mainly composed of pectins, various hemicelluloses and cellulose (Bauch et al. 1968). Degrading the pits by commercial enzyme preparations, such as pectinases and cellulases, has been attempted in order to improve the permeability of several European, American and Japanese wood species (Nicholas and Thomas 1968; Bauch et al. 1970, 1973; Meyer 1974; Adolf 1975, 1976; Morishita et al. 1986; Ohkoshi et al. 1987; Militz 1993a). The permeability of spruce (*Picea abies*) sapwood and heartwood increased most with mixtures of different hydrolytic enzymes giving a broad spectrum of enzymatic activities. Scanning electron micrographs showed the expected degradation of pits, considerably improving the wood permeability. In addition, the opening of other liquid pathways (resin channels, intercellular spaces) contributed to the refinements (Militz 1993b). However, for a technically feasible improvement, several weeks of treatment time were needed. From a practical point of view, it would be advantageous to have a fast, one-step process that uses a mixture of hydrolytic enzymes diluted in a liquid wood preservative which does not negatively affect the enzyme activity (Militz and Homan 1993).

The influence of micro-organisms on the permeability of wood has been known for some decades. Suolahti and Wallen (1958) found that certain bacteria improved the permeability of softwood after storage of the wood under wet conditions. Bacterially degraded wood had a lower pectin content than sound wood and, as shown by scanning electron microscopy, the pectin-rich bordered

pits of softwood species were broken down (Greaves 1970; Adolf et al. 1972). However, these findings did not lead to a commercial process, mainly due to the relative long storage time (months or years) and the uneven wood preservative distribution and surface coating uptake.

Besides bacteria, moulds are able to colonise wood without attacking the structural wood components. Wood infected by *Trichoderma* species was reported to have an increased permeability (Johnson and Giovik 1970). An extensive screening programme examined the production of cellulase, pectinase and amylase enzymes by *Trichoderma* species. *T. viride* and *T. aureoviride* were selected as the most effective for further permeability studies. A much higher preservative uptake was recorded in the sapwood of spruce (*P. abies*) logs stored for 4 weeks at 25 °C after fungal inoculation. The heartwood, however, remained unaffected, even after longer exposure times (Rosner et al. 1998). In further laboratory experiments, spores of the selective delignifying white-rot basidiomycetes *Dichomitus squalens* and *Phanerochaete chrysosporum* were suspended in a nutrient solution and used to inoculate spruce wood surfaces. Preservative uptake in both sapwood and heartwood increased considerably after a relatively short period (2–3 weeks; Rosner et al. 1998; Tucker et al. 1998).Thermal sterilisation of the wood surface enables rapid colonisation by selected fungi (*Trichoderma*, *Gliocladium*, *Phanerochaete*, *Dichomitus* species). An improved process for wood treatability by fungi was recently patented which includes a thermal sterilisation step prior to fungal inoculation (Messner et al. 2002).

Biological protection of wood against decay, microbial staining, fungal moulds and insect pests

The major biological threats and pests of wood belong to the fungi and insects. These organisms cause detrimental effects in wood quality, including unwanted staining and various forms and levels of decay and browsing damage. Bacterial degradation of cell wall polymers occurs when wood is exposed to soil contact (tunnelling and erosion bacteria), while only the pit structures are degraded during storage in water (Clausen 1996). In addition, bacterial and particularly mould infestations give rise to hygiene problems. At harvest time, particular precaution is needed. Further handling depends on the usage and environmental circumstances in which the wood and wood products are kept. Chemical methods of wood protection are powerful (Crawford et al. 2000; Peylo and Willeitner 2001; Humphrey 2002), but incur certain environmental and health risks (for examples, see Woods 1994; Westlund and Nohrstedt 2000; Decker et al. 2002; Hingston et al. 2002). Strategies for the biological control of microbes and insects offer environmentally friendly wood protection with low health risks. Biological control agents (BCAs) are advantageous in that they are specific in targeting a biological hazard and are readily biodegradable. However, in contrast to chemical agents, BCAs might be more sensitive to climatic conditions and adequate handling requires a greater knowledge. Since they are mostly living materials, BCAs have a limited shelf-life, but this problem might be corrected by improving their formulation.

Biological control after felling

Following cutting and during the period of drying and storage in the forest and sawmill yard, which lasts several months, timber is specifically susceptible to decay and microbial staining until the water content within the wood decreases to about or below 25%. Timely felling during the winter and suitable storage can prevent microbial infection, in particular mould, that easily occurs in very humid climates and poor aeration. In addition, biological measures are feasible against unwanted surface discoloration, sapwood-staining and decay (Graf 2001).

There is an increasing list of microbes reported from laboratory tests to have antagonistic effects against various kinds of decay and stain fungi (Graf 1990). Unfortunately, experience shows that observations made in laboratory tests are often not reliable when transferred to outdoor conditions (Graf 2001). Nevertheless, screening for micro-organisms suitable for developing efficiently functional and reliable BCAs is ongoing, due to political favour towards environmentally friendly protection measures. Application of suspensions of 10^6 spores/ml of the filamentous ascomycete *G. roseum* in wood wafer tests provided satisfactory protection against colonisation by a number of unwanted fungi in western hemlock, white spruce, jack pine, amabilis and balsam fir but not in Douglas fir, white birch and trembling aspen (Yang and Rossignol 1999). Under laboratory conditions, the visual deteriotation of wood blocks of *Pinus sylvestris* by mould and sapwood-staining was found to be reduced by some bacterial isolates, due to the production of antifungal volatiles (Payne et al. 2000). Isolates of the yeast *Debaryomyces* reduced fungal growth on wood blocks in vitro under conditions of high relative humidity and a variety of temperatures at 15–25 °C, conditions that are favourable to fungal development. Protection is reported to be successful against a mixture of wood-mould fungi at a spraying rate of 10^8 colony-forming units (CFU)/cm^2 and against sapwood-staining fungi at 10^6–10^8 CFU/cm^2 (Payne and Bruce 2001). The treatment of wood with bacteria or yeasts before, during or even after infection by the target fungi was found to prevent wood spoilage. These single-celled, easy to produce and formulate microbes are therefore good candidates for application as short-term BCAs, particularly during kiln-drying and short-term storage at sawmills (Payne et al. 2000).

Microbial sapwood-staining is mostly caused by ascomycetes and related hyphomycetes belonging to *Ophiostoma*, *Ceratocystis*, *Cladosporium*, *Alternaria* and other genera, but it can also be due to infection by the basidiomycete *Aureobasidium pullulans* (Yang and

Rossignol 1999; Fleet et al. 2001). Remarkably, many sapstain fungi cause little or no destruction and strength loss in the wood (Blanchette et al. 1992a). However, unsightly staining represents a loss of value for the two main uses of wood: the production of solid wooden objects and the production of paper and cardboard, in which wood is pulped and fiberised (Vanneste et al. 2002). Sapstain fungi live on sugars, lipids and resins present as storage and protective materials within the ray parenchyma and resin canals of the sapwood (Martinez-Inigo et al. 1999; Fleet et al. 2001). Most sapwood-staining is within the grey/blue/brown/black range. This type of discoloration (commonly referred to as bluestain) comes from the melanin incorporated in the fungal cell walls of hyphae colonising the ray parenchyma (Brisson et al. 1996). The secretion of secondary fungal metabolites, like naphthoquinone pigments in the case of pink-staining *Arthrographis cuboidea* (Golinski et al. 1995) and extended quinones in the case of green-staining *Chlorociboria* species (Saikawa et al. 2000), also causes sapwood discoloration. Analysis of Italian intarsia panels of the fifteenth century showed that green *Populus* wood stained by *Chlorociboria* found some use in former times (Blanchette et al. 1992b). In Canada, marketing strategies have now started to make bluestain wood acceptable and fashionable as "Denim Wood" or "Denim Pine" for the furniture industry (Tracy 2002). If broadly successful, natural fungal colouring of wood might even be provoked on purpose. However, in most wood applications, fungal sapwood-staining is still unwanted. Adverse fungal sapwood infection might be inhibited either by the colonisation of sapwood by micro-organisms competing for the nutrient source or by the production of suitable metabolites preventing fungal growth. *Trichoderma* species have been shown to control sapstain fungi by both devices. Vanneste et al. (2002) report that *T. harzianum* is significantly better in the internal tissues of debarked logs than a conventional chemical fungicide (NP-1) but is not as good as wood treatment with plant oils containing oxygenated alcohols and phenolic monoterpenes.

A problem with potential fungal control agents competing for the sapwood nutrients is that some themselves may disfigure the timber, due to their own pigments (Smouse et al. 1999; Payne et al. 2000). Agar screening tests promise that melanin produced by infecting bluestain fungi might later be removable from wood for example by enzymes of the yeast *Galactomyces geotrichum* (Ratto et al. 2001). The white-rot fungus *Phlebiopsis gigantea* is shown to effectively inhibit bluestain *Ophiostoma* species in wood colonisation in both laboratory and field trials. Moreover, *P. gigantea* is able to decolourise previously stained sapwood by parasitising *Ophiostoma* hyphae (Behrendt and Blanchette 2001).

The currently most elegant biological solution to deal with the sapstain problem is the inoculation of freshly sawn timber by melanin-deficient mutants of *O. piliferum* (Behrendt et al. 1995). The United States product Cartapip 97 is a market formulation of such an albino mutant. Originally, it was designed to remove the pitch from wood chips (resins, fatty acids) in pulping, in order to save on bleach chemicals and decrease effluent toxicity in pulp- and paper-making. As a side-effect, treated wood chips show better resistance to infection by other micro-organisms, including sapstain fungi (Blanchette et al. 1992a; Farrell et al. 1993; Wang et al. 1997; Dorado et al. 2000). When applied in the laboratory or in the field on freshly sawn wood before challenging with other fungi, wood colonisation by sapstain fungi is strongly inhibited (up to 100%). Moreover, some protection still takes place when simultaneously applied with other fungi but, once the wood is colonised by other strains, Cartapip 97 has little effect (Behrendt et al. 1995; White-McDougall et al. 1998).

To understand wood colonisation and challenging sapstain fungi by Cartapip 97, the white *Ophiostoma* mutant was transformed with the green fluorescent protein (GFP) gene *gfp* of the jellyfish *Aequorea victoria* and the transformants were monitored within wood by GFP expression (Lee et al. 2002). Since genetically modified organisms (GMOs) should not be carelessly released into the environment, other markers must be identified for detection in the field experiments needed for evaluating ecological safety before Cartapip 97 can be registered as a BCA in various States. Biological field trials of Cartapip 97 were recently carried out in Germany under the special legislative and controlled conditions of this country. Here, the endogenous β-tubulin gene, together with two sequence-specific DNA primers (Cat1, Cat2), served as a molecular marker to follow-up Cartapip 97 in field-tested logs. The marker also appears to be useful in other European countries and in New Zealand, Alberta and British Columbia, but it has limitations in monitoring Cartapip 97 in certain regions of the United States and Canada (Schroeder et al. 2002). In South Africa, quarantine restrictions have so far blocked the import of Cartapip 97. Dunn et al. (2002) analysed the pathogenic potential of Cartapip 97 to native pine species. The authors claim Cartapip 97 is not a virulent pathogen to local pines and conclude it is therefore safe for use in South Africa.

Biological control of timber in service

Wood has many potential functions in human societies, for example as an indoor building material and in outdoor constructions. The moisture content of timber is crucial with respect to destructive microbial and insecticidal confrontations. Wood with a water content of 12% or below, as typically found within buildings, is not susceptible to biological threats. Wood moisture of 12–18% enables attack by insects. Infestation by fungi (moulds, staining fungi) is also possible when the moisture content rises (sporadically) above 18%, as for example observed outdoors for roof-protected wood and indoors in humid rooms. Wood degradation by basidiomycetes (white-rot, brown-rot) requires a moisture content above the fibre saturation point (about 28–33%).

Beyond this point, unbound water is found in cell lumina. Permanently wet wood in soil contact is vulnerable to fungi (soft-rot) and bacteria (Graf 2001).

Building mycology and microbial biodeterioration is a matter of the internal environmental conditions: humidity, temperature and ventilation. Continuous monitoring and preferentially environmentally friendly preventative maintenance is needed for conservation, particularly in rooms regularly exposed to higher humidity (Singh 1999; Graf 2001; Palfreyman et al. 2001). The dry-rot fungus *Serpula lacrymans* is a typical brown-rot basidiomycete found uniquely destructive in buildings under certain environmental conditions (Palfreyman et al. 1995). Some *Trichoderma* isolates prevented *Serpula* colonisation in laboratory wood-block tests, independently of whether they were viable or non-viable. However, these ascomycetes could not stop decay of already infected blocks, making the isolates only valid for prevention (Score et al. 1998). One mode of antagonism appears to employ inhibitory volatile organic compounds (VOCs) produced by the *Trichoderma* isolates (Humphries et al. 2002). In particular, the aldehydes heptanal, octanal, nonanal and decanal and related ketones were inhibitory to growth of a range of brown- and white-rot fungi, *Trametes versicolor*, *Neolentinus lepideus*, *Postia placenta* and *Gloeophyllum trabeum* (Bruce et al. 2000; Humphries et al. 2001). The production of VOCs by *Trichoderma* is determined by cultural age and medium composition, mainly by the amino acids available (Bruce et al. 2000) and less by the sugars available (Canessa and Morrell 1997). The amino acid content present in the various timbers to be protected therefore plays a decisive role in the effectiveness of antagonism of decay fungi by *Trichoderma* (Bruce et al. 2000). In a field and cellar trial set-up, the most promising *T. viride* strain (T60) had a protective effect against basidiomycete decay fungi and against soft-rot and sapstain development (Brown and Bruce 1999; Brown et al. 1999). *Trichoderma* strains are also bactericidal through the production of bacteriolytic enzymes (Manczinger et al. 2002), but their effects on the growth of bacteria in wood have not yet been evaluated.

Persistence in protection by *Trichoderma* or other fungal antagonists is likely not achievable, but there can be at least a certain prolongation of the life-time of wood products (Canessa and Morrell 1997; Graf 2001). Indoor or outdoor usage and the general moisture content of the timber can contribute to the economic success of a preventive *Trichoderma* BCA application; and it might be limited to wood less challenged by environmental conditions (Graf 2001). Application of the commercial agent BINAB FYT (pellets of spores and mycelial fragments of *T. polysporum*, *T. harzianum* and *Scytalidium* sp. FY) to impregnated distribution poles under environmentally demanding outdoor conditions gave variable results. The brown-rots *Lentinus lepideus* and *Antrodia carbonica* were inhibited even after 7 years, whereas protection of the pole interiors against the more powerful white-rot *T. versicolor* failed (Bruce et al. 1991).

In terms of the various kinds of insects attacking timber in service (for example ants, house longhorns, anobiid beetles), efforts to develop an efficient BCA focus on gluttonous subterranean termites (Graf 1990)—in the USA, losses due to termite damage and control is estimated to approach U.S. $ 2×10^9 annually (Culliney and Grace 2000; Rath 2000). The entomopathogenic hyphomycetes *Beauveria bassiana* and *Metarhizium anisopliae* are most promising in biological termite control (Culliney and Grace 2000; Le Bayon et al. 2000; Grace 2003; Milner 2003; Wang and Powell 2003), but pathogenic bacteria (for example *Serratia marcescens* Bizio) causing insect mortality upon ingestion are also available (Graf 1990; Connick et al. 2001; Grace 2003). The most virulent *Metarhizium* strains are isolated from termite-associated material (Milner 2003), whereas *Beauveria* isolates from other sources are equally as good as strains originating from termites (Wang and Powell 2003). Treatments might either be targeted on the whole colony or on individual termites that subsequently distribute the biocide in their communities (Rath 2000). In Petri dish experiments, spores of mycoinsecticides were transferred by infected workers to healthy termites and total mortality reached 50–100% within a few days to weeks (Le Bayon et al. 2000; Wright et al. 2002; Wang and Powell 2003). However, termites show strong alarm and defence responses towards spore-dusted individuals (rapid bursts of longitudinal oscillatory movement, aggregation of untreated insects, grooming, biting, defecation, burial of the infected termites). For successful fungal control in the field, this social behaviour must be overcome by strategies such as masking the repellency of spores, addition of attractants, definition of dosage levels below alarm thresholds and selection of less detectable strains. Once identified, a repellent might also be exploitable in the development of termite-repellent product additives and barriers (Staples and Milner 2000; Myles 2002; Milner 2003).

Beauveria and *Metarhizium* are amendable to DNA transformation (Valadares-Inglis and Inglis 1997; Sandhu et al. 2001), which should allow the construction of mycoinsecticides with better-defined characters. Increasing the copy number of the homologous protease gene *pri1* in *M. anisopliae* by transformation led to enhanced secretion of the cuticle-degrading protease and, when tested with the moth *Manduca sexta*, to a 25% reduction time to death and a 40% reduction in food consumption (St. Leger et al. 1996).

Mechanism of white- and brown-rot decay

A major part of the biotechnological approaches in the wood and forest product sector focuses on exploiting wood-decaying basidiomycetes and their extracellular enzymes. Wood decay by basidiomycetes can be generally divided into two groups: Some species utilise lignin to the same rate than cellulose and hemicellulose or preferentially remove lignin and hemicellulose from the

wood cell wall (Eriksson et al. 1990). This decay-type is referred to as white-rot, since wood in the later stages of attack essentially consists of white cellulose. It is mainly associated with hardwood decay. Predominant in softwoods, brown-rot fungi remove cellulose and hemicellulose, while lignin is mineralised to a minor degree. Nevertheless, chemical modification of lignin occurs, such as demethylation and oxidation (Kirk 1975; Jin et al. 1990a). Due to an increased proportion of oxidised brown lignin, the decayed wood darkens to a brown colour (Highley and Dashek 1998; Green and Highley 1997). The overall biochemical mechanisms of both white- and brown-rot decay are not fully elucidated.

In white-rot fungi, wood polysaccharides are degraded during the primary metabolism of the fungus, while lignin degradation due to the variety of chemical bonds occurs only during secondary metabolism and yields no net energy gain (Keyser et al. 1978). Degradation of cellulose is caused by a multi-enzyme complex of various endo-1,4-β-glucanases (EC 3.2.1.4), exo-β-glucanases (EC 3.2.1.91) and 1,4-β-glucosidases (EC 3.2.1.21). Endo-1,4-β-glucanases cleave cellulose randomly within the polymer chain. Starting from the non-reducing ends, the resulting chains are hydrolysed by exo-β-glucanases (cellobiohydrolases) to give cellobiose (Eriksson and Pettersson 1975a, 1975b). Cellobiose is further cleaved into two glucose units by 1,4-β-glucosidases (Deshpande et al. 1978). Alternatively, it can be oxidised to cellobionolactone by cellobiose dehydrogenases [e.g. cellobiose oxidase (EC 1.1.3.25), cellobiose-quinone oxidoreductase (EC 1.1.5.1); CBQ] using e.g. quinones, phenoxy radicals and Fe^{3+} as electron acceptors (Westermark and Eriksson 1974, 1975; Henriksson et al. 1995, 2000). Accordingly, quinone-reducing enzymes, especially CBQ, form a link between cellulose and lignin degradation. Cellobiose oxidase is assumed to take part in lignin degradation via the Fenton reaction. Hydroxyl radicals are formed via Fenton′s reagent (H_2O_2+Fe^{2+}) when cellulose, hemicellulose and lignin are simultaneously degraded (Henriksson et al. 1995, 2000). Hemicellulose degradation is performed by a multi-enzyme system of hydrolases comparable with that in cellulose degradation, whilst the mode of action is more complex. In wood, hemicelluloses (especially xylans) are closely associated to lignin, probably even covalently bound. Hemicelluloses are first cleaved by endo-enzymes (xylanase, mananase) and shorter chains are further hydrolysed to simple sugars by glycosidases (mannosidases, xylosidase, glucosidase; for a review, see Eriksson et al. 1990).

During the white-rot process, the initial attack on lignin is considered to be performed by extracellular phenoloxidases [laccase (EC 1.10.3.2)] and peroxidases [lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13); Evans 1991; Orth and Tien 1995]. Peroxidases use hydrogen peroxide as co-substrate, while laccases require molecular oxygen as electron acceptor (Eriksson et al. 1990). These enzymes catalyse the one-electron oxidation of e.g. lignin-related phenolic groups to relatively stable phenoxy radicals. These radicals undergo non-enzymatic reactions, such as: (1) radical coupling and polymerisation, (2) oxidation of α-carbinol to α-carbonyl groups, (3) alkyl–phenyl cleavage, (4) demethoxylation and (5) reactions initiated by quinone methide formation (Kirk and Shimada 1985). Repolymerisation of decayed lignin fragments is evidently prevented by the actions of CBQ (Ander et al. 1990; see above).

Brown-rot fungi are able to degrade cellulose and hemicelluloses without any preceding removal of lignin. The initial attack on crystalline cellulose is assumed to be caused by hydroxyl radicals (Fe^{2+}/H_2O_2 system; Koenigs 1974; Green and Highley 1997). Phenolic low-molecular-weight chelators of iron ions are proposed to produce hydroxyl radicals in the direct vicinity of cellulose (Jellison et al. 1997). As in white-rot fungi, hemicellulose degradation by brown rots is performed by a multi-enzyme system of hydrolases, whilst the mode of action is more complex (Eriksson et al. 1990). Brown-rot fungi mineralise lignin to a minor degree (Kirk and Highley 1973; Ander et al. 1988). They are reported to increase the number of phenolic groups in lignin mainly by demethylation of methoxyl groups and to a lesser extend by hydroxylation of aromatic sites. Compared with sound wood, the number of aliphatic hydroxyl groups seems to be slightly lower during early decay (Kirk 1975) and to increase during advanced stages (Jin et al. 1990a).

In addition to the application of whole cultures of wood-decaying fungi, isolated enzymes from white-rot fungi are utilised for technical purposes. Laccases, peroxidases and xylanes (carbohydrate hydrolases) mainly from white-rot fungi have been applied in bio-pulping (Bajpai 1999; Spiridon and Popa 2000), particle- and fibre-board manufacturing, improvement of preservative penetration in wood and waste bioremediation.

Fungal cultures and oxidative enzymes in the production of board material

The production of wood-based board materials implies that wooden particles (flakes, chips), fibres or strands are tightly glued together into panels through an operation at high temperature and pressure (Malony 1993). Fermentation of wood chips prior to pulping and pressing has found an application in mediating adhesion. In addition, two principle enzymatic strategies to enhance fibre adhesion are distinguished: Oxidative enzymes (laccase, peroxidase) in combination with lignin or lignin-like phenolics gives a 2-component glue, while enzymes alone give a 1-component system.

Enzymatically polymerised lignin as an adhesive for particle boards (2-component system)

In wood, lignin acts as a natural glue between its fibres and between fibres and other cell elements. In the particle-board industry, the addition of polyphenolic

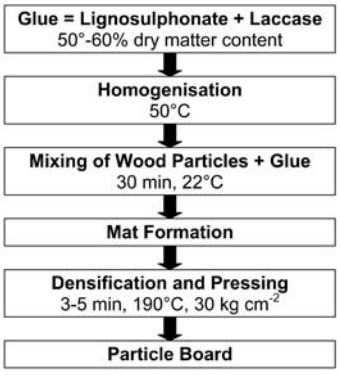

Fig. 2 The principle steps in the 2-component process for the manufacture of particle boards (Haars et al. 1989)

lignosulphonate to conventional phenol–formaldehyde (PF) glues is aimed at replacing in part the phenol component produced from petrochemicals. Formaldehyde may act as a cross-linker between synthetic phenols and lignosulphonate, a cheap by-product (spent sulphite liquor) from the pulp and paper industry (e.g. see Roffael and Rauch 1971; Shen 1974; Nimz 1983; Ayla and Nimz 1984). Radical polymerisation of lignosulphonate instigated by oxidative enzymes (laccase, peroxidase) is an alternative way of cross-linking, with the positive side-effect that no carcinogenic formaldehyde is emitted during the service life of the boards (Kharazipour et al. 1998b). Spent liquor composed of lignosulphonate (component 1) combined with peroxidase or laccase (component 2) was proposed as a binder for particle boards (Nimz et al. 1972, 1976). The required high quantities of cheap laccase can be obtained from the fungal fermentation of spent sulphite liquor that additionally contains easily accessible carbohydrates (Haars et al. 1987).

Haars et al. (1989) described a 2-component process whose principle steps are depicted in Fig. 2. Particle boards (19 mm) for indoor use were manufactured by gluing with a mixture of spray-dried sulphite liquor and concentrated culture filtrate (crude enzyme) of *T. versicolor*. With 0.42–0.47 MPa, these boards surpassed the requirements of transverse tensile strength (DIN 52365 test) of 0.35 MPa specified by European standard EN 312-4. However, the particle boards exceeded the standard values for dimensional stability (24 h swelling in water, DIN 52364 test), due to the hydrophilic nature of the sulphonate groups present in lignosulphonate. Such swelling in water can be reduced by the addition of hydrophobic types of lignin (Haars et al. 1989; Hüttermann et al. 1989a, 1989b). A more efficient process to reduce swelling combines a lignin-laccase-based adhesive with 1% methylene diphenyl diisocyanate (PMDI). The tensile strength was doubled and the swelling was halved, compared with individual treatments using either lignin-laccase or PMDI, respectively (Hüttermann and Kharazipour 1996; Hüttermann et al. 2001).

The idea of enzymatic 2-component adhesives was further pursued in paper-board manufacturing. Oxidative polymerisation of phenolics (dehydrogenation polymer, DHP), vanillic acid, catechol, mimosa tannin (*Acacia mollissima*) and tannic acid catalysed by crude peroxidase from madake bamboo (*Phyllastachys bambusoides*) shoots or by laccase from *T. versicolor* was evaluated for the gluing of thermo-mechanical pulp (TMP; Yamaguchi et al. 1991, 1992, 1994). The tensile and ply-bond strength of hot-pressed TMP–phenol paper boards were significantly higher than those of TMP papers produced in the absence of polymeric phenols. Pre-treatment of TMP fibres with laccase further increased the ply-bond strength. It was thought that lignin located on the fibre surface was activated by laccase through the loosening of its macromolecular structure (Yamaguchi et al. 1992, 1994).

Brown-rotted lignin as an adhesive

In several studies, lignin from technical processes such as sulphonate or Kraft pulping (Fengel and Wegener 1984) served for partial substitution of the phenol component in phenol formaldehyde (PF) adhesives. Such lignins have low formaldehyde reactivity, due to a limited number of free phenolic groups and because methoxyl groups and aliphatic side-chains block possible reaction sites. In contrast, brown-rotted lignin (BRL) isolated from decayed wood displays a reactivity with formaldehyde which was more than 50% higher than that of Kraft lignin. Substituting the phenol component in PF adhesives by 35% BRL resulted in flake boards with a strength comparable with that of flake boards glued by conventional PF resins. Water uptake and swelling were not more than 5% higher in BRL-PF-bonded boards (Jin et al. 1990b).

In a 2-component system, BRL and oxidative enzymes (horseradish peroxidase, laccase) were tested for bonding wood laminates. The efficiency of the enzymatic activation of BRL strongly depended on the wood species used in the gluing test. However, the shear strength of enzymatically bonded laminates was always lower than that of laminates glued by conventional adhesives. Furthermore, none of the BRL-bonded samples were water proof—they delaminated in boiling water (Jin et al. 1991). In addition, the application of BRL in gluing appears hardly feasible in practice, since a long fungal incubation and further elaborative extraction steps are necessary to obtain BRL.

Enzymatic activation of auto-adhesion of wood fibres (1-component system)

Fibres used for medium-density fibre (MDF) boards are commonly produced by thermo-mechanical pulping of wood chips. The TMP chips are heated to temperatures above the glass-transition point of lignin, where it begins to melt and thus can be separated mechanically. While the separated fibres are cooling down to room temperature,

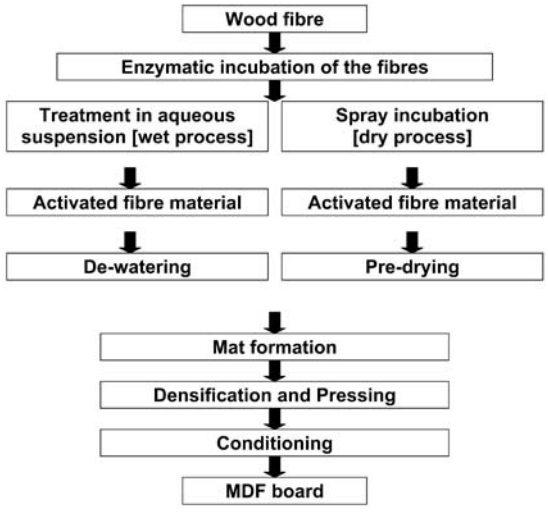

Fig. 3 The principal steps in the wet and dry treatments for manufacturing medium-density fibre (MDF) board from wood fibre

lignin solidifies into a plasticised, glassy layer on the surface of the fibres. Thus, lignin loses its auto-adhesive properties. For MDF board manufacturing, the auto-adhesion of lignin can be reactivated by oxidative enzymes in a 1-component system without any additional substrate as gluing material (Unbehaun et al. 2000). Without enzymes, the self-bonding of wood fibres occurs after hot-pressing above a critical temperature, but the surface reactivity of the fibres is not sufficient to abandon the application of synthetic binders (Back 1987).

The regeneration of auto-adhesive properties by oxidative enzymes has been analysed by two research groups (Felby et al. 1997b, 1998; Kharazipour et al. 1997, 1998a). Both groups distinguished between a wet and a dry treatment (Fig. 3; Table 1). Enzyme incubation of fibres was performed by Kharazipour et al. (1997) either in aqueous suspension (wet process) or the enzyme solution was sprayed onto the fibre (dry process). In the wet process, according to Felby et al. (1997a), the fibre mat is de-watered through cold pressing, while in the dry process the mat is forced-air-dried.

In the enzymatic reaction step, both groups made use of the same crude commercial *Trametes* laccase (SP 504, Novo-Nordisk; Felby et al. 1997a; Kharazipour et al. 1997; Table 1). Kharazipour et al. (1998a) additionally applied peroxidase SP 502 (Novo-Nordisk) together with hydrogen peroxide (15 mM added at intervals of 10 min over the incubation period).

In laboratory-scale experiments using laccase, the strength properties of MDF boards were significantly increased, compared with control boards incubated with thermo-inactivated enzyme or no enzyme. Kharazipour et al. (1997) and Kharazipour and Hüttermann (1998) obtained the best board properties (internal bond strength, swelling) after an incubation of 4 h at pH 5.0 (Table 1). The type of enzyme application, wet or dry, had no fundamental influence (Table 1). Felby et al. (1997b, 1998) reached comparable board properties with a significantly lower enzyme activity after just 1 h of incubation, but at pH 4.5 (Table 1), the pH optimum of the enzyme (Mai et al. 2001). The type of mat drying caused no major difference in the internal bond strength. However, the swelling in water was significantly lower when the mat was forced-air-dried (Table 1). Peroxidase SP 502 was only tested in a "wet" process (Kharazipour et al. 1998a). As with laccase, the main properties of peroxidase-bonded MDF boards from wood fibres reached the requirements of the European standard CIN DIN 622-5 (Table 1). However, the strength properties of peroxidase-bonded boards just met the minimum limits. As a further disadvantage, peroxidase requires hydrogen peroxide as co-substrate. Special caution during the storage and application of hydrogen peroxide is necessary, due to its high oxidising power and explosion risk.

Accompanying investigations showed that, in fibre suspensions, the generation of stable phenoxy radicals correlated with the oxygen consumption by laccase (Felby et al. 1997a, 1997b). The concentration of these radicals was 5–6 times higher than that in parallel samples with untreated fibres and did not decrease over a period of more than 2 weeks (Felby et al. 1997b, 1998). The authors assume that colloidal lignin acts as a charge mediator between laccase and lignin on the fibre surface (Felby and Hassingboe 1996).

Up-scaling of the laccase-mediated process on a pilot plant was recently reported, using thermostable enzyme from the ascomycete *Myceliophthora thermophila* (Felby et al. 2002; Table 1). The strength properties of the enzyme-bonded boards produced were comparable with those of conventional urea–formaldehyde (UF)-bonded boards (e.g. internal bond strength 0.93 MPa, compared with 0.99 MPa). However, the longer pressing times needed to obtain comparable bond strengths and the lower moisture resistance of enzyme-bonded boards require some improvements to make the process economically feasible in practice. The addition of wax in order to improve moisture resistance prevented proper gluing and considerably reduced the strength properties of the boards (Felby et al. 2002).

In addition to purified enzymes, whole culture media from the fermentation of malt or wastewater from fibre board manufacturing by brown- and white-rot fungi were studied in the MDF board production of rape-straw fibres. Strength properties were reported to be almost twice as high as those of corresponding boards bonded by purified laccase from *T. versicolor* (Kühne and Dittler 1999; Unbehaun et al. 2000). This increase was explained by the additional contribution of hydrolytic enzymes (Unbehaun et al. 2000).

Fungal transformation of wood to produce adhesive-free boards

Fungal pre-treatment of wood chips reduces the refining energy in thermo-mechanical pulping. Wood chips fer-

Table 1 Process conditions and properties reported for medium-density fibre boards glued by auto-activation with oxidative enzymes (1-component system). Data in square brackets indicate the requirements according to European Standard CIN DIN 622-5. *TMP* Thermo-mechanical pulp

Process conditions and board properties	Report					
	Kharazipour et al. (1997)		Kharazipour et al. (1998a)	Felby et al. (1997a)		Felby et al. (2002)
Incubation type/curing condition	Wet	Dry	Wet	Wet	Dry	Dry
Fibre	80% spruce/pine mixture, 20% beech (TMP)		80% spruce/pine mixture, 20% beech (TMP)	Beech (TMP)		Beech (TMP)
Enzyme	Laccase SP 504		Peroxidase SP 502	Laccase SP 504		*Myceliophthora thermophila* laccase
Fibre content in solution/moisture content of fibre	3.6% w/v in buffer	50, 60, 66% moisture content	6.6% w/v in buffer	5% w/v in water		Water added in the blow line to reach 55% moisture content
pH	5.0 (optimum)		6.0 (optimum)	4.5		7.0
Enzyme activity	1,000 units/ml, 27,000 units/g (fibre)		300 units/ml, 4,500 units/g (fibre)	3.5 units/g (fibre)		6–24 units/g (fibre)
Incubation temperature	35 °C		20 °C	20 °C		50 °C
Incubation time	4 h	12 h	4 h	1 h	1 h	30 min
Reduction of water content	De-watering by filtration	Pre-drying at 50 °C to 20–25% moisture content	De-watering by filtration	Cold press to ~33% moisture content	Forced-air-drying at 40 °C to 12% moisture content	Flash-drying to 14–18% moisture content
Pressing	5 min, 190 °C, 2.5 MPa		5 min, 190 °C, 2.5 MPa	5 min, 180 °C; target density: 1.000 g/cm^3	5 min, 200 °C; target density: 0.850 g/cm^3	5 min, 200 °C; target density: 850 kg/m^3; moisture content of fibres before pressing: 11–13%
Board thickness	5 mm		5 mm	3 mm		8 mm
Board density	0.78 g/cm^3 (wet process)		0.76 g/cm^3	1.037 g/cm^3	0.895 g/cm^3	0.86 g/cm^3
Board thickness swelling (24 h)	23% [22%] (wet process)		28% [22%]	57% [27%]	19% [27%]	46% at 24 units/g_{fibre}, 69% at 6 units/g_{fibre} [15%]
Board internal bond strength	0.7–1.4 MPa [0.70 MPa] (wet process)		~0.6 MPa [0.70 MPa]	1.55 MPa [0.70 MPa]	1.57 MPa [0.70 MPa]	0.82 MPa at 6 units/g_{fibre}, 0.93 MPa at 24 units/g_{fibre} [0.65 MPa]

mented with the white-rot fungus *Trametes hirsuta* and the brown-rots *G. trabeum* (Unbehaun et al. 1999, 2000), *Coniophora puteana* and *Fomitopsis pinicola* (Körner et al. 2001) at a temperature of 19–26 °C for 3 weeks required 40% less refining energy, compared with untreated wood chips. Importantly, the weight loss of the chips was less than 4%. Fungally pretreated wood chips were able to bond without extra added adhesives (glue, enzyme) under pressing conditions usually applied for conventional gluing. Fibres obtained from wood chips after 9 days of fungal fermentation gave rise to MDF boards that displayed a 3.5-times higher bending strength and a 3-times higher modulus of elasticity than boards pressed from untreated fibres. Thickness swelling in water (24 h) was reduced by 60–70% after application of the mycological treatment (Unbehaun et al. 2000; Körner et al. 2001).

"Myco-wood"

The incubation of beech wood with the white-rot fungi *Pleurotus ostreatus* and *T. versicolor* has been applied to produce so-called "myco-wood" (Luthardt 1963; Schmidt 1994; Unbehaun et al. 2000). Depending on the incubation time, the fungal treatment reduced the density and loosened the structure of the wood. In consequence, the machinability and rate of water sorption were improved. ″Myco-wood″ was mostly used for pencils, rulers, drawing tables and wooden forms in the glass industry. During 1958–1965, 55×10^6 "myco-wood" pencils were produced in the former German Democratic Republic. At the moment, this technique is not applied in practice, but production is under consideration by some German pencil producers.

Disposal of treated wood waste

Conventional wood preservatives are toxic, causing environmental problems and high costs during their disposal. Most inorganic and organic wood preservatives are highly resistant to biodegradation and present the risk of leaching when disposed on landfills. Combustion of treated wood is a relatively effective way of disposal, although, due to the need for special furnaces, the cost are rather high. Micro-organisms offer environmentally solutions to the problem (Table 2).

The microbial detoxification of wood treated with organic preservatives focuses on creosotes and pentachlorophenol (PCP). Various types of pro- and eukaryotic micro-organisms are principally able to degrade these components under specific culture conditions (for a review, see Juhasz and Naidu 2000; Litchfield and Rao 1998). However, the bioremediation of impregnated wood is restricted to those organisms that penetrate wood, tolerate preservatives and thus actively grow on the treated substrate.

Creosote is a coal-tar distillation product composed of more than 200 highly heterogeneous polycyclic aromatic hydrocarbons (PAHs) and various phenolic compounds. It is exclusively used for outdoor applications such as railway sleepers and poles. Various bacteria and fungi have been isolated from creosote-treated timber. The PAHs in creosote are degraded by both groups of micro-organism, although bacteria show a much higher toxic threshold than fungi for PAHs (Kerner-Gang 1975). *Pseudomonas creosotensis*, for example, metabolises neutral constituents of creosote in treated wood (Drisko and O′Neil 1966). However, bacteria only degrade PAHs with up to four aromatic rings. Degradation occurs intracellularly and larger molecules do not pass the cell membrane (Weißenfels et al. 1990; Cerniglia 1992). The degradation of pure mixtures of highly condensed PAHs (more than four rings) by white-rot fungi in liquid cultures was first reported by Bumpus et al. (1985). The most toxic part of creosotes for basidiomycetes is the phenolic fraction (Da Costa et al. 1969). Soft-rot fungi (ascomycetes, deuteromycetes) isolated from creosote-treated timber were shown to actively grow on filter paper impregnated with creosote (Ribichich and Lopez 1996). Thus, the preceding removal of phenolics by bacteria, ascomycetes and deuteromycetes enables the subsequent colonisation of creosote-treated wood by basidiomycetes.

For the remediation of chipped creosote-treated railway sleepers, the toxic threshold of PAHs for various white-rot fungi was determined to be 3900 $\mu g_{PAH}/g_{wood}$. This value represents about one-tenth of the actual concentration in impregnated sleepers. Of 89 white-rot tested basidiomycetes, *Bjerkandera adusta* was the most effective in decontaminating pre-extracted wood with reduced PAH content (Messner and Böhmer 1998). In another study, the total creosote content of chipped, not pre-extracted railway sleepers was reduced by about 65% after 3 weeks of incubation with an isolate of *Polyporus pinsitus*. Non-sterile inoculation of treated wood was possible due to the addition of potato pulp as an easy nutrient source for fungal establishment or, alternatively, due to pre-treatment of the substrate with detergent solution (Majcherczyk and Hüttermann 1998).

PCP constitutes a toxic, highly recalcitrant bactericidal and fungicidal compound which is now prohibited in many countries. Due to considerable amounts of residual waste and due to its persistency, its disposal is still a major concern. PCP-degrading bacteria isolated from contaminated soil failed to remove PCP from wood (McBain et al. 1995). In contrast, *T. viride* specifically depleted 62% of Na-PCP from impregnated pine sapwood in soil-block tests without causing considerable weight loss (Unligil 1968). In another study, a *Trichoderma* species were shown to degrade PCP, with the consequence that *G. trabeum* caused a considerable weight loss at subsequent inoculation (Duncan and Deverall 1964). The rotting fungi *Coniophora puteana* and *T. hirsuta* caused comparable or somewhat higher Na-PCP removal than *Trichoderma*. However, this was accompanied by a high weight loss of wood (Unligil 1968; Lamar and

Table 2 Approaches to bioremediate preservative-treated waste wood

Preservative	Experimental conditions	Organism(s)	References
Creosote	Shaken cultures (not tested on treated wood)	*Pseudomonas creosotensis*	Drisko and O′Neil (1966)
	Pre-extracted chipped treated railway sleepers	*Bjerkandera adusta* (most effective of 89 white-rot basidiomycetes tested)	Messner and Böhmer (1998)
	Chipped treated railway sleepers, not pre-extracted	*Polyporus pinsitus* (most effective of ten white-rot basidiomycetes tested)	Majcherczyk and Hüttermann (1998)
Polycyclic aromatic hydrocarbons	Treated wood chips (laboratory scale)	Fungus, species not indicated	Legay et al. (1998)
Pentachlorophenol (PCP)	Impregnated pine sapwood (laboratory standard soil block tests)	*Trichoderma viride*, *Coniophora puteana*	Unligil (1968)
	Wood chips from treated ammunition boxes (laboratory and pilot-plant scale)	*Trametes hirsuta*	Lamar and Dietrich (1992), Lamar (1995)
	Treated wood chips (laboratory scale)	*Trichoderma* species and *Gloeophyllum trabeum* in succession	Duncan and Deverall (1964)
	Treated wood chips (laboratory scale)	Fungus, species not indicated	Legay et al. (1998)
Formulation of PCP, γ-hexachlorocyclo-hexane and DDT	Treated wood chips (laboratory scale)	*Pleurotus ostreatus*	Majcherczyk and Hüttermann (1998)
Organoiodine fungicides	Shaken cultures (not tested on treated wood)	*Tyromyces palustris*, *Serpula lacrymans*, *Trametes versicolor*	Lee et al. (1992a, 1992b)
Chromated copper arsenate (CCA)	Treated saw dust or chips (laboratory scale)	*Bacillus licheniformis*	Clausen (1997, 2000b), Clausen and Smith (1998a, 1998b)
	Treated wood chips (laboratory scale)	*Acinetobacter calcoaceticus*, *Aureobacterium esteroaromaticum*, *Klebsiella oxytoca* (most effective of 28 isolated bacteria)	Clausen (2000a)
	CCA type C-treated wood stakes (laboratory scale)	*B. licheniformis*	Crawford and Clausen (1999)
	Treated wood blocks (laboratory standard soil block tests)	*Meruliporia incrassata*, *Antrodia radiculosa* (most effective of 95 isolates of wood-decaying fungi)	Illman and Highley (1996), Illman et al. (2000)
	Treated wood chips (laboratory scale)	*Antrodia vaillantii*, *Poria placenta*	Stephan and Peek (1992), Peek et al. (1993), Stephan et al. (1996)
	Chipped CCA-treated poles (pilot-plant scale)	*A. vaillantii*	Leithoff and Peek (1997, 1998)
Copper chromium boron	Treated wood chips (laboratory scale)	*A. vaillantii*, *P. placenta*	Stephan and Peek (1992), Peek et al. (1993), Stephan et al. (1996)
Copper chromium fluorine	Treated wood chips (laboratory scale)	*A. vaillantii*, *P. placenta*	Stephan and Peek (1992), Peek et al. (1993)
Ammoniacal copper quaternary ammonia	Treated wood blocks (laboratory standard soil block tests)	*M. incrassata*, *A. radiculosa* (most effective of 95 isolates of wood-decaying fungi)	Illman and Highley (1996), Illman et al. (2000)
	ACQ type D-treated wood stakes (laboratory scale)	*B. licheniformis*	Crawford and Clausen (1999)
	ACQ-B	*Wolfiporia cocos*, *P. placenta*	De Groot and Woodward (1998)
Ammoniacal copper citrate	Treated wood chips (laboratory scale)	*W. cocos*, *P. placenta*	De Groot and Woodward (1998)
	Treated wood stakes (laboratory scale)	*B. licheniformis*	Crawford and Clausen (1999)
Copper naphthenate	Treated wood chips (laboratory scale)	*W. cocos*, *P. placenta*	De Groot and Woodward (1998)
	Treated wood stakes (laboratory scale)	*B. licheniformis*	Crawford and Clausen (1999)
Oxine copper	Treated wood stakes (laboratory scale)	*B. licheniformis*	Crawford and Clausen (1999)
Ammoniacal copper zinc arsenate	Treated wood chips (laboratory scale)	*W. cocos*, *P. placenta*	De Groot and Woodward (1998)
Copper chrome	Treated wood chips (laboratory scale)	*A. vaillantii*, *P. placenta* (most effective of 40 mainly brown-rot isolates)	Stephan and Peek (1992), Peek et al. (1993), Leithoff et al. (1995), Kortekaas and Sierra-Alvarez (1996), Stephan et al. (1996)
Borax	Treated saw dust (laboratory scale)	*Paecilomyces variotii*	Parker et al. (1999)

Dietrich 1992). Likewise, with unnamed fungi Legay et al. (1998) observed high decay rates of PCP (also PAHs) in treated wood together with considerable weight loss. Based on laboratory studies with *T. hirsuta* showing 84% decrease in PCP content and 25% weight loss of wood chips from PCP-treated ammunition boxes (Lamar and Dietrich 1992), scaling-up was attempted. On a pilot scale of 35 t, PCP-treated chips of western red cedar and southern pine utility poles displayed after 8 weeks a PCP depletion of only 30%. The failure of the scale-up treatment was explained by the inhibition of fungal growth, due to high heat production in the wood pile (Lamar 1995).

Many commercial wood preservatives are formulations of both fungicides (PCP, γ-hexachlorocyclohexane) and insecticides (DDT). Majcherczyk and Hüttermann (1998) achieved the degradation of such a formulation in treated wood, using the white-rot fungus *P. ostreatus*. In shaken cultures, two brown-rots (*Tyromyces palustris, S. lacrymans*) and a white-rot fungus (*T. versicolor*) were active in the fungal decomposition of organoiodine fungicides. *T. versicolor* was more effective than the brown-rot fungi but decontamination of the wood was not studied (Lee et al. 1992a, 1992b).

Inorganic preservatives, particularly heavy metals, are the most persistent in the environment and cannot be degraded by biological consortia. However, some bacteria and fungi are tolerant to copper, chromium arsenate, tin, zinc or fluorine (Silver 1996; Gadd 2000). Some of these micro-organisms are able to detoxify or even remove the preservatives from wood. Metal ions in wood might be methylated or chelated by extracellular organic acids. Removal occurs by remobilisation and subsequent leaching or by binding to fungal hyphae (Stranks and Hulme 1975; Gadd 1999; Gadd and Sayer 2000).

In CCA, chrome exists in two oxidation states: Cr(III) and Cr(VI). Cr(III) is the desired state, since it enhances the fixation of the salts in wood and it is much less toxic than Cr(VI). Some *Pseudomonas* species were shown to be able to detoxify Cr(VI) by enzymatic reduction to Cr(III) (e.g. McLean and Beveridge 2001). During fermentation of CCA-treated wood, the soil-inhabiting bacteria *Acinetobacter calcoaceticus*, *Aureobacterium esteroaromaticum* and *Klebsiella oxytoca* were able to release 98% of the chromium, while *Bacillus licheniformis* leached the highest percentage of copper (93%). Arsenate was released to 44–48% by *B. licheniformis* and *A. calcoaceticus* (Clausen 2000a). The high potential of *B. licheniformis* for the remediation of wood treated with copper-based preservatives was studied in detail (Clausen 1997; Crawford and Clausen 1999). Compared with bigger wood chips, copper removal was much more efficient when sawdust was tested as substrate. To remediate larger wood chips, fermentation with *B. licheniformis* was combined with either steam explosion or acid extraction. Steam explosion did not enhance the removal of CCA either in conjunction with acetic acid extraction or bacterial fermentation. The most effective removal was obtained by a 2-step process consisting of oxalic acid extraction followed by fermentation with *B. licheniformis* (Clausen and Smith, 1998a, 1998b; Clausen 2000b). The manufacturing of particle boards made from bioremediated (2-step process) CCA-treated wood chips was evaluated. The modulus of rupture and internal bond strength were reduced compared with boards obtained with untreated chips, probably due to residual oxalic acid rather than bacterial action (Clausen et al. 2001; Kartal and Clausen 2001).

Compared with other microbial groups, fungi can be very tolerant to toxic metals (Gadd 1993). Preceding exposure of CCA-treated wood to ascomycete and deuteromycete species detoxified the preservatives and enabled subsequent colonisation and wood degradation by less tolerant brown-rot basidiomycetes (Duncan and Deverall 1964). Due to their high production of complexing oxalic acid, brown-rot fungi often exhibit a higher tolerance towards copper and other heavy metals than white-rots. Screening 95 isolates of wood-decay fungi revealed 17 species to be tolerant to CCA and 21 to ammoniacal copper quaternary ammonium (ACQ) formulations. In CCA-treated wood, *Meruliporia incrassata* caused highest weight loss, while *Antrodia radiculosa* gave highest percentage degradation of ACQ (Illman and Highley 1996; Illman et al. 2000). X-ray spectroscopy did not reveal oxidation of Cr(III) to the more toxic Cr(VI) during the decay of CCA-treated wood by *M. incrassata* (Illman et al. 1996).

A tremendously high copper resistance and substantial weight loss of CCA-treated wood was observed for the brown-rots *A. vaillantii*, *Wolfiporia cocos* and *Poria placenta* (Da Costa 1959; Da Costa and Kerruish 1964; Woodward and De Groot 1999; Collett 1992). In laboratory experiments with *W. cocos* and *P. placenta*, decontamination of wood treated with various copper-containing preservatives, such as ammoniacal copper zinc arsenate, ACQ type B, ammoniacal copper citrate (CC) and copper naphthenate, was strongly dependent on the type of preservative tested. The median percentage weight loss caused by *W. cocos* for all kinds of treated wood was 4 times higher than by *P. placenta*. In contrast, the decay of untreated wood due to *P. placenta* was more than twice as high as that due to *W. cocos*. The concentration of copper in the decayed wood was similar to that before decay, since the removal of copper occurred to the same extent as the degradation of wood. In both species, copper accumulated in the fungal hyphae (De Groot and Woodward 1998, 1999).

Fermentation of copper-chrome-treated wood with *A. vaillantii* and *P. placenta* resulted in almost complete removal of chrome, whereas copper removal was lower due to formation of a water in-soluble oxalate complex (Stephan and Peek 1992; Peek et al. 1993; Kortekaas and Sierra-Alvarez 1996). Interestingly, the mass loss of copper-chrome-treated wood by *A. vaillantii* was slightly higher than that of untreated wood. When easily accessible nutrients were added, the decay of CC-treated wood proceeded at an even higher rate (Leithoff et al. 1995; Stephan et al. 1996). In a first pilot-plant experiment,

1 m^3 units of chipped CCA-treated poles were fermented with *A. vaillantii* (Leithoff and Peek 1997). However, technical implementation of the process was prevented by severe problems in controlling the moisture and heat in the infested wood pile and by infections of moulds and bacteria (Leithoff and Peek 1998).

On borax-treated wood, the mould *Paecilomyces variotii* was able to grow, excreting increased amounts of β-D-1,4-glucosidase. Obviously, boron was partly detoxified by complexing with glucose. A white sporulation-deficient mutant was isolated that showed high release of β-D-1,4-glucosidase. The mutant was proposed for both remediation and as a biocontrol agent for wood in service, following the established example of Cartapip (Parker et al. 1999).

Conclusions and outlook

Various biotechnological approaches have been intensively studied in the pulp and paper industry for more than 30 years. Biotechnological techniques, such as biopulping, bio-bleaching, pitch reduction and wastewater treatment have been accompanied by new developments of conventional methods within a given production scheme. Thus, advances in biotechnological and conventional techniques need to be adapted to each other.

In the wood and wood-based industry, biotechnological approaches have so far been little explored. In some cases, biotechnological processes developed for the pulp and paper industry have been transferred to the wood sector. An intriguing example is Cartapip 97, the white mutant of the sap-stain fungus *O. piliferum* that has been developed to control pitch during pulping and now is in use as an effective biocontrol agent to prevent bluestain of wood after felling. Studies presented in this review are biotechnological developments in the utilisation of antagonistic organisms for wood protection, improvements in the treatability with preservatives, the production of bio-glued or adhesive-free board materials and the disposal of preservative-treated wood. Most of these techniques are yet not feasible in practice. However, high prices for energy and petrochemical products, legal requirements and increasing environmental concern promote such applications.

The broadest future sector in biotechnology in the wood industry is probably the application of enzymes and fungal cultures, both in the alteration of wood properties and in adhesion promotion. Isolated enzymes promise to effectively substitute chemicals, thereby reducing the energy required in the processes. The application of cheap ″crude″ enzyme mixtures can be more efficient than isolated enzymes in cases where high substrate specificity is not required. In addition, the product properties may even improve (Kühne and Dittler 1999). Genetic engineering provides the opportunity for enzyme overexpression in easily manageable micro-organisms, such as *Aspergillus* and ascomycete yeast species (Berka et al. 1997; Conesa et al. 2002; Record et al. 2002; Soden et al. 2002). Currently available enzymes with their natural properties may not be of optimal quality for application, but protein engineering may optimise enzyme activity, substrate spectrum, temperature tolerance or pH optimum (Butler et al. 2003).

This review focuses on micro-organisms and their enzymes and thus on the biotechnological targets in the near future of the wood industry. In the long term, the genetic engineering of trees has a high potential to improve the properties of both standing trees (fungal/insect resistance) and wood in service (physical properties, increased durability), as reviewed by Pena and Seguin (2001) and by Fenning and Gershenzon (2002). Transgenic trees with decreased or altered lignin content have been produced in order to reduce the consumption of energy and chemicals for the delignification and refining of wood chips. However, the genetic stability of these transformations and the impact of transgenic trees on the ecosystem have to be evaluated in long-term studies. Due to the long period needed to conduct these studies and the time additionally required for growth, wood products from transgenic trees cannot be expected to enter the market within the next two decades. In addition, social acceptance is needed and must be guaranteed before releasing genetically modified trees into the environment.

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