LIGNIN BIODEGRADATION—PRESENT STATUS AND FUTURE

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ABSTRACT

Though the Basidiomycetes fungi are mainly responsible for degrading the lignocellulosic substances in nature, several Ascomycetes fungi belonging to Xylariaceae and also certain bacteria are claimed to show degrading activity on lignin. But the actual process of lignin degradation is still cloudy. Inoculum source, nutrient nitrogen source, oxygen supply, various growth substrates such as glucose, cellulose etc are reported to exert effects on lignin degradation. There is possibility of commercial establishment of lignocellulose based industries.

MAINTENANCE of life on earth is dependent on the continuous operations of many biogeochemical cycles. Of these, one of the most important biospheric cycles is the carbon cycle. Trapping the sun's radiant energy by photosynthesis, the autotrophic higher land plants fix about half of the earth's carbon annually which is then incorporated in their body. After the death of these plants, the dead organic matters are decomposed by microorganisms and ultimately the fixed carbon is released primarily as CO_2, thus completing the carbon cycle.

But the decomposition of plant residues which contains abundant lignocellulosic tissues is a very complex process. Most microbes degrade isolated wood carbohydrates, but are unable to decompose lignin which protects the carbohydrate, or unless the tissue is degraded to very small particles (kraft lignin) so that the lignin barrier is overcome. It is evident, therefore, that the microbial degradation of lignin is the most important biodegradative event in the earth's carbon cycle. However, though efforts are on the way for over three decades all over the world to find out the actual mechanism of biodegradation of lignin, the knowledge gained is still far behind satisfactory. Kirk[1] has given a nice review on this aspect. The present paper gives an overview of the findings obtained so far from the study of the microbial degradation of lignin.

The lignocellulosic tissues of higher land plants are the major repository of photosynthetic energy and renewable organic matter. The stems of woody angiosperms contain 18–25% lignin (on a dry weight basis), while the gymnosperms and monocotyledons contain 25–35% and 10–30% lignin respectively. The rest of the plant tissue consists mostly of cellulose with lesser amounts of protein, ash and various extractives. Chemically lignin is an amorphous three dimensional aromatic polymer composed of oxyphenylpropane units. Three major groups of lignins are recognized[2]: guaiacyl lignin—found in most conifers, guaiacyl-syringyl lignin—found usually in dicotyledons and a few gymnosperms, and guaiacyl-syringyl-*p*-hydroxyphenyl lignin—found in some grasses and in compression woods of conifers. Guaiacyl lignin consists primarily of coniferyl alcohol units with small amounts of coumaryl and sinapyl alcohol-derivative units. Guaiacyl-syringyl lignins contain monomeric units derived from approximately equal amounts of coniferyl alcohol and sinapyl alcohol with only minor amounts of coumaryl alcohol-based units. Guaiacyl-syringyl-*p*-hydroxyphenyl lignin is supposed to contain equal amounts of all three cinnamyl alcohols[2].

The white rot fungi belonging to Basidiomycetes play a predominant role in the complete degradation of lignin. The white rot fungi are defined as the fungi which are able to decompose all the structural components of wood including both cellulose and lignin. Several hundred species belonging to the families Agaricaceae, Corticiaceae, Hydnaceae, Polyporaceae and Thelephoraceae are white rotters[1]. Under suitable environmental conditions, they are able to degrade all structural components of wood including lignin with ultimate liberations of CO_2 and water[3]. Of course different species decay the various components of wood at different rates. *Polyporus berkeleyi* removes the lignin from wood in preference to cellulose or hemicellulose[4] and so also *Pycnoporus cinnabarinus*[5]. Similar results have been obtained by other workers such as Kirk[6] with *Rigidoporus ulmarius* and *Polyporus resinosus*, and by Ander and Eriksson[5] with *Pleurotus ostreatus; Phlebia radiata* and *Merulius tremellosus*. But some

white rot fungi are also known to degrade lignin and cellulose components of wood at about the same rate such as, Coriolus versicolor[3] and Ganoderma applanatum[6]. Kawase[4], however, found a strain of Ganoderma applanatum degrading the carbohydrate components of wood more rapidly than the lignin part.

Another group of wood decaying Basidiomycetes fungi also decompose the carbohydrate components of wood causing only limited degradation of lignin. These fungi are called brown rot fungi[1,7,8]. In brown rot decay, a residue of modified lignin that is typically 'dark brown' is left behind. It is evident, therefore, that a vast number of Basidiomycetes fungi take part in degrading the lignocellulosic substances. Here lies the great ecological importance of the group.

Several members of Ascomycetes belonging to the family Xylariaceae e.g. Xylaria polymorpha, Hypoxylon deustum (= Ustulina deusta) have been reported to degrade wood causing a typical white rot type of decay. That U. deusta produces chemical changes in decayed wood like a typical white rot was reported first by Campbell and Weirtelak[9]. Long after, Merrill et al[10] showed that several species of Xylariaceae could decay wood but comparatively at slower rate than Basidiomycetes. Presently some soil-inhabiting Ascomycetes and Fungi Imperfectii are known to attack moist wood and produce a characteristic softening of surfaces of the woody tissues[8,11,12]. These fungi, called soft-rot fungi, are able to cause extensive weight loss in wood[13]. Levi and Preston[14] reported extensive delignification of beechwood by Chaetomium globossum where lignin losses were found to be up to 45%. Earlier Savory and Pinion[15] also recorded about 92% weight loss in beechwood decayed by C. globossum, implying very extensive lignin degradation. By using[14]C-lignins, it was proved[16,17] that the soft rot fungi can oxidise lignin to CO_2. Several workers[18-23] have shown that some soil fungi such as strains of Aspergillus, Penicillium, Fusarium and Alternaria are able to degrade lignin to some extent. Bacteria, particularly certain species belonging to Bacillus, Nocardia, Streptomyces and Xanthomyces, are also claimed to show slow degrading activity on lignin[24-28].

It is thought that fungal-bacterial association speeds up the degradation of lignocellulosic materials as compared to degradation rates of fungi alone. Blanchette and Shaw[29] noted significant increase in wood decay during 5 months of decay treatments by combining bacteria and yeasts with several wood rotting fungi such as Coriolus versicolor, Hirschioporus abietinus and Poria placenta. It is believed that in this mutualistic relationship, the bacteria increase fungal growth by supplying vitamins and other growth promoting substances to the fungi. In return, the bacteria utilize the wood decay products released by fungal attack on the woody cell wall. According to Kirk et al[30] the white rot fungi, which are able to attack the unaltered lignin polymer, are to be regarded as primary invaders, while the bacteria probably play the secondary role and they further degrade the lignin after it has been altered by primary degraders.

Effects of environment — Effects of environments on lignin degradation have been studied by some workers recently. Studies of Crawford and coworkers[31,32] demonstrated that the rates of lignin degradation vary substantially with the inoculum source. After 30 to 35 days, 45% conversion to CO_2 was observed in one soil sample, while in one water sample, 30% conversion to CO_2 was noted. Hackett et al[33] examined lignin biodegradation in a variety of natural materials including soils, lake sediments, silage, animal beddings and rumen contents, employing aerobic and anaerobic conditions. Degradation was found to occur only under aerobic conditions. Moreover, the degree of aerobic degradation also varied greatly with the type of material employed, site, soil type and temperature. Extensive studies by Kirk and his colleagues on lignin degradation by Phanerochaete chrysosporium have shown that lignin cannot serve as a sufficient carbon and energy source for its catabolising. The fungus degrades lignin only in presence of an additional growth substrate such as cellulose, glucose or succinate[34]. A high percentage of oxygen also is found necessary for this degradation[30]. Another interesting observation with P. chrysosporium is that the level of nutrient nitrogen in the substrate exerts a profound effect on its rate of lignin degradation. Low concentration of nitrogen nutrient is found to be favourable for lignin degradation, while this degradation is suppressed if the concentration of nutrient nitrogen is rather high[30,34,35].

Enzymes and lignin degradation — The possible role of enzymes in lignin degradation has been studied more extensively than any other aspect of lignin degradation, but the knowledge gained so far is still in infancy. Lignin being polymeric, remains outside microbial cell walls and therefore it must be attacked by extra-cellular enzymes. Several investigators[16,38] have shown that phenol oxidases

are always present in lignin degrading fungi. Ander and Eriksson[39] obtained strong evidence that phenol oxidases are required for fungal lignin degradation and perhaps in a regulatory role. Three kinds of phenol oxidases namely laccase, peroxidase and tyrosinase are believed to be greatly involved in lignin degradation; but the true role of these enzymes in degradation is yet to be defined. According to Eriksson and Lindholm[40], phenol oxidases can mediate the direct oxidative degradation of lignin to certain extent, but their prime function is to further polymerize lignin and lignin degradation products. Kirk[41] reported that as the lignin polymer is attacked by an extra-cellular non-specific oxidising agent(s), the enzymes may not be directly involved. Some other suggestions have also been put forward. Hall[42] speculated that 'diffusible species' are derived from molecular oxygen. Zeikus[43] similarly suggested the involvement of some 'chemical agents'. Koenings[44] apprehended that H_2O_2 might in some way be involved. Though substantial work has been done on various aspects of biodegradation of lignin, the exact mechanism of the process is still cloudy. However, recent years have seen an increased interest in this area of research. The ability of the wood-rotting fungi to lignocellulose conversion is now being exploited commercially in the development of lignocellulose based industries, chief among these industries are agriculture, lumbering and paper-making. By-product lignins from the chemical pulping of wood are also used to make various commercial products such as dispersants, binders, oil well drilling muds etc and via chemical degradation to make vanillin, dimethyl sulphoxide and a few other chemicals[45-47]. There are also reports of bioconversion of industrial lignins to polyphenols[48]; bioconversion of lignocellulosics to organic acids[49, 50], methane[51-56], glucose[57-59], alcohols, or single cell protein[60-63]. But due to insufficient production, the practical use of these degradation products has been proved to be difficult. Industrially fruitful utilization of lignin biodegradation products requires further research on the physiology of the biodegrading organisms, detailed biochemistry, and enzymology of lignin degradation process and also genetic control over lignolytic enzyme system. Genetical technique may also be used in such investigations, utility of which has already been evidenced by some workers[39, 64, 65]. Undoubtedly real success in developing industrially valuable commodities by bioconversions of lignin and lignocellulosic can only be achieved by applying knowledge from collaborative research on these different aspects of lignin degradation process.

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