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FUNGAL DEGRADATION OF WOOD COMPONENTS

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Abstract - A survey is given of the microorganisms involved in the degradation of dead plant material. The white-rot fungi, which can degrade all the major wood components, have been discussed in detail and their way of attacking wood has been studied by scanning electron microscopy as well as by transmission electron microscopy.

A review of the present knowledge of enzyme mechanisms involved in fungal degradation of cellulose, hemicelluloses and lignin is given. To develop biotechnical processes based on lignocellulosic materials the need for a deeper understanding of these mechanisms is stressed.

INTRODUCTION

In nature there is a continuous degradation of dead plant material by saprophytic microorganisms. To degrade woody materials is mainly a task for fungi. The strong wood-degrading effect that fungi have depends, in part, upon the organization of their hyphae, which give the organisms a penetrating capacity. Different types of fungi give rise to different types of wood rot. One normally distinguishes between soft-rot, brown-rot and white-rot fungi. The blue staining fungi are also associated with wood damage. They do not, however, cause wood-degradation.

The term soft-rot emanates from the fact that there is a softening of the surface layer when wood is attacked by this group of fungi. In the secondary wall of the attacked wood cylindrical cavities with conical ends appear. The term soft-rot is now used whenever this characteristic cavity pattern occurs, even if no softening of the surface layer has taken place. Soft-rot is more common in hardwood than in softwood. It has been suggested that the reason for this is the quality differences in the lignin of hard- and softwood. The methoxyl content of hardwood lignin is usually higher, about 21 %, than in softwood lignin where the methoxyl content is about 14 %. Soft-rot is caused by fungi belonging to Ascomycetes and Fungi Imperfecti (1).

Brown-rot and white-rot attack on wood is mainly caused by fungi belonging to Basidiomycetes. The hyphae of the fungi are normally localized in the cell lumen and these hyphae penetrate from one cell to another through openings, or by producing bore holes in the cell walls. Brown-rot fungi mainly degrade cellulose and the hemicelluloses in wood. In an early stage of degradation they depolymerize cellulose faster than the degradation products are utilized. Brown-rotters begin the degradation process from the cell lumen and first degrade the S_2 and then, later, the S_1 wall (2).

The white-rot group of fungi is a rather heterogeneous group of organisms. They have in common the capacity to degrade lignin as well as the other wood components. They also have in common the ability to produce extracellular enzymes which oxidize phenolic compounds related to lignin. This is the reason why phenolic compounds have been utilized for the identification of white-rot fungi. The relative amounts of lignin and polysaccharides degraded and utilized by these fungi vary, and so does the order of preferential attack. The normal method of wood degradation by white-rot fungi is for the celluloses and the lignin to be attacked simultaneously. However, there are examples of a specific degradation of the middle lamella lignin (1). It has been demonstrated by several workers (3) that a totally specific attack on the lignin by white-rot fungi cannot be undertaken. This is most likely so because so much energy is required to degrade lignin that an additional, more easily accessible energy source is also necessary. Low molecular weight sugars as well as the polysaccharides

in wood serve as co-substrates. This is demonstrated in Fig. 1, where the loss of wood components after 10 weeks rotting with a wild-type and a cellulase-less mutant strain, Cel 44, of the white-rot fungus Sporotrichum pulverulentum is shown. The rotting with the wild-type fungus involves a decrease in all three wood components, cellulose, hemicellulose and lignin, while the cellulase-less mutant causes a degradation of only xylan and lignin.

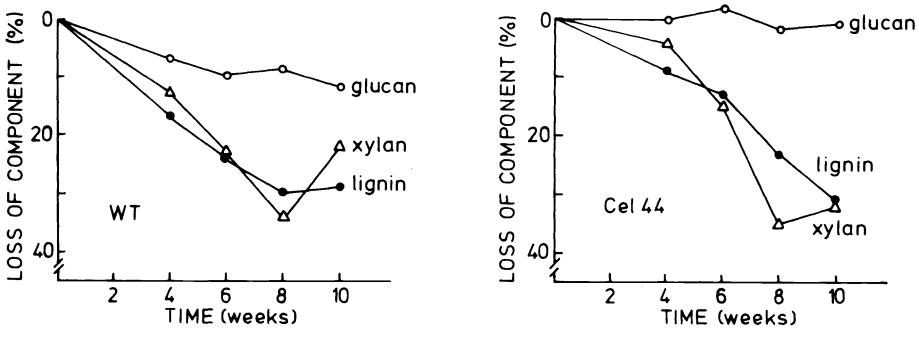

Fig. 1 Degradation of wood components in birch by WT S. pulverulentum and its cellulase-less mutant Cel. 44.
o Glucan ● Lignin Δ Xylan

INVESTIGATION OF FUNGAL DEGRADATION OF WOOD BY ELECTRON MICROSCOPY

To better understand how the wood components are degraded by fungi on the molecular level it is important to investigate the morphology of fungal attack on wood. Such studies have been undertaken both on the micromorphological level and on the ultrastructural level using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Before these studies were undertaken it was important to determine the optimal growth conditions for the fungi in wood (4). Rypáček and Navrátilová (5) had earlier reported that the white-rot fungus Trametes versicolor colonizes wood very densely. In one cubic cm of wood up to 1300 m of fungal hyphae can be produced. This must mean that the diffusion distances of the enzymes degrading the wood are very short. We have demonstrated that the white-rot fungus S. pulverulentum grows 1 mm per hour on a malt agar plate surface and 0.5 mm an hour in either birch- or spruce-wood (4). Thus, it seems likely that the rate limiting steps in fungal degradation of wood are the enzymic reactions involving the polymeric wood components rather than the growth capability of the fungus.

Mutants of microorganisms have been widely used for metabolic studies. However, to my knowledge fungal mutants have never been utilized for morphological studies of wood degradation. In our laboratory we are developing a microbiological process for specific delignification of wood chips in order to save energy in mechanical pulping. The delignification is carried out by using cellulase-less mutants of white-rot fungi (6). The different morphological changes brought about in wood during attack by wild-type and mutant strains of different white-rot fungi have been studied by both SEM- and TEM-techniques (7,8). The white-rot fungus S. pulverulentum and its cellulase-less mutant Cel 44 have featured most prominently in these studies. In Figs. 2 and 3 it is demonstrated that Cel 44 grows from one wood cell to another by utilizing existing holes (7). In Fig. 4 it is shown that the wild-type S. pulverulentum can grow from one wood cell to another by penetration of the wood cell walls while cellulase-less mutants cannot. It is seen from Fig. 5 that a substance covering the tracheid cell wall disappears at a distance of 3-5 µm from the fungal cell wall. This shows that enzymes attacking wood components diffuse from the fungal cell wall out into the surrounding medium.

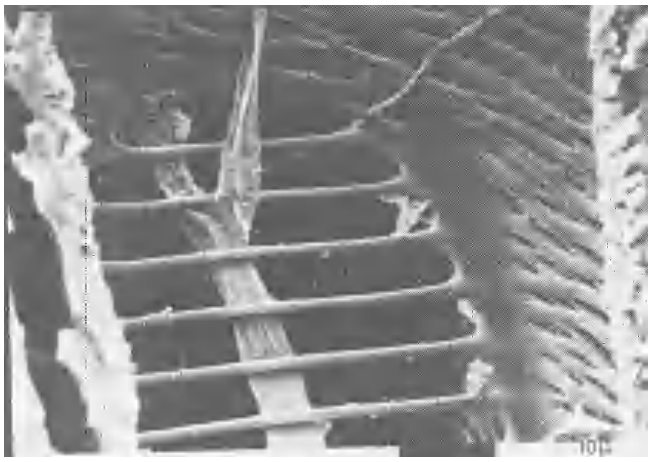

Fig. 2 Growth of Cel 44 through the perforated plate between vessels in birch.

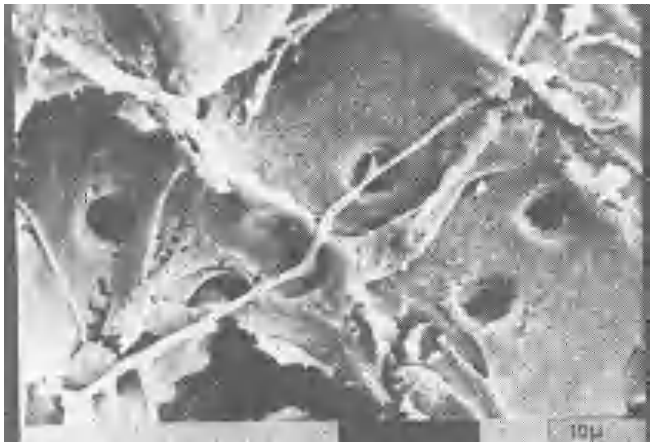

Fig. 3 Growth of Cel 44 through the pits between ray cells and between a ray cell and a tracheid cell in pine.

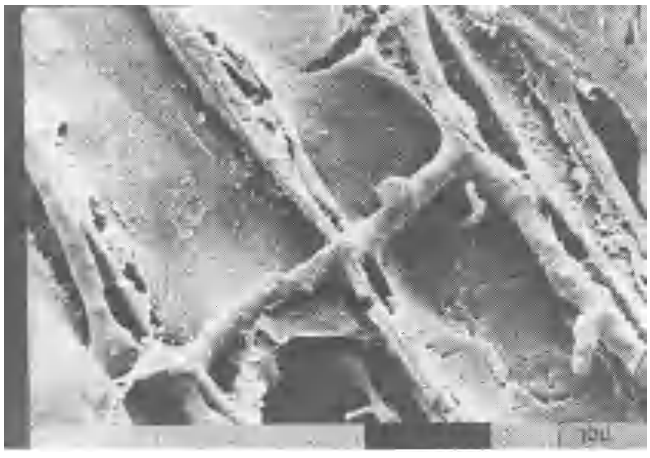

Fig. 4 The wall of a ray cell penetrated by S. pulverulentum.

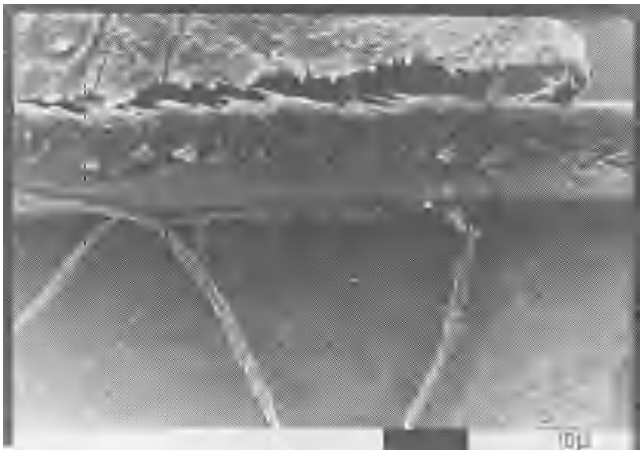

Fig. 5 A substance covering the tracheid cell wall disappears at a distance of 3-5 µm from the fungal cell wall. Phlebia radiata in spruce wood.

While the use of the SEM technique has given valuable information on the mode of growth of white-rot fungi in wood, TEM offers further possibilities. Ruel et al. (8) has used this technique to study how spruce wood changes after rotting with either the wild-type S. pulverulentum or its cellulase-less mutant Cel 44. These studies have given information both at the micromorphological level and at the ultrastructural level, where changes in the organization of the three main fibre cell wall components, cellulose, hemicellulose and lignin, have been investigated. The most important of the results obtained can be summarized as follows: Confirmation that the wild-type of S. pulverulentum can grow through the fibre cell walls, mainly the S_2 layer, while Cel 44 cannot. The capacity to bore holes through the fibre cell wall must be related to the power of the wild-type fungus to attack the cellulose. In Fig. 6 it can be seen that the mutant has mainly attacked the layer between S_1 and S_2.

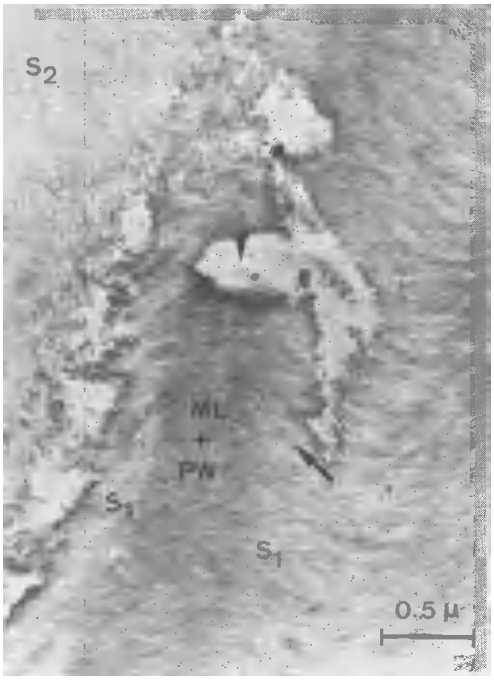

Fig. 6 Vertical progression decay through the S_1/S_2 layer. From (8).

In the transition layers the cellulose microfibrils change direction and this seems to make these layers more susceptible to attack by the mutant. However, the transition layers are easier to degrade for both wild-type and cellulase-less mutants. The transition layer between S_2 and S_3 is also easily degraded while the S_3-layer is surprisingly resistant to microbial attack. Our studies have clearly demonstrated that the parts most resistant to microbial degradation are the S_3 layer and the middle lamella.

The average distance from the fungal cell wall where changes in the macromolecular organization of the wood due to the diffusion of fungal enzymes can be observed has been shown to be 1-3μ m for the wild-type and around 0.2-0.3μ m for the mutant. In these areas around the fungal hyphae, it seems as if the hemicelluloses are the first wood components to be degraded. This has been shown using cytochemical tests. However, it is also clear that the lignin is changed morphologically at a distance from the fungal cell wall. Instead of being layered in the wood in the form of longish barriers the diffusion of the enzymes cause the lignin to be aggregated in the form of granulae. This granule formation can be seen in Figs. 7 and 8.

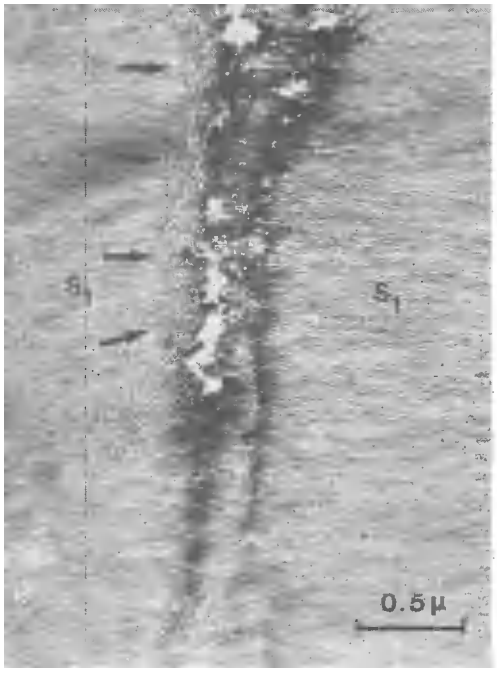

Fig. 7 Diffusion of enzymes from the fungal cell wall tears down "the lignin-lamella" in the wood.
S. pulverulentum. From (8).

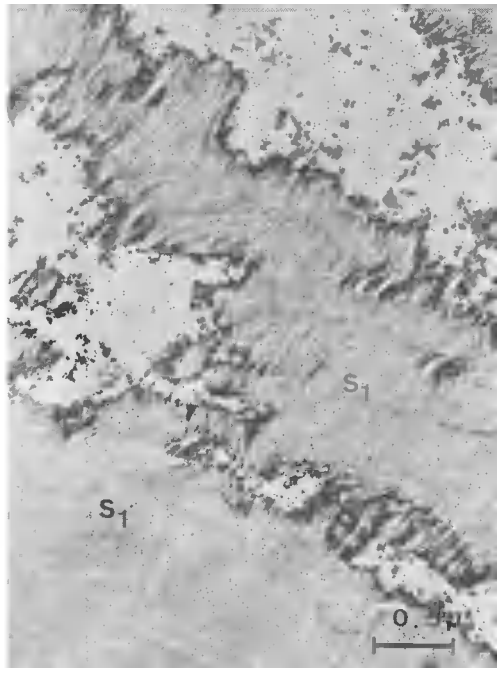

Fig. 8 At a prolonged rotting the lignin aggregates and forms piles 40-80 nm in size.
S. pulverulentum. From (8).

As far as is known this is the first case where lignin modifying enzymes have been visibly shown to diffuse from the fungal cell wall. It seems possible, therefore, to say that lignin modification is not to be considered as purely a contact phenomenon, i.e. that such a modification is possible only on the establishment of direct contact between the fungal cell wall and the lignin substrate.

DEGRADATION OF CELLULOSE

The enzyme mechanisms involved in cellulose degradation have been particularly well studied for two fungi, namely the white-rot fungus S. pulverulentum (9) and the mould Trichoderma reesei (10) (the fungus T. viride QM 6a and strains derived from it are now referred to as T. reesei).

The fungus S. pulverulentum produces three different types of hydrolytic enzymes namely a) five different endo-1,4-β-glucanases which attack at random the 1,4-β-linkages along the cellulose chain; b) one exo-1,4-β-glucanase which splits off cellobiose or glucose units from the non-reducing end of the cellulose; c) two 1,4-β-glucosidases which hydrolyse cellobiose and water soluble cellodextrins to glucose and cellobionic acid to glucose and glucono lactone (9).

It has been generally accepted that essentially the same picture is also true for cellulose hydrolysis by T. reesei (11). However, a few differences have been recognized such as the

number of the various hydrolytic enzymes, the degree to which the β-glucosidase activity is bound to the fungal cell wall etc. The action of the exo-glucanase in S. pulverulentum differs from the action of the corresponding enzymes in T. reesei in that the exo-glucanase from S. pulverulentum splits off both glucose and cellobiose while the exo-glucanases from T. reesei only split off cellobiose (9).

However, to degrade crystalline cellulose a synergistic action between endo- and exo-glucanases is taking place in both fungi. Crystalline cellulose is not attacked by one of these types of enzymes alone. Amorphous cellulose is, however, degraded by both types of enzymes (9,10).

In a recent paper by Gritzali and Brown (12) a much simpler enzymic pattern of the fungus T. reesei QM 9414 has been found compared with that previously reported (11). Using a different cultivation technique only one endo-glucanase and two exo-glucanases were now obtained (12). Instead of being grown on cellulose, glucose was used as carbon source. After washing, the glucose grown cells were transferred to a buffer where the hydrolytic cellulose-degrading enzymes were induced by the addition of sophorose. Polyacrylamide gel electrophoresis of the concentrated culture solution revealed that the separation pattern of cellulases induced by sophorose is much simpler than the separation pattern of enzymes obtained after several days of cultivation on cellulose. It seems likely, therefore, that the multiplicity of cellulases in the cellulose culture is caused by proteolytic degradation. In S. pulverulentum the five endo-glucanases are very similar in molecular weight, amino acid composition etc. However, they differ somewhat in function (13). Recent investigations of culture solutions after growth of S. pulverulentum on cellulose have demorstrated the existence of two different proteases of the carboxy-peptidase and chymotrypsin type. These enzymes seem to influence the release of endo-1,4-β-glucanases from the fungal cell wall (14). They also appear to modify the fungal cell wall (15). Whether or not these enzymes are responsible for the complex pattern of the endo-glucanases in S. pulverulentum is not known. However, the recent finding of Gritzali and Brown (12) concerning the very simple enzyme picture in T. reesei QM 9414 when the cellulases are induced by sophorose in a short term culture point to this possibility. An investigation into the effect of these conditions on cellulase production in S. pulverulentum is under way.

In S. pulverulentum, an oxidative enzyme of importance for cellulose degradation has been discovered in addition to the hydrolytic enzymes described above (16). The enzyme has been purified and characterized and found to be a cellobiose oxidase, which oxidizes cellobiose and higher dextrins to their corresponding onic acids using molecular oxygen. The enzyme is a hemoprotein and also contains a FAD group. It is not yet known whether this enzyme also oxidizes the reducing end-group formed when a 1,4-β-glycosidic bond is split in insoluble cellulose through the action of the endo-glucanases. It was recently reported by Vaheri (17) that culture solutions of T. reesei grown on cellulose also contain gluconolactone, cellobionolactone and cellobionic acid. These findings indicate that T.reesei also produces an oxidative enzyme involved in cellulose degradation. However, further confirmation is necessary in this case.

The fungus S. pulverulentum has two pathways to oxidize cellobiose. The first is through the enzyme cellobiose oxidase while the other is through the enzyme cellobiose:quinone oxidoreductase (18,19). This enzyme reduces quinones and phenoxy radicals in the presence of cellobiose and is of importance for the degradation of both cellulose and lignin. Although the enzyme seems to be involved in both lignin and cellulose degradation the highest yields of the enzyme were obtained when cellulose powder was used as a carbon source. In S. pulverulentum development of cellobiose:quinone oxidoreductase activity and cellulolytic activity occurred simultaneously. The enzyme is relatively specific for its disaccharide substrate while the requirements of the quinone structure are less specific and the enzyme is able to reduce both ortho- and para-quinones. A reaction scheme for the enzyme is presented in (9).

Regulation of endo-1,4-β-glucanases in the white-rot fungus S. pulverulentum has recently been investigated using a newly developed sensitive method (20). The method is based upon the viscosity lowering effect of endo-1,4-β-glucanases on solutions of carboxy-methyl cellulose (CMC). The effect of inducers and repressors can be determined as well as whether the enzymes are localized on the cell wall surfaces or actively released into the surrounding medium. The results show that cellobiose causes induction at concentrations as low as 1 mg/l and that glucose concentrations as low as 50 mg/l cause repression of enzyme formation. Mixtures of inducer and repressor give rise to a delayed enzyme production compared with solutions of inducer only.

Studies of the mould T. reesei QM 6a using the same technique show that cellobiose is not an efficient inducer. However, sophorose causes induction of endo-1,4-β-glucanases at a concentration of 1 mg/l. The comparison between the regulation of endo-1,4-β-glucanase production in the two fungi also demonstrates several other important differences. Thus, a solution of CMC alone induces enzyme formation in S. pulverulentum but not in the T. reesei strain. Under our experimental conditions no endo-1,4-β-glucanases were actively excreted into the solution by T. reesei. The enzymes were bound to the cell wall. However, S. pulverulentum released the enzymes into the medium although they first appeared bound to the

cell wall. It was recently shown by Gritzali and Brown (12) that sophorose gives rise to active excretion of endo-1,4-β-glucanases into the culture solution of T. reesei QM 9414. The differences in the results of the two studies must be due either to differences in the fungal strains or in cultivation conditions.

The production of cellulases in fungi can also be hampered by factors other than catabolite repression. Thus, it was demonstrated by Váradi (21) that a wide variety of phenols repress the production of both cellulases and xylanases in the fungi Schizophyllum commune and Chaetomium globosum. At concentrations of less than 1 mM, vanillic acid, vanillyl alcohol and vanillin considerably repress the production of these enzymes. In recent studies in our laboratory (22) it was shown that in a phenol oxidase-less mutant (Phe 3) of S. pulverulentum the production of endo-1,4-β-glucanases was drastically repressed in the presence of kraft lignin and phenols at a concentration of 10^{-3} M. However, both the wild-type (WT) and a phenoloxidase-positive revertant (Rev 9), obtained by U.V. irradiation of Phe 3, produced the enzymes without significant repression by phenols. Furthermore, if a highly purified laccase preparation was added to the growth medium of Phe 3 in the presence of phenols, the endo-1,4-β-glucanase production increased to normal. These results indicate that kraft lignin and phenols decrease endo-1,4-β-glucanase synthesis in Phe 3 due to the absence of phenoloxidizing enzymes. Phenoloxidases may thus function in regulating the production of cellulases by oxidizing lignin-related phenols which act as repressors of enzyme production when S. pulverulentum is growing on wood.

The activity of cellulose hydrolyzing enzymes in culture filtrates of S. pulverulentum is dependent not only on mechanisms regulating their biosynthesis but also on the presence of specific regulatory inhibitors of the enzymes themselves. One such inhibitor is gluconolactone produced by oxidation of glucose by the enzyme glucose-oxidase or by hydrolytic cleavage of cellobionolactone (9). The importance of gluconolactone for the regulation of 1,4-β-glucosidases from S. pulverulentum has recently been studied in our laboratory (23). The extracellular β-glucosidase activity can be split into two main peaks. Both free 1,4-β-glucosidase and cell-bound enzymes appear in S. pulverulentum. The k_i-values for the two free β-glucosidases were $3{,}5 \times 10^{-7}$ and 15×10^{-7} respectively. A K_i-value of 3.2×10^{-5} M is obtained for the T. reesel QM 9414 β-glucosidase with gluconolactone as an inhibitor. The corresponding K_i-value for the same enzyme by glucose is 1×10^{-3} M (12).

DEGRADATION OF HEMICELLULOSES

An extensive review article on hemicellulases was published in 1976 by Dekker and Richards (24). This article summarizes in great detail what was known to that date concerning the degradation of the different hemicelluloses.

Endo-1,4-β-mannanases are hydrolytic enzymes capable of hydrolyzing the 1,4-β-D-mannopyranosyl linkages of D-mannans and D-galacto-D-mannans. However, enzyme preparations have been described which have the capability of degrading mannans with other structures (25). The mannanases produced by different microorganisms have been reported to be both induced and constitutive enzymes (24). All fungal mannanases have been shown to degrade the mannans in a random manner and thus to be of the endo-type.

In addition to the endo-1,4-β-mannanases, 1,4-β-mannosidases are also produced (26). These enzymes have a similar function in mannan degradation to the 1,4-β-glucosidases in cellulose degradation, that is, the release of monomeric sugars from water soluble dextrins. As far as is known mannan degrading enzymes of the exo-type do not exist (24).

A variety of different xylan degrading enzymes do exist. Thus, both the endo-1,3-β-xylanases and endo-1,4-β-xylanases are produced by both fungi and bacteria. Since the structure of xylan polysaccharides vary a great deal the xylanases also show a variety of different activities (24). Xylanases seem to be produced both constitutively and by induction. It has repeatedly been demonstrated by different workers (27,28) that xylanases are produced when a cellulose preparation, free from xylan, is used as the sole carbon source for fungal growth. Cellulases and xylanases seem to be different enxymes and, in the few cases where one enzyme has been reported to have two activities, it seems likely that one is dealing with an impure enzyme preparation. It was demonstrated by Björndal [cited in (29,30)] that D-xylan was present in the mycelium of Stereum sanguinolentum. It is suggested by these workers that formation of D-xylanase in this organism, when grown on cellulose, may be self-induced. The xylanase may then be induced for the lysis of old cell wall material, which then supplies the organism with an endogenous form of energy. Thus, it appears that in fungi, at least for degradation of xylan, there is often no strict differentiation between adaptive and constitutive D-xylanases.

Xylosidases have also been reported to be produced by fungi (26). Their function is to release monomeric sugars from water soluble xylo-dextrins. However, as far as is known, no reports are published on the existence of exo-xylanases (24). As stated above crystalline cellulose can only be enzymatically degraded by the synergistic action of endo- and exo-1,4-

-β-glucanases. Since neither mannan nor xylan are crystalline in structure there should be no need in these cases for the existence of an endo- and an exo-pair of enzymes.

DEGRADATION OF LIGNIN

Lignin is a phenyl propanoid structural polymer of vascular plants which gives the plants rigidity and binds plant cells together (31). Lignin also decreases water permeation across cell walls of xylem tissue and protects plant tissues from invasion by pathogenic microorganisms. This very complex polymer is not readily attacked by microorganisms. The most successful group of organisms in lignin degradation, the white-rot fungi, remain the only microorganisms shown capable of totally degrading all the major wood components.

A considerable amount of information has now accumulated concerning mechanisms of lignin degradation by white-rot fungi. To a large extent this is the result of comparative chemical analysis of sound lignins and lignins decayed for varying times by specific white-rot fungi. Lignins from spruce, heavily degraded by Coriolus versicolor and Polyporus anceps, have been characterized in great detail. Analysis has shown that there is a decrease in both aliphatic and phenolic hydroxyls while the number of carboxyl groups has increased. There is also an increase in the number of carbonyl groups in the side chain carbon next to the aromatic ring (32). In order to cleave an aromatic ring microorganisms normally require two hydroxyls ortho or para to each other, or three hydroxyls, attached to the ring. An increased number of phenolic hydroxyl groups during an early stage of lignin degradation would thus indicate that aromatic rings are prepared for cleavage. Ring cleavage would then result in enrichment of degraded lignin with carboxyl groups simultaneously with the loss of phenolic hydroxyls.

Kirk and Chang (32) distinguished between α, β-unsaturated and aromatic carboxyls within the lignins and observed that a large percentage of the carboxylic groups were not attached to an aromatic ring. This work has also shown that the attacked lignins have been extensively demethylated in spite of a low number of phenolic hydroxyls. Aromatic rings were also depleted in the degraded lignins, although the lignins were greater than 1700 dalton in mol. weight (33).

From these data a sequence of degradative reactions for lignin by white-rot fungi was proposed. Kirk et al. (33) suggest that fungi first attack exposed surfaces of the lignin polymer. Demethylation reactions, acting upon methoxyl groups within guaiacyl and syringyl units are the principal degradative reactions of early attack. As a result, the methoxyl content decreases and ortho-diphenolic moieties are formed. These in turn are attacked by fungal dioxygenases which open the aromatic ring in the polymer with the formation of aliphatic carboxylic groups.

Recent investigations have demonstrated the critical importance of several culture parameters in lignin metabolism by wood-rotting fungi (34). The studies by Keyser et al. (35) have shown that metabolism of lignin is maximal at low concentrations (approximately 2 mM) of nutrient nitrogen. It has also been demonstrated by several workers that lignin is metabolized only in the presence of an easily metabolized additional carbon source, such as glucose, cellobiose or cellulose (3). Furthermore, the degradation of the lignin polymer takes place only in standing cultures (34).

It is generally accepted that lignin biosynthesis is catalysed by phenol oxidases (36). The involvement of phenol oxidases in the degradation of lignin has also been discussed ever since Bavendamm (37) used gallic and tannic acids to differentiate between white-rot and brown-rot fungi. The reason for this interest may be that white-rot fungi, which degrade and utilize lignin, also produce extracellular phenol oxidases in contrast to brown-rot fungi. Furthermore, lignin contains phenolic units which constitute a substrate for phenol oxidases.

In view of the above, it seemed natural for us to study the importance of phenol oxidases in lignin degradation (22). For these studies three different strains of S. pulverulentum were used, namely a) the wild-type, b) a phenol oxidase-less mutant, Phe 3 (obtained by UV-irradiation of wild-type spores) and c) a phenol oxidase-positive revertant, Rev 9 (obtained by UV-irradiation of spores from Phe 3). The phenol oxidase-less mutant did not degrade lignin or any other wood components. However, the revertant degraded all wood components, including lignin, to the same extent as did the wild-type strain. After addition of purified laccase to kraft lignin agar plates, the phenol oxidase-less mutant could again degrade lignin almost as well as the wild-type, indicating that only the gene controlling the synthesis of phenol oxidase was affected by the mutagenic treatment. These results point to an obligatory role of phenol oxidases in lignin degradation.

It has been demonstrated in several studies that vanillic acid is always a metabolic product of lignin degradation by white-rot fungi (38). Since our efforts to use different isolated lignins as substrate for submerged growth of S. pulverulentum in order to evaluate the enzyme mechanisms involved in the degradation of lignin had failed, partly due to the difficulties in obtaining conditions for a degradation, we decided to use vanillic acid as substrate. The

strategy has been to approach the problem "enzyme mechanisms involved in lignin degradation by white-rot fungi" by working initially with small molecules, lignin models, approaching the lignin polymer as such by using more and more complex substances.

The results from the studies of vanillic acid degradation by S. pulverulentum are presented in Fig. 9 (39). It can be seen here that vanillic acid is simultaneously oxidatively decarboxylated to methoxyhydroquinone (MHQ) and reduced to vanillin and vanillyl alcohol. The decarboxylation pathway is more predominant in shake cultures, while there is more reduction in standing cultures. The reduction steps also seem to require energy in the form of an externally supplied, easily metabolized, carbon source such as glucose or cellobiose.

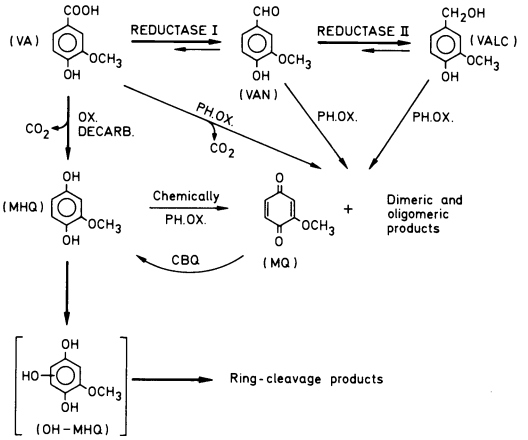

Fig. 9 Proposed routes for the metabolism of vanillic acid by S. pulverulentum. OH-MHQ is a hydroxylated MHQ which may be the true substrate for ring-cleavage. From (39).

The amounts of degradation products found in the culture media are generally low and never exceed 30 % of the initial vanillic acid concentration. Vanillate seems to be metabolized inside the fungal cell. The intracellular vanillate hydroxylase (catalyzing decarboxylation) has been isolated and purified and some of its characteristics described by Buswell et al. (40). The phenol oxidases laccase and peroxidase can also decarboxylate vanillic acid but are not dependent on NAD(P)H for activity (39,41).

It has also been demonstrated that S. pulverulentum produces an aromatic ring-cleaving enzyme (42). This enzyme does not cleave the ring unless three hydroxyl groups are attached. By using differently labelled vanillic acids it was possible to show that decarboxylation takes place before the ring is cleaved which in turn occurs earlier than the release of $^{14}CO_2$ from $O^{14}CH_3$-vanillate, Fig. 10. The results presented in Fig. 10 suggest that a third hydroxyl group is introduced into the ring via direct hydroxylation rather than via demethylation which does not appear to take place until after the ring is cleaved (39).

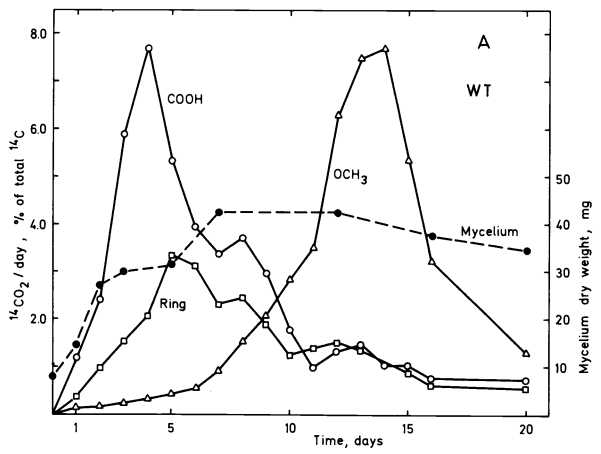

Fig. 10 $^{14}CO_2$ evolution from $^{14}COOH$-vanillate, $O^{14}CH_3$-vanillate and $^{14}Ring$-vanillate by S. pulverulentum in standing cultivation on 0.5 % cellobiose, 0.05 % yeast extract and aconitate medium at pH 4.5. From (39).

Quinones are readily formed by the action of phenol oxidases induced during the growth of white-rot fungi on both low molecular weight phenolic compounds and lignin. Although certain quinones are normal components of cellular electron-transport systems, quinones generally are highly reactive and are known to inhibit a wide range of metabolic processes. Therefore, reduction of quinoid intermediates would be essential. We have earlier reported that extracellular reduction of quinones and phenoxy radicals take place via the extracellular enzyme cellobiose:quinone oxidoreductase (18,19). This enzyme makes use of cellobiose as a co-substrate. The cellobiose is thereby oxidized to the corresponding lactone. In our work with vanillic acid metabolism, we have now found a second, intracellular, quinone oxidoreductase system from S. pulverulentum which reduces quinones to hydroquinones using pyridine nucleotides as electron donors (43).

In another study, ferulic acid has been used as substrate for S. pulverulentum (44). Several compounds such as vanillic acid, vanillin and methoxyhydroquinone have been identified as metabolic products. In addition, large amounts of reduced products such as coniferaldehyde and dihydroconiferyl alcohol are formed.

The approach of using lignin model substances to study lignin metabolism has been adopted by several other laboratories (3). Gold and coworkers (45,46) have studied both vanillic acid and dimer degradation by the white-rot fungus Phanerochaete chrysosporium (synonymous to S. pulverulentum). They were able to show direct cleavage of the α, β-bond of the β-ether type dimers (46). Dimers have also been studied by Higuchi's group in Japan (47).

In our laboratory we have now developed growth systems that will allow us to study more efficiently the degradation of the lignin polymer. The technique involves the use of labelled lignin and of a solid state growth system. This growth system will hopefully also allow us to characterize the enzymes involved in the attack on the lignin polymer.

BIOTECHNOLOGY BASED ON LIGNOCELLULOSIC MATERIALS

The increasing pressure on fossil fuel resources has given rise to a world-wide interest in the production of fuels and chemicals from renewable resources via biotechnological processes. A prerequisite for the development of biotechnology based on lignocellulosic materials is to have access to microorganisms that can attack materials such as wood, straw, sugarcane bagasse etc. and also to understand how such microorganisms carry out the decomposition processes at the molecular level.

This review was intended to present existing knowledge of enzyme mechanisms involved in the degradation of wood components. With an even better understanding for instance of the enzymes involved in lignin degradation, it may be possible to modify water soluble lignins from pulping processes into useful products by the use of specific matrix bound enzymes. For saccharification of cellulose to glucose and further fermentation to ethanol it is important not only to know which enzymes are carrying out the hydrolysis but also to understand, at the gene level, how the production of these important enzymes can be regulated and increased. With the intensive work that is going on in laboratories all over the world it seems inevitable that at least some of the petrochemistry can be substituted by wood chemistry and especially perhaps by wood biochemistry.

REFERENCES

1. A. Käärik, in C.H. Dickinson and G.J.F. Pugh (Eds.), Biology of Plant Litter Decomposition 1, Academic Press, London-New York, p. 129 (1974).
2. W.W. Wilcox, Bot. Rev. 36, 1 (1970)
3. P. Ander and K.-E. Eriksson, Prog. Ind. Microbiol. 14, 1 (1978).
4. K.-E. Eriksson, A. Grünewald and L. Vallander, Biotechnol. Bioeng. 22, 363 (1980).
5. V. Rypáček and Z. Navrátilová, Drev.Výskum 16, 115 (1971).
6. K.-E. Eriksson and L. Vallander, Biomechanical Pulping. In: Lignin Biodegradation: Microbiology and Applications, T.K. Kirk, T. Higuchi, H,-M. Chang (Eds.), Boca Raton: CRC Press (1980).
7. K.-E. Eriksson, A. Grünewald, T. Nilsson and L. Vallander, Holzforschung. In press.
8. K. Ruel, F. Barnoud and K.-E. Eriksson. To be published.
9. K.-E. Eriksson, Biotechnol. Bioeng. 20, 317 (1978).
10. D.D.Y. Ryn and M. Mandels, Enzyme Microb. Technol. 2, 91 (1980).
11. G.H. Emert, E.K. Gum, Jr., J.A. Lang, T.H. Lin and R.D. Brown, Jr., Adv. Chem. Ser. 136, 76 (1974).
12. M.Gritzali and R.D. Brown, Jr., Adv. Chem. Ser. 181, 237 (1979).
13. M. Streamer, K.-E. Eriksson and B. Pettersson, Eur. J. Biochem. 59, 607 (1975).
14. K.-E. Eriksson and B. Pettersson. To be published.
15. K.-E. Eriksson, A. von Hofsten and B. Pettersson. To be published.
16. A.R. Ayers, S.B. Ayers and K.-E. Eriksson, Eur. J. Biochem. 90, 171 (1978).

17. M. Vaheri, Nordforsks Arbetsseminarium, May 6-7, 1980, VTT:s Biotechnical Laboratory, Otnäs, Helsinki.
18. U. Westermark and K.-E. Eriksson, Acta Chem. Scand. B 28, 204 (1974).
19. U. Westermark and K.-E. Eriksson, Acta Chem. Scand. B 28, 209 (1974).
20. K.-E. Eriksson and S.G. Hamp, Eur. J. Biochem. 90, 183 (1978).
21. J. Váradi. In: Biodeterioration of Materials 2, 129 (1972). Eds. A.H. Walters and E.H. Hueck-van der Plas, London: Appl. Science Publishers.
22. P. Ander and K.-E. Eriksson, Arch. Microbiol. 109, 1 (1976).
23. V. Deshpande, K.-E. Eriksson and B. Pettersson, Eur. J. Biochem. 90, 191 (1978).
24. R.F.H. Dekker and G.N. Richards. In: Adv. Carbohydr. Chem. 32, 277 (1976).
25. Y. Tsugisaka, K. Hiyama, S. Takenishi and J. Fukumoto, Nippon Nogei Kagaku Kaishi 46, 155 (1972).
26. E. Ahlgren and K.-E. Eriksson, Acta Chem. Scand. 21, 1193 (1967).
27. H. Lyr, Arch. Microbiol. 34, 418 (1959).
28. B. Bucht and K.-E. Eriksson, Arch. Biochem. Biophys. 124, 135 (1968).
29. K.-E. Eriksson and W. Rzedowski, Arch. Biochem. Biophys. 129, 683 (1969).
30. K.-E. Eriksson and B. Pettersson, Int. Biodeterior Bull. 7, 3, 115 (1971).
31. K.V. Sarkanen and C.H. Ludwig. In: K.V. Sarkanen och C.H. Ludwig (Eds.), Lignins: occurrence, formation, structure and reactions,Wiley-Interscience, New York, p. 43 (1971).
32. T.K. Kirk and H.-M. Chang, Holzforschung 28, 217 (1974).
33. T.K. Kirk, H.H. Yang and P. Keyser, Dev. Ind. Microbiol. 19, 51 (1978).
34. T.K. Kirk, E. Schultz, W.J. Connors, L.F. Lorenz and J.G. Zeikus, Arch. Microbiol. 117, 277 (1978).
35. P. Keyser, T.K. Kirk and J.G. Zeikus, J. Bacteriol. 135, 790 (1978).
36. K. Freudenberg and A.C. Neish, Constitution and Biosynthesis of Lignin, Springer, Berlin, Heidelberg, New York (1968).
37. W. Bavendamm, Z. Pflanzenkr. 38, 257 (1928).
38. T.K. Kirk, W.J. Connors and J.G. Zeikus. In: Recent Adv. Phytochem. 11, The Structure, Biosynthesis, and Degradation of Wood. Eds.: F.A. Loewus and V.C. Runeckles, Plenum Press, New York, p. 369 (1977).
39. P. Ander, A. Hatakka and K.-E. Eriksson, Arch. Microbiol. 125, 181 (1980).
40. J.A. Buswell, P. Ander, B. Pettersson and K.-E. Eriksson, FEBS Lett. 103, 98 (1979).
41. K. Krisnangkura and M.H. Gold, Phytochemistry 18, 2019 (1979).
42. J.A. Buswell and K.-E. Eriksson, FEBS Lett. 104, 258 (1979).
43. J.A. Buswell, S. Hamp and K.-E. Eriksson, FEBS Lett. 108, 229 (1979).
44. J.K. Gupta, S.G. Hamp, J.A. Buswell and K.-E. Eriksson, Arch. Microbiol. In press.
45. Y. Yajima, A. Enoki, M.B. Mayfield and M.H. Gold, Arch. Microbiol. 123, 319 (1979).
46. A. Enoki, G.P. Goldsby and M.H. Gold, Arch. Microbiol. 125, 227 (1980).
47. M. Ohto, T. Higuchi and S. Iwahara, Arch. Microbiol. 121, 23 (1979).