Critical Review

Lignin—Designed Randomness

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Abstract

Humans have long used wood as a structural material for some of the same reasons that trees use it—it combines great strength, flexibility and durability with a relatively low density. These desirable properties depend partly on *lignin*, a major chemical constituent of many plants, including trees. Lignin is the most abundant aromatic polymer on earth and the second most abundant organic polymer of any kind, exceeded only by cellulose. It is estimated that 30% of the earth's non-fossil organic carbon is in the form of lignin. Considering its massive abundance and its high energy content (40% higher than cellulose, gram for gram), it is striking that no organism seems to have tapped it as an energy source. After posing this as an evolutionary enigma, we prepare to address it by reviewing what is known about the structure, biosynthesis, and biodegradation of wood in general and of lignin in particular. Then, returning to the enigma, we ask whether it is more readily explained within a Darwinian framework or a design framework. The Darwinian account must somehow reconcile 400 million years of failure to evolve a relatively modest innovation—growth on lignin—with a long list of spectacular innovations thought to have evolved in a fraction of that time. How can one mechanism have been at the same time so effective and so ineffective? That tension vanishes completely when the design perspective is adopted. Terrestrial animal life is crucially dependent on terrestrial plant life, which is crucially dependent on soil, which is crucially dependent on the *gradual* photo- and biodegradation of lignin. Fungi accomplish the biodegradation, and the surprising fact that it costs them energy to do so keeps the process gradual. The peculiar properties of lignin therefore make perfect sense when seen as part of a coherent design for the entire ecosystem of our planet.

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INTRODUCTION

When we think of natural polymers, the first things that come to mind are structural specificity and the properties that usually accompany this, like the optical activity that chirality produces and the information-carrying capacity that becomes possible when multiple monomer types are used. Typical examples are DNA, RNA, proteins, and polysaccharides of different kinds. Along with their structural specificity, these molecules are synthesized and degraded in a highly orderly fashion by specific enzymes. If these expectations are the rule, then lignin is the strange exception. As one of the major constituents of wood, lignin is random in the sense that it has no specific structure. It lacks optical activity and is neither made nor degraded by the direct action of enzymes. Perhaps the strangest thing about lignin, however, is that no living organism is able to use it as a sole carbon and energy source despite the fact that it is the most abundant energy-rich aromatic polymer on earth [1].

The cell walls within wood consist of a complex of three polymer types: cellulose, hemicellulose, and lignin. Trees depend on the extraordinary structural properties of this complex for their growth and survival, and people have long taken advantage of these properties as well. Wood combines biological and chemical stability, hydrophobicity, strength, flexibility, lightness, ease of manipulation, low heat conductivity, acoustic properties, and beauty. It is hardly surprising that these qualities have for millennia made wood a choice material for housing, furniture, art, musical instruments, and boats, and in more recent times for processed materials like fiberboard, cardboard, and paper as well.

In this review, we will focus mainly on the causes and implications of the biological stability of wood. None of the structural components of wood is easily biodegradable. Cellulose and hemicellulose are polysaccharides that form plant cell walls. The first of these is present in the form of insoluble crystalline fibers that resist degradation. Although both it and hemicellulose are degraded, the process is complex, requiring several enzymatic steps for completion and probably also active oxygen species

like hydroxyl radicals for its initiation [2]. The third wood polymer, lignin, is a disordered aromatic polymer that protects the cellulose and hemicellulose against microbial attack. The combination of these materials in wood is even less biodegradable than the individual components. There is, however, a group of microorganisms called *white-rot fungi* that can degrade all three structural components of wood. As will be discussed in detail, these fungi degrade cellulose and hemicellulose mainly by the direct action of hydrolytic enzymes (although oxidative radical reactions may also have a role [2]), and lignin via non-specific chemical reactions initiated by oxidative enzymes like peroxidases, phenol oxidases, and laccases.

The aspects of wood that resist biological degradation have also presented challenges for industrial processes involving wood-derived materials. The combination of lignin and polysaccharides found in wood, called *lignocellulose*, is a complex and recalcitrant solid that is difficult to separate into homogeneous fractions and from which it is difficult to produce pure chemicals. As we will see, much of this difficulty is attributable to lignin itself. Despite the tremendous amount of chemical energy stored in lignocellulose, its properties present major challenges for the change from hydrocarbon-based fuels to lignocellulose-based biofuels that has been proposed as part of a Europe-wide vision for 2025 [3].

Here, we review what is currently known about the structural components of wood that make these materials so difficult to process industrially and so difficult to degrade biologically. We then move to a more philosophical level by considering whether the existence of lignin and the absence of an organism that can grow on lignin are more readily explained from the Darwinian perspective or from the design perspective.

Structure of wood

Trees are both physically and chemically extremely complex. Their structure must be strong enough to support tremendous loads on cantilevered branches, flexible enough to bend in wind and under snow load, hydrophobic enough to transport aqueous nutrients long distances between roots and leaves, and durable enough to resist continual attack from pests and microbes for decades or centuries. Trees are divided into two distinct groups. Hardwoods belong to the angiosperms (dicotyledons), which typically have broad leaves. Softwoods belong to the gymnosperms (conifers), which usually have needles. In order to limit the complexity of the subject, we will narrow the discussion here to hardwood trees and specifically to birch (kingdom: Plantae, order: Fagales, family: Betulaceae), which is the national tree of Finland.

A cross-section of birch (Fig. 1) reveals the major macroscopic features of wood. In temperate climates, growth rings result from differences between the wood formed during the early and late growth seasons. The cambium layer is extremely thin (little more than a monolayer of cells) but as the active growth tissue it is responsible for the annual thickening of the trunk and branches. The new wood added each year by the cambium layer pushes the bark outwards. The inner bark layer contains living tissue (phloem) which is responsible for carrying

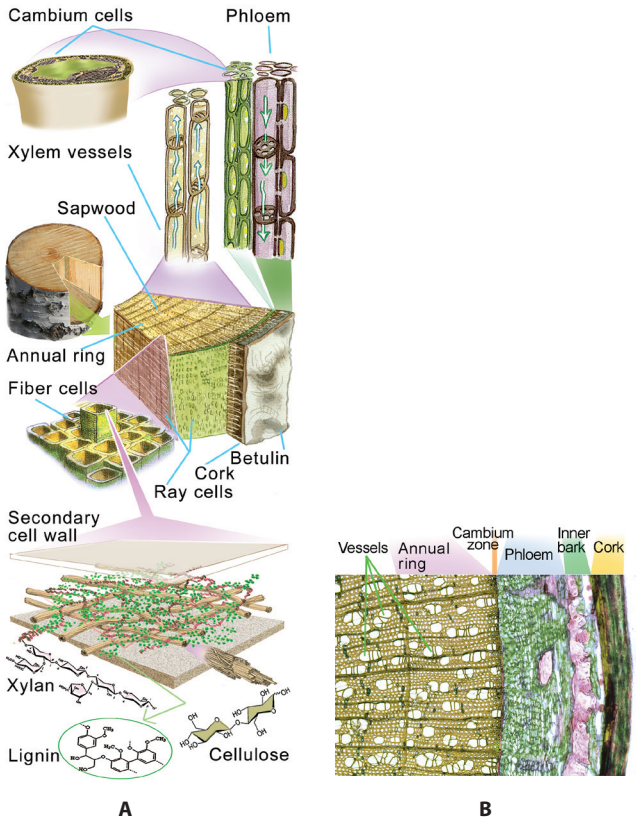


Figure 1: Structural features of wood. A) General structural features; B) micrograph of birch surface structures. **doi:** 10.5048/BIO-C.2012.3.f1

the products of photosynthesis from the leaves to all parts of a tree, while the outer bark is dead tissue that protects the wood against external injuries and microbial attacks. Sapwood is composed of both living and dead tissues. Sap is transported from roots to leaves through xylem vessels located in this part of the tree. Unlike gymnosperms, birch has no heartwood consisting of inactive cells. No new cells are added to sapwood or heartwood [4].

Mature wood cells have primary and secondary cell walls with interstitial space between the cells, called middle lamella. Wood cells are of various shapes and sizes, with elongated fibers or tracheids being the predominant forms. Their length varies from one millimeter in hardwood to three to eight millimeters in softwood. Young cells have porous cell walls that enable nutrients and water to flow through them, whereas the cell walls in mature tissues must be hydrophobic in order to transport water and nutrients over long distances. Ray cells are grouped into structures or tissues that extend horizontally and conduct sap radially across the tree. Mature wood cells of all kinds are firmly cemented together and remain so even after they cease to be alive. Dead wood cells are either empty or partly filled with deposits (gums and resins).

Chemical composition of wood

The chemical structure of wood cells varies with tree species, growth phase, and location within the tree and even within an individual cell. Trees contain a complex mixture of chemical

compounds like pectin, proteins, nucleic acids, suberin, and various extractives, but the interest here is on the three main structural components mentioned above: cellulose, hemicellulose, and lignin (Fig. 1A). The composition of birch is roughly 40% cellulose, 35% hemicellulose, and 20% lignin (spruce and pine having less hemicellulose and more lignin).

In 1836, French chemist Anselme Payen realized that most plant materials contain a fraction with a similar chemical composition that resists extraction. The resistant substance turned out to be cellulose, which is a linear polymer of β-(1,4)-linked glucopyranose units, as confirmed by Staudinger in 1953 [5]. Its seemingly simple molecular structure forms a surprisingly sophisticated complex—insoluble fibers made of microfibrils that are composed of elementary fibrils held together by hydrogen bonds. Native cellulose is a continuous crystalline polymer with occasional dislocations. Cellulose fibrils have the high tensile strength needed to form the support structure of the cell wall.

Cellulose is surrounded by a gel-like hemicellulose matrix that gives flexibility to the wall and, because of its porosity, allows nutrient transport in young cells. Hemicellulose is not a specific compound but rather a group of chemically heterogeneous polymers. Burton *et al*. [6] give a good review of the present understanding of plant cell polysaccharide heterogeneity and its possible causes. Various hemicellulose structures were determined by Timel in the 1950s [5]. Hemicelluloses are composed of pentoses (D-xylose and L-arabinose), hexoses (D-glucose, D-galactose and D-mannose) and uronic acids (D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid) with partially acetylated hydroxyl groups. Birch hemicellulose is mainly partially acetylated 4-*O*-methylglucuronoxylan, the major backbone being β-(1,4)-linked xylopyranose units with α-(1,2)-linked 4-*O*-methylglucuronic acid side groups (pine and spruce hemicelluloses are rich in partially acetylated galactoglucomannans). The side chains are functionally important because they prevent aggregation of the individual molecules and make them more water soluble, thus allowing formation of the gel-like porous structure of young plant cell walls.

Lignin is an aromatic and hydrophobic polymer that functions as glue that binds the individual plant cells and the carbohydrate polymers in the complex secondary cell wall. The three major roles of lignin are to protect plants against microbial attack, to give them the stiffness needed for structural stability, and to provide the hydrophobic capillary surface needed for the transport of aqueous nutrients from the roots to the leaves [7]. Water and salts are continuously transported by capillary forces at substantial rates (a large birch evaporates several hundred liters of water on a warm day) and, thanks to lignin, the upward and downward streams do not mix.

Without lignin, trees as we know them could not exist. It is lignin that provides the combination of flexibility and strength needed for erect growth and appropriate bending in response to wind or snow loads. Lignin is also the major industrial obstacle to converting plant carbohydrate polymers via simple sugars to biofuels like ethanol, butanol, or biodiesel, or to chemicals (e.g. organic acids, rare sugars). Although lignin has long been recognized as a potential source of a wide range of chemicals, attempts to make use of this potential have more or less failed.

BIOSYNTHESIS OF STRUCTURAL POLYMERS IN PLANTS

The most studied plant, *Arabidopsis thaliana*, has about 1000 genes responsible for producing the lignocellulosic cell wall structure. The complexity of this structure and the processes that form it have been reviewed recently [8], as has the regulation of plant cell wall formation [9]. The polysaccharides of woody plants are synthesized by glycosyltransferases. One of these, a very large enzyme complex called cellulose synthase, is located in the plasma membrane [10]. Each of the six subunits of this complex is thought to contain about six proteins, each being responsible for forming one of the glycan chains that combine to form a microfibril. Biosynthesis of other plant cell wall polysaccharides is less well understood, but recent biochemical and genetic studies are beginning to shed light on some of these processes. For example, the glycosyltransferases responsible for biosynthesis of xyloglucans and mannans are now known to be located in the membranes of the Golgi apparatus.

In the 1890s, Peter Klason was the first to suggest that lignin is a polymerized form of coniferyl alcohol. Half a century later, Karl Freudenberg began to elucidate the details of lignin structure [11], with Vanholme and coworkers having provided a recent update [12]. Lignin is an aromatic polymer that is derived from phenylalanine, one of the natural amino acids (Fig. 2A). It is generally thought to be racemic, which implies that even a dimer can exist as different stereoisomers. Lignin contains different functional groups such as hydroxyl, methoxyl, carboxyl and carbonyl groups, the relative amounts of which depend on the source and isolation method. It is almost impossible to purify lignin intact from plant material because of its covalent bonding to cellulose and hemicellulose. Because of this, efforts to characterize natural lignin typically aim at determining its average composition after crude extraction from plant biomass. Most of the covalent bonds that join lignin to plant polysaccharides are thought to be ether and ester bonds [13]. Typical isolation methods are alkali extraction (resulting in high purity and yields but with structural modifications), extensive milling (with low yields and partial degradation) and enzymatic isolation (resulting in high yields of intact lignin, but with contaminating proteins and carbohydrates). Synthetic lignins can be made from monolignols by enzymatically initiated radical polymerization, which allows their specific radioactive labeling. The fact that these are not complexed with polysaccharides means they can be obtained in pure form for various studies, but they likewise differ substantially from natural lignins.

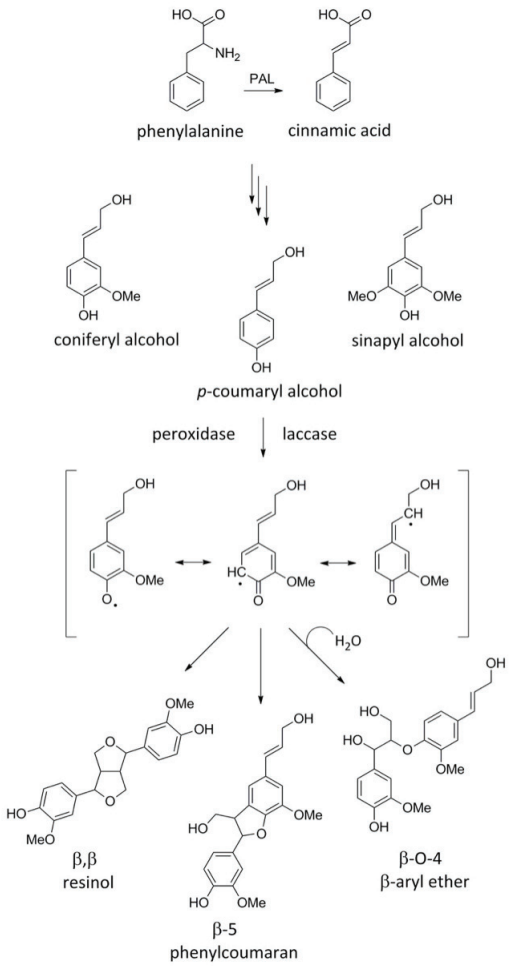
Lignin is actually not one specific molecule but many different variations on a theme. The basic structure of natural lignin is complex (see Fig. 2B and ref. [12]), even though it is formed from only one, two, or in some cases three different phenylpropanoids (monolignols). These monolignol building blocks are *p*-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol, which are derived from phenylalanine by several enzymatic steps (Fig.

2A). The composition, concentration, and molecular weight of lignin (ranging from thousands to tens of thousands daltons) depend on the tree species, the position in the tree, and even the cellular location. Genes for each of the biosynthetic steps leading to monolignols have been identified in different plants via the effects of their mutations, making genetic manipulation of the composition and amount of lignin possible [e.g. 14, 15].

The mechanism by which monolignols are secreted from the cytoplasmic compartment of plant cells is still unclear [12]. Once these building blocks reach the secondary cell wall, though, lignin polymer is formed from them by radical polymerization after oxidation either by peroxidases (heme-containing enzymes that use hydrogen peroxide for activation) or by laccases (copper-containing enzymes that use molecular oxygen for activation). Monolignol radicals can react in different ways to form dimers. For example, two coniferyl alcohol radicals can react to form fifteen different dimers, of which only five are stable. These dimers are themselves oxidized to form radicals that can react either with a monolignol radical or with another dimeric radical, and so on, resulting in very large and complex polymeric species. Since monolignols favor coupling in their β-positions, the dimers are mainly β-β, β-*O*-4 and β-5 linked molecules (Fig. 2A). Considering the difficulty of the oxidative enzymes gaining direct access to the growing lignin polymer within the crowded cell wall matrix, it seems likely that the dehydrogenation occurs at least partly by a radical transfer mechanism via compounds of low molecular weight. Since peroxidases and laccases are rather unspecific, any phenolic compound present in the cell wall may be incorporated into the lignin structure. Together with the amorphous and non-optical nature of lignin, this lack of enzymatic specificity has led to the view that lignin is fundamentally random in its polymeric structure. However, the fact that plants seem to be able to tailor the amount of lignin and its structure for different locations in the plant and in the cell wall suggests otherwise. The degree to which this complex polymer is random is therefore a matter of ongoing debate.

BIODEGRADATION OF LIGNOCELLULOSIC MATERIALS

Trees can live for hundreds or even thousands of years. Among the factors that enable this longevity are the physical protection of bark, the chemical protection of fungicidal compounds like pinosylvin, and the extraordinary durability of lignin. Damage that penetrates the bark presents an opportunity for fungal attack, but trees (especially conifers) respond to this by producing resins and fungicides to protect the wound area. Dead trees do undergo degradation, though. Photodegradation occurs at a rate that is roughly proportional to the lignin content while biodegradation shows inverse dependence [16]. It is nonetheless clear that the structural polymers of wood are intrinsically



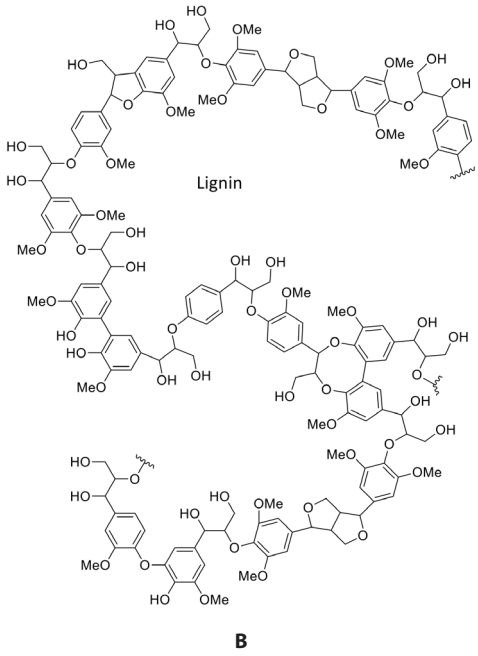


Figure 2: Lignin biosynthesis and structure. A) Cinnamic acid is formed from phenylalanine by phenylalanine ammonia lyase (PAL). From this the monolignols are formed by several enzymatic steps. Peroxidases or laccases initiate the radical polymerization of monolignols. B) Lignin structure as adopted from Vanholme *et al.* [12]. **doi:** 10.5048/BIO-C.2012.3.f2

less biodegradable than other natural polymers are. Under dry and/or anaerobic conditions they last for centuries.

When a birch tree dies, the bark remains practically untouched while rot fairly quickly causes softening of the inner wood (about 20 cm per year). This is probably because the inner wood in birch has no fungicidal compounds and its lignin is only partly polymeric [17].

Because of its low economic value and its extremely complex chemical nature, birch bark has not been studied extensively. It is made of multiple layers. The outer layer contains betulin (triterpene), which gives the white color and imparts fungal resistance. Below that is a highly hydrophobic corky layer made partly of suberin, which has both poly-aromatic and poly-aliphatic domains [18]. Birch bark is so hydrophobic and resistant to biodegradation that it has been used to make roofs and canoes which last for a century or more. Analysis of archeological tars found at Neolithic sites shows that betulin is a major component, its extraordinary longevity resulting from its resistance to microbial degradation [19]. Likewise, the root system of birch is very resistant to biodegradation despite the fact that it is in continual contact with all kinds of microorganisms in a damp environment.

The relative susceptibility of moist birch wood to biodegradation is atypical. Lignocellulose itself is remarkably resistant to biodegradation if the lignin glue is highly polymerized. Even industrial enzymatic degradation of lignocellulose requires extensive pretreatment in order for the cellulose and hemicellulose to be broken down [20]. Pretreatment methods include mechanical grinding, chemical pulping, and physicochemical processes like steam explosion in the presence of alkali or acid. It is the low degree of polymerization and lack of fungicidal extractives in birch that accounts for its relatively rapid degradation.

The first studies of cellulose and lignin degradation by fungi were performed in the 1920s by Falck and Haag [21]. As summarized by Lyr [22], it became clear in those early studies that fungi were able to attack all the structural components of wood. In all cases the polysaccharides were degraded simultaneously with lignin. Thus while some fungi, such as mycorrhiza, live in a symbiotic relationship with plants, others provide the essential function of enriching the soil with the products of decomposed plants.

Cellulose and hemicellulose degradation

The mechanism by which fungi hydrolyze cellulose and hemicellulose has been studied since the 1950s, with the soft-rot fungus *Trichoderma reesei* serving as the model organism [23]. *T. reesei* is used for industrial production of cellulose-degrading and xylan-degrading enzymes because it is one of the best known secretors of proteins, capable of producing up to 100 grams per liter of extracellular enzymes under optimized conditions. The recent publication of its genomic sequence enhances our ability to understand how it works [24]. Degradation of crystalline cellulose is the rate-limiting step in enzymatic lignocellulose degradation. This challenging process requires the concerted action of a whole battery of enzymes: endo-β-1,4-glucanases, cellobiohydrolases and β-glucosidases. A recent paper shows how the rate of hydrolysis of crystalline cellulose is retarded by the jamming of enzymes on its surface [25]. In intact wood, lignin effectively blocks the action of these cellulolytic enzymes by making the cellulose inaccessible to them.

Hemicelluloses are degraded by the same type of organisms as cellulose, but the heterogeneous nature of these molecules requires many different hydrolytic enzymes. Radicals can also react with hemicelluloses, especially pectins, when ferulic acid is one of the constituents in the structure [2]. The complete hydrolysis of birch xylan needs endo-1,4-β-xylanases, β-D-xylosidases, α-glucuronidases, acetyl xylan esterases, and ferulic /coumaric acid esterases [26]. In woody tissues lignin again blocks the hydrolysis.

Lignin degradation

Although plant and wood eaters are common among animals, no higher organisms are known to actually digest lignin. Ruminants eat lignin-containing grasses but can only digest the polysaccharide part, this being accomplished with the help of cellulose-degrading and hemicellulose-degrading microorganisms in their rumens. Termites eat wood, but again lignin is not consumed in the process (though gut microbes may alter it to some extent [27]). Only microorganisms are capable of degrading lignin. Some bacteria [28] can use monolignols as an energy and carbon source, and there are reports that some bacteria degrade plant cell walls by mechanisms called tunneling, erosion, and cavitation [29]. However, since lignin almost always contains sugars, it is likely that these processes depend on energy derived from the sugars. Using ^{14}C-labelled lignins, it has been possible to show that bacteria from some genera, like *Streptomyces*, *Nocardia*, and *Rhodococcus*, do degrade lignin, which is consistent with the identification of a bacterial lignin peroxidase [30]. Degradation is slow and proceeds to a limited extent, with most of the metabolized ^{14}C being in the side chains. Typically several weeks are needed to achieve roughly 10% degradation of lignins below 1,000 daltons in molecular weight, and oxygen is always needed. In view of this, the answer that a *Nature* news article gave several years ago to the question "What can't bacteria do?" still looks to be correct—they cannot metabolize lignin, because "the molecule is too large for most bacteria to handle, and its activation energy is too high" [31].

Fungi are the real lignin degraders

The major degraders of fully lignified plant tissues (>15% lignin) are filamentous fungi. Although there are over 2,000 species of wood-rotting fungi in diverse taxa, the substantial majority of these (over 90%) are white-rot fungi. Many review articles discuss the mechanism of lignin degradation by these organisms [e.g. 32–34]. Other wood-rotting fungi include the brown-rot basidiomycetes, which attack fully lignified tissues but without substantially depleting lignin. Soft-rot decay is caused by various ascomycetes and Fungi imperfecti. This type of decay is generally limited to outer surfaces of wood and occurs only under special environmental conditions.

To simplify the discussion, we concentrate here on white-rot fungi, since they are the most proficient lignin degraders. The fact

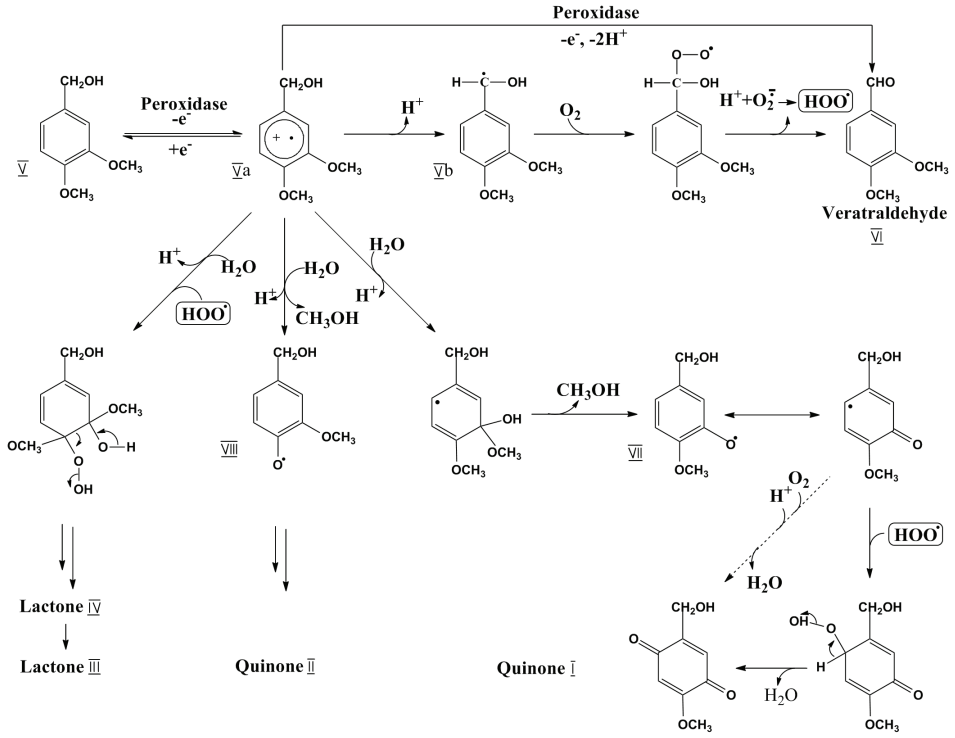


Figure 3: Proposed mechanism of the role of activated oxygen in degradation of a monomeric lignin model compound, veratryl alcohol (V), by lignin peroxidase. In the absence of oxygen, veratraldehyde (VI) is the only product formed from the cation radical. In the presence of oxygen, seven other products resulting from radical chemical reactions were detected. Redrawn from Haemmerli *et al.* [48]. **doi:** 10.5048/BIO-C.2012.3.f3

that even they show a low degree of proficiency underscores the general difficulty of lignin biodegradation. Under ideal aerobic conditions, the most extensively studied white-rot fungus, *Phanerochaete chrysosporium*, degrades one gram of various isolated lignins per gram of fungus (dry weight) in 48 hours, producing about 70% CO_2 and 30% low-molecular-weight water-soluble compounds [35]. To understand the mechanism of this degradation, monomeric and dimeric aromatic model compounds have been used [36]. In 1980, Hall [37] reasoned that the direct attack on lignin is probably from activated oxygen species rather than enzymes. In 1983, two research groups [38,39] isolated an extracellular enzyme from *P. chrysosporium* cultures. It was initially called H_2O_2-dependent dioxygenase, then diarylpropane oxygenase and ligninase before finally being shown to be a peroxidase (lignin peroxidase, LiP) with an exceptionally low pH optimum and high redox potential. This discovery created huge interest both in scientific and industrial circles. One of the discoverers was awarded the Marcus Wallenberg prize, the most prestigious in wood science. Biotech companies as well as pulp and paper companies invested heavily in the potential applications of these "ligninases," hoping that they would tame the untamable lignin. But signs that this was perhaps too optimistic began to appear soon after.

In 1985, another peroxidase (Mn-dependent peroxidase, MnP) was characterized [40]. It oxidizes Mn^{2+} to Mn^{3+}, which is then capable of oxidizing phenolic lignin structures. But the attack on lignin, being indirect, lacks the efficiency and thoroughness of direct enzymatic reactions. Likewise, although lignin peroxidase produces some low-molecular-weight compounds (like methanol from methoxyl groups) from lignin, its major effect is actually lignin *polymerization* [41]. So, in retrospect, the discovery of lignin oxidizing peroxidases was not as spectacular as first thought and still often claimed. By 1928 Bavendamm [42] had already demonstrated a close association between oxidation and lignin degradation. Later Higuchi and Kitamura [43], and Lyr [44] showed that peroxidase and laccase are involved in lignin degradation. Some further studies at that time, reviewed by Lyr [22], give evidence that the idea of peroxidases causing non-specific attack on lignin was already known in late 1940s. Glucose and xylose oxidases are pointed to in that review as sources of the hydrogen peroxide that is needed to activate the peroxidases.

What have more recent studies shown about lignin degradation? Experiments with dimers proved that lignin peroxidase attacks the dimer bond in lignin via a cation radical mechanism [45]. Studies performed first with monomeric 3,4-dimethoxybenzyl alcohol [46] and later with the β-*O*-4 dimer showed that lignin peroxidase opens aromatic rings [47]. Later Haemmerli *et al.* [48] showed by some elegant experiments that activated oxygen species are involved in the ring opening, which results in lactone and quinone formation (Fig. 3). Thus Hall [37] was right in thinking that activated oxygen species have a role. Reduction is also important, as became clear with the discovery that a broad-specificity NADPH-dependent aryl-alcohol dehydrogenase is produced by fungi during lignin degradation [49, 50]. Other achievements include the crystallization [51] and structural characterization of lignin peroxidase [52] and the characterization of several peroxidase isoenzymes [53,54].

Based on the model-compound studies of the 1980s, a model of lignin biodegradation was proposed by Schoemaker and Leisola in 1990 [55]. Since then, a third type of peroxidase (versatile peroxidase, a hybrid of LiP and MnP) has been detected (see [33] for references). MnP was shown to mineralize lignin to a small extent to CO_2 [56]. In white rot fungi, small metabolites from the initial enzyme reaction (including Mn^{3+}, lipid peroxides, and veratryl alcohol radical) play a major role in lignin degradation. The ability of these to enter the lignin matrix by diffusion explains how degradation is initiated even where enzymes cannot penetrate [2].

The complete sequencing of the *P. chrysosporium* genome has made detailed comparison with other fungi possible, which has expanded the number of known peroxidase isoenzymes [57]. Although laccase has been thought for many years to have a role in lignin degradation, little is known of this beyond its ability to oxidize phenolic rings [58,59]. To add to the puzzle, some laccase-producing fungi do not degrade lignin [60]. Whatever the precise function of laccase may be, it clearly differs from that of lignin peroxidase, which has a substantially lower pH optimum (around 3) and higher redox potential [61]. The review by Wong thoroughly discusses what is currently known about the molecular mechanisms of the ligninolytic enzymes and the role of peroxidases [33].

THE LIGNIN ENIGMA

The degradation of lignin by white-rot fungi has some special and even strange features. Firstly, lignin is not degraded during fungal growth but only after nutrient depletion triggers secondary metabolism. This is strange since secondary metabolism is usually connected to biosynthetic reactions rather than degradative processes. Secondly, despite the fact that complete oxidation of lignin is highly exothermic, fungal degradation of lignin actually needs an energy source. It has been postulated that lignin degradation is too slow to serve as a source of metabolic energy. Hatakka and Hammel [34] ponder the possibility that "if lignin fragments were metabolized intracellularly, at least some energy and carbon should be gained from lignin for the fungus, and the fungus should be able to grow on lignin." This is questionable since it is apparent that the faster lignin is degraded the more energy is needed [35]. Under optimal aerobic culture conditions, one gram of fungal mycelia degrades one gram of lignin in about 48 hours, *consuming one gram of glucose in the process* (as an energy source). Once glucose is depleted, lignin degradation ceases completely. And finally, fungi use the same kinds of enzymes (peroxidases and laccases) to initiate lignin degradation that plants use to *make* lignin. We call these curious features *the lignin enigma*.

While the extracellular lignin peroxidases from white-rot fungi mainly polymerize and condense isolated lignins [41] as do their plant counterparts, the fungus is able to change the direction of this process to degradation. How exactly it achieves this is not understood. During the growth phase of *P. chrysosporium*, the insoluble lignin is not attacked by the fungus (Fig. 4A). The secondary phase is initiated by secretion of veratryl alcohol, a secondary metabolite, followed by secretion of LiP and MnP and biosynthesis of intracellular reductases. At the same time lignin is attacked, which quickly becomes evident from a change to its color (Fig. 4B). The color change results from solubilization of lignin in the polysaccharide matrix on the cell wall, as seen in electron micrographs (Fig. 4C). What happens next is not understood. We postulate that lignin becomes linked to surface polysaccharides that prohibit polymerization. Evidence in support of this has been given by Kondo and coworkers [62], who showed that a glycosylated synthetic lignin was depolymerized by LiP while the non-glycosylated form was polymerized.

But whatever the mechanistic details turn out to be, the simple fact that no organism exploits the vast reservoir of energy stored up in lignin is remarkable. As the second most abundant biological material on earth, after cellulose, lignin is produced at a staggering rate of 60 billion metric tons per

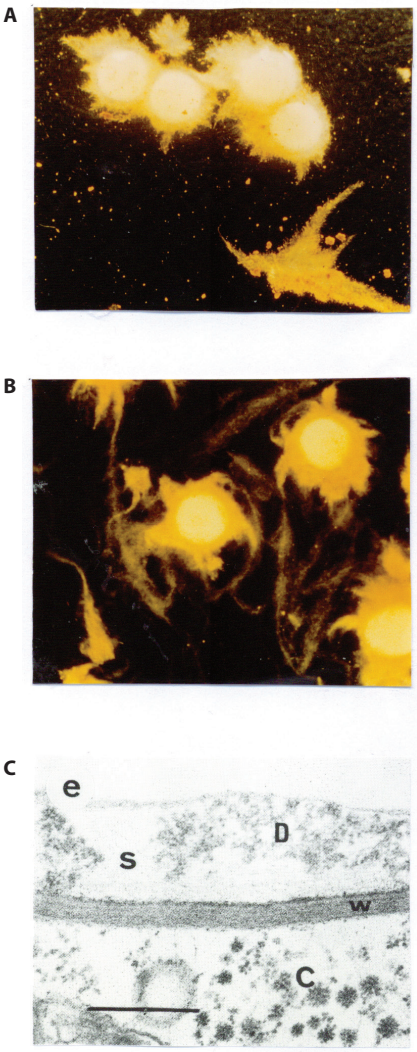


Figure 4: Lignin degradation by *P. chrysosporium*. A) Yellow insoluble lignin fragments during the primary growth phase; B) during secondary phase lignin is solubilized in the mycelium. C) Electron micrograph of lignin buried inside the polysaccharide layer during the secondary metabolic phase (e = extracellular space, s = cell surface polysaccharide, D = lignin, w = cell wall, c = intracellular space, scale bar = 1 µm). The cultures were grown as described by Muheim *et al.* [50]. **doi:** 10.5048/BIO-C.2012.3.f4

year [34] and accounts for 30% of the earth's non-fossil organic carbon [16]. Although not quite as abundant as cellulose, it is more energy-rich, at ~25 kJ/g compared to ~17 kJ/g. So, considering that lignin is generally assumed to have been in abundant supply since the late Silurian period, about 400 million years ago [63], the absence of an organism that lives on it should trouble evolutionary biologists. Darwin's evolutionary mechanism is widely assumed to have produced innumerable innovations well within that timeframe, many of these meeting challenges that seem much more difficult than dining on lignin. Virtually all the complex interactions between the plant kingdom and the other kingdoms of life, for example, are thought to have evolved within that period. Among these are nodulation, a complex symbiotic relationship between plants and bacteria thought to have arisen independently several times in different angiosperm families [64], birds capable of extracting nectar from flowers in flight (hummingbirds), orchids with blooms that mimic the appearance, feel, and pheromonal scent of female bees so well that males attempt to mate with them (accomplishing pollination in the process [65]), and plants equipped with the motor and digestive functions needed to trap and eat live insects, along with sophisticated contact sensors that enable them to distinguish food from non-food [66, 67]. The point becomes even more striking when we consider that remarkable innovations like these not only appeared in higher life forms with much smaller populations and much longer generation times than those of filamentous fungi (both factors greatly impeding Darwinian evolution), but often also many times independently. The complex C4 photosynthesis pathway, for example, is thought to have evolved some thirty-two separate times [68]. All of this poses a profound paradox for evolutionary biology: *How can microorganisms have failed to exploit lignin as an energy source while much less evolvable species have, on innumerable occasions, acquired solutions to problems that appear to be considerably harder?*

EVOLUTIONARY PERSPECTIVE

The origin of terrestrial plant life is sometimes considered to be as problematic as the origin of life itself. A recent discussion of the evolutionary origin of land plants is given by Wodniok *et al.* [69]. The usual assumption is that plant life can be traced back to algae. The first terrestrial plants, however, have complex structures of unknown origin, and the fossil record does nothing to clarify their origin. Among these structures are (a) the sporangium, (b) spores covered by a protective layer, (c) stomata for gas exchange, (d) xylem cells for transport of water and nutrients, (e) evaporation barriers to restrict water loss, and (f) complex three-dimensional mature forms. The birch family is believed to have originated about 70 million years ago in China [70]. This evaluation is based on comparative analysis of DNA sequences, morphology, and paleobotanical finds of pollen and seeds. Evidence for an evolutionary origin of terrestrial plant life and specifically of lignin is fragmentary and based on comparative analysis of structures and molecules and on succession in the fossil record. However, that record shows that species appear abruptly in the fossil record, telling us almost nothing about *how* they appeared. Similarly, as helpful as comparative analysis is for understanding similarities and differences among species, it says nothing about mechanisms of change. The belief that Darwin's mechanism explains what needs to be explained is therefore largely an assumption.

The origin of fungi is commonly assumed to have happened in line with the evolution and diversification of vascular plants and terrestrial ecosystems from macro-algae and early non-vascular land plants. However, molecular clock analyses have suggested a much earlier date for the first fungi, between 660 million and 2.15 billion years ago, with the major fungal phylum Basidiomycota appearing between 390 million and 1.5 billion years ago [71]. A recent study recalibrates these results using the most important fungal fossil *Paleopyrenomycites* and ends up with the conclusion that "fungi evolved right on track" [72]. Biochemist Rolf Thauer argues that accumulation of coal in the Carboniferous period is evidence that organisms could not degrade lignin when it first appeared as a component of hardwoods around 375 million years ago [31]. But there are other plausible reasons for the accumulation. The conditions for coal formation—high temperature and pressure with low oxygen—preclude lignin degradation whether or not lignin-degrading fungi existed. This suggests that coal may be the result of sudden large-scale burial of lignified tissues.

More significant than gaps in evolutionary understanding are the growing number of scientific observations that seem to call the whole theory into question. For example, gene duplication followed by adaptive divergence is assumed to be responsible for the development of the monolignol biosynthetic pathway [63]. However, the actual ability of this mechanism to develop new enzyme activities remains the subject of vigorous scientific debate. Gauger and Axe [73] have shown that the Darwinian mechanism would be hard-pressed to convert a single enzyme to a new activity in the history of life, even in bacteria—the most evolvable free-living life form. Turunen *et al.* [74] studied genome evolution in a yeast strain that has recently experienced whole-genome duplication. Their results indicate that the major effect has been degenerative evolution, in which unneeded functions were destroyed by mutations [see also 75,76]. Consistent with this, Gauger and coworkers have shown that reductive evolution (shutting down useless genes) can prevent constructive evolution even when ideal pathways for the latter are known to exist [77], and Behe [78] has convincingly demonstrated in a recent analysis that molecular evidence shows the net effect of the Darwinian mechanism to be *loss* of function.

The severe deficiency of the neo-Darwinian mechanism is actually fully consistent with the absence of a life form that lives on lignin. If evolutionary innovation is not nearly as easy as has been supposed, then we should not be surprised to find 'low-hanging fruit' that the evolutionary mechanism has failed to pick. The inconsistency arises only when this observation is conjoined with the claim that Darwin's mechanism adequately accounts for all the innovations that have appeared in life. A mechanism that leaves low-hanging fruit unpicked can hardly explain how so much high fruit *has* been picked.

DESIGN PERSPECTIVE

The paradox vanishes completely when we adopt a design perspective. Notice in particular that the relationship between which features get included in a complex system and the ease with which they are included is strikingly different for designed systems than for evolved systems. In evolved systems we expect the simplest features that benefit the system to be implemented, with the caveats that: 1) they must be so simple that they can be stumbled upon by a succession of accidents, and 2) their benefit must be reckoned in terms of immediate functional effect rather than any long-term goal. This explains why bacterial populations that are just two adaptive steps away from recovering a major metabolic function by repairing a mutant gene are found instead to eliminate that possibility by shutting the faulty gene down [77]. This type of cost-cutting turns out to be easier than repairing the gene, and because cost reduction is better than nothing, that becomes the favored option. Indeed, Darwin's mechanism will often forfeit opportunities for large gains because it has no ability to resist 'selling out' for smaller gains that are more immediate.

Contrast this with deliberate design. Here any number of opportunities to choose immediate advantage over long-term advantage must be forgone if anything of significance is to be accomplished. Research, testing, and design modification all require investment of resources with a willingness to wait for any benefit to be realized, but no one doubts that the reward for that kind of commitment more than justifies the cost. And while feasibility certainly constrains deliberate design, it is not at all the case that easier options are always preferred over more demanding ones. The opposite tends to be true, in fact. The most successful designs are those that push the limits of feasibility rather than those that settle for what is easiest.

When it comes to using lignin as food, it is clear that several major technical challenges would have to be overcome for this to happen, but that certainly does not make it impossible. Indeed, if anything qualifies as a universal hallmark of life, it is that it uniformly displays remarkable solutions to major technical challenges. Having summarized the difficulties, we acknowledge that we have no clear idea how an organism would make effective use of lignin as an energy source. Since its synthesis is apparently carried out not by enzymes but by random chemical processes, lignin catabolism cannot be envisioned as a simple reversal of the steps of synthesis. Instead, it would presumably require several new enzymes to hydrolyze the major bonds in lignin to make it water-soluble, which, in conjunction with a system for transporting the solubilized species, would enable catabolism via intracellular reactions. This is admittedly vague, but our inability to go beyond such a vague description says nothing about whether such a process is possible. After all, no scientist would have had any clear understanding of how the energy of sunlight is harnessed to build trees (complete with lignin) apart from our ability to examine that process in operation. The same can be said of all the remarkable processes we study as life scientists—none of us could have imagined them in any detail before we had the opportunity to observe them.

In the end, it seems plausible that dining on lignin is only difficult, not impossible, but either way the design view seems to offer a more satisfactory account of what we know. We know that all complex terrestrial life depends on land plants and that land plants depend on soil. We also know that humus, the organic component of soil, is generated by the continual, gradual decomposition of plant material, and we know that lignin is what forces that decomposition to be gradual. Without lignin, the polysaccharide components of dead plant material would be consumed too rapidly for plant-supporting soil to exist, and life as we know it would not be possible. Only in a world with lignin can terrestrial plant life have the rich diversity that we see, and without that diversity animal life as we know it could not exist.

So, while all other common organic polymers enter the food chain as energy sources, lignin *has* to be the exception in order for life to exist in anything like its present fullness. The fact that it also *is* the exception is therefore significant enough that it calls for explanation. Under the Darwinian view, the biosphere seems remarkably well-poised for the emergence of a microorganism capable of using lignin as its primary energy source. Indeed, since resource exploitation is thought to be a major evolutionary driving force, it is hard to imagine more favorable circumstances for evolutionary innovation. The first organism capable of growing on lignin, should it appear, would have to itself the second most abundant biological material on the planet to fuel its reproductive success. That success would change the biosphere so dramatically that widespread extinctions would occur, ultimately posing a severe threat even to its own survival. And as catastrophic as this would be, the only thing preventing it is its apparent lack of feasibility. The evolutionary account of life on earth must therefore acknowledge such a thing to be beyond the creative capacity of Darwin's mechanism, while attempting to reconcile this with the claim that all manner of more astonishing things were not beyond its creative capacity. Whatever words we choose for this, it inevitably has the suspicious convenience of retrospective story-telling—Darwinian evolution did whatever was done, while avoiding everything that had to be avoided along the way.

Perhaps the oddest aspect of this is that Darwin's theory is unable to make sense of a situation that otherwise makes perfect sense. If life is the product of intelligent design, it stands to reason that the whole design must be considered—not just the functions of molecules and cells and tissues and organs and organisms, but also the functions of entire ecosystems, all the way up to the global ecosystem. One of Darwinism's great deficiencies is that it cannot embrace such a comprehensive view of life. To the extent that reproductive fitness is a property of genotypes, it is a short-term property that manifests itself over the timescale of selective sweeps. It cannot look beyond the genotype in question to consider the effects its carriers have on other species, nor can it peer beyond the temporal horizon of the next selective sweep to consider the long-term effects that event might have. Deliberate design, on the other hand, routinely takes the broad view and the long-term view, and because of this it alone makes sense of life. From the design perspective,

the fact that a lignin-devouring microorganism would have disastrous consequences for life on Earth *is* the reason that no such organism exists, just as the fact that complex life depends on a long list of atypical planetary features *is* the reason that our planet has precisely those features. No other view makes so much sense of so much.

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