

BIOSOLUBILIZATION OF COAL IN AQUEOUS AND NON-AQUEOUS MEDIA

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ABSTRACT

Biocatalytic solubilization of coal can be achieved by microorganisms in aqueous medium or by enzymes in aqueous or organic media. In these systems, coal is converted to a liquid product through a variety of oxidative and/or hydrogenative reactions.

The mechanism and control of coal solubilization activity have been studied in organisms associated with coal in nature. The solubilization apparently involves catalysis by alkaline cell metabolites. The aqueous product of this microbial action may be a suitable substrate for other biological interactions such as biogas production by methanogenic organisms.

Enzymatic coal solubilization has been demonstrated in both shake flasks and fluidized-bed reactors. The process has been carried out under both anaerobic and aerobic conditions, with aqueous and hydrous organic solvents. The solubilization product of the anaerobic process is much less polar than that from microbial solubilization.

INTRODUCTION

The application of biotechnology to the utilization of low-ranked coals has become the focus of intense research activity in recent years. Biological systems -- whole organisms or fractions thereof -- have been shown to catalyze a variety of industrially significant reactions. These systems have potential utility in the upgrading and/or transformation of coal.

Biocatalysis generally occurs under mild, "physiological" conditions, e.g. at relatively low temperatures and pressures, and at pH near neutrality. Biological treatments of coal could therefore be conducted under mild reaction conditions relative to those under which conventional thermo/chemical processes are carried out. Biocatalysts characteristically demonstrate high specificity with respect to the products generated. Biological treatment of coal would thus promote the formation of specific products, including perhaps products with higher fuel value than that of the original substrate.

Biotechnology can be applied to various aspects of coal processing. The application which has received the greatest attention is the removal of contaminating heteroatoms, such as sulfur, from low-ranked coals by microorganisms. The use of biocatalysts to modify coal's carbon skeleton is, however, a rapidly developing area of great potential importance. Research at Oak Ridge National Laboratory has focused on the biotransformation of lignite and subbituminous coals by microorganisms or by isolated biochemical catalysts (enzymes). The

synthesis of liquid products from coal by biological systems, i.e. coal biosolubilization, is the subject of this exploratory research. This work will form a basis for the development of technologies for the production of clean-burning liquid or gaseous fuel products from coal substrates. The results of current initiatives in this area are summarized below.

Biosolubilization of coal in aqueous media

The solubilization of coal by microorganisms was first reported in 1982 by Cohen and Gabrielle (1). These and other workers noted the production of liquid droplets from coal, associated with the growth of mycellial organisms on the coal surface. Organisms competent to solubilize coal were isolated from coal in the environment (2) or in the laboratory (3). It was subsequently shown that these organisms solubilized coal when cultured on the surface of common, organic microbiological media. Suitable coal substrates for this activity were leonardite, lignites, and subbituminous coals. In the latter cases, the coals required oxidative pretreatment either through natural weathering or by chemical agents [hydrogen peroxide, ozone, nitric acid, etc.; (4)]. The product of this microbial activity was a water-soluble mixture of oxidized compounds of moderate molecular weight [30,000-300,000 daltons; (3)]. The material was enriched in carbonyl and hydroxyl functions relative to the coal substrate, and was precipitable at pH 1. Its characteristics resembled those of humic acids, except for its water-solubility.

Recent work (5,6) in this laboratory has sought to determine the mechanism by which microorganisms solubilize coal in vivo. Superior isolates (fungi) have been cultivated in a defined growth medium in both surface culture on agar and submerged culture in liquid medium. These culture methods simulate fixed-bed and fluidized-bed bioreactor configurations respectively, which have been proposed for use with this technology (Figure 1). The defined media developed for use in this work consisted of inorganic salts, supplying the organism's mineral requirements, plus a sole carbohydrate carbon source (5). These media support coal solubilization in vivo (Table 1). The use of defined media has minimized contamination of the liquid coal product with organic medium components, and will contribute to further product analysis. product recovery has been expedited by growth and solubilization under submerged culture conditions, i.e. in shake flasks. use of this system has also permitted the development of a spectrophotometric assay for coal solubilization, based on the appearance of chromophoric material (absorbing in the 420-450 nm spectral range) in cultures incubated with coal. Its spectral characteristics were identical to those of the material formed by the nonbiological action of alkali on coal.

The solubilization of coal by dilute alkali had been demonstrated previously, and had been implicated in the activity in vivo (6). Specifically, it was thought that microbial coal solubilization occurred as a fortuitous consequence of pH increases associated with growth. Evidence in support of this conclusion had been obtained in alkaligenic systems substantially contaminated with protein and other basic components. In the present work, activity was detected in an acidogenic system of known biochemical composition (Figure 2). These data support an involvement of alkaline catalysis in the microbial activity. The data suggest further that the proposed alkaline catalyst is produced by cultures in specific response to the presence of coal.

The liquid product generated by fungal action on coal may have some utility as

a feed stream for the biological production of other combustible fuels. Possible products are biogas (methane) and fuel alcohols.

Methane production from partially-oxidized compounds analogous to coal substructures has been demonstrated elsewhere. Based on this knowledge, a two-step process for conversion of coal to methane has been proposed. Coal solubilization would be carried out by aerobic microorganisms as described above. Subsequent biogas production would be accomplished through the action of anaerobic organisms. Preliminary tests have been conducted in order to determine the feasibility of the proposed process.

Microbial consortia isolated from a municipal sewage treatment plant or from a coal fly ash settling pond were incubated with biosolubilized coal under anaerobic conditions. The liquid coal product was nontoxic at low concentration toward microbial growth or metabolism. After acclimation, methanogenesis from methanol occurred with the same yield with or without the coal liquids present. Model compound studies were carried out with vanillin, which was shown to disappear from metabolically active cultures. A defined growth medium was designed for use with these methanogenic consortia. In that system, either vanillin or the coal liquid could serve as sole carbon source for growth and metabolism. Preliminary data from total organic carbon analysis with the coal liquid as the sole carbon source indicated at least partial degradation. These findings suggest that biosolubilized coal can be utilized by methanogenic consortia for biogas production, accomplishing a two-step conversion of coal to clean-burning gaseous product. These data also support the feasibility of using anaerobic microorganisms for other treatments of liquid coal product, possibly including the conversion of coal to fuel alcohol.

The feasibility of treating coal with enzymes in aqueous buffer has been explored. Preliminary trials were conducted with horseradish peroxidase and laccase from *Pyricularia oryzae*. These enzymes are a nonspecific oxidoreductase, transferring electrons from H_2O_2 , and an oxygenase respectively. The reactions were carried out in shake flasks under aerobic conditions. A measurable increase in coal solubilization occurred when laccase was used, although the extent of solubilization was somewhat low (Table 2). Horseradish peroxidase also increased solubilization, but to a smaller extent. The material yielded by enzymatic solubilization was water-soluble, and of a polarity similar to that of the product of microbial action.

Biosolubilization of coal in non-aqueous media

Bioprocesses involving intact microorganisms generally are carried out in aqueous environments, i.e. growth media or buffers. Considerable excitement has been generated by the finding that isolated enzymes can be used in organic solvents to catalyze certain reactions (8). The bioprocessing of coal in non-aqueous media would favor the formation of nonpolar products, possibly including reduced compounds with high fuel value.

Oxidizing enzymes would be required to catalyze the various reactions involved in coal depolymerization. Additional treatment with reducing enzymes may result in the production of low-molecular-weight nonpolar compounds similar to those generated by nonbiological coal liquefaction. These enzymes would cause a net increase in hydrogen content and a reduction in oxygen content. Recovery of

these products would be facilitated if these reactions were carried out in a non-polar organic solvent. The use of enzymes in organic solvents is complicated by the need to maintain the enzyme within an aqueous microenvironment in order to support activity (8). Hydrous organic media are therefore used in these systems.

The potential for enzymatic coal solubilization in nonaqueous media was evaluated via tests with horseradish peroxidase, alcohol dehydrogenase from yeast, and/or bacterial hydrogenase. Preliminary experiments were performed under aerobic conditions (air) using peroxidase dissolved in hydrous dioxane. The extent of coal solubilization was somewhat increased by this treatment, and was much greater than that previously measured in buffer alone (data not shown). Subsequent tests were carried out with enzyme mixtures in dioxane or pyridine, under a reducing atmosphere of 100% hydrogen. Solubilization of a leonardite was substantially enhanced by enzyme treatment (Table 3). A much smaller positive effect was seen in tests with bituminous coal.

The liquid product from anaerobic coal solubilization in organic solvent was found to be soluble in benzene (data not shown). This product thus differed from that obtained by aerobic treatment with enzymes or intact microorganisms in aqueous systems with respect to polarity and solubility behavior.

Additional studies were carried out in a fluidized-bed bioreactor under aerobic conditions, in which coal was treated with peroxidase in hydrous dioxane. Solubilization was very rapid and was enzyme-dependent (Figure 3).

SUMMARY

Biological treatments with whole cells or isolated enzymes has the potential to yield useful products from low-ranked coals. These products may include clean-burning gaseous or liquid fuels. Coal solubilization is a common element in these conversions. Exploratory research indicates that the characteristics of the product(s) obtained are dependent on the choice of operating conditions, i.e. whether in aqueous or organic solvent, and in the presence or absence of air. The development of future bioprocesses for coal utilization must build upon these findings, to achieve the directed synthesis of desired products.

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Table 1. Coal solubilization by Paecilomyces TLI (3; surface cultures)^a

Medium ^b	Coal solubilization ^c (% weight loss)
Complex:	
Sabouraud maltose	12.4
Defined:	
Czapek's	25.9
Minimal I	[5.3]

^aCultures were grown at 30°C, 80% RH for 7 d. Coal was added (0.5 g) and cultures reincubated for 7 d under the same conditions.

^bMedia contained 0.1% malose.

^cControls (uninoculated medium) exhibit <5% weight loss. Weight loss measurements incorporate <20% error.

Table 2. Enzymatic solubilization of acid-treated subbituminous coal in the presence of 0.05 M phosphate aqueous buffer at pH 6.0

Aqueous constituents	Average weight loss (%)
Buffer only	2.5
Buffer + 1625 units of peroxidase/mL	4.4
Buffer + 700 units of laccase/mL	6.8

Table 3. Anaerobic solubilization of coal in a hydrogen environment at ambient temperature and pressure by enzymes in organic solvents^a

Liquid phase	Type of coal	Enzymes	Weight loss of coal (%)
Dioxane + buffer	Lenordite	None	19.6
Dioxane + buffer	Lenordite	Mixed ^b	86.4
Dioxane + buffer	Bituminous	None	1.2
Dioxane + buffer	Bituminous	Mixed ^b	3.7
Pyridine + buffer	Bituminous	None	3.0
Pyridine + buffer	Bituminous	Mixed ^b	6.7
Pyridine + tetralin + buffer	Bituminous	None	4.6
Pyridine + tetralin + buffer	Bituminous	Dehydrogenase	6.0

^aThe tests were carried out for 48 h with 20 mL of liquid and 0.3 g of coal size-reduced to a range of -10 to +30 mesh in 50-mL shake flasks with a controlled gas environment in the flask headspace. When enzymes were used, they were introduced with an activity of 400 units/mL for peroxidase, 0.325 units/mL for hydrogenase, and 3640 units/mL for dehydrogenase. A 0.1 M aqueous phosphate buffer, pH 5.6, that constituted 5% (v/v) of the liquid solution was used.

^bThe enzyme mixture included peroxidase, hydrogenase, and dehydrogenase in equal weight proportions.

Figure 1

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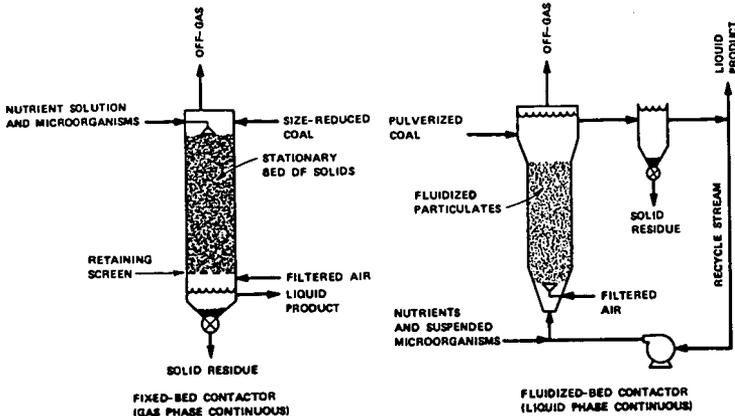


Fig. 1 Two possible bioreactor configurations for the microbial liquefaction of coal.

Figure 2

ORNL DWG 88-9880

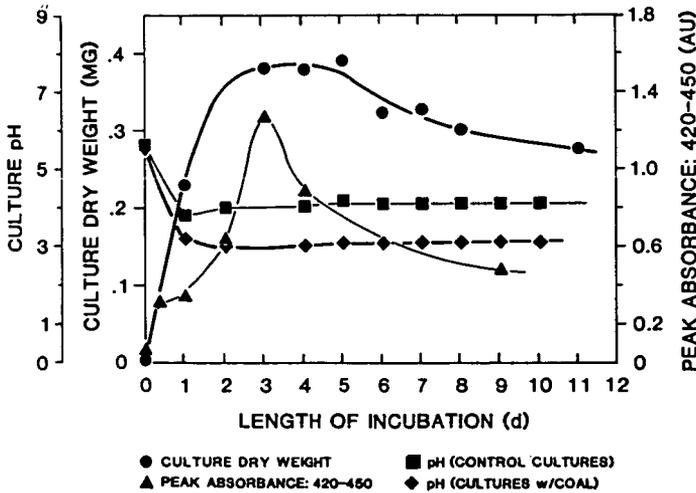


Fig. 2 Coal solubilization in an acidogenic system.

DISSOLUTION OF PREOXIDIZED SUBBITUMINOUS COAL
IN HYDROUS DIOXANE IN A FLUIDIZED BED

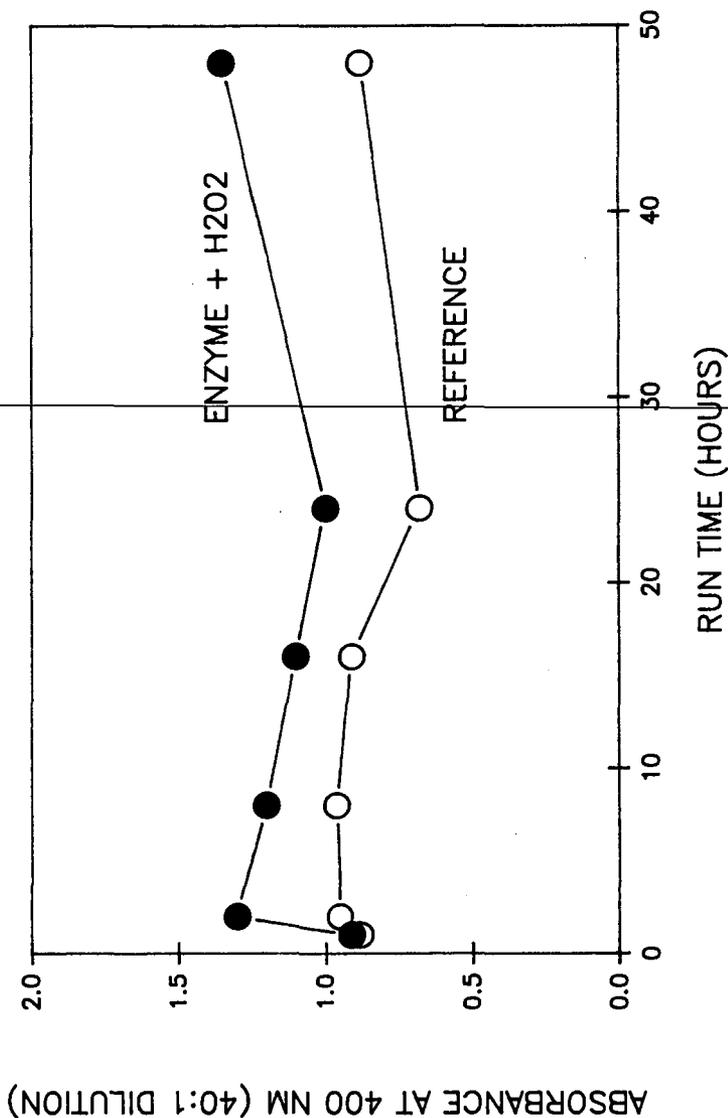


FIGURE 3