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M.J. BULL

*Gist-Brocades N.V., Research Department
P.O. Box 1, Delft (The Netherlands)*



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REFACE

In the 18 years since *Progress in Industrial Microbiology* first appeared, industrial microbiology has greatly spread its wings. Fermentation in particular has gone through a period of rapid growth and development. Some of the most recent developments in fermentation are in diametrically opposite directions. On the one hand we have the development of increasingly sophisticated fermentation plant - of which the airlift fermenter developed by I.C.I. - is a good example. An article on the fluid dynamics of this type of fermenter, to be written by Dr. Roessler, was scheduled for publication in this volume.

Due to the tragic and sudden death of Dr. Roessler, we have been robbed of an undoubtedly fascinating article. At the other end of the spectrum are the developments with simple fermentation equipment for developing countries. This village technology approach is discussed by the Drs. Forage and Righelato in their article over Microbial Protein from Carbohydrate Wastes.

Over recent years much publicity has been given to the work being done on the utilization of cellulose waste. The second most important natural waste compound in terms of abundance is lignin. The work on lignin has tended to be overshadowed by the flood of articles over cellulose. This situation has now been corrected by the excellent and comprehensive review, given by the Drs. Eriksson and Ander, in this volume. The group of compounds, that fall under the heading heterocyclics, is enormous. Undaunted by the enormity of this task Dr. Callely has succeeded in giving a wide-ranging and scholarly survey of the main routes by which micro-organisms attack the principal types of compound in this heterogeneous group.

Microbiologists working in the fermentation industry have received over the last few years an enormous stimulus out of other fields of study, such as computer technology and systems analysis. One particularly fruitful development has been the application of mathematical/computer modelling techniques. There has been a large volume of literature from all over the world, emphasizing different approaches and types of model.

Ir. Roels and Professor Kossen in their magnum opus, provide for the first time a critical survey of the literature and give furthermore some pointers as to where this type of work can be usefully expanded and in which direction.

Finally as the new editor I would like to express my thanks to the previous and founding editor of the series, Professor Hockenhull, not only for entrusting me with his "baby", but also for six happy years when it was my good fortune to be working with him.

M.J. BULL

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LIGNIN DEGRADATION AND UTILIZATION BY MICRO-ORGANISMS

PAUL ANDER and KARL-ERIK ERIKSSON

Swedish Forest Products Research Laboratory, Chemistry Department,
Stockholm, Sweden

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LIGNIN DEGRADATION AND UTILIZATION BY MICRO-ORGANISMS

1. INTRODUCTION

This review presents and discusses some of the recent work carried out in the field of lignin degradation and utilization by micro-organisms. The main emphasis is on the white-rot fungi and their production of different enzymes involved in lignin degradation, although other types of fungi as well as bacteria will also be dealt with. New methods in lignin research and the production of lignin-degrading enzymes will also be discussed, as well as new aspects of the use of micro-organisms for the bioconversion of wood and other lignocellulosic materials.

In 1971, several review articles concerning lignin degradation by micro-organisms were published [1-4]. Since then no extensive review articles have appeared. We have decided to devote our attention primarily to the progress made since these review articles were published. The reader is therefore advised to consult earlier reviews also in order to obtain more complete information.

A thorough knowledge of the mechanisms of microbial degradation of lignin is important for several reasons. One is that lignin is the major waste product in wood pulp production, and for the bulk of it no intelligent use has been found. With a better knowledge of the enzyme mechanisms involved in lignin degradation, it may be possible to modify the water-soluble lignin at least, into useful products by the use of specific matrix-bound enzymes. Another reason is that there is all over the world an increasing interest in the development of technical bioconversion processes by which waste lignocellulosic material can be processed into sugar, alcohol, other organic solvents or protein. These processes are based mainly on knowledge gained in basic research into the enzyme mechanisms involved in the microbial degradation of cellulose [5]. Very little is known about the corresponding mechanisms in lignin degradation. It is clear, however, that degradation of the lignin is the rate-determining step in these processes. A better knowledge of how lignin is attacked and metabolized is therefore an absolute necessity if the bioconversion processes are to be made economical.

Lignin is a phenylpropanoid structural polymer of vascular plants which gives the plants rigidity and binds plant cells together [6]. Lignin also decreases water permeation across cell walls of xylem tissue and protects plant tissues from invasion by pathogenic micro-organisms. Next to cellulose, lignin is probably the most common organic compound cycled on earth. The amount of lignin in different softwood and hardwood species in North

America and Scandinavia lies in the range of 18-33% [7,8]. Much of this lignin, as well as lignin in other kinds of plants, is converted to humus by different micro-organisms on the death of the plant tissues [9,1]. The humus found in the upper layer of soils performs several important functions in nature [1]. It influences the structure of the soil, increasing its aeration and moisture holding capacity. It also functions as a basic ion exchanger and is able to store and release nutrients to the surroundings. Humus, during its degradation, further releases carbon dioxide which can be used by growing plants. The degradation of humus is, however, a very slow process, which may in part be due to oxygen deficiency in lower parts of the soil. The lifetime of humus has been calculated to be between 360 and 2860 years depending on the type of soil investigated [9]. The nature and characterization of humus as well as its biological decomposition has been discussed in more detail by Christman and Oglesby [1] and by Hurst and Burges [9].

2. THE STRUCTURE OF LIGNIN

According to the definition of Sarkanen and Ludwig [6], lignin is a natural polymeric product arising from an enzyme-initiated dehydrogenative polymerization of three primary precursors: trans-*p*-coumaryl alcohol, trans-coniferyl alcohol and trans-sinapyl alcohol (Fig. 1). A typical softwood lignin contains approximately 80% coniferyl alcohol, 14% *p*-coumaryl alcohol and 6% sinapyl alcohol [2]. Hardwood lignin, on the other hand, contains similar amounts of coniferyl and sinapyl alcohol and a minor amount of *p*-coumaryl alcohol. In bamboo and grass lignins the amount of *p*-coumaryl alcohol is higher than in softwood and hardwood lignins [4].

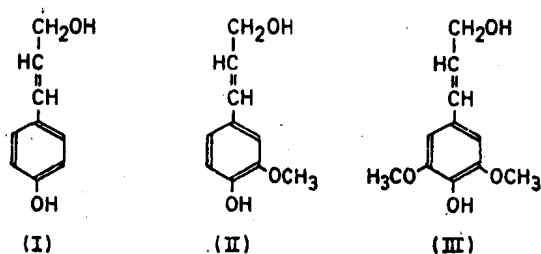


Fig. 1. *p*-Coumaryl alcohol (I), coniferyl alcohol (II) and sinapyl alcohol (III).

It is now generally accepted that the cinnamyl alcohols (I, II, III) are dehydrogenated by phenol oxidases to give free radicals (Fig. 2), which spontaneously polymerize [3, 10-12]. In this way the highly branched lignin polymer is formed with the phenylpropane units covalently bonded to each other in a number of different ways (Fig. 3).

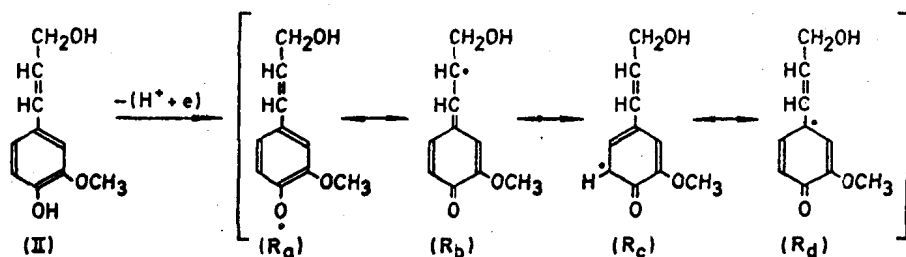


Fig. 2. Resonance structures of the coniferyl alcohol radical.

Both laccase and peroxidase have been implicated in this polymerization with the weight of the evidence in favour of peroxidase [3, 6, 11]. Harkin and Obst [13] investigated lignifying cells using syringaldazine with and without the addition of H_2O_2 to detect laccase or peroxidase activity. Application of 0.1% solution of syringaldazine to cross sections of freshly cut surfaces of sample stubs produced no coloration to indicate the presence of laccase. After the addition of H_2O_2 , however, an intense purple ring formed almost immediately in the xylem tissue adjacent to the cambium. The authors conclude that it therefore seems certain that the phenol oxidase in the zone of lignification is exclusively peroxidase. Synthetic radioactively labelled dehydrogenative polymerizates (DHP) made by polymerization of coniferyl alcohol with peroxidase are now used in biological lignin research [14, 15].

Schematic formulae for spruce lignin have been presented by Freudenberg [11, 16], Harkin [12] and Adler [10]. More recently a formula for beech lignin has been presented by Nimz [17]. The formula for spruce lignin suggested by Adler [10], containing 16 units, is presented in Fig. 3. According to Kirk [4] there are three major intermonomer linkages in lignin:

- The arylglycerol- β -aryl ether type involves about 40% of the phenylpropane units of spruce lignin and about 60% of these units in birch lignin.
- The phenylcoumaran structure involves about 20% of the phenylpropane units in spruce lignin and about 10% of those in birch lignin.
- The

biphenyl structures may involve 25% of the phenylpropane units of spruce lignin and about 10% of those in birch lignin. Diaryl ether structures, pinoresinol structures and benzylether bonds are also present in Fig. 3 [4]. It must be pointed out that this formula for lignin, like other lignin formulae, shows only an arbitrary sequence of phenylpropane units from a very limited part of the lignin macromolecule.

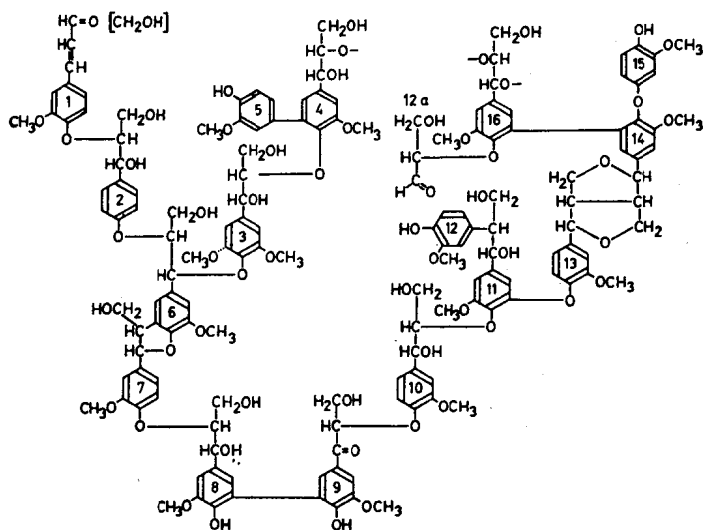


Fig. 3. Schematic formula for a section of spruce lignin consisting of 16 units, according to Adler [10].

3. LIGNIN-DEGRADING MICRO-ORGANISMS

There are three types of fungi living on dead wood which actually degrade one or more of the wood components: soft-rot fungi, brown-rot fungi and white-rot fungi. Mould and stain fungi, belonging to the Ascomycetes and the Fungi Imperfecti use only pectin, sugars and starch in wood and cause discoloration by pigments in the hyphae (stain fungi) and by pigments in the conidia (mould fungi) [18]. The degradation of lignin by bacteria is still an uncertain question but it will also be discussed here.

3.1. SOFT-ROT FUNGI

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 belonging to the Ascomycetes and Fungi Imperfecti species. In the secondary wall of the attacked wood, cylindrical cavities with conical ends appear [19]. Some soft-rot fungi also cause an erosion of the wood cell walls starting from the cell lumen (Fig.23). This decay is more common in hardwood than in softwood. The formation of soft-rot cavities in the secondary cell wall is called Type 1 attack, while Type 2 attack involves erosion of the cell wall [20]. Available knowledge concerning decay of wood by soft-rot fungi has been summarized by several authors during the last six years [19, 21, 22].

The lignin-degrading and metabolizing abilities of soft-rot fungi have been discussed by Kirk [4]. Recently Eslyn et al. [23] calculated the decrease of lignin, cellulose and hemicellulose of red alder, balsam poplar and western white pine after decay to different weight losses by six soft-rot fungi. Lignin, which was analysed by the "sulfuric acid" method, was attacked by all these fungi. Western white pine was not decayed significantly by three of the fungi, and only to low weight losses (15% or less) by the other three fungi. In red alder and balsam poplar the carbohydrates were depleted faster than the lignin [23].

In order to ascertain whether soft-rot fungi actually utilize lignin, Haider and Trojanowski [14] measured the release of $^{14}\text{CO}_2$ from DHP's which were ^{14}C -labelled in the methoxyl groups, in the side chains and in the aromatic rings, respectively. Six soft-rot and two white-rot fungi (Polyporus versicolor* and Pleurotus ostreatus) were included in this study. They found that soft-rot as well as white-rot fungi had the ability to release $^{14}\text{CO}_2$ from all the differently labelled DHP's. The $^{14}\text{CO}_2$ was released from the side chains and from the aromatic rings earlier than from the methoxyl groups. Later, however, more $^{14}\text{CO}_2$ was released from methoxyl groups than from side chains and aromatic rings. From these results it was concluded that soft-rot fungi can attack methoxyl groups, β -ether linkages of the side chain, and also the aromatic ring structures of DHP and of lignin [14]. The figures obtained for $^{14}\text{CO}_2$ -release after growth of the soft-rot fungi for 10 or 15 days were normally only about 2-4% of the applied activity. Kirk et al. [15], in a similar investigation using two brown-rot fungi, obtained a release of 1-8% of the applied activity as $^{14}\text{CO}_2$ after 25 days. This indicates that soft-rot and brown-rot fungi have a similar effect on lignin - i.e. a rather limited attack. This should be compared with the results obtained with white-rot fungi which release much more $^{14}\text{CO}_2$ (15-40%) from the

*Synonyms: Coriolus, Polystictus or Trametes versicolor.

differently labelled DHP's (see below) [15]. Due to different cultivation techniques and different incubation times a direct comparison is, however, difficult.

In conclusion: Soft-rot fungi can degrade lignin to a certain extent, especially in hardwoods. They seem to be able to cause demethylation of lignin, and possibly also to degrade the side chains and the aromatic rings somewhat, although the extent of degradation is low.

3.2. BROWN-ROT FUNGI

Brown-rot fungi mainly decompose the polysaccharides in wood and usually cause only a slight loss of lignin [24]. Kirk and Highley [25] measured the removal of lignin, cellulose (glucan) and hemicellulose from five conifer woods by the brown-rot fungi Poria monticola, Lenzites trabea and Lenzites lepidus. The lignin content was generally only slightly reduced by these fungi. Lignin analyses were performed by the sulfuric acid method which gives reproducible values at least in the case of white-rot fungi. It is, however, difficult to analyse samples of brown-rotted wood by this method. This was reflected by both apparent decreases and increases in the lignin content of the brown-rotted samples. White-rotted wood, containing only small amounts of lignin degradation products, gives more reliable analyses [25].

During the decay, the removal of cell wall substance by brown-rot fungi begins in the S2 layer of the secondary wall. The S1 layer of this wall may also be destroyed but, due to their high lignin content [8], the primary wall and the middle lamella are very resistant to degradation by brown-rot fungi [18]. In advanced stages of decay, when most of the polysaccharides are consumed, the cell wall collapses. This causes a decrease in the wood volume.

The most noted change in the lignin on attack by brown-rot fungi is a decrease in the methoxyl content [4]. As a result of decay of sweetgum by Lenzites trabea, methoxyl-deficient units, containing phenolic hydroxyl groups, were formed in the lignin skeleton [26]. The brown colour of brown-rotted wood may be the result of both spontaneous and phenol oxidase-catalysed oxidation of catechol (o-diphenol) units to quinoid or melanin-type chromophores. Phenol oxidases are probably released during the autolysis of old fungal mycelium.

L. trabea was also used more recently by Kirk [27] to decay spruce wood. From this decayed wood, lignin was isolated and compared with milled

wood lignin (MWL) and with "extractive" lignin from sound wood. It was found that 35% of the methoxyl groups in the C₆-units were degraded. The demethylation was greatest in units bearing phenolic hydroxyl groups. The demethylation may be performed by phenol oxidases (oxidative demethoxylation) or by mono-oxygenases (oxidative demethylation). The expression "demethylation" is here used for both reactions. About twice as many α -carbonyl groups were present in the degraded lignin as in the sound sample [27].

One of the differences between brown-rot and white-rot fungi as regards their mode of attack seems to be that brown-rot fungi lack an efficient ring-cleaving enzyme system, although this has not as yet been definitely established [27]. They may also lack the ability further to metabolize ring-cleavage fragments if these are formed. Nor do brown-rot fungi lead to oxidative shortening of the side chains in terminal phenylpropane units as do white-rot fungi. Brown-rot fungi, in contrast to white-rot fungi, also lack the enzyme cellobiose:quinone oxidoreductase [28]. This was confirmed by cultivating the brown-rot fungi Coniophora puteana, Daedalea quercina, Laetiporus sulphureus, Poria oleracea and Tyromyces sericeomollis on cellulose, on wood meal and on kraft lignin-cellulose agar plates, using methods as in reference [29].

That brown-rot fungi demethylate but do not substantially degrade lignin was also confirmed using radioactive DHP's labelled in the methoxyl groups, in the side chains or in the aromatic rings [15]. The two brown-rot fungi Gleophyllum trabeum (=Lentzites trabea) and Poria cocos were used. Although there was good growth of the two fungi, only a low degradation of the side chain and of the ring-labelled lignins was obtained compared with white-rot fungi. Thus the two white-rot fungi P. versicolor and Phanerochaete chrysosporium both released 15% of the applied radioactivity in the aromatic ring as ¹⁴CO₂, while the two brown-rot fungi only released 1-2% [15].

In conclusion: Brown-rot fungi, as well as soft-rot fungi, can cause only a limited degradation of lignin. They demethylate lignin and introduce α -carbonyl groups into the propane side chains. Ortho-diphenolic structures can also be detected in brown-rotted lignin, but brown-rot fungi seem to lack ring-cleaving enzymes as well as the enzyme cellobiose:quinone oxidoreductase.

3.3. WHITE-ROT FUNGI

Lignins attacked by white-rot fungi contain more oxygen than the correspon-

ing sound lignin [4]. The content of total carbonyl, carboxyl, and hydroxyl in degraded lignin is somewhat higher than in sound lignin [30, 31]. Nitrobenzene oxidation of lignin from white-rotted wood gives less vanillin and more vanillic acid than that of the corresponding lignin from sound wood. Hata [30] suggested that this higher yield of vanillic acid was due to a shortening and oxidation of some terminal side chains in phenylpropane units in the lignin. Fig. 4 shows such a degradation of coniferyl alcohol end-groups in lignin [30]. These groups are converted to vanillic acid groups in a multi-step enzymatic process. The ether-linkages between vanillic acid groups and the phenylpropane units are then broken, resulting in liberation of vanillic acid. By further enzymatic action, new end-groups may appear which can be degraded as outlined above. This process requires enzymes cleaving ether-linkages in the lignin. Such enzymes are produced mainly by bacteria but possibly also by white-rot fungi [32-36]. It may be of importance to note that the degradation pathway proposed by Hata [30] in Fig. 4 coincides partly with the bacterial degradation pathway for veratrylglycerol- β -(*o*-methoxyphenyl) ether (Fig. 6) proposed much later by Crawford [33].

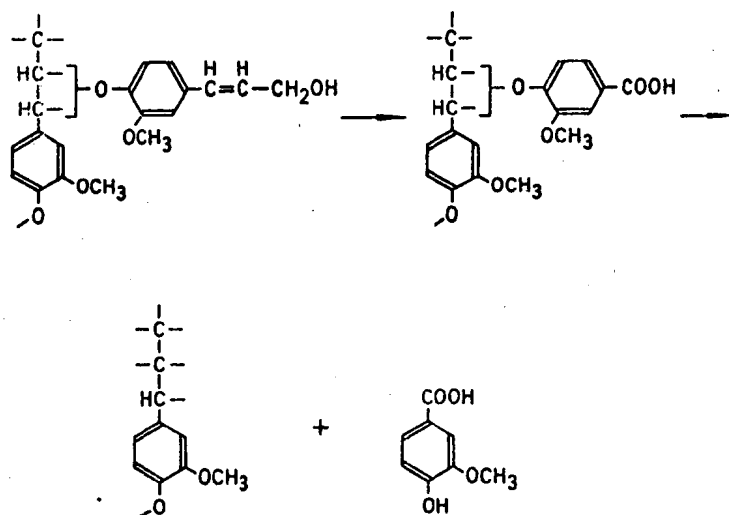


Fig. 4. Degradation of coniferyl alcohol end-groups in spruce lignin by the white-rot fungus *Poria subacida* [30].

MWL's and extractive lignins from sound and decayed wood were also compared by Kirk and Lundquist [37]. MWL was prepared from sapwood of sweetgum (Liquidambar styraciflua L.) which had been decayed to an average weight loss of 32% by Polyporus versicolor, and from the corresponding sound wood. The authors found that the MWL's from sound and decayed wood did not differ significantly. The decayed wood and the sound wood were also extracted with benzene-ethanol and ethanol. It was shown that the extract from the sound wood contained tannin materials which, at least in part, were removed by the fungus so that the wood became bleached, thus giving rise to the name "white-rot". The analyses of the extracts indicated that the fungal attack had not led to any accumulation of low molecular weight phenolic compounds. It was further suggested that in the decay of sweetgum wood by P. versicolor a limited part of the lignin is first attacked and utilized before the decay proceeds to other parts of the lignin [37].

In order to study still more carefully the changes in lignin caused by the attack of white-rot fungi, Kirk and Chang [38] extracted degraded lignins from more severely decayed wood. The lignins were isolated from spruce wood decayed to 45% weight loss by Polyporus anceps and from spruce wood decayed to 50% weight loss by P. versicolor. These two extracted degraded lignins (purified using gel filtration with Sephadex G 25) were compared with MWL and two extractive lignins from sound wood [38, 39]. The extracted, degraded lignins had a molecular weight of not less than 600, as shown by gel filtration using Sephadex G 50. Monomeric lignin model compounds and a lignin model with $M_n = 638$ were used as reference substances [38].

Analysis showed a 25% decrease in methoxyl content in the extractive lignins attacked by the two fungi, but nevertheless this, a very low content of aromatic (phenolic) hydroxyl groups was detected. This is in contrast to decay by brown-rot fungi, where an increase in phenolic hydroxyl content in the lignin has been observed [26, 27]. These results point to a fast degradation of ortho-diphenolic structures by white-rot fungi and suggest that brown-rot fungi lack the ability to degrade such structures. Although the methoxyl content decreases, the amount of vanillic acid released on acidolysis of the degraded lignin with HCl/dioxane increased, compared with sound lignin. The increase in vanillic acid may be due to oxidative shortening of terminal coniferyl alcohol units as in Fig. 4. Acidolysis of degraded lignin gave only 10% as much ketol (II in Fig. 5) as did the acidolysis of the sound lignin. This points to a substantial decrease in structures of type I via cleavage of aromatic rings and oxida-

tion of side chain in these structures.

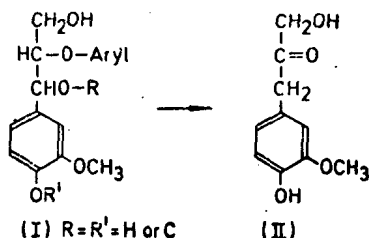


Fig. 5. Structure of the ketol (II), 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-2-propanone, a major product of the acidolysis of sound lignin [39].

It was also shown [39] that the number of aromatic carboxyl groups was 0.2 or less per C_9 -unit in the degraded lignin. The total number of carboxyl groups were, however, 0.55 per C_9 -unit. Thus more than half of the carboxyl groups were not conjugated to aromatic ring. By spectrometric investigations, Kirk and Chang concluded that the non-aromatic carboxyl groups were mainly α,β -unsaturated and that they were not derived from the side chains. Since it is known that compounds containing α,β -unsaturated carboxyl groups are released on cleavage of aromatic rings [40-42], and since it was shown [38] that the extracted, degraded lignins did not contain lignin compounds with MW less than 600, this result may indicate that cleavage of the aromatic rings occurs while they still are bound in the polymer. A similar suggestion was also made by Haider and Grabbe [43]. They found, using labelled DHP that the cleavage of aromatic rings proceeded faster than the cleavage of side chains. A decreased number of aromatic rings was also indicated by spectroscopic studies as well as by the decrease of ketol (II) [39]. Together with the 25% decrease in the methoxyl content and the absence of ortho-diphenolic structures, this also points to cleavage of aromatic rings bound in the polymer.

On the basis of the results obtained by Kirk et al. [44] at the Forest Products Laboratory in Madison, these authors have recently [44] summarized their knowledge of the chemistry of degraded lignins and also discussed the mode of lignin degradation and metabolism by white-rot fungi. According to these authors, lignin degradation starts in various parts of the polymer by demethylation of guaiacyl and syringyl units to yield ortho-diphenolic units. Demethylation may be effected by phenol oxidases and/or mono-oxyge-

nases [45, 46] (see also Section 4). Aryl- β -ether cleavages may also occur. Then cleavage of aromatic rings may occur while they are still bound in the polymer.

If this is true, it is possible that there are three carbon atoms in the propane side chain on cleavage of the ring. However, only cleavage of protocatechuic acid (see 4.3.), containing a carboxyl group in the side chain, has so far been reported to be performed by white-rot fungi [40, 47, 48]. Cis, cis-muconic acids which are formed on cleavage of ortho-diphenols by bacteria [42] could not be detected in white-rotted lignin [49]. The above mentioned reactions gradually expose new parts of the polymer to attack by the fungus. During this attack some terminal side chains are re-oxidized to release vanillic acid [30, 39], while phenylpropane side chains are oxidized in the α -position to yield α -carbonyl groups [39, 44, 45],

Since 1971 very few articles have been published dealing with the degradation of lignin model substances by white-rot fungi. Some earlier publications will, however, be discussed in section 4.

Vanillic acid is degraded via protocatechuic acid by the fungus P. versicolor (Flaig and Haider [47] and Cain et al. [40]). Kirk and Lorenz [50], using another white-rot fungus, Polyporus dichrous, did not, however, find any intracellular, extracellular, or even significant whole cell activity against protocatechuic acid. Vanillate was metabolized by this fungus via methoxyhydroquinone (1,4-dihydroxy-3-methoxybenzene) instead. The further metabolism of methoxyhydroquinone was not elucidated. This result may indicate that P. versicolor and P. dichrous are two different types of white-rot fungi with certain differences in selected metabolic pathways. A recent investigation by Ander and Eriksson [29] supports the possibility of different metabolic pathways for different white-rot fungi (cf. Table 4 and section 5.1.).

The degradation by P. dichrous of several alkyl ethers of vanillic acid was also studied [51]. The reactions performed by the fungus included 4-dealkylation, hydroxylation of the 4-alkoxyl groups and reduction of carboxyl groups. Syringic acid and its ethers were, however, resistant to degradation. Since P. dichrous mainly degrades hardwood containing syringyl structures, it would be expected that such structures would also be degraded in liquid cultures. This discrepancy shows that metabolism of lignin in wood may be different from metabolism of lignin model substrates in liquid cultures (cf. Ref. 29).

In conclusion: Lignin degradation and utilization by white-rot fungi is

mainly an oxidative process. During this degradation lignin is demethylated to ortho-diphenols, which in turn are probably immediately degraded by dioxygenases. Indications have been given that the cleavage may already occur while the ring is bound in the lignin polymer. Terminal end-groups are probably degraded with the liberation of vanillic acid after cleavage of β -aryl ether bonds. The part played by phenol oxidases in the different steps has not been elucidated (see 4.4.).

3.4. BACTERIA

The investigation of the problem of whether bacteria are ~~able to de-~~ grade and metabolize lignin has often been associated with ~~weaknesses in~~ experimental techniques. These difficulties have been pointed out ~~espe-~~cially by Kirk [4]. However, some evidence seems to have accumulated showing that some bacteria can cause a certain amount of lignin degradation. It is, however, evident that uncertainties still remain.

Greaves [52] concluded in 1971 that there is no direct evidence to implicate any particular species of bacteria in the breakdown of lignin in situ. Crawford et al. [53] reported that a strain of Nocardia corallina produces no detectable structural changes in a lignin isolated from spruce. The bacterium could, however, utilize aromatic compounds such as *p*-anisic and veratric acid. Cartwright and Holdom [54] used an enzymatically released lignin from birch wood meal in a screening for lignin-utilizing bacteria. They found only one bacterium (an Arthrobacter sp.) which survived repeated subculture on the lignin substrate. They reported a low decrease in lignin content and concluded that bacteria have no major role in the degradation of lignin in situ.

More recently Kawakami [55] showed that Pseudomonas ovalis degraded both pine and beech MWL in a stationary culture for 60 days. The molecular weight of the MWL was shown by Sephadex gel filtration to have decreased after growth of the bacterium. A decrease of β -aryl ether bonds in MWL was also reported [55, 56]. The coniferyl aldehyde content of pine and beech MWL decreased strongly (70 and 80% respectively).

The ability of bacteria to degrade different phenols, some of them related to lignin, is very well established [41, 42, 46] and will be discussed later in connection with ring-cleaving oxygenases.

The ability of bacteria to cleave arylglycerol- β -aryl ether bonds may be of importance in lignin degradation, since this linkage bonds together 30-50% of the phenylpropane units [1]. Crawford et al. [32] in 1973 re-