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Biotransformation of Coal Substructure Model Compounds by Microbial Enzymes

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ABSTRACT

Research with both coal substructure model compounds and macromolecular coal has shown that intermonomeric chemical bonds within coal are susceptible to cleavage by microbial enzymes from bacterial and fungal sources. This is particularly true when low rank lignite coals are used as substrates for enzymes after first being solubilized under alkaline conditions to form water-soluble coal polymers. When these soluble polymers are used as substrates, high-performance liquid chromatography (HPLC) methods are used to show that particular enzymes catalyze their depolymerization. By using chemical presolubilization followed by enzymatic depolymerization, it may be possible to develop commercial processes for the enzymatic depolymerization of coal into useful low-mol-wt chemicals, or into liquid or gaseous fuels. Evidence indicates that oxidative enzymes, such as peroxidases or etherases, and hydrolytic enzymes, such as esterases, have the best potential for effectively depolymerizing coal. Recent findings in our laboratory also suggest that certain hydrolases from saprophytic soil fungi may also work well.

Index Entries: Biotransformation; coal; lignite; enzymes; esterases; peroxidases; etherases.

INTRODUCTION

Current Status of Research on Biological Coal Liquefaction

Considerable research has been devoted to studies of the microbial solubilization, liquefaction, and gasifaction of coal (*1*). A principal goal of this research has been to develop biological processes to convert coal into

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liquid or gaseous fuels efficiently using either microorganisms or their enzymes. Although there has been some success in developing microbiological processes for removing pyritic sulfur from coals (2), few promising biotechnologies have been found for liquefying or gasifying coal. This is despite the findings that some bacteria and fungi can solubilize and partially depolymerize low-rank coals (3,4), and that certain strictly anaerobic bacteria may partially gasify coal (5). Limited coal depolymerization has been shown to occur during growth of some fungi and bacteria on either solid coal or alkali-solubilized coal polymers (3). The microbial enzymes most likely involved include extracellular esterases and peroxidases (1,3). Although this research has led to valuable additional knowledge about the biochemistry of coal biotransformation (6), economically feasible and effective coal liquefaction processes have not yet resulted from the research.

Coal is a complex three-dimensional condensed polyaromatic macromolecule that also contains various kinds of carbon–oxygen and carbon–oxygen–carbon bonds (7). This is a significant problem that must be overcome if extensive biological depolymerization of coal is to be achieved. In addition, the relative abundance of the different macromolecular structures varies not only with the rank of the coal (e.g., lignite, subbituminous, bituminous), but also with the type of biomass from which it was derived (7). Even macromolecular bonds that might be susceptible to biodegradation are often physically unavailable to microbial enzymes.

Attempts to increase the susceptibility of coal to enzymatic attack have met with limited success. They have primarily involved presolubilizing the coal by treatment with strong bases (e.g., NaOH) and/or preoxidizing it with acids (e.g., nitric acid) or by aerated heating (1,6). Acid oxidation has been popular, since it partially degrades the coal while introducing oxygen (primarily as new carboxyl groups). Microbes attack these pretreated coals more readily, but still do not extensively depolymerize them (3). Sufficient numbers of susceptible bonds apparently are still not present, at least at the macromolecular surfaces where enzymes most likely function. A second problem stems from the complex macromolecular chemistry of coals. Enzymes, even those that have broad substrate specificities, are probably unable to depolymerize coals extensively because of the condensed polyaromatic nature of coal, and because of the diversity of chemical bonds present within the macromolecule. A large percentage of these intramolecular bonds may not be readily susceptible to enzymatic cleavage. In essence, there are insufficient enzyme access points within the coal molecular structure. Thus, a research breakthrough is needed if we are to find a way to bioliquefy coals using microbial enzymes without first using harsh chemicals to destroy the basic integrity of the coal.

The Biological Degradation of Lignin

Though low-rank coals, particularly lignites, contain abundant condensed polyaromatic structures, they are similar to lignin in structure in that they also contain carbon–oxygen bonds, including phenolic, carboxyl, ether, and ester groups (*8*). Lignin is a biodegradable polymer decomposed by ligninolytic fungi and bacteria. These microorganisms utilize extracellular peroxidases to initiate the degradative process (*8–10*). Peroxidases of the fungus *Phanerochaete chrysosporium* and several bacteria of the genus *Streptomyces* are the most studied. They initiate degradation by oxidizing specific phenolic and/or nonphenolic aromatic groups within the lignin molecule, removing a single electron in the process (*8,11*). Removal of the electron generates a free radical within the conjugated aromatic ring structure of the lingin, and as this radical moves into the lignin macromolecule, the molecule spontaneously fragments and depolymerizes. This is one of the most unique and important degradative processes known in nature.

The Microbial Depolymerization of Coal

If coal is to be liquified, it must also be depolymerized (*3*), particularly if depolymerization can be accomplished by nonoxidative or reductive mechanisms (*4*). To be extensively degraded under anaerobic conditions (e.g., converted to ethanol, methane, phenols, and so forth), prior depolymerization must be extensive enough to reduce the average molecular weight of the depolymerized coal to below 1000 (*4*). However, as discussed above, if coal is to be significantly depolymerized, a great many linkages within its macromolecular structure must be cleaved. Low-rank lignite coals are abundant and are good candidates for depolymerization. They are more aliphatic, are richer in oxygen, contain fewer condensed ring structures than do higher-rank coals, and contain more intermacromolecular linkages that may be susceptible to enzymatic cleavage (*6,7*). Yet, lignite, like other forms of coal, is a complex three-dimensional polymer containing a diversity of structures. In addition, the macromolecular structure of the lignite is influenced greatly by the inorganic cations present within the matrix (*6,7*). These ions help hold the macromolecule together, via charge interactions between the ions and functional groups in coal. Researchers need to be cognizant of these and other basic facts of coal chemistry when developing projects aimed at using enzymes to depolymerize coal.

One of the most promising approaches to developing coal biodepolymerization processes is to utilize a two-stage chemical/enzymatic process in which the coal is first converted into a soluble form prior to being subjected to enzyme action (*3,6,12–14*). Solubilization makes the macromolecular structure of the coal more accessible to enzymes as compared to

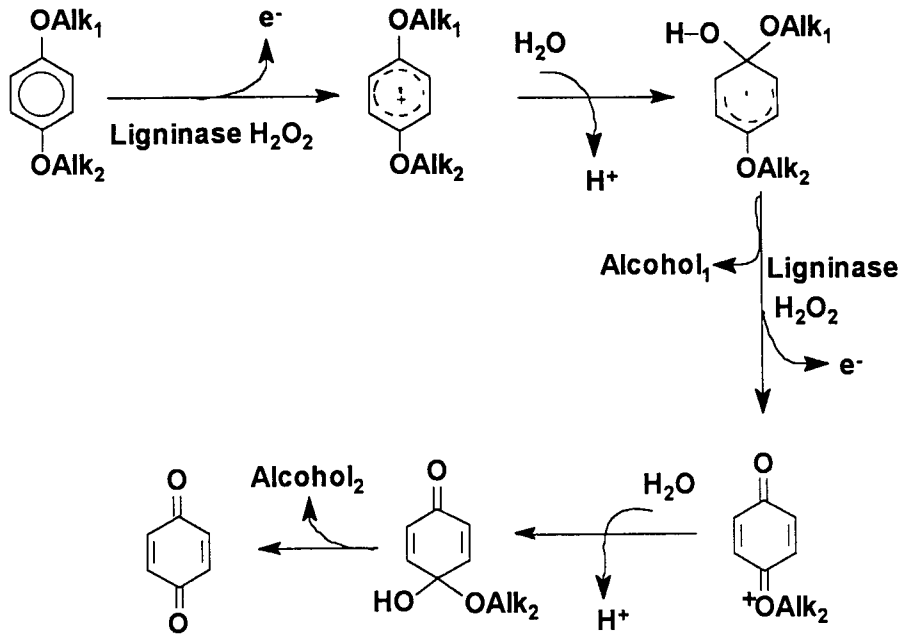

Fig. 1. An example of the free radical mechanism of lignin peroxidase.

insoluble coal, where only surface structures are readily accessible. Many lignite coals are quite soluble in basic solutions (*6,12–14*). Once solubilized, the coal can be maintained as a water-soluble polymer even at pHs as low as 4 or 5 (*13,14*). At these pHs, oxidative and hydrolytic enzymes can function.

Enzymes Involved in Coal Depolymerization

A variety of oxidative, reductive, and/or hydrolytic enzymes may be useful for depolymerizing lignites. Oxidative etherases may be important, but remain unstudied. Extracellular peroxidases have been shown to oxidize coal, including, for example, the peroxidases of the actinomycete *Streptomyces viridosporus* (*10,11*), the white-rot fungus *Phanerochaete chrysosporium* (*8,15*), and of eubacteria, such as *Pseudomonas cepacia* (D. L. Crawford, unpublished results). Peroxidases catalyze one-electron oxidations, which may generate cation-free radicals within lignite coal, in much the same way they do when oxidizing lignin (*8*). Within polymers like lignin, which contain conjugated bond structures, these unstable radicals move freely and cause a variety of spontaneous decomposition reactions that cleave carbon–oxygen–carbon bonds, such as those found in low-rank coals. Figure 1 shows a representation of this type of free radical decomposition reaction. Figure 2 shows how the generation of a free radical by lignin peroxidase results in the decomposition of fully conjugated structures, such as those in azo dyes. Peroxidase-initiated free

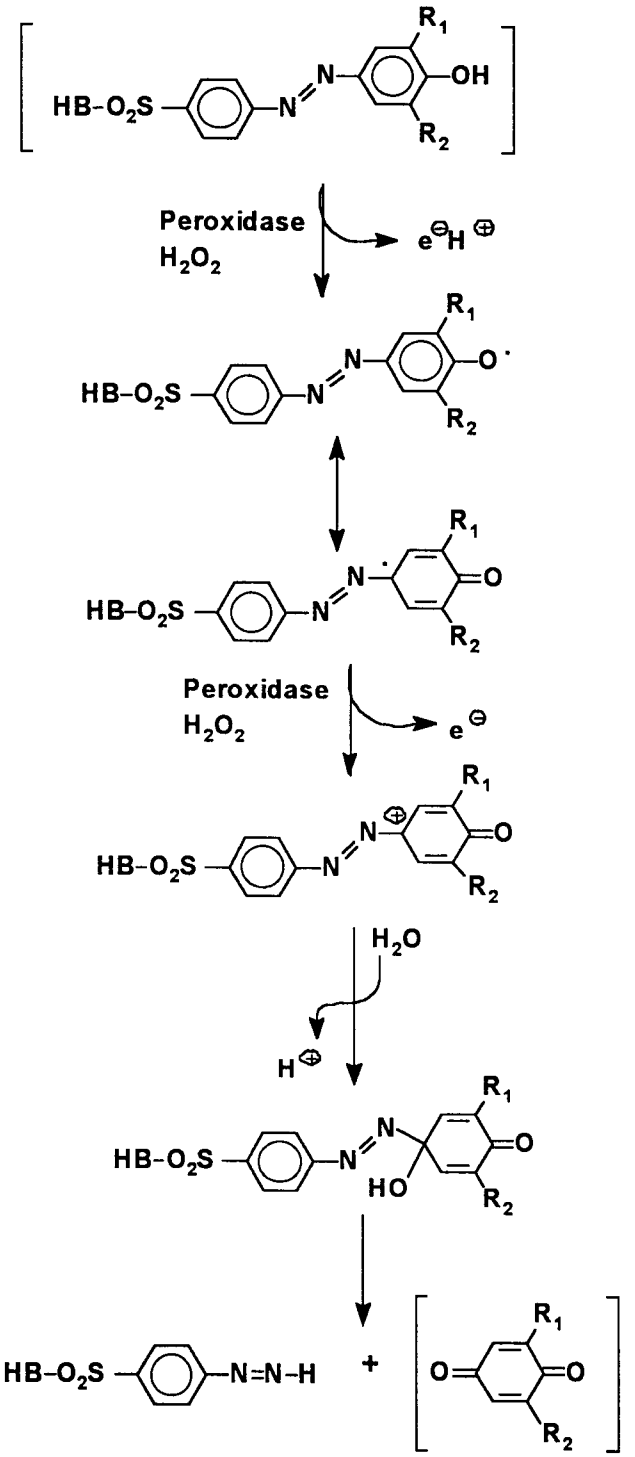

Fig. 2. A proposed pathway for cleavage of sulfonated monoazo dyes by the lignin peroxidases of *P. chrysosporium* and *S. viridosporus*. Taken from Goszczynski et al. (*16*).

radical decomposition is one of the unique degradative processes in nature and might be successfully applied to the depolymerization of coal.

Although few reductive enzymes have been reported to act on coal, they may ultimately prove valuable in the production of liquid fuels from coal (*4*). Reductases require cofactors, such as reduced pyridine nucleotides, to supply the reducing power needed for the reactions they catalyze. Such enzymes are typically intracellular and would not necessarily be expected to act on highly polymeric molecules, such as coal. However, certain enzymes might be useful once extracted from microbial cells, for example, when used to reduce carbon–carbon double bonds within aliphatic structures in lignite coals or to demethoxylate aromatic rings within the coals. These types of reactions would increase the hydrogen content of the coal and/or reduce its oxygen content, thereby increasing its fuel value. In particular, reductive enzymes might be useful in converting low-mol-wt, depolymerized coal fragments to more reduced, possibly liquid products. A more complete review of this topic has recently been published by Deobald (*4*). Anaerobic reductive enzymes that might be useful in the depolymerization and/or reduction of coal include decarboxylases, dehydroxylases, quinone/and ketone reductases, and dehydrogenases that reduce carbon–carbon double bonds. To date, enzymes catalyzing these types of reactions have not been examined for their coal biotransforming potential.

Next to peroxidases, hydrolytic enzymes appear to have the greatest potential for use in the depolymerization of lignite coals. Lignites, for example, contain significant amounts of oxygen present within ester, carboxyl, and carbonyl groups (*7*). Many of these, particularly esters, may be susceptible to hydrolytic cleavage. Extracellular esterases have been clearly implicated in the depolymerization of lignite coals (*3*) and appear to be a most promising family of enzymes for further examination. Most of the coal depolymerizing bacteria thus far studied in our laboratory produce multiple extracellular esterases when growing in the presence of soluble coal polymers (*3,13*, and unpublished results), and the ester bonds, along with ether bonds, become significantly depleted within coals depolymerized by bacterial cultures and/or enzyme preparations from those bacterial cultures (*3,13*).

In work not yet published, we have recently been examining a *Cephalosporium* fungal strain for its ability to hydrolyze ketone-substituted carbon–carbon bonds similar to those present in lignite coals. This fungus was isolated from soil obtained at an old lumber mill site that contained large amounts of decomposing sawdust and other lignocellulosic material. We have found that this strain produces a novel enzyme that hydrolytically cleaves carbon–carbon bonds containing ketone structures like those found in coal. Preliminary data indicate that the *Cephalosporium* produces the enzyme intracellularly, but extracellular activity is also found at low levels when cells are grown in a liquid medium containing the coal sub-

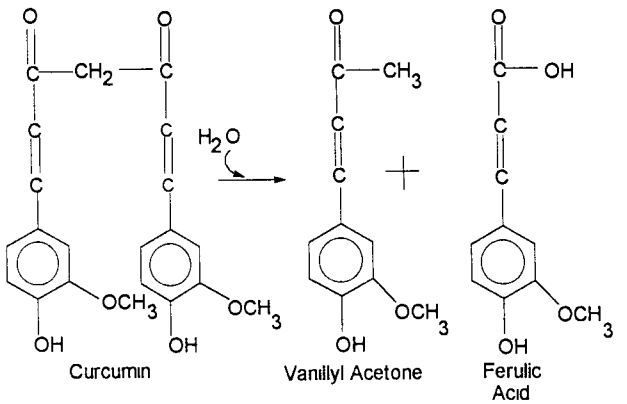

Fig. 3. The reaction catalyzed by the *Cephalosporium* curcumin hydrolase.

structure model compound curcumin (1,6-heptadiene-3,5-dione 1,7-bis [4 hydroxy-3-methoxyphenol); *see* Figure 3) as a sole source of carbon and energy for growth. We feel that this enzyme, or similar ones from other organisms, may prove useful for the depolymerization of coal. We have also determined the pathway by which the *Cephalosporium* strain degrades curcumin (*17*). The first reaction in the pathway utilizes the hydrolase, which hydrolytically cleaves the carbon–carbon bond associated with the β-diketone structure of curcumin (Fig. 3). On hydrolysis, a carboxylic acid (ferulic acid) and an aldehyde (vanillyl acetone) are formed. The *Cephalosporium* degrades the ferulic acid through protocatechuic acid (3,4-dihydroxybenzoic acid) and reduces the vanillyl acetone's carbon–carbon double bond to form zingerone. Zingerone is either degraded slowly through unknown intermediates, or it may be a dead-end product of the pathway.

Although hydrolases that cleave carbon–carbon bonds are rare, they have been reported to be involved in a variety of biodegradative pathways (*17*). Curcumin contains a ketone bond similar to those common in lignite coals. We are presently determining whether the enzyme can hydrolyze similar bonds within the macromolecular structure of soluble lignite coal polymers.

FUTURE RESEARCH DIRECTIONS

It will be a challenge to develop efficient, economic coal bioconversion processes, particularly if the goal is to produce liquid or gaseous fuels from coal. Fuels are low-value products that must be produced very cheaply in large amounts. Considering the typical costs of microbial or enzymological conversions, even the economic production of value-added chemicals from coal will prove difficult. In order to maximize the chances of

success, it is vital that an emphasis be placed on interdisciplinary research that closely links coal chemistry with microbiology and enzymology. The combined chemical solubilization/biological depolymerization approach using lignite coals appears promising and is well worth continued emphasis. Enzymes similar to the peroxidases, esterases, and hydrolases discussed above have the greatest potential for significantly depolymerizing lignite, because they target the most common "enzymatically cleavable" intermonomer bonds in low-rank coals.

Researchers must also develop some completely new approaches to solving this problem. It is unlikely that classical microbial cell and/or enzymatic bioconversion processes can be developed that will be both sufficiently efficient, yet economic, as well as scaleable to the level needed for commercial implementation. For example, the solution may lie in devising some novel way to utilize peroxidase-initiated "free radical-mediated decomposition" to depolymerize coals completely and quickly into sufficiently low-mol-wt products (averaging $\leq$ 1000 Dalton). If this can be accomplished, other chemical, biological, and/or biomimetic approaches can be used to generate final products, such as liquid or gaseous fuels and/or chemical feed stocks.

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