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#### SUMMARY

Lignin biodegradation occupies a central position in the earth's carbon cycle, because most renewable carbon is either in lignin or in compounds protected by lignin from enzymatic degradation (cellulose and hemicelluloses). Lignin biodegradation is responsible for much of the natural destruction of wood in use, and thus is responsible for substantial economic losses. Furthermore, lignin degradation may have a causal role in plant pathogenesis. Finally, potential biotechnical applications of lignin-degrading enzymes or organisms promise to add another dimension to the importance of the process. Despite its significance, however, lignin biodegradation is only slowly being defined chemically and biochemically. Major reasons for this slowness include the relatively recent description of the chemical structure of lignin, its inherent complexity, and the several unusual features of lignin biodegradation, which have only been described very recently. Review articles published periodically during the past 50 years trace the progress of research on lignin biodegradation. Since about 1970 the research effort has accelerated greatly, and progress has been commensurate. The immediate future promises additional major breakthroughs, both in basic understanding and in biotechnical application of bio-ligninolytic systems.

#### INTRODUCTION

Almost 200 research papers on the microbial degradation of lignin have been published since the first international seminar (1) was held in 1978. In addition, ten review articles (2 - 11), the book of proceedings of the first seminar, and R. L. Crawford's book (12) have been published. Significant advances have been made since 1978, and the field is now more active than it has ever been, with approximately 20 laboratories around the world contributing.

In this opening chapter, my first purpose is to point out why there is an accelerating interest in this area of research--i.e. why lignin biodegradation is becoming recognized as a centrally important process. My second purpose is to review briefly the 60-year development of knowledge in this field, in an attempt to put our present efforts, described in subsequent chapters, into an historical perspective.

#### Importance of Lignin Biodegradation

The earth's carbon cycle. Lignin biodegradation has assumed through evolution a disproportionate and central role in the earth's carbon cycle. In

the first place, lignin is very abundant. It is probably second only to cellulose among renewable organic compounds, accounting for perhaps 15-20% of all photosynthetically fixed carbon. But of even greater significance is the fact that in woody plant tissues the lignin forms a protective sheath around the cellulose and the hemicelluloses. This sheath must be disrupted before the polysaccharides are accessible to enzymatic attack. Thus, most of the earth's renewable carbon is either in lignin or in compounds protected by lignin. In natural decomposition, the lignin sheath is disrupted either mechanically--by insects and marine borers, or biochemically--by microorganisms (see 13). It is not possible at present to estimate the contributions of these two types of processes, but I suspect that the biochemical processes are by far the more important, and that the biochemical decomposition of lignin is the key to the recycling of most of the earth's carbon.

Destruction of wood products, and plant diseases. Wood is our major building material, and is likely to remain so. Unfortunately, it decays fairly rapidly when used improperly in construction, as it often is. Replacing decayed structures costs several thousands of millions of dollars yearly. As will be apparent from the foregoing discussion, lignin biodegradation is the key to much of this destruction.

Related to the destruction of wood in service is the destruction by some plant pathogens of lignified tissues in living plants. Examples include certain white-rot fungi that are pathogenic; Rigidoporus lignorum, Armillaria mellea and Fomitopsis annosum are examples. In addition, various fusaria, which cause many plant diseases, have been shown to degrade lignin partially (17,18). Thus it is possible that lignin biodegradation is a component of pathogenesis in some plant diseases, caused not only by certain white-rot fungi, but also by members of the Ascomycetes and Fungi Imperfecti.

Potential technical applications. Recent research on lignin biodegradation has undoubtedly been stimulated more by the several tantalizing potential applications of bio-ligninolytic systems than by a desire to understand the carbon cycle, or to develop better control methods for wood decay and for plant diseases. Some potential applications of bio-ligninolytic systems are listed in Table 1. Of these, bio-mechanical pulping and waste treatment have received the most research attention (19). Both applications look quite promising and deserve additional research. Several of the potential uses of bio-ligninolytic systems can probably be more readily implemented if the enzyme system responsible for ligninolytic activity can be separated from the living microbial cells: e. g. bleaching of pulps, modifying pulp properties, and producing chemicals from lignin.

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<sup>a</sup>Lignin degradation may not be a requisite for all wood decay. The brown-rot fungi are a serious cause of destruction of wood in service, but it is not clear how important lignin degradation is in this type of decay. The initial attack of the cellulose is thought to be by small non-enzymatic agents that can penetrate the lignin barrier (14). Studies have shown, nevertheless, that brown-rot fungi, which are basidiomycetes closely related to the lignin-decomposing white-rot fungi, do cause limited degradation of lignin, and in fact slowly convert its carbon to CO<sub>2</sub> (15,16).

Table 1. Potential technical applications of  
bio-ligninolytic systems

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- 1) Partial delignification to increase ruminant digestibility
  - 2) Partial delignification as pretreatment for enzymatic saccharification
  - 3) Partial delignification and/or lignin modification to reduce energy requirements and improve pulp properties in mechanical pulping
  - 4) Lignin modification in mechanical pulps to improve pulp properties.
  - 5) Delignification to bleach pulps
  - 6) Modification of natural or industrial byproduct lignins to produce chemicals
  - 7) Treatment of lignin-derived wastes
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#### Historical Research Perspective

Our understanding of how lignin is biodegraded has lagged far behind our understanding of the biodegradation of all other studied biopolymers for several good reasons. One of the major ones is that the chemical structure of lignin became clear only in the late 1960s (Table 2). Research on biodegradation before the 1960s could only be incomplete. Of equal importance in hampering research on lignin biodegradation, however, has been the inherent complexity of the structure of lignin, and the attendant difficulties in its isolation and study--factors leading even now to substantial technical problems in lignin research. Microbiologists weak in organic chemistry or unable to involve interested chemists in their research have found the field frustratingly difficult. Finally a third factor--or more accurately, a third set of factors--has retarded progress on lignin biodegradation research: Lignin biodegradation is an unusual process, and scientists have had to work in large part without precedents from other biodegradation systems, and without experimental guidelines. For example, lignin is unique among biopolymers in part because its degradation involves extracellular oxidations.

Despite the problems, progress has been made through the years. In the following, I have attempted to summarize briefly the historical development of lignin biodegradation research.

Prior to the 1920s, little research was conducted on lignin biodegradation. The rather substantial amount of research during the 1920s and early 1930s was summarized in the first literature review devoted to lignin biodegradation, written by A. G. Norman in 1936 (22, Table 3). Two years before, in 1934, M. Phillips (23) had summarized briefly what was known about the subject in connection with an extensive review of lignin chemistry. Both of those reviews reached similar conclusions which have for the most part remained valid. Lignin was recognized to be the major plant component most resistant to biological degradation, although it was clear that it is biodegraded. It was recognized that white-rot wood decay fungi decompose lignin in wood, and that completely selective removal of lignin had not been observed--as it still has not. Reports of anaerobic biodegradation of lignin were seen as inconclusive, in part because of the overriding problems in the analytical determination of

Table 2. Some milestones in the elucidation of the lignin structure (see 21)

Investigator (Date)	Contribution
A. Payén (1838)	Showed that wood consists of cellulose plus an encrusting material
E. Erdmann (1868)	Showed that lignin (the encrusting material of wood) is aromatic
P. Klason (early 1900s)	Deduced that lignin is similar to coniferyl alcohol
K. Freudenberg (1926-1932)	Demonstrated through chemical analyses that lignin is formed from methoxylated arylpropane units
H. Erdtman (1933)	Hypothesized that lignin is formed by dehydrogenative polymerization of coniferyl alcohol
E. E. Harris (1938)	Proved that lignin has an arylpropane skeleton
K. Freudenberg (late 1930s-1960s)	Identified the major intermonomer linkage types and formulated the basic structure of lignin through experiments on the dehydrogenation of coniferyl alcohol
A. Björkman (1956)	Prepared the first good isolated lignin--milled wood lignin
E. Adler (1950s, 1960s)	Confirmed the structure of lignin and quantified the various substructures through analytical studies of milled wood lignin and lignin model compounds

Table 3. Developments in the understanding of lignin biodegradation prior to 1936 [from review by A. G. Norman (21)]

- 1) Lignin was seen to be by far the most resistant major constituent of plant materials, but slow biodegradation was shown to occur.
- 2) Lignin in plant materials was shown to be attacked fairly readily by basidiomycetes, but its removal bo always be accompanied by removal of cellulose and hemicelluloses. Lignin in wood was shown to be decomposed by white-rot fungi.
- 3) Pure culture studies were in their infancy.
- 4) The only report of extensive anaerobic decomposition of lignin had not been authenticated.
- 5) Isolated lignin was found to be unavailable to microbes.<sup>1</sup>
- 6) Quantitative estimation of lignin was recognized to be a serious experimental problem; poor methods had led to conflicting reports on the microbiological degradation of lignin.

<sup>1</sup>It was recognized, however, that the harsh methods used for isolating lignin might have altered its structure.

lignin. Isolated lignins were reported to be very resistant to biodegradation, but it was suspected that the method of isolation (with strong mineral acids) might have altered the lignin structure, which we now know to be the case. Importantly, few pure culture studies had been conducted.

S. A. Waksman, who was later to receive the Nobel Prize for his work on antibiotics, conducted a number of pioneering studies on lignin biodegradation in the 1920s and 1930s. His review in 1944 (24) echoed the conclusions of Norman (22), and in addition reached several more, including a) that isolated lignin added to other plant components does not inhibit their degradation; b) that older plant tissues are more resistant to biodegradation than immature ones because of changes in the lignin during maturation; and c) that lignin accumulates in soils, composts, and peat bogs, where it is gradually transformed into humus and perhaps coal. These three conclusions remain intact today. Waksman also reviewed his work with coworkers in which several studies were conducted with lignin isolated by heating plant tissues with phenol. Such "phenol lignin" contains a substantial percentage of covalently bound phenol, so that conclusions concerning biodegradation are virtually meaningless. This example is cited merely to illustrate the methodological difficulties in the early studies.

S. Gottlieb and M. Pelczar's extensive and excellent review was published seven years after Waksman's review (25). The authors recognized even then that ". . . few dogmatic generalizations can be made," in large part because of the incomplete knowledge of the structure of lignin, which they discussed at some length. Their major conclusions (Table 4) again echoed those of earlier reviews, but with refinements and some additions. They reviewed their own ongoing work reporting the first growth of microorganisms (white-rot fungi) on isolated lignin. In those studies they used "Brauns' native lignin," which is unaltered by the mild isolation procedure, but which is of relatively low molecular weight. Subsequent reports of growth on lignins have appeared, but the findings reported by Gottlieb and Pelczar have not been carefully verified, and good evidence indicates that some white-rot fungi cannot use lignin as sole

Table 4. Developments from 1936 to 1951  
[from review by S. Gottlieb  
and M. J. Pelczar (24)]

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- 1) Research confirmed that higher fungi degrade lignin in nature.
  - 2) A good correlation was found between formation of colored products from tannic acid and other phenols and lignin degradation among higher fungi.
  - 3) Polyporus versicolor<sup>1</sup> and other fungi reportedly used Brauns' native lignin as growth substrate.
  - 4) No identified bacterial species had been reliably associated with lignin degradation.
  - 5) No lignin-degrading enzyme activity had been demonstrated.
  - 6) Many faulty techniques had been used.
  - 7) Research had been hampered by the lack of understanding of lignin structure.
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<sup>1</sup>Now classified as Coriolus versicolor.

carbon and energy source (26). Nevertheless, the question of the energetics of lignin metabolism remains an intriguing one that deserves further investigation. The review of Gottlieb and Pelczar dealt at some length with the correlation among wood decay fungi between a positive phenol oxidase reaction and ability to decompose lignin. This subject has received considerable further study since that review, but even today the possible role of phenol oxidases in lignin degradation is unclear. Gottlieb and Pelczar also discussed the problems associated with methodology in this and other aspects of lignin biodegradation research.

In Japan, T. Higuchi exhaustively reviewed the status of lignin biodegradation research, including Japanese work, in 1954 (27). His major conclusions were similar to those of Gottlieb and Pelczar (25), and he, too, discussed the chemistry of lignin as known at that time. Higuchi also emphasized the problems with methodology that plagued research on lignin, particularly lignin isolation procedures. Importantly, he pointed to the virtual absence of any real understanding of the chemical and biochemical mechanism of lignin biodegradation. Phenol-oxidizing enzymes and their possible role in lignin biodegradation received considerable attention, as did the differences among wood decay fungi in their rates of decomposition of the major wood components.

Several developments in lignin biodegradation research occurred in the 1950s and 1960s (Table 5). Lindeberg (29) found that lignin in forest litter is decomposed by hymenomycetes that are not wood-decaying fungi. Even to date, however, the litter-decomposing hymenomycetes have received little additional attention. In other work, the soft-rot type of wood decay was discovered by Findlay and Savory (30). Savory and Pinion (31) later showed that lignin is degraded to some extent by this group of non-basidiomycetous fungi. Lignin in brown-rotted wood was first analyzed by Apenitis *et al.* (32) and by Enkvist *et al.* (33); both groups reported that methoxyl loss is a prominent characteristic. Higuchi *et al.* (34) reported the first studies of the chemical changes in lignin in wood during decay by white-rot fungi, noting especially the loss of structures yielding vanillin on nitrobenzene oxidation. In the 1960s the structure of lignin was becoming clear, putting research for the first time on a firm chemical foundation. The first studies of biodegradation using lignin model compounds were conducted by Russell *et al.* (35), and later studies

Table 5. Developments from 1951 through 1969 (see 28)

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- 1) Degradation of lignin by litter-decomposing hymenomycetes was described.
  - 2) The soft-rot type of wood decay was described.
  - 3) Some of the chemical changes caused by brown-rot and white-rot fungi in lignin were described, and degradation was seen as primarily oxidative.
  - 4) Model compounds were first used in biodegradation research.
  - 5) The possible role of phenol oxidases in lignin biodegradation was investigated.
  - 6) Additional evidence was presented that bacteria degrade lignin.
  - 7) The role of lignin in humus formation and in nitrogen sequestering was investigated.
  - 8) Differences between white-rot and brown-rot in their effects on wood were described.
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were reported by Ishikawa et al. (36), by Kirk et al. (36), and by Fukuzumi et al. (38). The differences between the white and brown rots in the effects on wood were clarified by Cowling (39). Phenol oxidases received considerable attention in the 1960s, as is evident in a section on "biological degradation of lignin" in a 1967 review on lignin by Harkin (20).

In 1971 I reviewed the literature on lignin biodegradation (28), having just spent 1-1/2 years in the lignin chemistry laboratory of Prof. E. Adler. My conclusions on reading the older literature were much the same as those reached in previous reviews. In addition, it had become clear by 1971 that the mode of lignin decomposition by white-rot fungi differed from that of other biopolymers in that it appeared to be primarily oxidative instead of hydrolytic. Further work had shown that the major effect of brown-rot fungi on lignin seemed to be demethylation of aryl methoxyl groups. Little was known about the effects of non-basidiomycetous fungi on lignin (which is still the case), even though there was good evidence that various soil fungi degrade lignin. Evidence was growing that some bacteria can at least modify lignin. Little was known about the biodegradation of industrial lignins. And still almost nothing had been learned about the detailed chemistry and biochemistry of ligninolysis.

The proceedings of the first seminar (1) document the substantial progress made between 1970 and 1978. Researchers then had the benefit of a knowledge of the chemical structure of lignin, and of new methods for isolating and studying lignin. Some of the substantial accomplishments during the period are given in Table 6. The development of assays for biodegradation based on  $^{14}\text{C}$ -lignins removed the uncertainties about both qualitative and quantitative analysis, and permitted progress on several fronts.

Progress since the first seminar has, as I mentioned at the outset, also been substantial; much of it is described in the following chapters. Table 7 lists several of the research accomplishments that seem particularly noteworthy. The list, however, reflects my own biases; it is really up to the reader of this book to judge for himself or herself just what the most notable recent advances have been.

In any event, one can only conclude that the rate of progress has continued to accelerate. We now know quite a bit about how lignin is biodegraded. The immediate future promises major breakthroughs in basic understanding, and hopefully in the first directed biotechnical applications of nature's lignin-degrading enzyme systems.

Table 6. Developments from 1970 through 1978 (see 1,2)

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- 1) Biodegradation assays based on  $^{14}\text{C}$ -lignins were developed.
  - 2) Evidence was presented that lignin is resistant to anaerobic degradation.
  - 3) Partial decomposition to  $\text{CO}_2$  by pure cultures of actinomycetes was demonstrated.
  - 4) Partial decomposition to  $\text{CO}_2$  by soft-rot and brown-rot fungi was demonstrated.
  - 5) Decomposition rates of lignin and model compounds in soils and other environments were measured.
  - 6) Culture conditions were defined and optimized for lignin degradation by white-rot fungi.
  - 7) The white-rot fungus Phanerochaete chrysosporium (=Sporotrichum pulverulentum) was studied and selected as a suitable experimental organism for detailed study.
  - 8) The relationship of lignin degradation to secondary metabolism was discovered in Phanerochaete chrysosporium.
  - 9) Degradation of lignin model compounds by bacteria, fusaria, and brown-, soft- and white-rot fungi were reported, and some transformations were described.
  - 10) The chemistry of white- and brown-rotted lignins was further elucidated.
  - 11) Theories about the possible role of phenol oxidases were advanced.
  - 12) The concepts of biomechanical pulping and wastewater cleanup by white-rot fungi were advanced.
  - 13) Ligninolytic cellulase-less mutants of white-rot fungi were developed.
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Table 7. Developments since 1978

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- 1) A possible basis for the apparent absence of anaerobic decomposition was presented (40,41).
  - 2) The first good evidence for degradation of lignin in an insect gut was reported (42).
  - 3) Many products formed from lignin during degradation by Phanerochaete chrysosporium were described and their probable origins deduced (43).
  - 4) Pathways for model compound metabolism by Phanerochaete chrysosporium, Fusarium solani, were described (see 10).
  - 5) The chemistry of Streptomyces degradation was partially elucidated (44).
  - 6) Physiological features of lignin metabolism by Phanerochaete chrysosporium and other white-rot fungi were described (6,11).
  - 7) An hypothesis was advanced (9) and tested (this book) that "diffusible" activated oxygen species are fungal agents of lignin degradation.
  - 8) The first ligninolytic enzyme was discovered in Phanerochaete chrysosporium (46, this book).
  - 9) Techniques were developed for mutation, selection, and genetic manipulation in Phanerochaete chrysosporium (47).
  - 10) Bio-mechanical pulping and wastewater purification with white-rot fungi were further investigated (see 19).
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