# Biodegradation of Lignin

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# Lignin

- Lignin is the most common aromatic organic compound found in the lignocellulose component of the plant cell wall.
- Lignin is the most abundant source of carbon in soil after cellulose.
- Its characteristic ability to absorb UV (ultraviolet) radiation makes it susceptible to degradation on being exposed to sunlight.
- The source of lignin in soil can be of plant origin or lignocellulosic waste from the food processing industry.
- Lignin is an amorphic three-dimensional polymer composed of phenylpropanoid subunits.
- It acts as a binding material and is involved in cross-linking of cellulose that provides extra strength, rigidity, and stiffness to the cell wall.
- Lignin protects plant cells from enzymatic hydrolysis and various other environmental stress conditions.
- The complex structure of lignin makes it recalcitrant to most degradation methods.

# Lignin Structure and Its Biosynthesis

- In the plant cell, lignin is biosynthesized by the combination of three basic hydroxycinnamoyl alcohol monomers or monolignols:

  1. p-Coumaryl alcohol
  2. Coniferyl alcohol
  3. Sinapyl alcohol

- These monolignols are often referred to as phenylpropanoids, which differ in the substitutions at the 3-C and 5-C positions in aromatic ring.
- The natural polymerization of monolignols (lignification) starts with the oxidative formation of phenoxy radicals catalyzed by peroxidases and/or laccases.
- It is followed by combinatorial radical coupling, generating carbon–carbon and carbon–oxygen (ether) bonds.
- The emerging crosslinked network and aromatic nature of lignin explain its recalcitrance towards degradation.

# Lignin degradation processes

- The Lignocellulosic complex in the plant cell wall contains approximately 40 to 60% cellulose, 20 to 40% hemicellulose, and 10 to 25% lignin.
- Certain enzymes from specialized bacteria and fungi have been identified by researchers that can catalyze a number of oxidative and hydroxylation reactions, depolymerize the phenolic and non-phenolic lignin polymer, and also mineralize the insoluble lignin.
- The biodegradation of lignocellulosic biomass has been widely studied in wood rotting Basidiomycetes microorganisms.
- These basidiomycetes are categorized as white-rot and brown-rot fungi.
- White-rot fungi are the most effective bio-degraders of lignocellulosic biomass (e.g., *Phanerochaete chrysosporium, Pleurotus ostreatus* and *Ceriporiopsis subvermispora*) and can degrade lignin faster than other microorganisms.

# White-rot fungi

- White-rot fungi produce a number of extracellular enzymes that directly attack lignin, cellulose, and hemicellulose of the plant cell wall to decompose it.
- These enzymes include laccases and peroxidases, such as lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP).
- **Laccases** catalyze the oxidation of polyphenols and methoxy-substituted phenols by generating free radicals.
- **Lignin peroxidases** catalyze the oxidative depolymerization of lignin with $H_2O_2$ acting as the oxidizing agent.
- These enzymes are relatively nonspecific, and can therefore oxidize phenolic aromatic substrates as well as various non-phenolic lignin model compounds.
- **Manganese peroxidases** use $H_2O_2$ to oxidize $Mn^{2+}$, which is present in wood and soils, thus generating reactive $Mn^{3+}$ ions, mediates oxidation of a large number of phenolic substrates.
- **Versatile peroxidases** combine the properties of lignin peroxidases and manganese peroxidases, conferring the catalytic versatility.
- They can oxidize $Mn^{2+}$ to $Mn^{3+}$ like manganese peroxidases, but can also oxidize non-phenolic compounds in the same manner as lignin peroxidases.

# Brown rot fungi

- Typical examples of brown-rot fungi include *Gloeophyllum trabeum*, *Postia placenta* and *Fomitopsis palustris*
- The brown-rot fungi are less efficient in degrading lignin compared to white-rot fungi.
- Lignin degradation by brown- rot fungi mainly involves non-enzymatic oxidation reactions producing hydroxyl radicals via Fenton chemistry.

$$[Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + \bullet OH]$$

- Brown-rot fungi partially oxidize lignin via aromatic ring demethylation.
- They do not degrade lignin directly, but rather modify it by partial oxidization and then preferentially degrade the polysaccharides.
- Wood decomposed by brown-rot fungi is therefore characterized by a brown color arising from the residual lignin.
- In addition to color changes, wood decayed by brown-rot fungi shrinks, breaks into brick-shaped pieces and crumbles to a brown powder.

# Bacterial lignin degradation

- Research in terms of lignin degradation by bacteria has been limited.
- Many soil bacteria such as Actinomycetes are also capable of mineralizing and solubilizing polymeric lignin and lignin-related compounds.
- Bacteria (e.g., *Streptomyces viridosporus, Brucella, Ochrobactrum, Sphingobium, Sphingomonas*, *Pseudomonas putida* and *P. fluorescens*) could oxidize phenolics but not the non-phenolic compounds.
- Various types of cleavages in lignin molecules, e.g., aromatic ring, demethylation, and oxidation, are catalyzed by bacterial enzymes.
- Bacteria are relatively rich in another type of peroxidase, the so-called dye-decolorizing peroxidases (DyP).
- DyPs are mainly active at acidic pH and show a very broad substrate profile, including several classes of synthetic dyes, monophenolic compounds, veratryl alcohol, β-carotenes, $Mn^{+2}$ and lignin model compounds.
- Laccases are ubiquitous in nature, found in plants, fungi, bacteria and insects.
- They are often secreted as extracellular catalysts and typically perform polymerization or depolymerization reactions.
- Similar to DyPs, the most studied bacterial laccases in lignin degradation are from actinomycetes, particularly from *Streptomyces* species.

# The process of catabolic lignin degradation

- Process involves:
  - **cleavage of ether bonds** between monomers;
  - **oxidative cleavage of the propane** side chain;
  - **demethylation**;
  - **benzene ring cleavage to ketoadipic acid** which is fed into the tricarboxylic acid cycle as a fatty acid.
- Most research has been concentrated on white-rot basidiomycete fungi, such as *Phanerochaete chrysosporium* (= *Sporotrichum pulverulentum*), which is able to **mineralise lignin** completely to carbon dioxide and water.
- The lignin-degradative system of *P. chrysosporium* appears after cessation of primary growth (that is, it is an aspect of the **secondary metabolism** of the organism) and can be induced by nitrogen starvation.

# Applications of lignin degrading enzymes

- Several biotechnological applications such as paper industry, textile industry, wastewater treatment and the degradation of herbicides.
- **Biopulping** is an industry where ligninolytic enzymes can improve the quality of pulp by releasing and purifying the cellulose.
- Laccases are extremely important biocatalysts, which can be exploited for:
  - paper pulp bleaching,
  - detoxification (particularly bioremediation of pulp mill effluents),
  - fibre modification,
  - dye decolourisation,
  - removal of phenolics from wines,
  - organic synthesis and chemical transformation of pharmaceuticals
- Persistent pesticides like **pentachlorophenol** (**PCP**) and **polychlorinated biphenyls** (**PCBs**) can be catabolized by lignin degrading organisms.

# Curial effect on environment

- Lignin degrading fungi produce small organic molecules to enhance activity of the lignin and manganese peroxidases incorporate chlorine from the wood into **chloroaromatics**, and even the synthesis of veratryl alcohol requires **chloromethane**.
- These volatile compounds are released into the atmosphere (thereby flushing the chlorine out of the substrate) and may themselves act as environmental pollutants.
- The annual global release to the atmosphere from this source has been estimated at 160,000 tonnes, 75% of which is released from tropical and subtropical forests, with 86% being attributable to *Phellinus* spp. alone.
- Chloromethane is a powerful **greenhouse gas** and atmospheric pollutant which can have adverse effects on stratospheric ozone, yet in this case it is the product of a natural ecosystem.

# Questions

- Write an essay on microbial degradation of lignin. Discuss advantages and disadvantages of lignin degradation by microorganisms.
- Write short note on :
  - Lignin biodegradation
  - Lignin degrading bacteria
  - Lignin degrading fungi
  - Applications of lignin degrading enzymes
  - Effect of lignin degradation on environment