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Mechanism and Potential Applications of Bio-Ligninolytic Systems in a CELSS

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ABSTRACT

A large amount of inedible plant material, generated as a result of plant growth in a Controlled Ecological Life Support System (CELSS), should be pretreated and converted into forms that can be recycled on earth as well as in space. The main portion of the inedible biomass is lignocellulosic material. Enzymatic hydrolysis of this cellulose would provide sugars for many other uses by recycling carbon, hydrogen, oxygen, and nitrogen through formation of carbon dioxide, heat, and sugars, which are potential foodstuffs. To obtain monosaccharides from cellulose, the protective effect of lignin should be removed. White-rot fungi degrade lignin more extensively and rapidly than other microorganisms. *Pleurotus ostreatus* degrades lignin effectively, and produces edible and flavorful mushrooms that increase the quality and nutritional value of the diet. This mushroom is also capable of metabolizing hemicellulose, thereby providing a food use of this pentose containing polysaccharide. This study presents the current knowledge of physiology and biochemistry of primary and secondary metabolisms of basidiomycetes, and degradation mechanism of lignin. A better understanding of the ligninolytic activity of white-rot fungi will impact the CELSS Program by providing insights on how edible fungi might be used to recycle the inedible portions of the crops.

Index Entries: CELSS; lignocellulosic material; lignin; white-rot fungi; *Pleurotus ostreatus*; lignin degrading enzyme system.

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INTRODUCTION

Bioregenerative life support systems will be needed in the future to support manned space flight involving long-duration missions and human visits to Mars *(1,2)*. Controlled Ecological Life Support Systems (CELSS) are being studied to establish the technological basis for the developments of life support systems that are relatively independent of resupply *(1)*. CELSS is defined as a self-sustained closed system within which biomass recycles in its various forms. Only energy enters or leaves a CELSS *(3)*.

Bioregenerative processes in CELSS will be very important to regenerate renewable resources as raw materials while recycling the waste components in the system. Food, water, and breathable atmosphere are the elements essential to human survival. An autonomous bioregenerative life support system that continually recycles the solid, liquid, and gaseous materials fundamental for human life is the goal of NASA's CELSS Program *(4)*. Areas of CELSS research include food production, nutritional requirements, waste management, and systems management and control *(4,5)*.

Food production in space requires that optimal plant species for a vegetarian diet, with a high percentage of edible plant biomass, maximum yield, and maximum nutrient value, while using a minimum of space and power. CELSS's environment will be affected and controlled by food production, such as temperature, airflow, humidity, carbon dioxide level, and illumination for the optimal production *(4)*. Selection criteria for candidate species are based on nutritional use and horticultural characteristics. It is more likely that traditional legume-cereal based vegetarian diets will be pursued for a CELSS. For example, legumes such as soybeans, peanuts, and cowpeas could be combined with cereals like wheat and rice to provide all the essential amino acids needed in the diet, and to decrease the total protein intake. A complex carbohydrate source such as potato, vegetables for vitamins, minerals, and fibers could be included to add a variety to the diet. *Brassica napus*, an oil crop, is another potential candidate for a CELSS with seed yields of 40–45% oil, 20–25% carbohydrate, and 20–25% high-quality protein without the beany flavor that soybeans have *(3,6)*.

Harvest index of a crop is defined as the percent edible portion. For example, the harvest index of brassica (rapeseed) plant is 20–25%. Stems, siliques (seed pods), leaves, and roots constitute the large quantity of inedible biomass. Rapeseed leaves have a higher protein content and comparatively lower carbohydrate content than stems or pods, and perhaps may be consumed directly. Converting the inedible biomass to edible forms will increase the harvest index value for the crop *(7,6)* while also providing a flexibility and variety in the diet. The purpose of recycling within the system is to supply human inhabitants with specific quantities and qualities of food, water and air, while eliminating the dangers of biological and chemical toxicity. This will require recycling waste materials to food, carbon dioxide to oxygen, waste water to potable water, and the removal of undesirable volatile, solu-

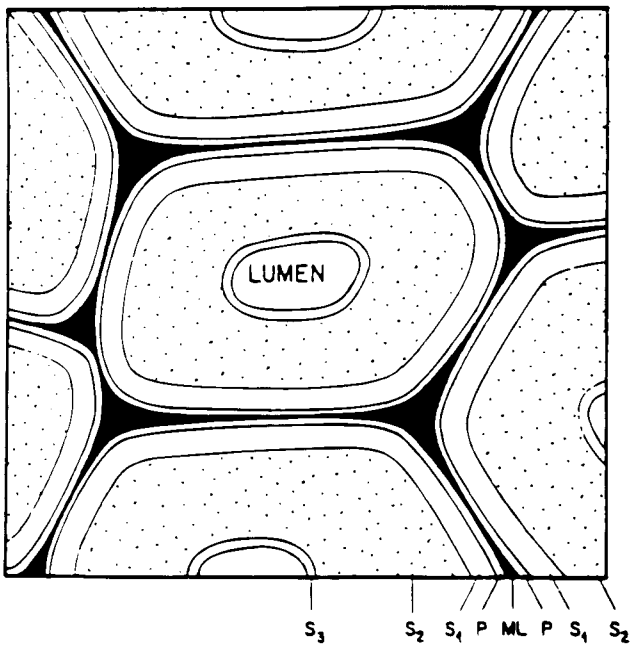

Fig. 1. Schematic structure of wood cell wall. Typical cell wall consists of middle lamella (ML), primary cell wall (P), and secondary wall (S), which is composed of outer layer (S_1), central layer (S_2), and inner layer (S_3) (from ref. *42* with permission).

ble, and solid inorganic and organic materials *(1)*. Recycling of lignocellulosic plant materials will increase the harvest index value for selected plant species, and it will decrease the storage volume required in a CELSS.

INEDIBLE PLANT BIOMASS

The inedible plant material is mostly carbohydrate, composed primarily of cellulose, and also contains considerable quantities of hemicellulose and lignin, and some protein and ash *(8)*. "Lignocellulosic" material is the term used to indicate that lignin is associated with the cellulose and hemicellulose.

Cellulose is a linear unbranched homopolymer of glucose units. In contrast to cellulose, which contains only 1,4-β-D-glycosidic linkages, hemicellulose is structurally more complex. It is a polymer of glucose, mannose, arabinose, galactose, and xylose. Hemicellulose has three basic forms: 1,4-β-D-xylans,1,3- and 1,4-β-D-galactans, and 1,4-β-D-mannans. The relatively short chains are frequently branched and the sugar residues are often acetylated or methylated *(9)*.

Lignin is a complex, three-dimensional aromatic polymer composed of phenylpropanoid units. It fills the spaces between cellulose fibrils, together with hemicellulose and pectin, in woody cell tissues. The concentration of lignin is the highest between the wood cells in the middle lamella (Fig. 1)

although the majority of the total lignin is in the thick, secondary cell walls. Lignin functions as binding material between cell wall components, and gives rigidity and strength to plant cell wall. It provides a protective barrier against biochemical and mechanical stresses. Plants usually react to injury with lignification *(10)*. Cellulose exists as "fibrils" embedded in a matrix of hemicellulose and lignin *(11)*. To be able to hydrolyze cellulose to glucose monomers effectively, lignin and hemicellulose associated with cellulose fibrils should be modified or removed. Removal of lignin will increase the recycling efficiency of plant polysaccharides.

Unlike cellulose and hemicellulose the lignin polymer is not linear, and it does not have a repeating, hydrolysable interunit bond. Lignin has a highly irregular structure resulting from free radical polymerization of the precursors, *p*-coumaryl, coniferyl, and sinapyl alcohols (Fig. 2). The relative proportions of these precursors depend on the type and age of plant and tissue *(12)*. Free radical copolymerization of these alcohols produces the heterogenous, cross-linked, and highly polydisperse polymer (Fig. 3). Over ten interphenylpropane linkage types occur. A few of these linkages predominate in the structure of the polymer *(9)*. The interunit bonds are characterized by the points of attachment. The standard nomenclature designates positions on the propyl side chains as α, β, and γ, (α being proximal to the aromatic ring) and positions on the aromatic ring as 1–6 (indicating the point of attachment of the propyl side chain. Thus, the β-O-4 ether bond is quantitatively one of the major interunit bond types in lignin structure (Fig. 2 and 3).

PRETREATMENT OF LIGNOCELLULOSIC MATERIAL

Polysaccharides from cellulose and hemicellulose can be enzymatically hydrolyzed to sugars that facilitate the microbial-based recycle of carbon, hydrogen, oxygen and nitrogen, giving carbon dioxide, heat, and potential foodstuffs. Lignin inhibits the efficiency of cellullases and hemicellulases by acting as a protective seal for the cellulose with which it is associated. Therefore, ways have to be found to efficiently overcome the protective effect of lignin. Pretreatment methods must be found to increase the bioavailability of the cellulose in a cost effective, safe, and environmentally compatible manner. Conversion of the indigestible cellulosic material into monosaccharides would provide sugars that could be used directly for human food or as a nutrient source for the growth of yeast, fungi, or plant cell cultures *(8)*.

Chemical, physical, and biological pretreatments are used to overcome the resistance to enzymatic hydrolysis and enhance the cellulase action by increasing the accessibility of the substrate to the enzyme *(11,13–15)*. It has been suggested that the difficulties in lignin removal are due to the ester and ether linkages forming between hemicellulose sugar hydroxyls and α-carbonyl of phenyl propane subunits of lignin *(16)*.

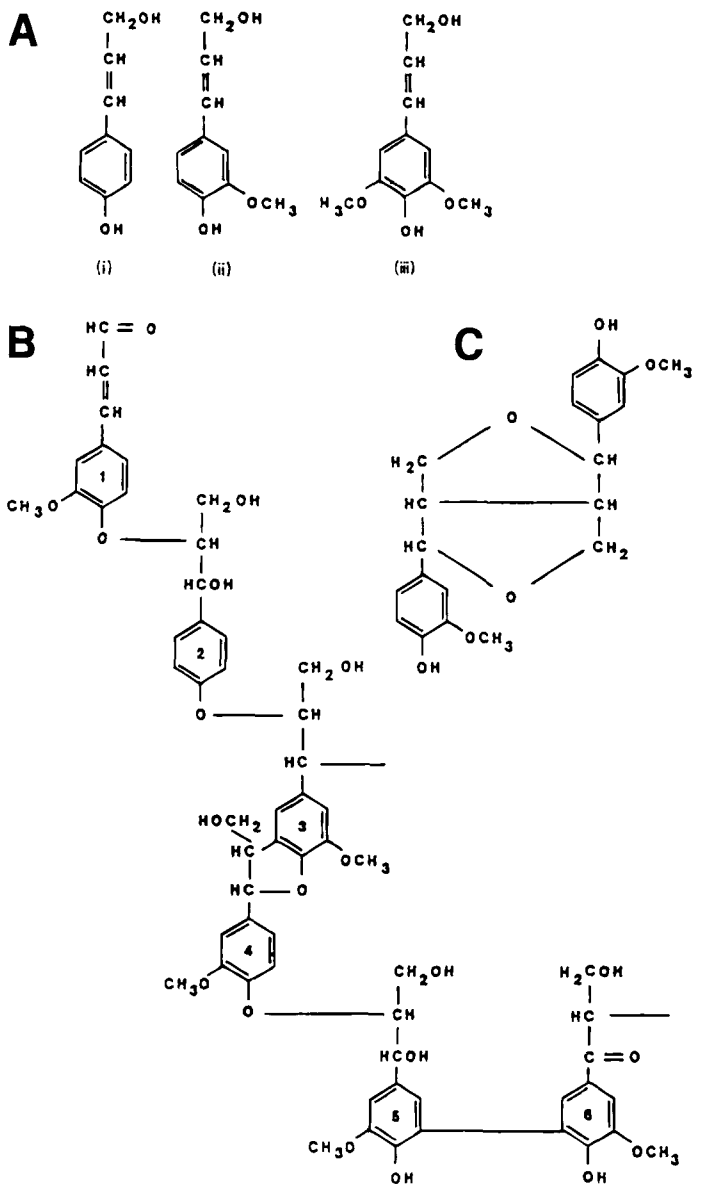

Fig. 2. **(A)** Building units of lignin: (i) coumaryl; (ii) coniferyl; and (iii) sinapyl alcohol. **(B)** Major linkage types of lignin. **(C)** Pinoresinol, a dilignol. (*12*, with permission).

The advantages of biological delignification over physical and chemical pretreatment methods are analogous to the advantages of enzymatic hydrolysis over acid hydrolysis of polysaccharides. The biological processes proceed under mild conditions, give higher product yields and fewer side reactions, and require less energy and less reactor resistance to pressure and corrosion. The disadvantages are also analogous. The biological processes are slower *(17)*.

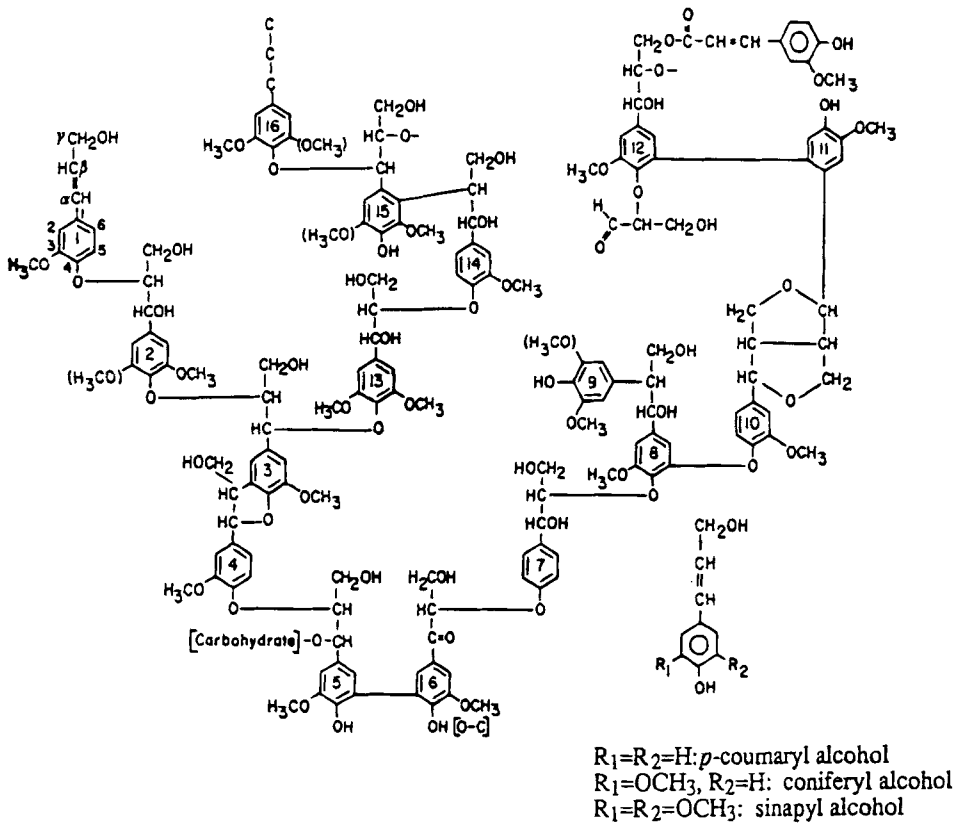

Fig. 3. Schematic structural formula for lignin. Polymerization of the three precursor alcohols, following one-electron oxidation, produces lignin. (*18*, with permission).

The complexity and irregular structure of lignin, and limited information about the ways and the mechanisms of lignin degradation present challenges in the study of biodegradation of lignocellulosic material. A better understanding of the mechanism of the lignin degradation method chosen would make it possible to increase the degradation rate, and/or degradation efficiency. The composition of the substrate needs to be defined, and the parameters that are thought to play key roles need to be studied separately as well as in relation to each other. Such studies could give the optimal values of the parameters, possibly to increase the degradation rate of lignin. Since lignin degradation is extremely important to the treatment and recycling of lignocellulosic materials, an in depth review of what is currently known about basic lignin biodegradation mechanisms is presented.

MICROBIAL LIGNIN DEGRADATION

Lignin is degraded by a narrower array of microbes than any other major biopolymer (*18*). The white-rot basidiomycetes degrade lignin more extensively and rapidly than any other known group of organisms. These

fungi are also well adapted for utilizing the other major plant components. Because the complex lignin polymer encrusts the cellulosic microfibrils of plants and is chemically bonded to the hemicelluloses, the fungi capable of lignin degradation in fully lignified tissue play a key role in the recycling of carbon, not only from the lignin polymer, but also from the plant polysaccharides (9). The growth of white-rot fungi as a pretreatment has been studied both for wood (19,20), and agricultural residues (11,21–24).

Fermentation technology using a wide range of fungal cultures are beneficial in many industrial areas of biotechnology, for example, enzymes such as amylase by *Asperigillus* species, cellulase by *Trichoderma* species, antibiotics (penicillin, cephalosporin, griseofulvin, and fumagillin), ergot alkaloids used in medicine, and organic acid production such as citric acid by *Aspergillus niger*. Cheese and oriental food (soy sauce, miso, tempeh, and tofu) production are other areas for fungal fermentations, together with mushroom production (25).

Several white-rot fungi produce edible fruiting bodies (mushrooms), for example *Pleurotus ostreatus*, the oyster mushroom, and *Lentinus idodes*, the shiitake mushroom. Pretreatment using these fungi would be beneficial to a CELSS, because the system would be a solid-state type of fermentation (minimal outside control and moisture required), and if fruiting bodies are desired, they could be grown to supplement an otherwise bland diet.

There are some advantages of using the *Pleurotus ostreatus* culture in conjunction with a CELSS. *Pleurotus* is able to degrade lignin effectively as well as hemicellulose and cellulose. Basic growth conditions for *Pleurotus ostreatus* to pretreat lignocellulosic agricultural wastes were developed by (26) and (23). There are some reports that lignin is preferentially degraded by *Pleurotus*, but it is possible that lignin simply appears to be preferentially degraded because lignin surrounds the cellulose and hemicellulose and must be removed to allow access to the hemicellulose and cellulose. Also, there is a possibility of using *Pleurotus ostreatus* in a CELSS, first to pretreat the plant inedibles, and then to produce mushrooms, a flavorful variation in a space diet (27). Mushrooms would also increase the nutritional quality of the diet. They are a good source of protein, vitamins, especially B1, B2, B12, and C, minerals, and carbohydrates (28).

Phenerochaete chrysosporium is possibly one of the best characterized white-rot fungi (10,12,29). The results obtained in defined cultures of *P. chrysosporium* have been widely accepted as a paradigm for the physiology of ligninolysis among most white-rot fungi (18). Studies with *P. chrysosporium*, together with *Pleurotus* species, and other white-rot fungi, showed that lignin degradation with these microorganisms is an oxidative process. Nitrogen limitation promotes extensive degradation of lignin, although some white-rot fungi, such as *Bjerkandera adusta* produced lignin-degrading enzymes in a nitrogen-rich medium (30). The enzyme system and mechanism of lignin degradation, studied mostly with *P. chrysosporium*, give some insights into the nutritional requirements and environmental control para-

meters that would play significant roles in delignification by basidiomycetes. Even though there are some differences between different fungi species, for example, *Pleurotus ostreatus* is more selective in lignin degradation than *Phenerochaete chrysosporium (29)*, the lignin degradation mechanism in both cases are believed to be similar, although the enzymes used for the degradation process might be different for different species *(31–34)*.

Some of the potential applications and earth benefits of ligninolytic cultures are:

1. partial delignification for the production of cellulosic products (bio-mechanical pulping, bio-bleaching);
2. conversion of lignocellulosics (improving ruminant digestibility, cultivating edible mushrooms) into feed and food;
3. treatment of lignin derived wastes;
4. biomodification of lignin by-products to yield valuable polymeric or low molecular weight chemicals *(35)*.

PHYSIOLOGY OF FUNGI

Nutrients might be assimilated either directly or after breakdown by extracellular enzymes secreted by fungi. A reduction in the growth rate can be brought about by limiting the supply of any of the essential nutrients. Under these conditions growth is no longer balanced, and nutrients are diverted into metabolic pathways that are not essential for growth.

Metabolic pathways that occur when the organism is growing at maximal rates have been referred to as primary pathways. Primary metabolites are those that have to be produced for growth to occur, such as nucleic acids, proteins, carbohydrates, and lipids, and their precursors. The metabolic pathways that produce energy have to be active, and intermediates or end products may accumulate usually at the end of rapid growth phase. In the stationary phase, these end products tend to be metabolized further *(25)*. Secondary metabolic pathways become functional at sub-maximal growth rates, such as for antibiotic production.

For white-rot fungi, nitrogen, in the form of ammonia or proteins, amino acids and nucleic acids, is the natural substrate. Lignin degradation proceeds during the secondary growth phase which is triggered by nitrogen, carbon, or sulfur limitation, but not phosphorus. Lignin degradation reactions require oxygen and there are a few enzymes recognized which play a role in lignin degradation, such as lignin peroxidase, manganase peroxidase, laccase, and H_2O_2 generating enzymes. Cation radical formation and enzymatic combustion are key degradation reactions. Depolymerization is kinetically favored because ligninases oxidize their substrates by one electron; the diversity of subsequent reactions of the unstable intermediates is a function of their structures. It is this nonspecific subsequent oxidation of lignin that leads us to refer to the process as enzymatic combustion *(18)*.

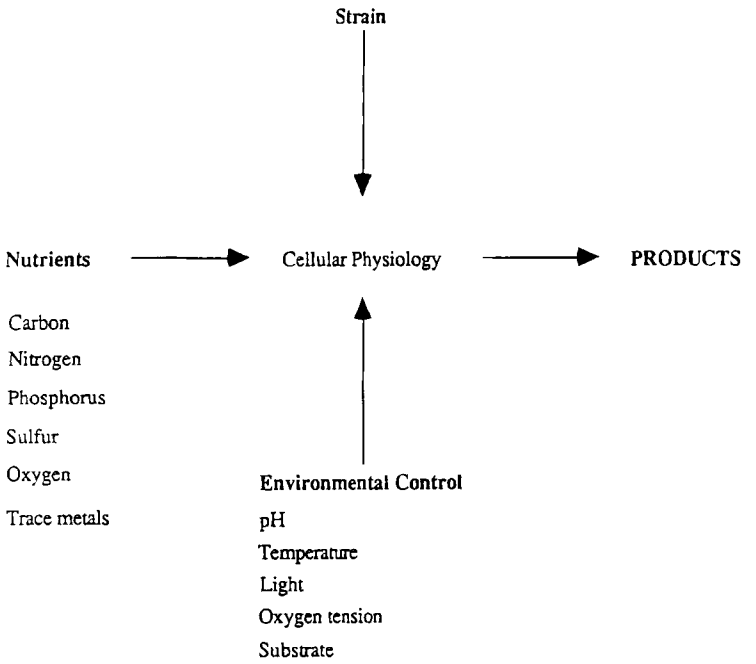

Fig. 4. Parameters affecting microbial metabolism, adapted from Berry, 1975 *(36)*.

Figure 4 gives a general scheme of the parameters that should be considered for the desired consequences, and the products for fermentation processes *(36)*. Lignocellulosic materials are natural substrates for basidiomycetes, but to increase the degradation rate of lignin, or efficiency of this process, some of the nutritional and environmental factors that are believed to play important roles in lignin degradation mechanism in these microorganisms should be studied and optimal conditions for biodelignification by *Pleurotus ostreatus* must be determined.

Carbon and Nitrogen Metabolism

Carbohydrates, fatty acids, and organic acids from deamination of amino acids are the major carbon sources. Most fungi can utilize a range of monosaccharides, oligo- and polysaccharides, but uptake is restricted to monosaccharides. Hydrolytic enzymes secreted or present on the cell surface including amylases, cellulases, and invertase break down polysaccharides to monosaccharides. Galactose is less readily metabolized. Glucose is metabolized the best in the case of *Pleurotus ostreatus*, and glucose, mannose, and xylose are used better than arabinose and galactose. Carbohydrate catabolism is divided into three phases: glycolysis; pyruvate metabolism and TCA cycle; and oxidative phosphorylation.

Glycolysis is defined as the conversion of glucose to pyruvate under aerobic conditions. In fungi, three pathways have been described: Embden-

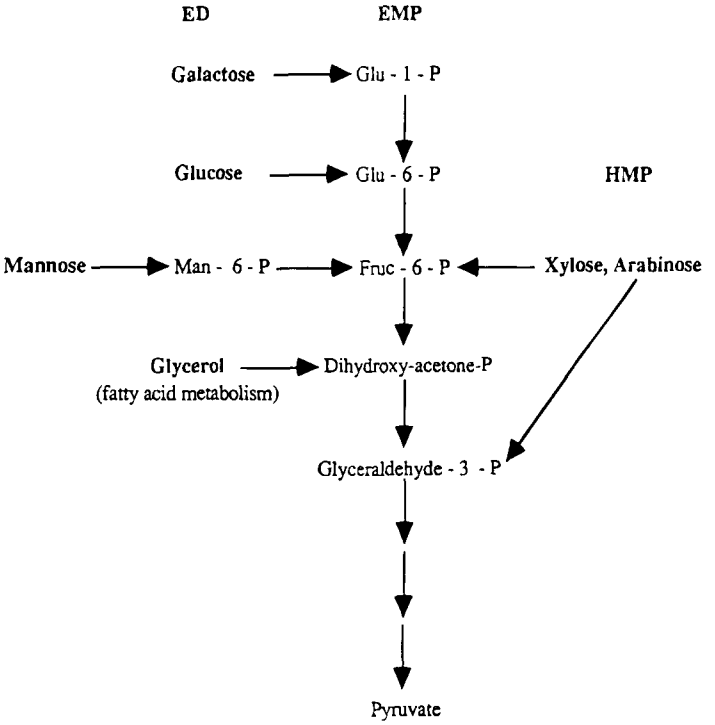

Fig. 5. Glycolysis, adapted from Berry, 1975 *(36)*.

Meyerhof-Parnas; hexose monophosphate; and Entner-Doudoroff. Depending on the substrate and other controlling factors, one or combination of these three pathways could be used (Fig. 5) *(36,37)*.

In the TCA cycle, pyruvate is oxidized to carbon dioxide. Figure 6 shows some of the intermediates of TCA cycle and their biosynthetic roles *(37)*.

Basidiomycetes do not utilize nitrite or nitrate. Nitrogen source should be in the form of proteins, amino acids, and nucleic acids or ammonia. Phenylalanine and tyrosine can be synthesized to fumarate, an intermediate of TCA cycle. Some of the amino acids can be converted to acetyl CoA by the microorganism and incorporated into TCA cycle that way. Fatty acids go to acetate and then, to acetyl CoA; isocitrate and acetate can be converted to oxaloacetate, another TCA cycle intermediate, through the glyoxylate cycle.

The substrate should be able to provide the primary growth elements such as carbon, nitrogen, sulfur, phosphorus, and trace elements. Temperature, pH and oxygen tension must be within desirable ranges for the microorganism. In the secondary growth phase, depending on the product desired, change in the control parameters could increase the fermentation rate by manipulating the secondary metabolic pathways.

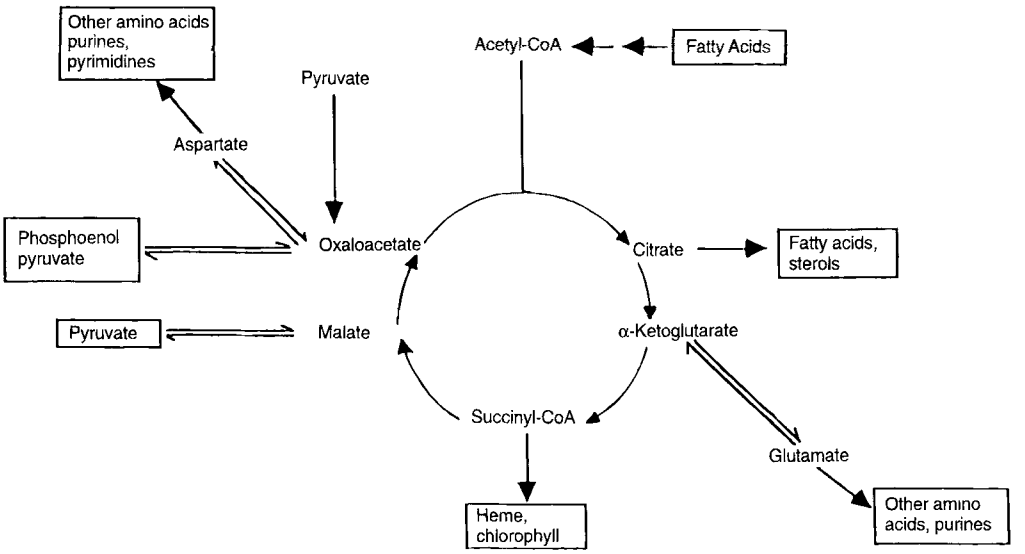

Fig. 6. Major biosynthetic roles of some TCA cycle intermediates, adapted from Mathews and van Holde, 1990 *(37)*.

Oxygen

Oxygen has a stimulatory effect on the ligninolytic systems. Lignin depolymerization is improved when oxygen is used rather than air to grow the culture. Molecular oxygen increases the production of H_2O_2 and veratryl alcohol, and increased veratryl alcohol concentration results in increased lignin peroxidase activity either through direct induction or as a result of protection against H_2O_2-mediated inactivation of the enzyme *(29,38)*.

Microelements

Another factor known to be associated with enhanced lignin peroxidase activity is the concentration of microelements. A 1.7-fold increase in enzyme activity was reported by incorporation of a trace metal solution containing Mn, Mg, Co, Ca, Zn, Cu, Mo, and Al into the culture medium of *P. chrysosporium*, and this effect was attributed to the Cu or Mn components *(39)*. Increased Fe also increases ligninolysis with only marginal effects on mycelium yields *(29)*.

LIGNIN DEGRADING SYSTEM

White-rot basidiomycetes degrade lignin more rapidly and extensively than other microbial groups. Recent intensive studies on the lignin degrading enzyme system of basidiomycetes have shown that the extracellular part of the system is comprised of the enzymes lignin peroxidase (LiP), manganese peroxidase (MnP), glyoxal oxidase and certain metabo-

Table 1
Enzymes Making up the Delignification System in White-Rot Fungi

Enzyme	Function
Lignin peroxidase	Oxidizes aromatic nuclei Cleaves propane side chains
Manganese peroxidase	Oxidizes phenols Generates H_2O_2
Laccase	Produces phenoxy radicals
Quinone-oxidoreductases	Prevents oxidative polymerization of the radicals produced
Glyoxal oxidase Sugar-oxidases Alcohol-oxidases	Generate H_2O_2

lites. Lignin is fragmented by this system, and the plethora of degradation products; partially degraded lignins, low molecular weight oligomeric and monomeric degradation products, and low molecular weight aromatic acids such as vanillic acid *(10)* are taken up by the hyphae and further metabolized by the intracellular system. Complex and heterogeneous structure of lignin requires the biodegradative systems to be extra- and intracellular, nonspecific and nonhydrolytic *(38,40)*. Table 1 summarizes the enzymes involved in lignin degradation by basidiomycetes.

Lignin degradation is observed in the secondary growth phase of white-rot fungi. When nitrogen is depleted, and the carbon:nitrogen ratio in the substrate increases, extensive lignin degradation starts *(41)*. After the readily available carbon and nitrogen sources are used, due to the ability of fungi to degrade less available nutrient sources such as lignin, fungal growth continues with a secondary mycelia. During this phase, lignin undergoes a number of oxidative changes including aromatic ring cleavage, giving a wide array of low molecular weight fragments.

Lignin Peroxidase (LiP)

The enzyme lignin peroxidase (LiP) has a ping-pong mechanism. First, hydrogen peroxide oxidizes the resting enzyme by two electrons to give Compound I, enzyme intermediate (Fig. 7). Oxidized LiP oxidizes aromatic nuclei in lignin by one electron, generating cation radicals. Then, these radicals react spontaneously with nucleophiles (primarily H_2O), and with molecular oxygen. The result is an enzymatic combustion in which C–O and C–C linkages are cleaved, depolymerizing the polymer and opening aromatic rings. These products enter the hyphae where they are further oxidized by as yet undescribed enzymes *(38)*. A proposed reaction sequence which gives insight into the electron balance:

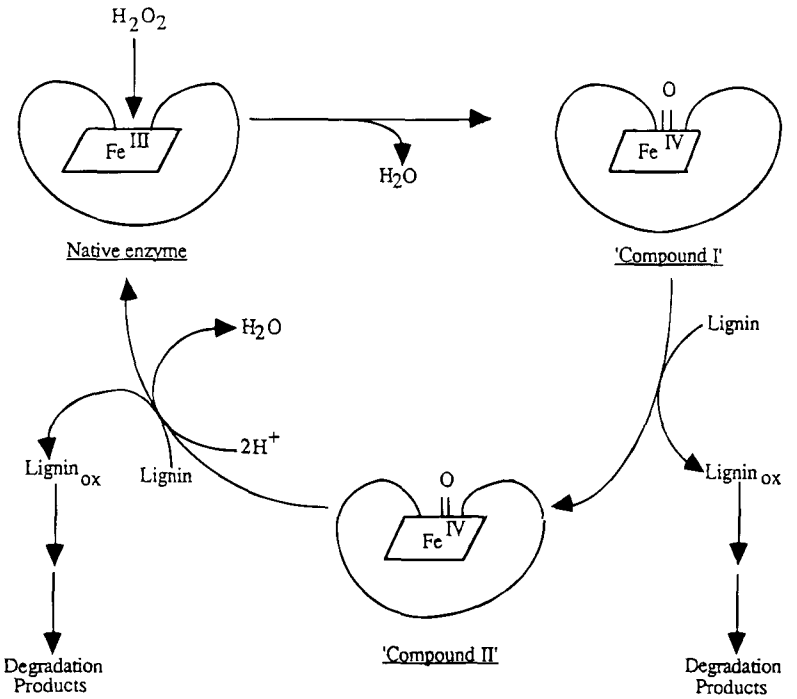

Fig. 7. Catalytic cycle of *Phanerochaete chrysosporium* lignin peroxidase (from ref. *29* with permission).

$$\text{LiP} + H_2O_2 \longrightarrow \text{Compounded I} + H_2O$$

$$\text{Ferric state of ligninase} + H_2O_2 \longrightarrow \text{Compounded I (two electron oxidized form of the enzyme)} + H_2O \quad (1)$$

$$\text{Compound I} + S \longrightarrow \text{Compounded II} + S^+$$

$$\text{Compound I + alkoxylated substrate} \longrightarrow \text{Compounded II (one electron oxidized form of the enzyme) + unstable radical cation} \quad (2)$$

$$\text{Compound II} + S \longrightarrow \text{Li} + S^{+\cdot}$$

In Cleland notation, the reaction can be written as *(43)*

$$\begin{array}{cccc} H_2O_2 + S & S^{+\cdot} & S & S^{+\cdot} \\ \downarrow & \uparrow & \downarrow & \uparrow \\ \text{LiP} & \text{Compound I-S} & \text{Compound II} & \text{LiP} \end{array} \quad (3)$$

Figure 7 represents the catalytic cycle of LiP for *Phanerochaete chrysosporium (29).*

The compound I form of the enzyme produces veratryl alcohol radical cations from veratryl alcohol molecules. Then, the veratryl alcohol radical cation removes one electron from the methoxylated aromatic ring of the lignin molecule, initiating bond cleavage. Finally the radicals react with oxygen molecule and give active oxygen species which then lead to auto-

oxidation of lignin. This sequence offers a possible explanation of why increased enzyme activity does not necessarily mean increased lignin degradation. The enzyme is not necessarily the rate limiting component, since active oxygen species and veratryl alcohol molecules are also required.

Manganese Peroxidase (MnP)

MnP oxidizes Mn (II) to Mn (III), which in turn can oxidize a variety of organic substrates. Data indicate that the enzyme undergoes a redox cycle similar to that found for LiP. However, while both Mn (II) and various phenols are able to reduce Compound I to Compound II, only Mn (II) can reduce Compound II back to the native enzyme, thereby accounting for the manganase dependence of the enzyme. MnP might be functioning in lignin degradation as phenol-oxidizing enzymes and possibly by generating H_2O_2 *(29)*.

Laccase

Laccase is an enzyme that catalyzes the one-electron oxidation of phenols to phenoxy radicals, which in turn undergo a variety of nonenzymic interactions leading to C_α-oxidation, alkyl-phenyl cleavage, and demethoxylation. Such reactions might be important in the degradation of phenolic units in lignin, and phenolic intermediates released during polymer decomposition.

Quinone-Oxidoreductases

Even though the mechanism has to be understood better, it is believed that enzymes such as cellobiose:quinone oxidoreductase, and NAD(P)H:quinone oxidoreductase are responsible from the reductive steps. These steps prevent oxidative polymerization of radical species and quinones generated during polymer decomposition. For lignin degradation to take place effectively, active degradation products of lignin should be prevented from polymerizing lignin. Quinone-oxidoreductases have a role in providing the necessary reductive steps.

H_2O_2-Generating Enzymes

Hydrogen peroxide is required for ligninase activity. It activates the peroxidases by oxidizing them. The oxidized enzyme generates cation radicals which in turn cause the degradation of lignin molecule. Therefore, a supply of extracellular H_2O_2 is an important factor in lignin depolymerization. Enzymes that play a role in generating H_2O_2 are: glucose 1-oxidase, glucose as substrate; glucose 2-oxidase, glucose and xylose as substrates; Mn (II)-dependent peroxidases, through oxidation of reduced glutathione, dithiotreitol, NADPH, dihydroxymaleic acid; glyoxal oxidase, aldehyde, α-hydroxy carbonyl, and α-dicarbonyl compounds as substrates.

3,4-Dimethoxybenzyl (veratryl) alcohol is a secondary metabolite of basidiomycetes. It is synthesized from phenylalanine. Veratryl alcohol is a substrate for the oxidized form of LiP, and veratryl alcohol radicals are obtained from this reaction. These cation radicals then react with lignin and remove one electron from the methoxylated aromatic ring, initiating bond cleavage. Oxygen is also thought to bind to veratryl alcohol cation radicals, giving rise to the production of active oxygen species which are capable of promoting extensive auto-oxidation of lignin.

Addition of veratryl alcohol to primary growth stage of the culture does not induce the enzyme LiP. The culture should be in its secondary, ligninolytic stage. It has been shown that addition of veratryl alcohol at concentrations above 0.2 m*M* increased LiP yields in cultures of *Phlebia radiata (29)*. In addition, veratryl alcohol has been shown to potentiate LiP oxidation of compounds which are not good LiP substrates *(40)*. Veratryl alcohol is metabolized to carbon dioxide; first it is oxidized to veratric acid, then methylated to vanillic acid. Vanillic acid is a major intermediate of lignin degradation. Figs. 8 and 9 summarize lignin degradation with respect to the role of veratryl alcohol and H_2O_2 *(10,38)*.

SUMMARY

Pretreatment and recycling of inedible plant materials will be necessary in a CELSS environment. The main portion of the inedible biomass is lignocellulosic material consisting of lignin, cellulose and hemicellulose. Lignin inhibits the efficiency of cellulose and hemicellulose pretreatment by acting as a protective seal for these polymers with which it is associated. Therefore, an efficient method of lignin removal will be critical in a CELSS. Further impacts of the conversion of inedible plant biomass to nutritious edible microbial biomass include: on-site generation of dietary supplements which would be difficult to derive by other means; and the processing of inedible plant material to CO_2, water, protein, and edible carbohydrate using a nonpathogenic microorganism. This would be of significance to a CELSS since it would provide a bioregenerative subsystem which is passive, safe, and food producing as well being complimentary to physical/chemical subsystems (i.e., combustion, pyrolysis and/or wet oxidation) being contemplated for destruction of underutilized plant biomass generated as a consequence of food production in a CELSS.

Several challenges face the implementation of fungal based, bioregenerative recycle of plant biomass. The long term stability of a biological subsystem must be demonstrated in the context of a functioning, extraterrestrial, waste processing system. Development of packaging technology is needed for delivering viable cultures of the microorganisms, nutrients, and promoters of lignin degradation at the point of use. Further, the application of microbial cultures by astronauts must lead to the desired rate of

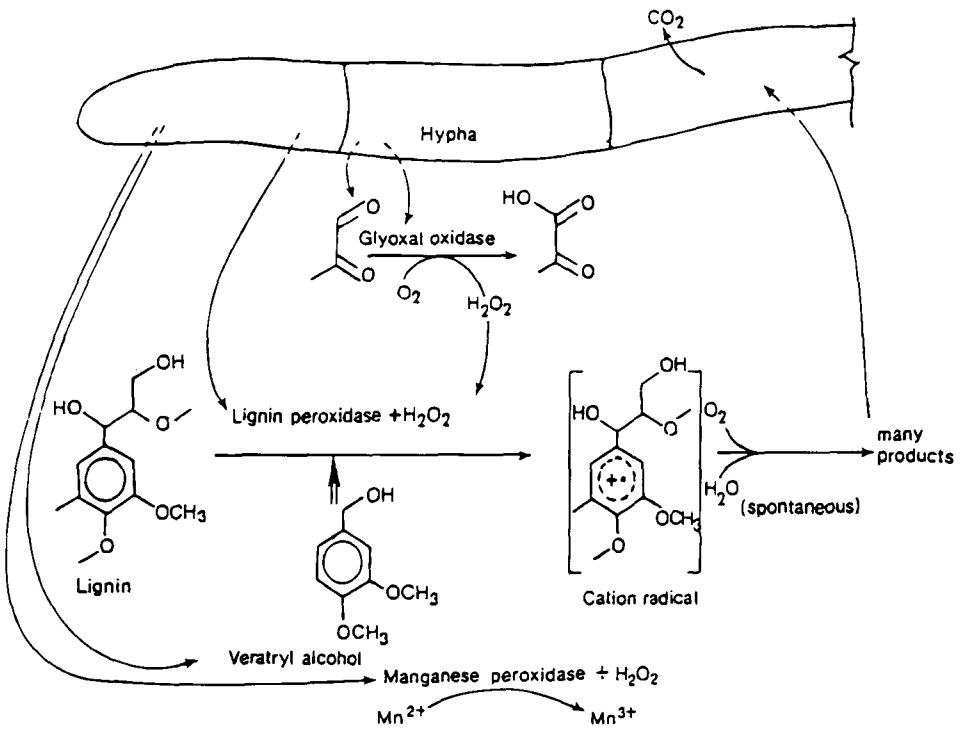

Fig. 8. Scheme illustrating the lignin-degrading system of *Phanerochaete chrysosporium* (from ref. *38* with permission).

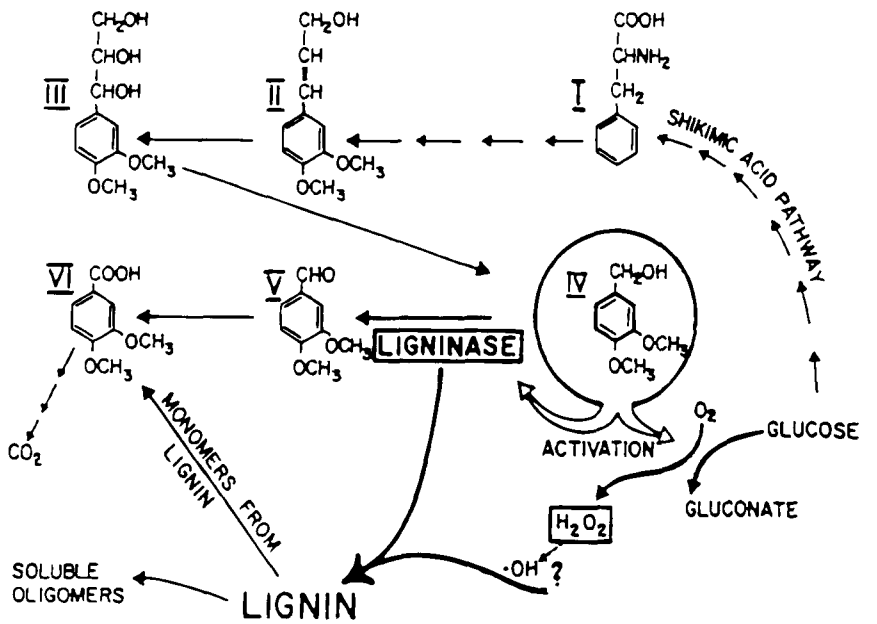

Fig. 9. Possible connection between veratryl alcohol metabolism and lignin degradation. (I) Phenyl alanine; (IV) Veratryl alcohol; (V) Veratraldehyde; (VI) Veratric acid (from ref. *10* with permission).

microbial activity and extent of lignin degradation at an extraterrestrial planetary outpost. Perhaps the most important immediate research concern is demonstration of complete degradation of all of the organic components of plant biomass (hemicellulose, cellulose, and lignin) with minimal preprocessing of the lignocellulosic material. The literature reviewed in this paper should prove to be helpful in indicating directions which the research should take in addressing these goals.

White-rot fungi degrades lignin more effectively than other microorganisms. The lignin degradation mechanisms of white-rot fungi are activated in secondary growth phase. Water, hydrogen peroxide, and veratryl alcohol are some of the necessary components of the mechanism. Enzymes, including lignin peroxidase, manganese peroxidase, laccase, and glyoxal oxidase are involved in the degradation process and are responsible for the formation of hydrogen peroxide and free cation radicals which in turn cause autodegradation of the lignin molecule. Oxygen has important roles in these reactions especially to produce hydrogen peroxide and to react with cation radicals. Water molecules react with cation radicals too. The increase in veratryl alcohol concentration increases lignin peroxidase activity. In addition, trace elements affect the process. It should be possible to increase lignin degradation by controlling and manipulating the parameters which are involved in lignin degrading system. A white-rot fungus such as *P. ostreatus* would produce edible mushrooms in addition to the ability to degrade lignin, so it should be effective and beneficial in a CELSS environment in terms of adding a variety to the diet of inhabitants, and increasing the efficiency of the recycling of inedible biomass.

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