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DEGRADATION OF WOOD BY MICROORGANISMS

by

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I - INTRODUCTION

In some unfavorable environmental conditions of use, wood can be severely degraded by microorganisms. The most efficient wood destroyers are the Fungi of the class of Basidiomycetes, from the white-rot or the brown-rot groups. Lignolytic activity has been found in more than 600 species. Other fungi, like Ascomycetes or even Fungi imperfecti are also able to damage wood although less severely. Some bacteria are known as agent of wood deterioration but the degradation process is usually limited and slow.

Because of the considerable importance that wood has gained in the life of man, all this biological causes of wood damaging are the subject of intensive investigations. Forest trees, timber, wood piles and mines, pulpwood have to be protected against degradation by microorganisms. An improved knowledge of the decaying processes will ensure progress in the present techniques of wood preservation which are of a very great economic importance.

In the past, excellent reviews have covered the considerable volume of publications dealing with the subject of microscopic study of wood degradation by microorganisms. The various types of decay caused by bacteria and fungi, the morphological changes provoked by the digestion of woody tissues (Meier 1955, Jurasek 1964, Liese 1970,

Wilcox 1968-1973) the various patterns of the propagation of the hyphae (Cowling 1961) are different topics which have been well reviewed in the past. The aim of this article is to present the main results which have been described at the ultrastructural level and obtained since 1970 in the field of wood degradation by fungi.

II - MICROMORPHOLOGICAL ASPECTS OF WOOD DECAY

A. Scanning electron microscopy

Since 1970, the scanning electron microscope (SEM) has been extensively used in the field of wood pathology (Blanchette et al., 1978). The possibility of tridimensional observations makes SEM particularly attractive for the exploration of infected wood. The most commonly investigated subjects were the size and shape of hyphae, the mode of microcolonization of tissues, the growth of the hyphae into tracheids, fibers, vessels, rays cells, the spreading from cell to cell in the woody tissues (Fukuda et al. 1975, Blanchette et al. 1981, Eriksson et al. 1980a, Dirol 1976, 1978, Ravilly and Dirol 1977, Blanchette 1980a, Otjen and Blanchette 1982).

The disorganization of wood by white-rot fungi, white-pocket rotters, brown-rotters or canker-rot fungi has been described in detail by several authors (Bravery 1971, Jutte and Zabel 1974, Dirol 1976, 1978, Blanchette 1980a, ab, 1982b, Otjen and Blanchette 1982, Blanchette 1983).

Erosion troughs formed beneath the hyphae are characteristic of the early stages of decay by the white-rot fungi (Blanchette 1980) whereas numerous holes within the tracheids are visible at more advanced stages of decay (Jutte and Zabel 1974, Blanchette 1977, 1979, Ravilly et Dirol 1977, Dirol et Ravilly 1979, Eriksson et al. 1980). Fig. 1 shows erosion troughs or holes typical of a white-rot pattern of decay in tracheids (Blanchette, 1982b). Spindle-shaped zones of cellulosic microfibrils surrounded by apparently sound wood are characteristic of the white-pocket type of decay (Blanchette 1980b, a, 1982b, 1983, Dirol 1980, Otjen and Blanchette 1982).

Brown-rot fungi remove cellulose and leave lignin relatively unaltered. In advanced stages of degradation, button-hole shaped cavities are visible (see fig. 2) (Dirol 1978). Upon drying the wood cracks, checks into cubical blocks and finally crumbles (Blanchette et al. 1978, Dirol 1978, Blanchette 1980b). SEM has also been used in the study of the associations among Bacteria, Yeasts and Basidiomycetes during natural wood decay processes (Kilbertus et al. 1976, Blanchette and Shaw 1978, Blanchette et al. 1978, Blanchette 1979, 1982b).

However, resolution in SEM is affected by the fact that samples have to be metallized before observation and by the lack of specific staining for wood components. Although limited to a bidimensional plan of observation, TEM offers over STEM the advantage of the sharpness of its images. Also specific staining can be used for the identification of the wood components.

B. Transmission electron microscopy

The micromorphological aspects of wood decay can be conveniently visualized by the observation at low magnification of ultra-thin sections of wood (Chou and Levi 1979, Wilcox et al. 1974, Ouelette 1978, Peterson and Thomas 1978) or other lignified tissues like barks (Parameswaran al. 1976). The pattern of degradation of the different layers of tracheids or fibers can be easily followed. The propagation of the hyphae across the cell walls of woody tissues is also conveniently studied.

Recently, Ruel et al. (1981) have studied ultra-thin sections of spruce and birch wood degraded by Sporotrichum pulverulentum. After a six-week incubation period the fungal hyphae and microhyphae are observed both in tracheids and parenchyma ray cells, the latter being usually the first infected. In spruce (fig. 3), the tracheids appear as the most altered structures. A quite characteristic feature of the propagation of the hyphae is the disorganization of the wood structure consecutive to the enzyme diffusion through the transition layers S_2/S_3 and S_1/S_2 of the tracheids walls. The easier fungal progression into these transition layers can be due to a looser fibrillar texture or a lower degree of lignification. *... a later study on wood decay*

The three layers of the tracheids are not degraded at the same rate. S_3 , in spruce tracheids appears particularly resistant. This

layer is progressively detached along the S_2/S_3 transition layer and finally torn off but is not lysed.

The S_2 layer mainly in its inner part is the most degraded. Several holes are bored concentrically to the lumen and tend to coalesce to form large galleries which result in the complete disorganization of the internal part of S_2 . It can be understood that wood suffers dramatic losses of its physical properties after such a complete disorganization of the internal part of the S_2 layer. The S_1 layer is degraded only in the later stages of decay.

In the case of a brown-rotter such as Coniophora puteana, the aspects of wood degradation are more discrete. It can be established by the observation of ultra-thin sections of Pinus sylvestris wood that the organization of the tissues is maintained after a four-week period of incubation. Nevertheless, radial dislocations turning into holes towards the lumen and outwards, towards the S_1 layer are visible (fig. 4). S_2 appears less affected by the enzymes of the brown-rotters than that of the white-rotters. In more advanced stages of degradation the wall disorganization progresses from the holes and concentrically modifies the lignin texture provoking a separation of a ring formed with S_3 and the internal part of S_2 . Such an observation was already noticed by Wilcox et al., in Douglas-fir tracheids (1974).

To summarize and as it has been already stated by Liese (1970), the differences in susceptibility of the three main layers of a tracheid (or fiber) wall according to the organism involved can be

ascribed to the different chemical composition and the architecture of these layers.

III - ULTRASTRUCTURAL ASPECTS OF THE DEGRADATION OF WOOD COMPONENTS

A major difficulty at this level of observation lies in the identification without ambiguity of the three main wall components cellulose, hemicelluloses and lignin. There are no specific stainings but for lignin (Hepler et al. 1969). Lignin can be visualized after oxidation by potassium permanganate by a deposition of manganese oxides wherever lignin is present (Kerr and Goring 1975). The polysaccharide part of the wood cell wall can be visualized by the PATAg reagent (Thierry 1969) provided a carefully controlled time of oxidation with periodate is applied (Ruel et al. 1977).

Nevertheless a clear distinction between hemicelluloses and cellulose is difficult to obtain because of their highly packed arrangement. In this respect the enzymic modifications undergone by lignin, cellulose and hemicelluloses on the site of the progression of the hyphae can provide an indirect approach to the visualization of the ultrastructural organization of the cell wall.

In order to precise the effect of the different degrading activities of the fungi on the respective cell wall constituents, four types of fungal strains have been used. One was the wild-type Sporotrichum pulverulentum selected for its high capability to degrade lignin and all other wood components (Ander and Eriksson 1978). The

second was the cellulase-less mutant Cel 44 (Ander and Eriksson 1975) which was found to be totally unable to degrade cellulose but could attack both xylan and glucomannan. The third was the wild-type Sporotrichum pulverulentum grown on glucose impregnated samples. In this condition of growth, cellulase activities are repressed (Eriksson and Goodell 1974, Eriksson and Hamp 1978) as well as hemicellulases activities (Eriksson, personal communication).

The fourth fungal species used in our experiments was the brown rotter, Coniophora puteana. The activity of this fungus is characterized by a two steps degradation. The first step consists in an intensive depolymerization of the cellulose. In a second step, there is a further degradation of the depolymerized cellulose and an attack of the hemicelluloses (Kirk 1975).

Owing to the particular enzymatic equipment of each of the four strains, information could be obtained both on the degradation and the localization of the wall components.

A. Aspects of lignin degradation

The action of the wild-type and that of Cel 44 were compared on ultra-thin sections of spruce and birch wood (Ruel et al. 1980). The changes brought about by the ligninolytic enzymes could be established by comparison of the lignin ultrastructural aspects in decayed and sound areas.

In transverse sections made in sound wood (fig. 5) lignin can be seen as a network of short and interrupted lamellae slightly varying in orientation or as random spots. This heterogeneity in the ultrastructural distribution of lignin (Ruel et Barnoud 1983) has to be related to the variations in the microfibrils orientation inside the S_2 layer at the level of microterritories of the wall (Revol et al. 1982).

In spruce wood decayed with Cel 44, the walls of the tracheids take a swollen and dislocated aspect particularly visible in the S_1 layer where cellulose microfibrils are lying in the plane of the section (fig. 6). Bundles of fibrillar material are separated by more or less important opened spaces. Whether the rupture of the cohesion factors between the wall components was due to ligninolytic or hemicellulolytic enzymes is still an open question although we have evidence that the breakdown of the hemicelluloses, or at least a part of them, is the first manifestation of the enzymes diffusing in the tracheid walls (Ruel et al. 1981). These observations confirmed the chemical datas suggested by Kirk and Highley in 1973 and later obtained by Ander and Eriksson (1975a).

These images of an initial swelling of the lignin network are only observed in wood decayed by the cellulase-less mutant Cel 44. It must be recalled that the lignin degrading activity of this strain is approximately five times slower than that of the wild-type (Ander and Eriksson, 1975a). This low degrading activity could be explained by the loss of the enzyme cellobiose quinone-oxydo-reductase which plays

an important role in lignin degradation (Westermarck and Eriksson 1974a and b, 1975). The fact that polysaccharides in wood must be degraded simultaneously with lignin (Ander and Eriksson 1975, 1977, 1978 ; Kirk and Moore, 1972 ; Kirk et al. 1976) could also explain the reduced activity of this mutant.

When spruce wood is decayed by the ligninolytic enzymes of the wild-type strain, lignin offers a markedly different aspect. Observations on cross-sections show, around the bore holes, an area extending on two to three micrometers in diameter presenting a granular structure. This area is more strongly contrasted by MnO_2 deposits than the surrounding sound part of the wall (fig. 7). These ultrastructural images of lignin degradation are to be related to the chemical data recently obtained by Kirk (1981), Chang et al. (1981), Higuchi (1982), Chen et al. (1983). The white-rot degradative processes of lignin give in part monomeric units bearing α -carboxyl and α -carbonyl groups as a result of $C\alpha-C\beta$ cleavage in the side chains as well as $C\alpha$ -oxidation (Kirk and Chang 1974, 1975, Kirk 1981, Chua et al. 1982, Chen and Chang 1982). Lignin is also partly degraded by cleavage of the aromatic rings when still bound in the polymer. Ring cleavage results in an enrichment of degraded lignin with carboxyl groups (Kirk and Chang 1975, Chua et al. 1981, Ellwardt et al. 1981, Kirk et al. 1978). Furthermore an increase in phenolic hydroxyl groups has been established at an early stage of lignin degradation (Eriksson 1981).

According to these chemical changes, one can expect the enzymatically degraded lignin to be more reactive to KMnO_4 . We have observed in fact that the form of lignin left aside the decayed zones is more contrasted as the degradation proceeds.

This fact was also observed on longitudinal sections where characteristic deposits of electron dense granules are abundant (fig. 8). These granules which are very reactive to potassium permanganate fail to stain with reagents for polysaccharides (neutral or acidic) or proteins. Accordingly they can be considered as degraded forms of lignin not metabolized by the fungus (Chen et al. 1983). When sections are cut tangentially through the S_1 layer (fig. 9) the dark granules of modified lignin can be seen still aligned. They are about 5 to 10 nm in diameter. At a more advanced stage of degradation they become isolated and then tend to aggregate to form large clusters of 30 to 50 nm in size.

The same aspects of ligninolysis can be seen on birch ultra-thin sections degraded by the Cel 44 strain which is more active on hardwoods than on softwoods. Lined up dark granules are formed before being detached from the wall (fig. 10).

The abundance of these lignin residues in the walls attacked by the enzymes of the wild-type strain of Sporotrichum pulverulentum must be correlated to Kirk's assumption (1981) that lignin degradation is a slow process relevant to the secondary stage of growth of the fungus (see also Chua et al. 1983).

It was thought for a long time that brown-rot fungi can degrade both cellulose and hemicelluloses but do not lyse the lignin part of the wall (Cowling 1967, Wilcox 1968, 1970, Liese 1970). In early stages of degradation, tracheids cell walls take a foamy aspect (fig. 11). The characteristic distribution in short and interrupted lamellae of the lignin in sound wood seen in transverse sections, is modified. Lignin repartition becomes both scattered and loosened, taking a granular aspect. However, although modified in its organisation, lignin remains as a coherent structure.

There is no apparent enhancement of the reactivity of the lignin to potassium permanganate oxidation as it was seen after white-rotting of the cell walls. However an important demethylation has been described by Kirk (1975) but this chemical modification seems to have no consequences on lignin susceptibility to permanganate.

B. Aspects of the degradation of the hemicelluloses and cellulose

The polysaccharide constituents of the walls are known to be degraded simultaneously with lignin by certain white-rotters (Cowling 1961, Liese 1970, Kirk and Moore 1972, Kirk 1973, Ander and Eriksson 1975, Eriksson 1980, 1981). The hemicelluloses are the first to be degraded as shown by chemical analysis (Kirk and Highley 1973, Eriksson 1975, Eriksson 1980, 1981, Kirk 1981, 1982) and confirmed by ultrastructural observations. This is illustrated in fig. 12 showing a section of spruce wood degraded by the wild-type strain and in which glucomannans and galactoglucomannans have been strongly solubilized.

This degradation took place principally around the bore holes. A crown failing to stain appears as a clear zone whereas the sound part is heavily deposited with silver grains. In the case of the walls degraded by Cel 44, the degradation being slower than with the wild-type, mannans and glucomannans depleted areas are essentially limited to the pits (fig. 13).

In fig. 14, the removal of hemicelluloses in S_2 created an opening in the compact structure of the wall exposing the cellulose framework. The weakly stained fibrillar structures can be regarded as pure cellulose. This resistance of cellulose to staining with PATAg in secondary wall was overcome by the use of the minimum beam exposure technique ("low dose" (Beer 1980)). This is a convenient method to prevent cellulose degradation under the electron beam. When applied to ray-parenchyma cells of birch degraded with Cel 44, long bundles of cellulose microfibrils still surrounded with matriciel components could be observed (fig. 15).

With the wild-type strain grown on glucose-impregnated wood which cannot degrade cellulose and hemicelluloses, the lignin solubilization lead to a "honeycomb" pattern which tends to give individualized microfibrillar structures (fig. 16 & 17). The finest fibrillar structures observed (35 to 40 nm in diameter) should correspond to pure cellulose microfibrils.

It is noteworthy that whereas the typical electron diffraction diagram of cellulose I was obtained on the parts of the wall where the matriciel components are still present (fig. 18), this typical diagram

disappeared in the strongly depleted areas, although they still kept a fibrillar aspect (fig. 19). The loss of the characteristic diffraction diagram of cellulose raises a problem about the nature of these fibrils.

IV - DIFFUSION OF WOOD DEGRADING ENZYMES

It has been proposed for a long time that the remarkable ability of the fungal mycelium to grow in the woody tissues was due to excreted enzymes which diffuse into the surrounding wall material (Cowling 1961, Jurasek 1964, Liese 1970, Wilcox 1968, 1970).

Such observations are consistent with the data obtained in scanning electron microscopy. The highly diffusible character of the cellulolytic enzymes is suggested essentially by the observation of degraded zones at large distances from the hyphae (Blanchette et al. 1978, Dirol 1978, Blanchette 1983, Eriksson et al. 1980). This is particularly evident in the case of brown-rotters which utilize the carbohydrates and leave the lignin only slightly affected.

This capability of the enzymes to act at a distance from the fungal cells was further evidenced by Eriksson's experiments (1981) showing the diffusion of purified enzymes isolated from Sporotrichum pulverulentum.

Ligninolytic systems have not yet been isolated and the more common hypothesis is that ligninolytic enzymes do not degrade lignin

unless in straight contact with their substrate (Kirk 1973, Chua et al. 1983).

At the present time there is no specific marker permitting the direct and specific visualization of the several proteins being present in the different groups of enzymes involved in the lysis of wood components. The nature of the diffusible enzymes can be deduced by the study of the micromodifications that they produce along the site of their progression. It has thus been possible to confirm that hemicellulases and cellulases migrate far from the tips of the hyphae which are known as the active site of the enzymes secretion.

It can be demonstrated in electron microscopy that enzymes migration, if it starts from the lumen as observed by other workers, (Cowling 1961, Wilcox 1970) can progress from a hole to another through thin channels. These channels are traced by the negative reaction with the PATAg staining, this data being due to their depletion in hemicelluloses (fig. 20).

As it has been chemically established (Ander et al. 1975), both the use of lignin (KMnO_4) and polysaccharide (PATAg) stains enabled to show that polysaccharide hydrolases are the first to act in the case of Sporotrichum pulverulentum decay (Ruel et al. 1981).

A more difficult situation arises about the elucidation of the diffusion of enzymes implicated in lignin degradation.

From the fig. 6 & 7 and 9 & 10 (e.g, section III.A) and from the fig. 21, it is obvious that chemical and ultrastructural changes occur in the lignified walls around the penetrating hyphae of Sporotrichum pulverulentum. These changes which have been already described (Ruel et al. 1981, 1983) are also observed in sections where no hyphae are present. The reasons of the modifications of the lignin molecular organization in the decayed areas are not clearly explained up to now.

Although several chemical data have shown that a true contact or a narrow binding are an necessary condition for the degradation of the lignin polymer (Rosenberg 1980, Chua et al. 1983), it is not yet established whether or not the components or some of the components of the ligninolytic system do diffuse or not from the point of their emission.

Two hypotheses can be proposed in relation with the data presented above. The first would be in favor of an initial enzymatic reaction requiring a contact between the hyphae and the wall components in their native state of molecular organization. From this initial reaction, other reactions non enzyme-mediated would be induced and propagated to the surrounding parts of the walls. The second hypothesis can be based on the fact that in most cases enzymes of the ligninolytic system diffuse to a certain distance from the hyphae in conjunction with hemicelluloses degrading enzymes. To our point of view a satisfactory answer will be obtained when specific markers of the several components of the ligninolytic system will be available.

At the present time the diffusion of the lignin-degrading enzymes remains an unsolved question.

V - DEGRADATION OF WOOD BY BACTERIA

Degradation of woody tissues by mixed bacterial flora or pure culture of bacteria has been the object of microbiological, chemical and microscopic investigations for many years. Several reviews on these subjects have been presented since 1970 (Liese 1970, Wilcox 1970, 1973, Liese and Greaves 1975). It is generally recognized that bacterial degradation of wood has a real economical importance only in special environmental conditions.

The presence of a large number of bacteria associated with micro and macrofungi in decayed wood has been recognized for a long time. No rigorous investigations were made until recently to establish clearly the ability of pure strains of bacteria to depolymerise and metabolize the wood components in their natural molecular association as they exist in situ in lignified walls.

Haider et al. (1978), Haider and Trojanowski (1980) Crawford et al. (1980) have established by the use of ^{14}C labelled lignins the capability of several species of bacteria to effectively degrade lignin in its isolated polymeric form or in lignified tissues. Pseudomonas, Bacillus and Nocardia strains were active in lignin degradation. Bacterial delignification of poplar wood sections has been investigated by Monties et al. (1981). These authors claim that high degradation rates of delignification (52 %) were obtained within

a one month incubation period with a Pseudomonas species. Studying the decomposition of lignified cell wall of Fagus sylvatica and Pinus sylvestris by 66 selected bacterial strains, Schmidt and Liese (1982) have shown that none of them could deteriorate sapwood cross sections (20 μm in thickness). After mild delignification with chlorite, several strains were able to heavily degrade the cell wall but without lignin metabolism (fig 22).

As it appears from the literature, a contradictory situation arises between the several authors who have studied wood degradation by bacteria. Additional data are to be obtained to show with clarity to what extent lignin can be effectively degraded and metabolized by the sole action of bacteria. By opposition, the degradation of cellulose and hemicelluloses when not protected by lignin is an easy process. Natural humification processes are quite evidently the result of an association between several groups of microorganisms (Blanchette 1979, Blanchette et al. 1978).

VI - CONCLUSION

In this chapter we have tried to cover some of the recent developpements obtained in the field of wood degradation by microorganisms. The results presented are mainly concerned with the most largely studied wood-decomposing fungus, the white-rotter Sporotrichum pulverulentum. In the lignified walls decayed by the penetrating hyphae of this fungus, investigations have been made on the ultrastructural changes provoked by the hyphae ; the modifications

of the supramolecular organization of the wood components have also been explored.

To have a more comprehensive view of the decaying process, it would be useful to extend this type of investigation to other fungi of the same group and also to fungi of the other groups of wood destroyers.

For the improvement of our knowledge in the field of wood degradation by micro-organisms, several developments are needed. For example, better contrasting reagents have to be developed for an accurate interpretation of the electron micrographs. It is also of the utmost importance to coordinate on the same wood samples the chemical analyses and the images of the changes in the macromolecular associations with the data given by the electron microscope.

In the field of the diffusion of enzymes in woody tissues, selective markers of proteins are needed to visualize the several fungal enzymes at work. Immuno-cytochemical markers could be the proper answer to this type of investigation.

It must be considered that improvements in our knowledge of the wall modifications resulting from the various and complex processes of the wood decay will be of importance to make progresses in two applied fields, such as biological delignification and wood preservation.

LEGENDSAbbreviations used in the figures

ML = middle lamelle

PW = primary wall

S₁ = outer layer of the secondary wall

S₂ = middle layer of the secondary wall

S₃ = inner layer of the secondary wall

H = hyphae

PC = parenchymatous cell

F = microfibrils

Fig. 1 - Scanning electron micrograph of white-fir wood decayed by P. Pini var. cancriformans. Erosion troughs or holes, typical of a white-rot pattern of decay in tracheids. (Blanchette 1982b)

Fig. 2 - Scanning electron micrograph of Scots pine sapwood cell walls degraded by the brown-rot fungus, Serpula lacrymans. Button-holes shaped cavities are visible. (Diro1 1978)

Fig. 3 - An example of the tracheid-walls degradation of Spruce due to the wild-type strain of the white-rotter, Sporotrichum pulverulentum. Bore holes tend to join and coalesce to forms large galleries. (Ruel unpublished)

Fig. 4 - Brown-rot degraded pine wood tracheids (Coniophora puteana). The organization of the wall is maintained but radial dislocations (arrow) giving rise to holes near S_1 and near the lumen are visible (arrow-heads). (Ruel unpublished)

Fig. 5 - Sound wall of a Spruce tracheid. Cross section through the S_2 layer after $KMnO_4$ staining. Lignin appears as short and interrupted lamellae varying in orientation under the region observed (arrows) and sometimes as random spots (framed in dotted lines). (Ruel et Barnoud 1983)

Fig. 6 - Spruce wall tracheid degraded by Cel 44 showing the swelling and the rupture of the lignin network in the S_1 layer. Bundles of fibrillar material are separated by more or less important opened spaces (arrow). (Ruel et al. 1981)

Fig. 7 - Wild-type Sporotrichum pulverulentum degraded tracheid wall. After $KMnO_4$ staining, bore holes are surrounded by a strongly contrasted crown of disorganized lignin (arrows). (Ruel and Barnoud 1981)

Fig. 8 - Longitudinal section through a wild-type decayed tracheid. A lot of characteristic deposits of electron dense granules border the decayed areas. (Ruel unpublished)

Fig. 9 - Longitudinal tangential section through the S_1 layer (wild-type decaying) dark granules of modified lignin can be seen still aligned (arrows). When detached, they tend to form large clusters (arrowheads). (Ruel et al. 1981)

Fig. 10 - Birch wood fibers decayed by the Cel 44 mutant of *Sporotrichum pulverulentum*. The images of lignin degradation are identical to those observed in Softwoods. Lined up dark granules are formed before being detached from the wall (arrows). (Ruel and Barnoud 1981)

Fig. 11 - Brown-rotted tracheid cell wall of Pine. Lignin network takes a foamy aspect and lignin repartition becomes both scattered and loosened. KMnO_4 fixed section. (Ruel unpublished).

Fig. 12 - Hemicelluloses degradation in a wild-type degraded tracheid spruce wall. A crown failing to stain with the PATAg reagent is clearly visible around the bore holes (arrows). (Ruel et al 1981).

Fig. 13 - Cel 44 pit degrading area (spruce wood) after PATAg staining. The solubilization of the hemicelluloses part leaves fibrillar structures. (Ruel unpublished).

Fig. 14 - Cel 44 degraded S2 layer stained with PATAG. In the clear area corresponding to hemicelluloses solubilization, weakly stained fibrillar structures (arrows) can be regarded as cellulose microfibrils. (Ruel unpublished).

Fig. 15 - Cel 44 degraded Birch ray-parenchyma cell observed in minimum beam exposure conditions (without any staining). Long bundles of cellulose microfibrils (arrows) still surrounded with matriciel components (arrow heads) are visible. (Ruel and Barnoud 1981).

Fig. 16 - Longitudinal section through a spruce tracheid cell wall degraded by the wild-type strain Sporotrichum pulverulentum (glucose impregnated wood). Lignin solubilization leads to a "honeycomb" structure clearly seen in these minimum beam exposure conditions of observations. (Ruel et al 1983).

Fig. 17 - Detail of the honeycomb structure of the S2 layer. This structure shows wrapped fibrils with ill defined edges in the upper part (arrow) and thinner fibrils with well defined edges in the lower part (double arrow). (Ruel et al 1983).

Fig. 18 - Transverse section as described in fig 16. The diffraction diagram realized in the outer part of the S2 layer (clear circle) is typical of cellulose I. (Ruel et al 1983).

Fig. 19 - Same type of degradation but in a more degraded area, near the lumen. The diffraction diagram shows an absence of cristallinity. (Ruel et al 1983).

Fig. 20 - Cross section of a PATAg stained tracheid of spruce degraded by the wild-type Sporotrichum pulverulentum showing the diffusion of hemicellulolytic enzymes from a hole to another. Traces of diffusion are materialized by thin PATAg negative channels (arrows). (Ruel and Barnoud 1981).

Fig. 21 - Longitudinal tangential section through the S1 layer of a tracheid wall showing lignin modifications due to ligninolytic enzymes after KMnO_4 staining. The modified area is about 0.2μ to 0.3μ in size (arrowhead). (Ruel et al 1981).

Fig 22 - Bacterial decomposition of the inner secondary wall layer of a pretreated beech wood section with adhering bacteria (arrows). (Schmidt and Liese 1982).

REFERENCES

- Ander, P. and Eriksson, K.E. (1975). Svensk Papperstidn., 78, 643-652
- Ander, P. and Eriksson, K.E. (1977). Physiol. Plant, 41, 239-248
- Ander, P. and Eriksson, K.E. (1978). Prog. Ind Microbiol., M.J. Bull, (Elsevier, Amsterdam, Ed.), 14, 1-58
- Ander, P., Hatakka, A. and Eriksson, K.E. (1980), Arch. Microbiol, 125, 189-202
- Beer, M. (1980). Anal. Chem. Rev., 52, 40R-42R
- Blanchette, R.A Canad. J. For. Res, vol 9, n°4 (1979) 464-469
- Blanchette, R.A (1980a) Canad. J. of Bot, 58-13, 1496-1503
- Blanchette, R.A (1980b) Journal of Forestry, 78-12, 734-737
- Blanchette, R.A (1982a) Phytopathology 72-10, 1272-1277
- Blanchette, R.A (1982b) Canad. J. Forest Res. 12 2, 304-310
- Blanchette, R.A (1982c) Can.J. Forest Res. 12, 3, 538-544
- Blanchette, R.A (1983) Mycolgia -in Press-
- Blanchette, R.A and Shaw, C.G (1978) Phytopathology, 68, 631-637
- Blanchette, R.A, Shaw, C.G and Cohen, A.L. (1978). In Scanning Electron Microscopy - vol II. SEM Inc., AMF O'Hare, IL, U.S.A., 61-67
- Blanchette R.A, Sutherland, J.B and Crawford, D.L (1981) Canad. J. of Bot. 59, 1, 1-7
- Bravery, A.F (1971) J. Inst. Wood Tech. 30, 13-17
- Chou, C.K and Levi, M.P (1971) Holzforschung 4, 107-112
- Chua, M.G.S., Chen, C.L., Chang, H.m and Kirk, T.K (1982), Holzforschung, 36, 165-172

- Chua, M.G.S., Choi, S. and Kirk, T.K. (1983) Holzforschung, 37, 55-61
- Chen, C.L., Chang, H.m, and Kirk, T.K (1981a) Holzforschung 35, 3, 99-103
- Chen, C.L., Chua, M.G.S., Evans, J.E., Chang, H.m ; Kirk, T.K. (1981b) Int. Symp. Wood and Pulping Chem. Stockholm. 3, 75-87
- Chen, L.C., Chang, H.m and Kirk, T.K. (1982a) Holzforschung, 36, 3-9
- Chen, C.L., Chua, M.G.S., Evans, J. and Chang, H.m. (1982b) Holzforschung, 36, 239-247
- Chen, C.L, Chang, H.m. and Kirk, T.K (1983), J. Wood Chem. and Technol. 3, 1, 35-57
- Cowling, E.B. (1961) Tech. Bull n°1258 Forest Products Laboratory, Forest Service, U.S. Department of Agriculture, 79 pp
- Cowling, E.B. and Kirk, T.K (1976), Biotechnol. Bioeng. Symp. n°6, 95-123
- Crawford, D.L. and Crawford, R.L. (1980), Enzyme Microb. Technol., 2, 11-22
- Dirol, D. (1976) Revue de Mycologie, Tome 40, 295-315
- Dirol, D. (1978) Revue de Mycol., Tome 42, 277-292
- Dirol, D. (1980) Cryptogam. Mycol. Tome I, 187-199
- Dirol, D. et Ravilly, F. (1979) Ann. Biol. Tome XVIII, Fasc. 11-12, 478-492
- Ellwardt, P. Chr., Haider, R. and Ernst, L. (1981), Holzforschung, 35, 3, 99-103
- Eriksson, K.E. (1978) Biotechnology Review in Biotechnology Bioengineering (John Wiley and Sons eds.) vol XX, pp 317-332

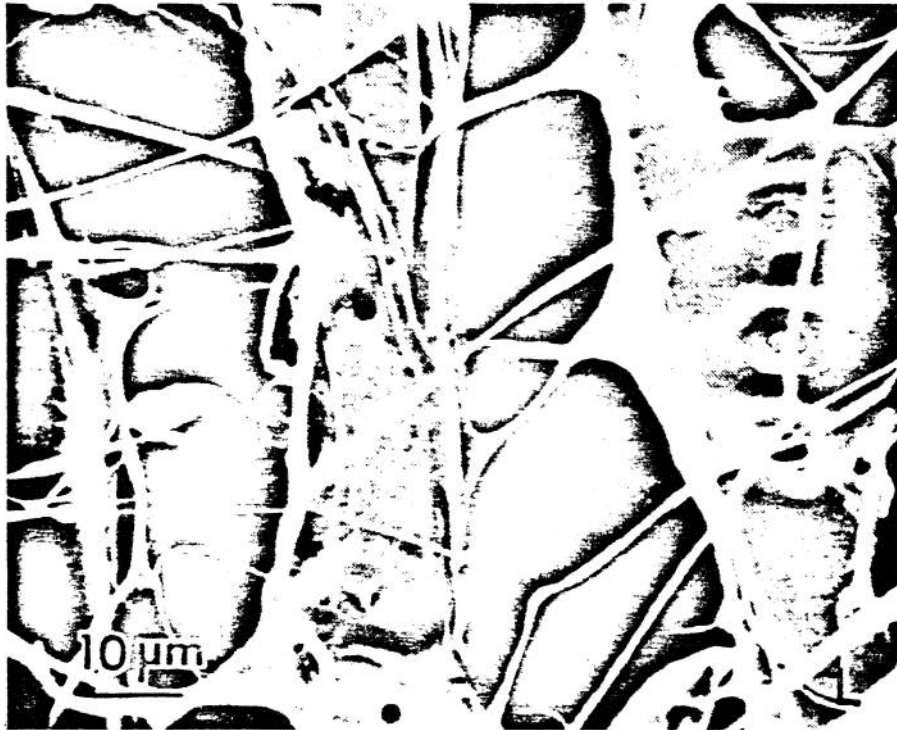
- Eriksson, K.E. (1981a) Pure Appl. Chem., 53, 33-43
- Eriksson, K.E. (1981b) Int. Symp. Wood and Pulping Chem. Stockholm., 3, 60-65
- Eriksson, K.E. and Goodell, E.W (1974) Can. J. Microbiol., 20, 371-378
- Eriksson, K.E. and Hamp, S.G. (1978) Papier (Darmstadt), 32, 12, 545-550

- Eriksson, K.E and Pettersson, B. (1975) Eur. J. Biochem., 51, 193-206
- Eriksson, K.E, Grünewald, A., Nilsson, T. and Vallander, L. (1980a) Holzforschung, 34, 207-213
- Eriksson, K.E., Grünewald, A., and Vallander, L. (1980b) Biotechnol. Bioeng. (John Wiley and Sons, Inc. eds) 22, 363-376
- Fukuda, K. and Haraguchi, T. (1975), J. Jap. Wood. Res. Soc. ; Tokyo, 21, (1), 43-46
- Haider, K. and Trojanowski, J. (1980), In "Lignin biodegradation : microbiology, chemistry and potential applications" CRC Press Inc. (Boca Raton FL. ed.) 111-134
- Haider, D., Trojanowski, J and Sundman, U. (1978) Arch. Microbiol., 119, 103-106
- Hepler, P.K., Fosket, D.E. and Newcomb, E.H. (1970) Am. J. Bot ; 57, 85-96
- Higuchi, T. and I. Kawamura (1955) J. Jap For. Soc., 37, 298-302
- Higuchi, T., Kawamura, I. and Kawamura, H. (1956) Mokuzaï Gakkaishi, 2, 31-35
- Higuchi, T. (1981) Int. Symp. Wood and Pulping Chem. Sotckholm, 3, 16-24

- Higuchi, T. (1982) Experientia, 38, 159-166
- Jurasek, L. (1964) Drev. Vyskum 3, (2), 126-141
- Jutte, S.M and Zabel, R.A (1974) IITRI/SEM, 445-452 (Available from IIT Research Institute, Chicago, IL 60616 U.S.A.)
- Kerr, A.J and Goring, D.A.I (1975) Cellulose Chem. and Technol, 9, 563-573
- Kilbertus, G., Rohr, R. et Mangenot, F. (1976) Holzforschung 30, 5, 156-164
- Kirk, T.K. (1973) In "Wood deterioration and its prevention by preservative treatment" (D.D. Nicholas, ed.), vol I, pp 149-181 ; Syracuse University Press, Syracuse, New-York
- Kirk, T.K. (1975) Holzforschung 29, 99-107
- Kirk, T.K. (1981) In "Biosynthesis and biodegradation of wood components" Int. Symp. Wood and Pulping Chem. Stockholm ; vol III, 66-70
- Kirk, T.K. and Chang, H.m (1974) Holzforschung 28, 217-222
- Kirk, T.K. and Chang, H.m (1975) Holzforschung, 29, 56-64
- Kirk, T.K. and Highley, T.L. (1973) Phytopathology, 63, 11, 1338-1342
- Kirk, T.K. and Moore, W.E. (1972) Wood Fiber, 4, 72-79
- Kirk, T.K., Connors, W.J. and Zeikus, I.G. (1976) Appl. Environ. Microbiol., 32, 192-194
- Kirk, T.K., Yang, H.H and Keyser, H.H. (1978) P. Dev. ind. Microbiol., 19, 51-61
- Liese, W. (1970) Ann. Rev. Phytopath., 8, 231-258

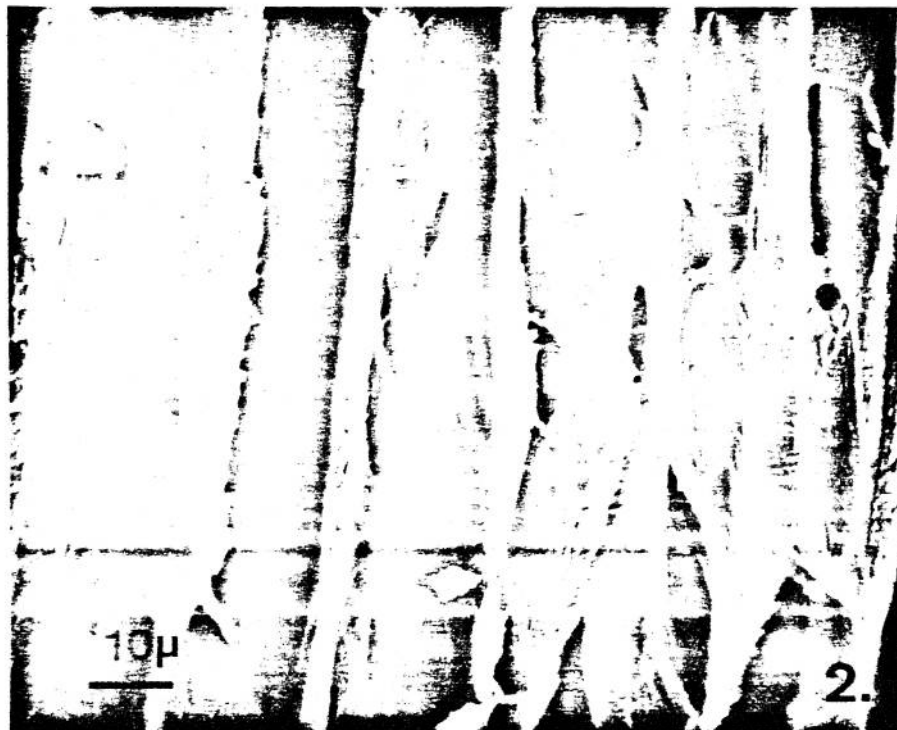
- Liese, W. and Greaves, H. (1975), In "Biological transformation of wood by microorganismes" (W. Liese, Ed.) Springer-Verlag, Berlin, 74-88
- Liese, W.G.E. and Parameswaran, N. (1976) Material und Organismen Beicheft 3, 63-75
- Meier, H. (1955) Holz Roh Werkst. 13, 323-338
- Monties, B., Odier, E., Janin, G. and Czaninski, Y. (1981) Holzforschung, 35, 217-222
- Otjen, L. and Blanchette, R.A. (1982) Canad. J. of Bot., 60, 12, 2770-2794
- Ouellette, G.B. (1978) Canad. J. of Bot., 56, 21, 2666-2693
- Parameswaran, N., Wilhelm, G.E. and Liese, W. (1976) J. of Forest Pathol., 5, 274-286
- Peterson, M.D and Thomas, R.J. (1978) Wood and fiber, Fall., 10, 3, 150-163
- Ravilly, F. et Dirol, D. (1977) Material und Organismen, 12, 1, 37-48
- Revol, J.F., Gancet, C. and Goring, D.A.I. (1982) Wood Sc., 14, 3, 120-126
- Rosenberg, S.L. (1980), Mycologia, 72, 798-812
- Ruel, K., Comtat, J. et Barnoud, F. (1977) C.R Acad. Sc. Paris, tome 284, série D, 1421-1424
- Ruel, K., Barnoud, F. and Eriksson, K.E. (1981), Holzforschung 35, 157-171
- Ruel, K. et Barnoud, F. (1983) C.R Acad. Sc. Paris, 296, III, 231-234
- Ruel, K., Barnoud, F. and Eriksson, K.E. (1983), Holzforschung ; In Press

- Shimada, M.F., Nakatsubo, F., Kirk, T.K. and Higuchi, T. (1981) Arch. Microbiol., 129, 4, 321-324
- Schmidt, O. and Liese, N. (1980), Holzforschung, 34, 67-72
- Schmidt, O. and Liese, W. (1982) Intern. J. of Wood Preserv., 2, 13-19
- Sutherland, J.B., Blanchette, R.A., Crawford, D.L. and Pometto, A.L. (1979), Curr. Microbiol., 2, 2, 123-126
- Takahashi, M. (1978) Wood Res., 63, 11-64
- Thiery, J.P. (1979) J. Microscopie, 6, 987-1018
- Westermark, U. and Eriksson, K.E. (1974a) Acta Chem. Scand. B28, 204-208
- Westermark, U. and Eriksson, K.E. (1974b) Acta Chem. Scand. B28, 209-214
- Westermark, U. and Eriksson, K.E. (1975) Acta Chem. Scand. B29, 419-424
- Wilcox, W.W. (1968) U.S. For Serv. Res. Note F.P.L., Report n°70 ; 42 pp
- Wilcox, W.W. (1970) Bot. Rev., 36, 1, 1-27
- Wilcox, W.W. (1973) In "Wood Deterioration and its Prevention by Preservative Treatments", (D.D. Nicholas, ed.), 1, 107-148, Syracuse University Press, Syracuse, New-York
- Wilcox, W.W., Parameswaran, N. and Liese, W. (1974) Holzforschung 6, 211-217



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