

## Use of Biocatalysts for the Solubilization/Liquefaction of Bituminous Coal in a Fluidized-Bed Bioreactor

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### **Abstract**

Biocatalysts allow the solubilization/liquefaction of coal at near-ambient temperatures. This research has focused on the chemical modification of enzymes to enhance their solubility and activity in organic media and on optimal reactor design for a biocatalyst coal-liquefaction process. Modification of hydrogenase and cytochrome *c* using dinitrofluorobenzene has effected increased solubilities up to 20 g/L in organic solvents ranging from dioxane to benzene. Use of these modified enzymes in a small fluidized-bed reactor (with H<sub>2</sub> sparge) resulted in greater than 35% conversion of coal. A new class of continuous columnar reactors will be necessary to achieve the high throughput and low inventory necessary for biocatalyst processes. The controlling mechanisms in fluidized-bed systems using very small coal particulates are being studied. This investigation has included the hydrodynamic modeling of coal segregation in fluidized-bed reactors, with direct microscopic visualization using fluorescence microscopy.

### **Introduction**

Biocatalysts allow the solubilization/liquefaction of coal at near-ambient temperatures. In such applications, it is apparent that enzymes must retain solubility and activity in organic media. This work summarizes our previous efforts on the modification of enzymes using dinitrofluorobenzene (DNFB) to enhance enzyme solubility and activity in organic media.<sup>1</sup> The ability of DNFB-modified hydrogenase and cytochrome *c* to solubilize lignite and bituminous coals in a fluidized-bed bioreactor is demonstrated. Optimal reactor design and scaleup will require detailed knowledge of coal segregation in the fluidized-bed bioreactor. A fluorescence visualization method is introduced to enable direct *in situ* observation of coal particles in operating fluidized bed reactors.<sup>2</sup> This information will be combined with a predictive mathematical model of coal fluidization<sup>3</sup> to enable efficient reactor design and operation.

## Methods

### Techniques for Enzyme Modification

Modification of hydrogenase and cytochrome c with DNFB was achieved by a significant revision of the method originally developed by Sanger.<sup>4-7</sup> Typically, the reaction solution was a mixture of water and ethyl alcohol, with the ethanol fraction varying from 28 to 65% that contained 0.1 to 3 vol% DNFB buffered to a pH of approximately 8.5 with a 7 mM phosphate buffer and/or NaHCO<sub>3</sub>. The reaction was carried out in shake flasks at ambient temperature ( $25 \pm 1^\circ\text{C}$ ) in the presence of air for reaction times of 0.5 to 2 h. After the primary reaction, the pH was reduced to 7 by the addition of HCl, and the reaction mixture was dialyzed overnight against 50 times the volume of the same ethanol-water-buffer mixture (without DNFB) at a pH of 7, using a cellulose membrane. After dialysis, the modified enzymes were converted to the reduced state by the addition of 5 mg/mL sodium dithionite and then lyophilized and stored under N<sub>2</sub> until used.

### Fluidized-Bed Tests

Tests with coal were made with a small, tapered fluidized-bed bioreactor that was fabricated from glass. The reaction chamber was a 15-cm-long column in the form of an inverse cone with a diameter varying from 1.25 cm at the entrance to 2.5 cm at the exit, and the reactor was temperature-controlled at 30°C by circulating fluid in a surrounding jacket. The 0.5- to 1.0-g samples of coal particles (North Dakota lignite and Illinois No. 6 bituminous coal, -270+325 mesh fraction) were fluidized by pumping the reaction fluid containing the biocatalyst through the column at the rate of 2 to 3 mL/min. The system operated with continuous liquid recycle, and there was a H<sub>2</sub> sparge in the upper part of the column. The course of the reaction was followed by periodically measuring the spectrophotometric properties of the liquid phase. There appears to be a broad increase in light absorption in the range of 250 to 450 nm as the coal disappears, although it is usually convenient to choose a single wavelength for the presentation of results. At the end of tests for coal conversion, the solid residue was removed by successive centrifugation and washing. The first wash solution was the same solvent as that used in the tests, and this was followed by acetone and, finally, water. The supernatants were removed by siphoning, and spectral measurements were made. Coal conversion was reported on a moisture and ash-free basis.

## Results

### Enzyme Modification with Dinitrofluorobenzene

Modification of enzymes with DNFB resulted in higher solubilization in organic solvents, which increased as the hydrophobicity of the solvent decreased. The solubility of the unmodified enzyme also increased as the hydrophobicity of the solvent decreased, but it varied from undetectable in benzene and toluene to barely detectable in pyridine and dioxane. Appreciable enzyme deactivation was noted in the solvent of lowest hydrophobicity, dioxane, although significant enzyme solubilization occurred in that solvent.

The degree of dinitrophenylation affects the solubilization of the modified enzyme in organic solvents. For example, with a 0.5-h reaction time, as the concentration of DNFB in the reaction solution was increased from 0 to 0.67 vol %, there was a corresponding increase in the dinitrophenyl (DNP) content and solubilization in benzene that reached a maximum value of 9.2 mg/ml.<sup>7</sup> However, there was also a corresponding decrease in the enzyme activity, presumably due to the addition of DNP in the region of the active sites. At the higher DNFB concentration, the activity was only 40% of the original value.<sup>7</sup> This could be partially alleviated by the adsorption of an appropriate substrate, such as benzyl viologen, to the enzyme mixture prior to the addition of the DNFB. For example, when 0.17% DNFB was used to react with hydrogenase for 30 min, the biocatalytic activity was only 86.5% that of the starting material. But, when 20 mM benzyl viologen was added to the reaction mixture, the resulting activity of the modified hydrogenase was increased to the original value. The preadsorption of the substrate apparently partially protected the active site from interaction with the phenylizing reagent.

### Coal Liquefaction/Solubilization Tests in Fluidized Beds

Two types of tests were made in small fluidized bed reactors: one in which the initial charge was maintained throughout the test, with periodic measurements of spectrophotometric changes in the organic solution, and one in which the solvent with modified enzymes was periodically replenished while the coal residue was maintained in the reactor. The latter type of test was somewhat representative of a continuous system in which some fresh biocatalyst was continuously introduced. Tests were made with pyridine as the solvent in contact with either bituminous or lignite coal.

In fluidized-bed tests with a single charge of the solvent containing DNP-enzymes, an apparent rapid conversion of the coal occurred during the first few hours, followed by a more gradual process throughout the remaining 24 h. This observation could be due to an initial use of the included reducing agent (DNP-cytochrome c) for the hydrogenation reaction, followed by the use of molecular H<sub>2</sub> for the succeeding hydrogenation at a lower rate. Without treatment for removing enzyme precipitation, the liquefaction/solubilization of the lignite was 23.9% and that of bituminous coal was 8.5%. The results were

somewhat higher than comparable shake-flask tests for both types of coal without HCl treatment.<sup>1</sup> Thus, the fluidized bed appears to be a more efficient contacting system.

One test was made with bituminous coal in pyridine in which the solvent containing DNP-mixed enzymes was replaced after 4 and 8 h in order to determine whether appreciable interaction could be induced throughout the course of the 24-h run. This was apparently the case since there was a significant increase in absorbance at 376 nm throughout a greater portion of the 24-h test. Apparent coal conversion without acid leaching of the residue to remove precipitated enzymes was 35.3%. This is the largest amount of biocatalyzed liquefaction/solubilization of a higher-rank coal that has been observed; it is a threefold increase over the fluidized-bed test in which there was a single charge of the solvent mixture.

#### Fluorescence Visualization of Coal Particles in a Fluidized Bed

Effective modeling, design and implementation of fluidized-bed reactors rely upon knowledge of bed expansion and segregation tendencies with particles of varying size and density. We have recently introduced a fluorescence method for the visualization of coal particles within a fluidized bed-reactor.<sup>2</sup> In this method, the dye fluorescein is added to the liquid phase and serves as a contrast agent. A modified fluorescence microscope abuts the column and is used to image the fluorescence emission in response to epi-illumination. The resulting image (dark particles on a bright field of fluorescent liquid) may be digitized and analyzed by using edge-detection algorithms to provide particle-area fraction and size distributions. The microscope may be positioned at any height relative to the column, thus producing particle segregation and expansion statistics as a function of bed height. Unlike previous attempts to visualize segregation in fluidized beds which require direct sampling, use of a modified ultra-thin column, low particle volume fraction, or radiation, the proposed method is non-invasive and may be conducted in an operational reactor at standard flow rates and particle loading. Further, unlike our previous attempts to directly label a bimodal distribution of coal particles,<sup>8</sup> the current method may be applied to any distribution of particle sizes and densities, without possible alteration of particle hydrodynamics.

Preliminary results have been obtained on a small (1-ft) column in which a bimodal distribution (53-63 and 150-250  $\mu\text{m}$ ) of coal was fluidized. Digitized images clearly show that particle segregation is occurring and that both large and small particles are present throughout the column. Figure 1 demonstrates the current abilities in bed visualization. Figure 1a is a view near the top of the column while Figure 1b displays conditions near the bottom. In these figures, the round, darker regions are coal particles fluidized by fluorescein/water. Even from these preliminary data, it is evident that more large particles reside at the bottom of the column. Digital filtering and edge-detection algorithms have been developed to convert such images into particle area fraction and size distribution data as a function of axial position. These data will be of use in the development of predictive mathematical models of bed expansion and segregation<sup>3</sup>, and will also be useful in assessing the accuracy of current models.

## Conclusions

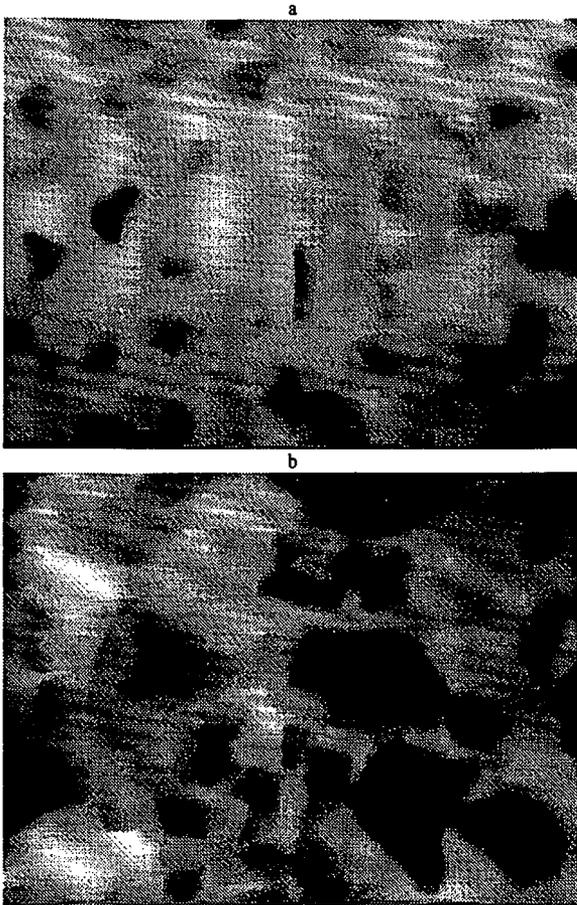
Reducing enzymes can be chemically modified by the addition of dinitrophenyl groups so that they will be soluble in organic solvents while still maintaining biocatalytic activity. A hydrogenase has been shown to enhance the liquefaction/ solubilization of bituminous and lignite coals in both pyridine and benzene at 30°C when a reducing reagent such as reduced cytochrome c and/or molecular H<sub>2</sub> is used. A less polar organic solvent such as benzene may be more effective, and a small fluidized-bed has been shown to be an efficient contactor, especially with sequential addition of the reaction liquids. Coal conversions of up to 35.3% have been observed. A fluorescence method has been introduced to enable the visualization of coal-particle segregation and degradation in a fluidized bed-reactor. This technique will greatly enhance the ability to design and efficiently utilize this reactor scheme.

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**Figure 1:** Visualization of fluidized coal particles using fluorescence microscopy. Coal particles appear dark on the otherwise bright background of fluorescent liquid. (a) Near top of column, the bed consists of only smaller particles (b) near bottom of column, the bed is a mixture of large and small coal particles. Video digitization, filtering and edge detection algorithms will be implemented to provide area fraction and particle size information from such images.