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Use of Modified Enzymes for the Solubilization/Liquefaction of Bituminous Coal in a Fluidized-Bed Reactor†

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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 (DNFB) or methoxypolyethylene glycol *p*-nitrophenyl carbonate (PEG-n) has effected increased solubilities up to 20 g/L in organic solvents ranging from dioxane to toluene. Use of these modified enzymes in a small fluidized-bed reactor (with H_2 sparge) resulted in >40% conversion of bituminous coal in 24 h. Research using model compounds suggests that the conversion process may be in part owing to splitting at methyl or ethyl bridges, and perhaps saturation of ring structures. 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 of particle transport in fluidized-bed systems using very small coal particulates are being studied. This has included the hydrodynamic modeling of coal segregation in fluidized-bed reactors, with

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direct microscopic visualization using fluorescence microscopy. A summary of our previously published work on enzyme modification and fluorescence visualization is presented.

Index Entries: Coal solubilization; enzyme modification; organic biocatalysis; fluidized bed; fluorescence visualization; segregation; reducing enzymes; solubility.

INTRODUCTION

There is increasing interest in utilizing biocatalytic systems (microorganisms or enzymes) for coal processing (*1*). This is primarily because bioprocesses are conducted at modest temperatures and pressures in a relatively benign chemical environment. The design and operation of such a biocatalytic process present obstacles in terms of both the chemistry of the catalytic interaction with coal and reactor design. Owing to the hydrophobic nature of coal, the chemical composition of the liquefaction product stream, and the increased penetration of coal allowed by organic solvents, it is desirable to conduct the biocatalysis in an organic media. It has been demonstrated that various enzymes can be effectively used as catalysts in conjunction with organic solvents for liquid/liquid interactions (*2–5*). However, when contact with a solid substrate in an organic solvent is desired, the most efficient form of the enzyme is a homogeneous catalyst that is soluble in the organic solvent. Most proteinaceous materials have only trace solubility in anhydrous organics, especially nonpolar organic solvents (*6,7*). Since enzymes are naturally hydrophilic molecules, they must be chemically modified to increase their hydrophobicity, and this must be achieved without significantly decreasing their catalytic activity. It has been shown that polyethylene glycol activated with cyanuric chloride (PEGc) can be attached to enzymes, resulting in increased organic solubilization (*7–9*). Recent work at this laboratory has demonstrated the use of the reagent 2,4-dinitrofluorobenzene (DNFB) to attach dinitrophenyl (DNP) groups to enzymes (*4,5*). DNFB and another modified polyethylene glycol, methoxypolyethylene glycol *p*-nitrophenyl carbonate (PEG-n), have been further investigated for enzyme modification with the subsequent study of conversion of coal to liquids enhanced by reducing enzymes with molecular hydrogen. The mechanisms for such a conversion have been studied by use of model compounds.

Liquid fluidized-bed reactors will be utilized in the envisioned biocatalytic process because of their efficient mass transport, ease of gas disengagement, and amenability to continuous operation. The efficient design, operation, and scale-up of such a reactor require detailed knowledge of the reactor axial pressure drop, dispersion of the solid and liquid

phases, and particle size distribution as a function of axial position and flow rate. Although such topics have been well studied for conventional gas fluidized beds, little information has existed regarding liquid fluidized beds of particles with small size and density (low Reynolds number regimes). In conjunction with Asif and Petersen, we have recently proposed a convection/diffusion model for the transient and steady-state behavior of these systems (*10,11*). This model is fully predictive and has been validated through its ability to forecast the transient pressure profiles caused by a step change in liquid flow rate (*12*). A fluorescence visualization method (*13*) has been introduced to validate further the model's ability to predict particle segregation within the bed. Once validated, this model will allow accurate design, operation, and scale-up of a fluidized-bed reactor for the bioconversion of coal.

ENZYME MODIFICATION AND COAL CONVERSION

Enzyme processes are catalyst-enhanced conversions that operate with reasonable specificity and at moderate operating conditions. The biocatalysts of interest for converting coal to liquids are reducing enzymes that can utilize molecular hydrogen or other inexpensive reducing agents. Specifically, hydrogenase enzymes isolated from bacteria that are relatively oxygen-stable (nickel-containing hydrogenases) are being studied for this application (*14*). Such enzymes not only can utilize molecular H_2 as the reducing agent, but can also use other artificial and natural electron acceptors and donors (*14*). Some heme-bearing molecules, such as cytochrome C, are known to mediate the catalytic process probably by enhancing electron transport (*7,14*). Reduced cytochrome c also appears to have some catalytic properties (*6,7*).

At least three biocatalytic approaches to the conversion of coal to liquids by reduction of the coal structure can be envisioned (*6,7*):

$$\text{Coal} + H_2\text{-hydrogenase} \rightarrow \text{reduced coal} + H_2O \quad (1)$$

$$\text{Coal} + H_2\text{-cytochrome c(?)} \rightarrow \text{reduced coal} + H_2O \quad (2)$$

$$\text{Coal} + \text{cytochrome c(H)-hydrogenase} \rightarrow \text{reduced coal} + \text{cytochrome c} + H_2 \quad (3)$$

Equation (1) represents the oxidation of H_2 while the coal structure is being reduced, Eq. (2) represents a similar reduction that may result from utilization of cytochrome c as the biocatalyst, and Eq. (3) indicates that other reducing agents (such as reduced cytochrome c) may be utilized for this reaction.

MATERIALS AND METHODS

DNFB, cytochrome c, and PEG-n were obtained from Sigma Chemical Co., St. Louis, MO, whereas organic solvents and other chemical reagents were analytical-grade and obtained from Fisher Scientific Co. (Pittsburgh, PA), EM Science Co. (Gibbstown, NJ), or Baker Chemical Company (Phillipsburg, NJ). The model compounds, 1,2-bis(4-pyridyl)ethane (BPE) and 1,2-bis(4-quinolyl)ethane (BQE) were provided by M. Farcasiu of the Pittsburgh Energy Technology Center and 1-[4-(2-phenylethyl)benzyl]naphthalene (PBN) was obtained from TCI America. Illinois #6 coal (PSOC 1493) was obtained from the Coal and Organic Petrology Laboratory at Penn State University. The coal was wet-sieved into the desired size ranges by Standard Laboratory Inc. Whitesburg, KY. The −275 + 325 mesh fraction was utilized in coal conversion studies. The hydrogenase isolated from *Proteus vulgaris* at Oak Ridge National Laboratory was recovered using an extraction and purification method previously described (*7,15*). The other hydrogenase used in this study was isolated from the hyperthermophilic Archaebacterium, *Pyrococcus furiosus* by M. W. W. Adams of the University of Georgia (*16*). The enzyme biocatalytic activities were determined by a spectrophotometric assay developed by Ballantine and Boxer (*17*).

Enzyme modification was carried out with either DNFB or PEG-n. Modification of the enzymes with DNFB was by a significant change in the technique originally developed by Sanger (*18*) and reported in detail elsewhere (*4*). This technique results in the introduction of dinitrobenzene (DNB) groups on free amino groups in the enzyme structure. Chemical modification with PEG-n was carried out by introducing the enzyme at a concentration of 5–10 mg/mL into an agitated, aqueous mixture buffered at a pH of 8.8 with a 0.1*M* phosphate buffer that contained 30–100 mg/mL of the activated PEG. The reaction proceeded for 2 h, after which the pH was adjusted to 7.8. After addition of 5 mg/mL sodium dithionite to maintain reducing conditions, the solution was lyophilized and the resulting solid material was stored at 0°C. The PEG-modified enzyme was contacted with the organic solvent of interest for 1 h at ambient conditions, after which the solid residue was removed by centrifugation.

Tests were made in shake flasks of 25–50 mL in a temperature-controlled water bath. Typically, 10 mL of liquid were used with 2–4 mg/mL of chemically modified, mixed enzymes (hydrogenase and cytochrome c). When model compounds were evaluated, they were present at a nominal 1 mg/mL. Tests for coal conversion were made with 50–100 mg of the coal sample. A hydrogen atmosphere was maintained by either continuous flow of the gas through the gas space of the flask after prior saturation of the gas with the liquid being used or by use of a sealed flask with periodic replenishment of the contained gas.

Some tests for coal conversion were made in a small, tapered fluidized-bed bioreactor (Fig. 1). This bioreactor consisted of a truncated cone that was 30 cm long with a 1.25-cm diameter inlet and a 2.5-cm diameter

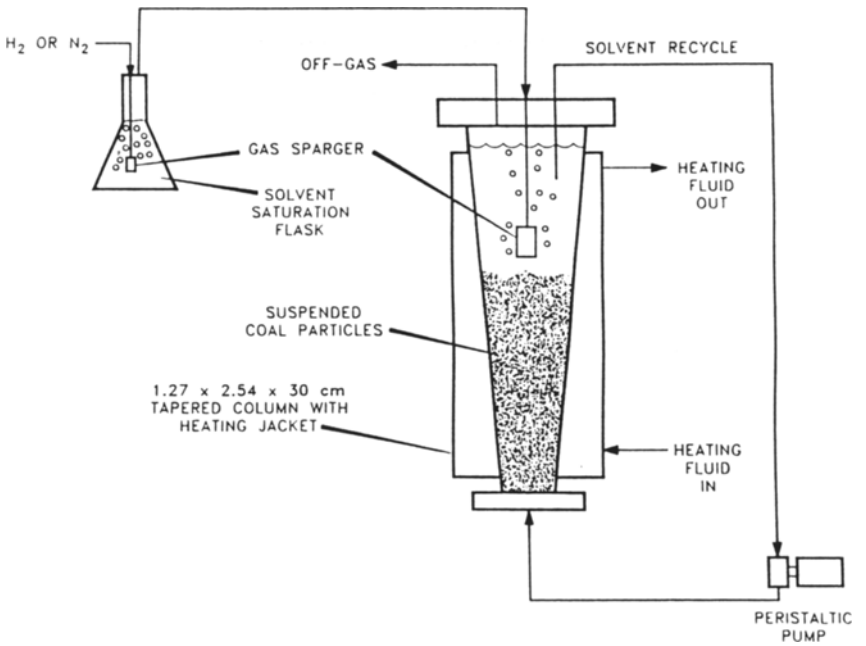

Fig. 1. A small fluidized-bed bioreactor used for determining coal conversion in organic media with modified enzymes. Figure adapted from (*19*).

outlet. Hydrogen was sparged through the liquid in the upper part of the reactor, and a circulating water bath provided temperature control.

The course of the coal conversion reaction was tracked by periodically measuring the spectrophotometric properties of the liquid phase and/or evaluation of the molecular constituents by gas chromatography (GC) utilizing a technique suggested by M. Farcasiu of the Pittsburgh Energy Technology Center. The chromatographic analysis was conducted using a Hewlett-Packard Model 5890 GC with a J&W 30 m × 0.248 mm fused silica capillary column coated with a 0.25 mm silicone SE-52, utilizing a head pressure of 20 psi helium and flame ionization for detection. The column temperature was ramped from 80 to 310°C. Total coal conversion was determined by using a measured amount of vacuum-dried (100°C) coal and then processing the solid residue by successive centrifugation, washing, and vacuum-drying. The first wash solution was the same solvent as that used in the tests, and this was followed by acetone and then water. Coal conversion was reported on a moisture- and ash-free basis.

RESULTS AND DISCUSSION

Chemical modification of the enzymes increases the solubility in organic media, although severe modification may also result in loss of activity (*4*). The modified enzymes in organic solvents enhance the con-

Table 1
Solubility of Modified Enzymes in Organic Solvents

Solvent	Hydrophobicity, Log P[b]	Average solubility, mg/mL[a]	
		DNP-hydrogenase	DNP-cytochrome c
Dioxane	−1.1	16	~20
Pyridine	0.7	9	~20
Synthetic solvent[c]	—	>2	>3
Benzene	2.0	2	3
Toluene	2.5	1	1

Adapted from (*19*).

[a]Approximately 0.2 g of lyophilized DNP-enzyme was contacted with 10 mL of dry solvent in a shake flask under nitrogen at 30°C for 1 h. The solid residue was removed by centrifugation, and a portion of the supernatant was dried to determine the amount of solubilization.

[b]Log P is defined as the logarithm of the partition coefficient in a standard octanol-water, two-phase system.

[c]A mixture of 45% tetralin, 40% 2-methylnaphthalene, and 15% *p*-cresol.

version of coal to liquids apparently by breaking aliphatic crosslinking and other reduction reactions.

Enzymes modified with DNFB or PEG-n had enhanced solubility in organic solvents ranging from levels as high as 20 mg/mL in relatively polar solvents, such as dioxane and pyridine, to <3 mg/mL in relatively nonpolar solvents, such as benzene and toluene (Table 1). Unmodified enzymes exhibited solubilities that were well below the detection limits of our assays. Unfortunately, there was a deleterious effect on enzyme activity as the level of DNFB reaction was increased, resulting in only 40% of the original at the highest level tested (*4*). Although the interaction of PEG-n was slightly less effective in enhancing enzyme solubility in organic solvents, it had only minimal impact on the enzyme activity. Enzymes modified with PEG-n retained as much as 90% of their original activity. The organic solvent also affected the enzyme activity, with irreversible loss especially in the more polar solvents. For example, even though the total quantity of modified enzymes in solution is greater in pyridine than in benzene, the remaining enzyme activity in benzene was 16-fold greater than that in pyridine (*19*).

An important aspect of the conversion of coal to liquids is the cleavage of covalent bonds that make up the three-dimensional structure. Thermal disruption of these bonds requires relatively high temperatures, but it is anticipated that biological catalysts will allow such interactions to occur at modest operating conditions. Model compounds were chosen with methylene and/or ethylene bridges between aromatic moieties to help evaluate the mechanisms of enzyme-enhanced coal conversion.

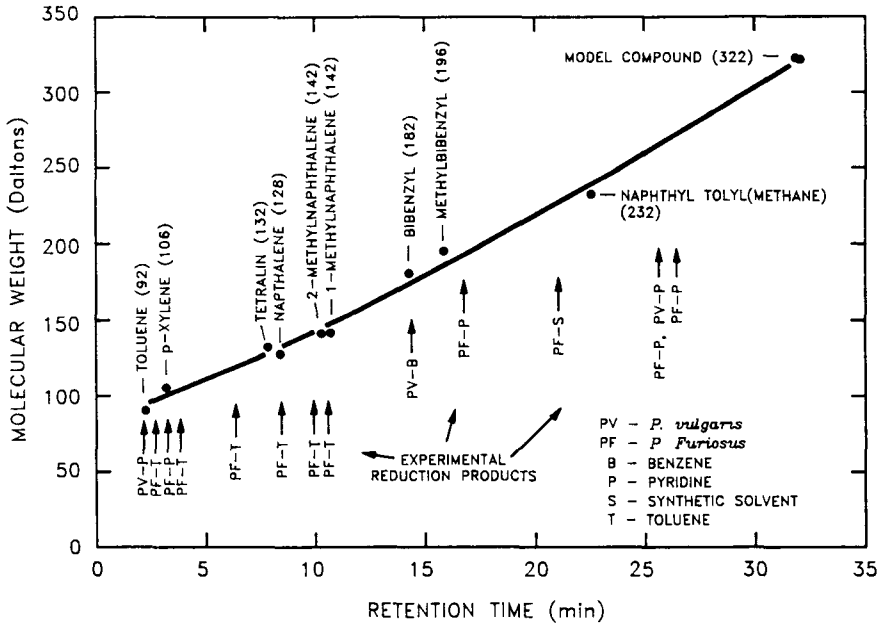

Fig. 2. GC retention times of the model compound, 1-[4-(2-phenylethyl) benzyl]naphthalene, and some possible reduction products with indicated molecular weights as compared to the products of enzyme-enhanced reduction. The tests were made in shake flasks at 30°C under a hydrogen atmosphere for a 24-h period with 2–4 mg/mL of PEG-n-modified mixed enzymes (hydrogenase from *P. vulgaris* or *P. furiosus* with approximately equal amounts of cytochrome c). None of the product peaks were detected when biocatalyst was not present. Figure adapted from (*19*).

The degradation of BPE and BQE was studied in benzene at 30°C with 2–4 mg/mL of a mixture of DNB-hydrogenase and DNB-cytochrome c under a hydrogen atmosphere in shake flasks. It was found that up to 52% of these model compounds could be degraded over a 12-h period. This could indicate that the ethylene bridging was being disrupted. More complete tests were made with PBN, a more complicated model compound with both methylene and ethylene bridging. Extensive GC analysis for the model compound and possible reduction products was performed during the course of the enzyme-enhanced interactions (Fig. 2). It has been previously shown that thermal degradation of this compound requires a temperature >400°C, whereas carbon black can reduce the required temperature to 320°C (*20*). However, chemically modified biocatalysts have a measurable effect at temperatures as low as 30°C. As indicated by the GC analysis of the reaction solutions, the experimental reduction products apparently included toluene, *p*-xylene, naphthalene, methylnaphthalene, and bibenzyl, all of which would be expected if there

was cleavage at the methylene and ethylene bridges. There were also unidentified reduction products that may represent some interaction with the ring structures. None of these peaks were detected when biocatalyst was not present in the solution. The two different hydrogenases produced different products, and there was also an apparent solvent effect with toluene producing the most products.

The thermophilic hydrogenase was apparently the most effective for interactions with PBN. As expected, that enzyme could effectively operate at a higher temperature with the maximim conversion occurring at 50°C (22%), but a noticeable decrease at 75°C (5%). It had previously been shown that the optimum temperature for the hydrogenase from *P. vulgaris* was 30°C (*6*).

Mixed modified enyzmes have been shown to enhance the conversion of coal to liquids in organic solvents under a hydrogen atmosphere (*4–7*). We now know that the characteristics of the enzymes and the solvents affect the conversion. As indicated by the increased light absorbance of the reacting fluid, the enzyme-enhanced interaction occurs throughout a 24-h period, but with the most rapid conversion reaction during the first 8 h (Fig. 3).

The most significant conversion of coal has been achieved in a fluidized-bed bioreactor utilizing pyridine at 30°C under a hydrogen atmosphere in which there were three additions of mixed enzymes (~4 mg/mL each of hydrogenase from *P. vulgaris* and cytochrome c modified with DNFB) at 0, 4, and 8 h (*4*). In this case, the conversion of the coal was >42% for the enzyme-enhanced test compared to <20% for the reference test.

HYDRODYNAMIC MODELING AND FLUORESCENCE VISUALIZATION

Liquid fluidized bed reactors will be utilized using either modified enzymes or whole microorganisms to achieve continuous coal conversion at a larger scale. The efficient design, operation, and scale-up of such a reactor requires detailed knowledge of the transient and steady-state expansion, and segregation of coal particles within the bed. Collaborating with Asif and Petersen at Washington State University (*10–12*), we have proposed a transient convection/diffusion model that is a modification of that proposed by Kennedy and Bretton (*21*). The governing equation is:

$$\frac{\partial C_i}{\partial t} = \frac{\partial}{\partial Z}\left[D_i\left(\frac{\partial C_i}{\partial Z} - \frac{C_i}{\rho}\frac{\partial \rho}{\partial Z}\right) - U_i C_i\right] \quad (4)$$

where the coal charge is divided into classes (denoted by subscript "i") based on size or density, and D_i is the class's dispersion coefficient, U_i its segregation velocity, and C_i its fractional volumetric concentration. Time is represented by "t", ρ is the liquid density, and "z" is the axial position

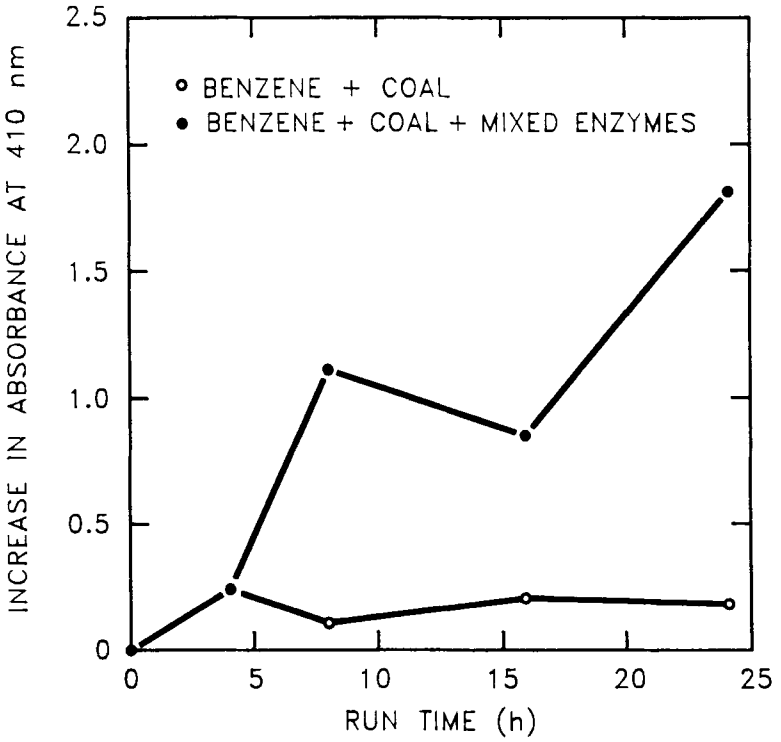

Fig. 3. Conversion of Illinois No. 6 bituminous coal to liquid enhanced with PEG-n-modified hydrogenase from *P. furiosus* (~2 mg/mL) and cytochrome c (~4 mg/mL) in a sealed shake container with an atmosphere of molecular hydrogen at 30°C. Greater than 15% of the coal was converted with the enzyme mixture compared to approx 7% conversion in the reference test. Figure adapted from (*19*).

within the reactor. Equation (4) is written for each subclass "i," resulting in a system of coupled, partial differential equations. The boundary conditions are that there is no flux at the base of the column where there exists a glass frit liquid distributor, and that there is no flux at the top of the column. The boundary condition at the top of the column may be modified to account for particle elutriation if desired. Key to solving this system of equations is the prediction of the dispersion coefficients and segregation velocities as functions of particle size, density and void fraction, and liquid superficial velocity, density, and viscosity. We have achieved this using the Richardson-Zaki relationship for the segregation velocity, and our own correlation for the dispersion coefficient (*11,12*). With these correlations, we have a fully predictive model (with no adjustable parameters) that results in a system of equations that must be solved numerically for a given set of operating conditions.

We have conducted a series of experiments measuring bed heights, steady-state axial pressure profiles, and transient pressure profiles in

order to verify the prediction of our model. The proposed model of fluidized-bed hydrodynamics may be more vigorously tested (at a microscopic level) through its ability to predict particle segregation. Previously developed experimental techniques to probe bed composition are either invasive or may not be used at high solids concentrations. We have introduced and validated a fluorescence visualization technique that allows bed segregation data to be obtained directly and noninvasively in an operational reactor (*13*). The proposed method, by causing the continuous phase to fluoresce, and observing the system using epi-illumination fluoroscence microscopy, increases the contrast between the dispersed and continuous phases. This enables direct visualization of discrete particulate entities at any volume fraction including close packed. Further, the technique is noninvasive and does not require specialized reactor systems. By not altering the dispersed phase through direct labeling, and without appreciably altering the chemistry of the continuous phase, this technique may be used *in situ* in actual operating reactors.

Illinois #6 coal (PSOC 1493) was obtained as described previously. The fluidizing media consisted of a 0.5% aqueous solution of the surfactant Tween 80 and 1 oz/5 gal Lab Algicide (PolyScience, Niles, IL) added to prevent bacterial and fungal growth on the coal and surfactant. Fluorescein was added at a concentration of 0.33 g/L. The reactor column consisted of 4, 1-ft sections of 1-in. id, 1.5 in OD Kimax glass pipe. Below the actual bed was a tapered subsection that served as a calming region and ended in a coarse glass frit liquid distributor. Pressure taps (2 mm id, 6 mm OD) were provided at 4-in. intervals along the 1-ft glass sections. The pressure taps were fitted with glass frits to prevent the entrance of coal particles. Pressure drop measurements were conducted using six high-sensitivity, variable inductance differential pressure transducers. Signals from the transducers were continuously monitored and stored by an A/D board housed in an NCR personal computer. Pressure readings were recorded at 5- or 10-s intervals.

The optical train used in this study is depicted in Fig. 4, and is described in detail in ref. (*13*). The illumination source consisted of a 100-W Hg bulb, and accessed the wall of the fluidized bed through the epi-illumination port of a Zeiss universal microscope head. The proper excitation wavelength for the fluorescein dye was selected using a fluorescein filter set. Subsequent to the fluorescent filters, the light was focused on the specimen using a 6.3× microscope objective. The focused excitation light (at ~480 nm) impinged the sample and excited the fluorescence (emitted at 520 nm) in the continuous phase. The dispersed coal particles lacked detectable fluorescence at this wavelength. Light emitted from the specimen was collected through the same objective used to focus the excitation light. The emitted light passed through a dichroic mirror housed in the microscope's epi-illumination head, and light at wavelengths below 500 nm was removed using a long-pass filter. The fluorescent signal was imaged using a video camera, and images were recorded for

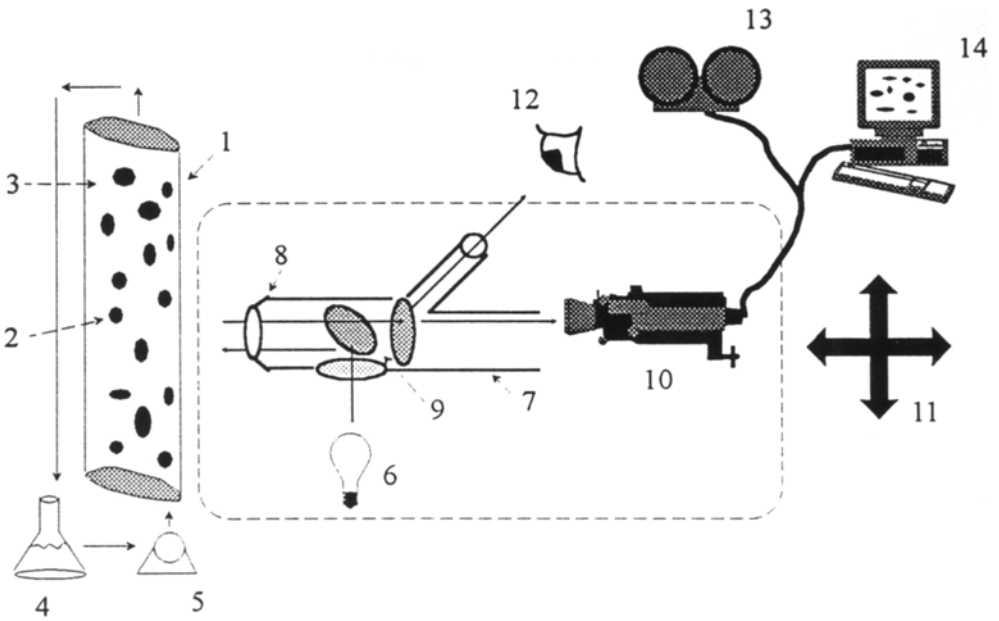

Fig. 4. Schematic of optical train used for the visualization of coal particles in a liquid fluidized bed: (1) fluidized-bed column; (2) dispersed phase; (3) continuous phase; (4) fluid reservoir; (5) pump; (6) light source; (7) microscope; (8) objective; (9) fluorescence filter set (band-pass filter, dichroic mirror, and long-pass filter); (10) video camera; (11) mobility of optical platform for focus and height adjustment; (12) observer; (13) VCR; (14) frame gabber/analysis computer. Figure adapted from (*13*).

later analysis using a VCR. The entire optical train was mounted on a custom-built optical platform, which enabled travel along the height of the bed, to record particle distributions at various axial positions, and movement perpendicular to the column to enable adequate focusing of the system. Images were analyzed using a video frame grabber (DT2867, Data Translation, Marlboro, MA) and commercially available video analysis software (Global Lab v2.0, Data Translation). Prior to particle sizing, the system was calibrated to determine the relationship between camera/video buffer pixel number and actual size. To obtain particle-size histograms, a video image was acquired from the VCR to the frame grabber, and particles were sized using a gray-level threshold method that detected particle edges. Additional video frames were acquired, and the protocol was repeated until >300 particles had been analyzed at a given bed height.

Two experimental runs are described here. The first utilized a monocomponent bed, and served to validate the fluorescence method and to provide transient pressure data for model verification. The second consisted of a bimodal size distribution to demonstrate the fluorescence visualization method's ability to detect segregation. In the unimodal fluid-

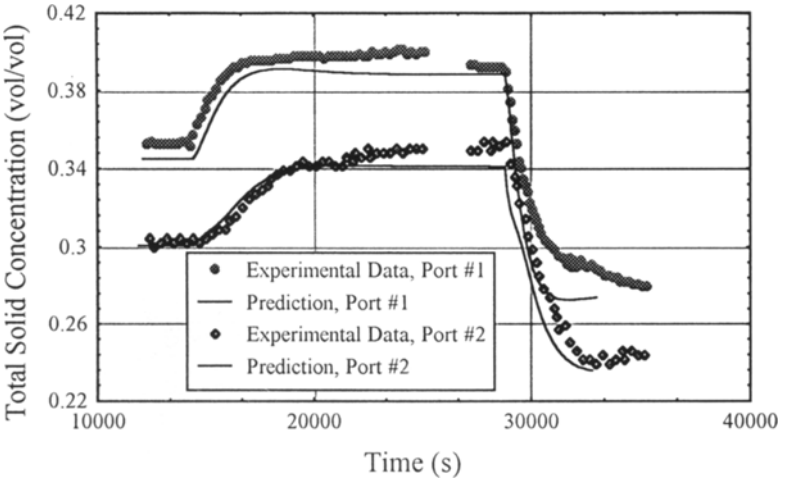

Fig. 5. Comparison of model predictions with the experimental transients of total solid concentration. The liquid velocity was first lowered from 0.0057 to 0.0037 cm/s at 14,500 s and then raised to 0.0109 cm/s at 28,900 s. Figure adapted from (*12*).

ization study, 100 g of oven-dried coal of a 45–63 μm diameter size fraction were added to the reactor column in a slurry of fluidizing media. These particles were allowed to pack under their own weight for 24 h. The pressure lines were zeroed on the bed in its settled state. The bed was fluidized by increasing the liquid flow rate through a peristaltic pump. The overall pressure drop as a function of liqud superficial velocity was recorded to ensure that the bed was fully fluidized. The cumulative pressure drop at a volumetric flow rate of 0.048 cm^3/s was verified to be linear (*13*), and visualization of the bed commenced. For the pressure transients presented here, the coal fluidized bed was allowed to reach a steady state at a liquid superficial velocity of 0.0057 cm/s. Then, a step change in the liquid velocity was introduced by lowering the flow rate to 0.0037 cm/s. Once the bed reached a steady state, the liquid velocity was again changed by raising it to 0.0109 cm/s. In this way, the pressure transients were recorded for two different cases, i.e., for a decrease in the flow rate and for an increase in the flow rate. Differential pressure drops were converted to particle concentrations as described previously (*12*). In the bimodal-particle fluidization study, 50.4 g oven-dry weight of a 45–63 μm diameter size fraction and 129.2 g dry wt of a 106–150 μm size fraction of coal were added to the reactor. Visualization of the column was performed at a superficial velocity of 0.015 cm/s at a variety of axial locations.

Figure 5 displays the experimental data of particle concentration obtained at two locations above the liquid distributor and compares them to the predictions of our mathematical model with no adjustable parameters. That is, Fig. 5 does not represent a fit of the data, but a prediction. The overall agreement between the model predictions and the experimental

data is excellent, and demonstrates that the model may be used to predict the macroscopic steady-state and transient behavior of the reactor.

Fluorescence visualization performed on the unimodal coal distribution served to validate the measurement technique (*13*). The method was able to reproduce the known distribution of particles at a series of axial positions along the bed, and its results corresponded to those obtained from grab sampling and light-scattering sizing analysis (*13*). The technique's capabilities were further demonstrated by its ability to detect segregation in a bimodal liquid fluidized bed of bituminous coal. Fluorescence visualization was performed at four axial positions: 8.4, 21.5, 52.1, and 72.5 cm above the liquid distributor. Figures 6 (A and B) display representative images obtained at heights of 8.4 and 72.5 cm, respectively. These images are both displayed at the same scale. Qualitative segregation may be detected by visual inspection of these images, which indicates that although small particles exist at both the upper and lower sections of the bed, larger particles are absent from the uppermost sections. The size histograms as a function of axial position obtained through fluorescence imaging are shown in Fig. 7. Qualitatively, it appears that the size distributions obtained at 8.4, 21.5, and 52.1 cm are identical, with both large and small particles present at these locations. At a height of 72.5 cm, however, large particles are absent. Future research will compare model predictions of particle segregation to that observed experimentally using fluorescence visualization.

CONCLUSIONS

Techniques for chemically modifying enzymes to increase their solubility and activity in organic media have been introduced for the purposes of biologically mediated coal conversion. Conversion of both model compounds and bituminous coal have been demonstrated at the bench-top scale. A mathematical model of fluidized-bed expansion and segregation has been introduced to allow the design and operation of a larger-scale process. This model has been validated at the macroscopic level using transient pressure profiles and will be tested at the microscopic level using fluorescence visualization of particle segregation.

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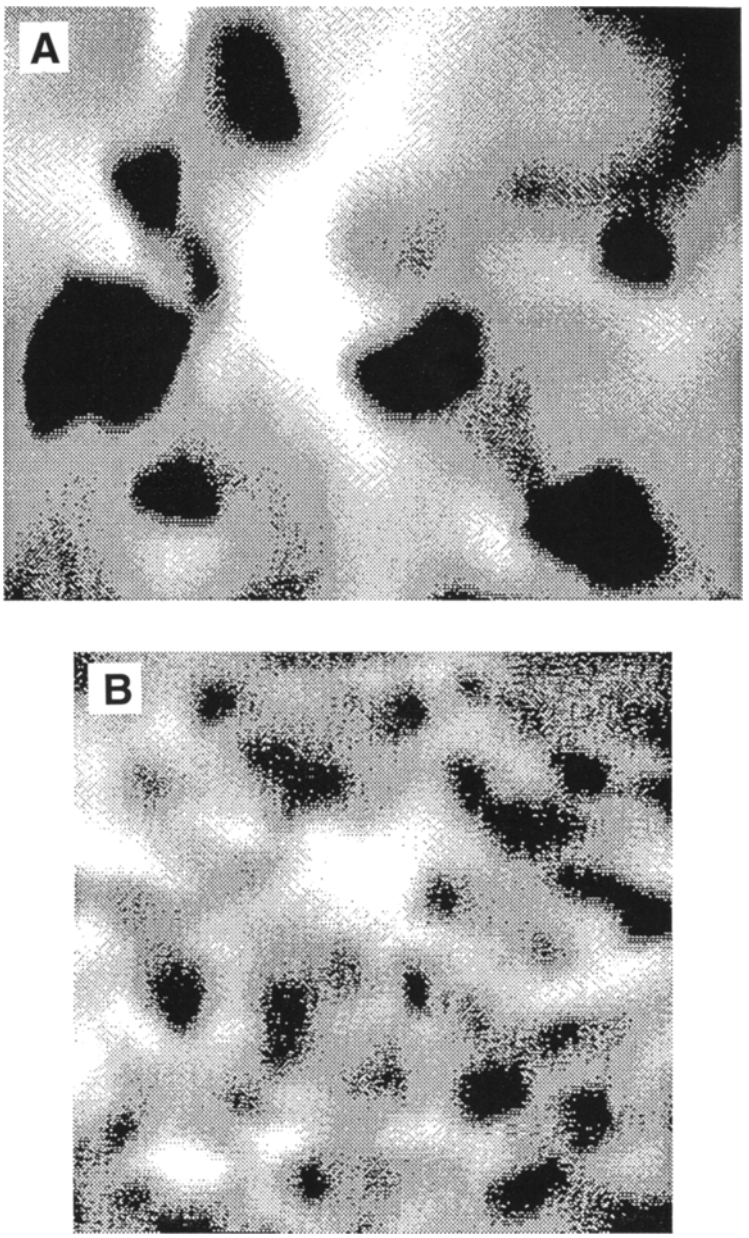

Fig. 6. *In situ* images of coal particles in the fluidized bed at heights of 8.4 (**A**) and 72.5 (**B**) cm above the liquid distributor. Using such images, coal particles may be sized using edge-detection algorithms. Both images are shown at an identical scale.

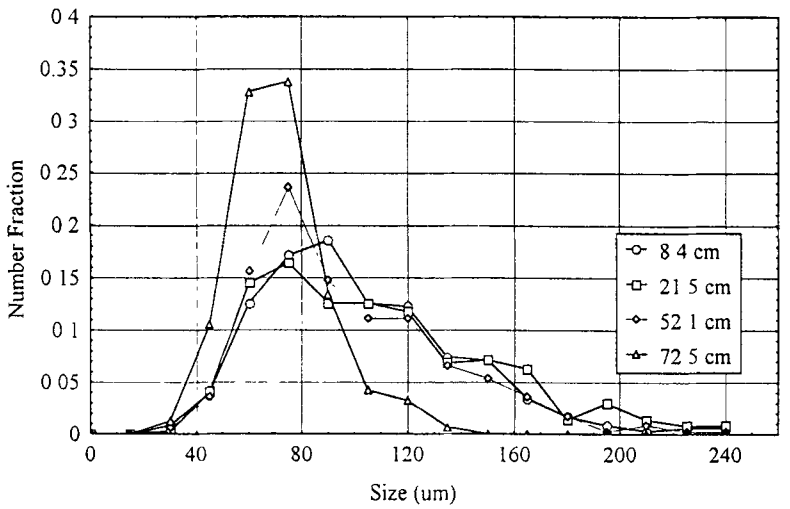

Fig. 7. Size histograms obtained using fluorescence microscopy in bimodal-particle fluidization experiment. Fluorescence visualization was performed at 8.4, 21.5, 52.1, and 72.5 cm above the distributor. Histograms obtained from the lower regions of the bed (< 60 cm) are statistically identical, indicating no detectable segregation in this section. The histogram obtained at 72.5 cm indicates few large particles existing at this height.

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