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BIODESULFURIZATION OF WATER-SOLUBLE COAL-DERIVED MATERIAL BY *Rhodococcus rhodochrous* IGTS8

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BIODESULFURIZATION OF WATER-SOLUBLE COAL-DERIVED MATERIAL BY *Rhodococcus rhodochrous* IGTS8

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ABSTRACT

Rhodococcus rhodochrous IGTS8 was previously isolated because of its ability to use coal as its sole source of sulfur for growth. Subsequent growth studies have revealed that IGTS8 is capable of using a variety of organosulfur compounds as sources of sulfur but not carbon. In this paper, the ability of IGTS8 to selectively remove organic sulfur from water-soluble coal-derived material is investigated. The microbial removal of organic sulfur from coal requires microorganisms capable of cleaving carbon-sulfur bonds and the accessibility of these bonds to microorganisms. The use of water-soluble coal-derived material effectively overcomes the problem of accessibility and allows the ability of microorganisms to cleave carbon-sulfur bonds present in coal-derived material to be assessed directly. Three coals, two coal solubilization procedures, and two methods of biodesulfurization were examined. The results of these experiments reveal that the microbial removal of significant amounts of organic sulfur from water-soluble coal-derived material with treatment times as brief as 24 hours is possible. Moreover, the carbon content and calorific value of biotreated products are largely unaffected. Biotreatment does, however, result in increases in the hydrogen and nitrogen content and a decreased oxygen content of the coal-derived material. The aqueous supernatant obtained from biodesulfurization experiments does not contain sulfate, sulfite, or other forms of soluble sulfur at increased concentrations in comparison with control samples. Sulfur removed from water-soluble coal-derived material appears to be incorporated into biomass.

BIODESULFURIZATION OF WATER-SOLUBLE COAL-DERIVED MATERIAL BY *Rhodococcus rhodochrous* IGTS8

INTRODUCTION

Microorganisms capable of selectively oxidizing inorganic/pyritic sulfur in coal are well known,[1] whereas the selective removal of organic sulfur from coal has received comparatively little study and is less well documented. (See Kilbane[2] for a recent review.) Most research concerning the microbial removal of organic sulfur from coal has focused on microorganisms capable of metabolizing dibenzothiophene (DBT), a compound that is widely regarded as representative of a portion of the organic sulfur compounds present in coal. *Acinetobacter*,[3] *Arthrobacter*,[4] *Brevibacterium*,[5] *Pseudomonas*,[4] and *Rhizobium*[3] cultures have been reported to be capable of using dibenzothiophene as carbon, energy, and sulfur sources for growth. An even larger number of microorganisms, chiefly *Pseudomonas* cultures, have been reported capable of co-metabolizing dibenzothiophene.[6-13] The vast majority of these microorganisms metabolize dibenzothiophene via the carbon-destructive pathway originally established by Kodama.[9] However, the desired reaction for the biodesulfurization of coal is the removal of sulfur with the retention of carbon -- and hence the calorific/fuel value. Microorganisms that metabolize dibenzothiophene via a carbon-destructive pathway would be predicted to be incapable of selectively removing sulfur from coal. A relatively large collection of such microorganisms, predominantly *Pseudomonas* cultures, was recently tested for the ability to desulfurize water-soluble coal-derived products, and it was found that extensive degradation of carbon was concurrent with the loss of sulfur.[12]

A microbial culture -- *Rhodococcus rhodochrous* IGTS8, isolated because of its ability to use coal as a source of sulfur for growth -- was subsequently shown to use a variety of organosulfur compounds, including dibenzothiophene, as sulfur sources but not as carbon sources.[2,14,15] IGTS8 has been shown to convert dibenzothiophene to 2-hydroxybiphenyl: sulfur is removed yet the carbon remains intact.[2,14,15] The data reported in this paper assess the ability of IGTS8 to selectively remove sulfur from water-soluble coal-derived material. Water-soluble coal-derived material was used because it is essentially free of inorganic sulfur and therefore contains only organic sulfur, it effectively overcomes accessibility limitations of experiments involving solid coal and experiments with water-soluble coal-derived material may provide data of greater relevance to the biodesulfurization of coal than experiments which employ model compounds.

MATERIALS AND METHODS

Growth Media and Culture Preparation

Rhodococcus rhodochrous IGTS8 has been described previously[2,14,15] and was used throughout this study. A defined basal salts medium (BSM) consisting of 2.44 g KH_2PO_4, 5.57 g Na_2HPO_4, 2 g NH_4Cl, 0.2 g $MgCl_2 + 6H_2O$, 0.001 g $CaCl_2 + 2H_2O$, 0.001 g $FeCl_3 + 6H_2O$, and 20-mM glycerol in 1 liter of sterile distilled, deionized water was used. The pH of BSM was 7.0. In some experiments the organosulfur compound trithiane (Fairfield Chemical Co.) supplied as crystals at a concentration of 20 mM was used as a sulfur source to supplement BSM for the growth of IGTS8. Nutrient agar (Difco Laboratories) was used in the determinations of colony-forming units (CFUs). Incubations were performed at room temperature.

Biodesulfurization experiments employing growing-cell cultures were performed by placing 2 grams of water-soluble coal-derived material in 20 liters of BSM, inoculating with IGTS8 at an initial cell density of 10^5 cells/ml, and incubating at room temperature for 3 weeks to allow bacterial growth to occur at the expense of the sulfur in coal. Microbial growth was assessed daily using one of two methods: examination of cell pellets and determination of CFUs. Cell pellets were obtained by centrifuging 1.5 ml of culture broth in a tared Eppendorf microfuge tube (Brinkman Instruments) at 14,000 rpm for 1 minute. The size of the cell pellet was assessed volumetrically and by weight. After observations on 3 consecutive days indicated that microbial growth had ceased, the experiments were terminated. Incubation times ranged from 3 to 6 weeks.

In experiments employing pre-grown cells, IGTS8 was grown to a cell density of 1×10^9 cells/ml in 10 liters of BSM containing trithiane. The culture was centrifuged at 10,000 X g for 10 minutes. The bacterial pellet was resuspended in 100 mls of BSM and centrifuged at 100 X g for 1 minute to pellet residual trithiane crystals. Washed cells were recovered by aspiration and resuspended in a final volume of 100 milliliters of BSM containing 2 grams of water-soluble coal-derived material. Control samples for both growing and pre-grown cell experiments were incubated in sterile BSM (without glycerol) and processed in a fashion identical to samples that contained bacteria.

Preparation of Water-Soluble Coal-Derived Material

This study used three coals from which water-soluble material was derived: two coals from the Illinois Basin Coal Sample Program, IBC-101 and IBC-107; and an Illinois No. 6 coal from St. Clair County, Illinois. All three coals were ground to 200 mesh topsize. Water-soluble material derived from each of these coals is designated as IBC-101(S), IBC-107(S), and Illinois No. 6(S), respectively. Water-soluble coal-derived material was prepared either by a mild alkali treatment according to the method of Stoner et al.[12] or by a modification of that method. In the modified method, coal was treated with four volumes (wt/vol) of 7-M HNO_3 for 48 hours at 90°C followed by addition of 20 volumes of 6-M NaOH and autoclaving for 15 minutes. The supernatant was decanted and saved while the coal residue was resuspended in 20 volumes of 3-M NaOH and autoclaved. This step was repeated. The combined supernatants were then processed by centrifugation and filtration using filters containing 0.45-micron-diameter pores. The soluble coal is recovered from the filtrate by adjusting the pH to 1.5 with HCl and centrifuging at 10,000 X g for 10 minutes. The pelleted material was washed with distilled water and dried at 105°C in a ceramic crucible. Salt crystals that formed on the sides of the crucible above the solid coal material were removed. To further remove salts, the solid coal matter was slurried in 100 mls of 1-N HCl and centrifuged. The supernatant was discarded, whereas the coal pellet was dried at 105°C and salt crystals were removed. This procedure was repeated six times, at which point salt crystals were no longer observed upon drying.

The recovery of water-soluble coal-derived material from biodesulfurization experiments entailed adjusting the pH to 8.5 with NaOH to maximize the solubility of water-soluble coal-derived material. The solution was then centrifuged at 10,000 X g for 10 minutes to pellet bacterial cells that were dried and analyzed for total sulfur content. The supernatant was then adjusted to pH 1.5 using concentrated HCl and centrifuged at 10,000 X g for 10 minutes. The pelleted material was processed to remove salts as described above, dried, and submitted for analysis. Supernatants were analyzed for the presence of soluble forms of sulfur.

Coal and Coal Product Analyses

Elemental analyses for all raw, soluble, and biotreated coal samples were performed by Huffman Laboratories of Golden, Colorado. Samples were analyzed for carbon and hydrogen with a custom-built analyzer that uses combustion and coulometric detection. Oxygen was ascertained on a modified oxygen analyzer (Model 5060; Coulometrics, Inc.). Nitrogen was determined on a nitrogen analyzer (Model NA 1500; Carbo Erba Instruments/Fison). Total sulfur was determined by using a sulfur analyzer (Model SC 132; LECO Corp.) and following American Society for Testing Materials (ASTM) procedure D-4239. Sulfur-forms analyses were performed following ASTM procedure D-2492. Analyses for moisture, ash, and calorific value (Btu/lb) were performed according to ASTM procedures D-3173, D-3174, and D-2015, respectively. All coal data are reported on a moisture-free basis.

Dried biomass samples were analyzed for total sulfur content using a LECO SC132 analyzer. Aqueous supernatants were analyzed for sulfate and sulfite using a Dionex 2010i (Dionex Corp.) ion chromatograph, and for total soluble sulfur using a Thermo Jarrell Ash Atom Scan 25 (Thermo Jarrell Ash Corp.) inductively coupled plasma emission spectrometer.

Determination of Biomass Concentration in IBC-101(S) Using a Modified Protein Assay

A modified version of a standard protein assay (reagents provided by the Pierce Chemical Co.) was employed for spectrophotometric determination of residual biomass concentration in recovered biotreated IBC-101(S) samples. This reagent system combines the reaction of protein with Cu^{2+} in an alkaline medium (yielding Cu^{1+}) with a highly sensitive and selective detection reagent for Cu^{1+}, namely bicinchoninic acid.

Known mixtures of 0-100% biomass and untreated IBC-101(S) were prepared to construct a standard curve relating protein concentration and % biomass (Figure 1). Samples of biotreated IBC-101(S) and sterile controls, were tested simultaneously to determine the amount of associated biomass as compared to the standard curve. Blank-corrected IBC-101(S) sterile controls and untreated samples show minimal interference with assay reagents using the modified protein assay method.

Samples to be assayed were dissolved in 2 ml BSM, 50 μl 0.1N NaOH was added and the samples were placed in a boiling water bath for 30 minutes. Upon cooling to room temperature, a 25 μl addition of 1N HCl was made to precipitate soluble material and the samples were centrifuged at 10,000 x g for 15 minutes. Immediately following centrifugation 0.1 ml of supernatant was transferred to a clean test tube containing 2 ml of Pierce working reagent. The solution was mixed and incubated for 30 minutes at 60°C, cooled to room temperature and the absorbance was measured on a spectrophotometer (Beckman DU-65) at 562 nm versus a water reference.

RESULTS AND DISCUSSION

Preparation of Water-Soluble Coal-Derived Material

The mild alkali coal solubilization method of Stoner et al.[12] gave disappointing yields of less than 1% by weight of water-soluble products derived from IBC-101, IBC-107, and

Illinois No. 6 coals (see Table 1). Total sulfur in the water-soluble material differed from the parent coal by 46% and 93% in the Illinois No. 6(S) and the IBC-107(S) material, respectively. An attempt to produce water-soluble material from IBC-101 coal by mild alkali treatment was aborted because of the exceedingly low yield, so no data are available for IBC-101(S) produced by this method.

The yield of water-soluble material produced from these coals by mild alkali treatment was insufficient to allow a thorough analysis of the product much less to perform biodesulfurization experiments with multiple samples and controls. Therefore, a modified method of producing water-soluble material from coal was developed to produce a greater yield of product with an organic sulfur content that is more representative of the original coal. This modified method is described in detail in the Materials and Methods section, but briefly it consists of treatment with a small volume of nitric acid followed by neutralization and three successive treatments with large volumes of strong solutions of sodium hydroxide. Supernatants are centrifuged and filtered to remove solid coal particles, then acid precipitation and centrifugation allows the recovery of soluble coal-derived material. As shown in Table 1, this method of solubilization was applied to IBC-101 coal and achieved a greatly enhanced yield of soluble product that more closely approximated the organic sulfur content of the original coal than the mild alkali treatment method.

The data in Table 1 indicate that IBC-101(S) is reasonably representative of the IBC-101 coal, at least as regards organic sulfur. The data in Table 2 contain the results of more thorough analyses and confirm that IBC-101(S) is generally representative of IBC-101 coal but does exhibit significant differences. An oxidized/weathered sample of IBC-101 was used to produce IBC-101(S) so that changes specific to the solubilization (and subsequent biodesulfurization) treatment could be more readily appreciated (see Table 2). Elemental analyses showed that compared to IBC-101 coal, the IBC-101(S) material had a much greater oxygen content, a higher nitrogen content, a significantly lower ash content, and a reduced sulfur carbon and heating value (Btu/lb) content. IBC-101 coal contains average values of 15.56% oxygen, 1.22% nitrogen, 10.66% ash, 4.17% sulfur, 65.28% carbon, and a calorific value of 11,268 Btu/lb. The corresponding values for IBC-101(S) are 25.39% oxygen, 4.18% nitrogen, 1.71% ash, 2.53% sulfur, 60.67% carbon, and a heating value of 9935 Btu/lb. The higher oxygen content is an expected consequence of the solubilization procedure, and the increased nitrogen content results from the treatment with nitric acid. The solubilization procedure removes pyrite and other inorganic matter resulting in a decreased ash content. Although the sulfur content of IBC-101(S) is considerably lower than IBC-101

Table 1. PRODUCTION OF WATER-SOLUBLE COAL-DERIVED MATERIAL USING TWO PROCEDURES

Coal and Derived Products	Solubilization Procedure	% Yield (by wt) Soluble Coal-Derived Material	% Total Sulfur	% Organic Sulfur
IBC-107	--	--	3.70	2.98
IBC-107(S)	a	<1	0.26	(0.26)[b]
Illinois No. 6	--	--	3.06	2.53
Illinois No. 6(S)	a	<1	1.65	(1.65)[b]
IBC-101	--	--	4.17	2.93
IBC-101(S)	c	63.4	2.53	2.37

[a] The method of Stoner _et al._, 1990.

[b] These organic sulfur values are estimated as the limited samples size available permitted only total sulfur determinations.

[c] The alkali solubilization procedure developed in this work (Materials and Methods).

Table 2. CHARACTERIZATION OF WATER-SOLUBLE MATERIAL DERIVED FROM WEATHERED IBC-101 COAL

Coal and Derived Products	Elemental Analysis					Sulfur Forms				
	Carbon	Hydrogen	Oxygen	Nitrogen	Sulfur	Sulfate	Pyritic Sulfur	Organic Sulfur	% Ash	Btu/lb
IBC-101	65.33	4.61	15.41	1.23	4.11	0.42	0.81	2.88	10.48	11336
	65.22	4.57	15.81	1.20	4.16	0.45	0.78	2.93	11.19	11051
	65.28	4.71	15.46	1.21	4.23	0.48	0.78	2.97	10.30	11416
IBC-101(S)	60.74	4.31	26.34	4.11	2.57	0.16	0.02	2.39	1.83	9812
	60.59	3.75	26.45	4.24	2.49	0.10	0.01	2.38	1.59	10057

coal, the majority of change is due to reductions of pyritic and sulfate sulfur such that the majority of organic sulfur present in IBC-101 coal is preserved in the IBC-101(S) product. The reduced carbon content in IBC-101(S) resulting from the solubilization procedure contributes to the reduced heating value. In general then, while no water-soluble coal-derived material can be completely representative of the original coal the solubilization method developed here succeeded in converting the majority of the organic material in IBC-101 coal into water-soluble material that had an organic sulfur content similar to IBC-101 coal. Accordingly the complex mixture of compounds present in IBC-101(S) is thought to be a good substrate for biodesulfurization experiments.

Biodesulfurization of Material Produced by Mild Alkali Solubilization of Coal

The IBC-107(S) and Illinois No. 6(S) material produced by mild alkali treatment was included in biodesulfurization experiments employing pre-grown and growing cultures of IGTS8 cells, respectively. This allowed a direct comparison of the two biotreatment approaches. These experiments were performed as described in Materials and Methods except that culture volumes were adjusted according to the small amount of material available. Although greater than 90% recovery of the biotreated coal samples and controls was achieved, small sample size allowed analysis of total sulfur only. The results of these analyses are shown in Table 3. Biotreated IBC-107(S) showed an 85% reduction of total sulfur with a treatment time of only 24 hours using pre-grown cells, whereas 39% sulfur removal was observed for Illinois No. 6(S) with a 6-week treatment time using growing cells. These results suggested that IGTS8 is capable of removing sulfur from water-soluble coal-derived material and that pre-grown cells may be more efficient than growing-cell cultures.

Biodesulfurization of Material Produced by Strong Alkali Solubilization of Coal

The results obtained in biodesulfurization experiments with IBC-107(S) and Illinois No. 6(S), as reported in Table 3, must be considered as tentative since these water-soluble products differ markedly from their parent coal samples particularly as regards to organic sulfur. Moreover, the quantity of material available allowed only limited biodesulfurization tests. Water-soluble material derived from IBC-101 coal by the strong alkali treatment described above was included in a variety of experiments employing both pre-grown IGTS8 cells and growing cultures of IGTS8. Because IBC-101(S) is more representative of its parent coal than are either IBC-107(S) or Illinois No. 6(S) and because multiple replicates and more thorough analyses

Table 3. BIODESULFURIZATION OF MATERIAL PRODUCED BY MILD ALKALI SOLUBILIZATION OF COAL

Coal and Derived Products	% Recovery	% Total Sulfur	% Biodesulfurization
IBC-107		3.70	
IBC-107(S) Untreated	100	0.26	
IBC-107(S) Biotreated[a]	97	0.04	85
Illinois No. 6		2.51	
Illinois No. 6(S) Untreated	100	1.65	
Illinois No. 6(S) Biotreated[b]	91	1.01	39

[a] Biotreatment involved pre-grown IGTS8 cells exposed to IBC-107(S) for 24 hours.

[b] Biotreatment involved using Illinois No. 6(S) as the sole sulfur source for the growth of IGTS8.

were performed, experiments with the IBC-101(S) material produced more conclusive results than experiments involving coal solubilized by the mild alkali treatment. IBC-101(S) obtained from sterile BSM control samples show no significant differences from untreated IBC-101(S) material, with the exception of an increase in carbon content from 60.67% to 61.66% (Table 4). This is most probably an apparent rather than a real change resulting from the limited amount of data.

The data in Table 4 indicate that biotreatment of IBC-101(S) with pre-grown cultures of IGTS8 decreased the amount of organic sulfur by 21%, 54%, and 72% after 48 hours of incubation in three independent experiments. Biodesulfurization experiments of 3 to 6 weeks duration, in which IBC-101(S) material served as the sole source of sulfur for the growth of IGTS8, resulted in organic sulfur reductions of 40%, 48%, and 67% in three independent experiments. Culture supernatants did not contain detectable/appreciable amounts of sulfate, sulfite, or other forms of soluble sulfur. Sulfur liberated from IBC-101(S) samples appears to be accounted for by the sulfur content of biomass. The variation reported here in results from biodesulfurization experiments employing IBC-101(S) has also been observed in experiments employing model compounds (data not shown). Although standardized conditions were used in these experiments, the desulfurization activity of IGTS8 pre-grown cultures varies from batch to batch; the rate and extent of growth of IGTS8 cultures using organosulfur compounds as sole sulfur sources are also variable. At this stage of the research IGT can not account for the reaction efficiency differences observed. Nonetheless, biotreated IBC-101(S) samples uniformly exhibit reductions in organic sulfur in comparison with the sterile controls.

There are, however, changes in the biotreated IBC-101(S) samples other than a reduction in organic sulfur. Biotreated samples of IBC-101(S) show increased hydrogen and nitrogen content and decreased oxygen content in comparison with untreated IBC-101(S) or with the sterile controls. Furthermore, biotreated samples from growing cell experiments show an increased ash content. The increased hydrogen and nitrogen content may be due to the presence of biomass in the treated coal samples despite thorough washing procedures. The decreased oxygen content of biotreated samples is probably a reflection of the metabolism of these aerobic microorganisms and the increased ash content of some samples is probably due to the adsorption of mineral matter from the bacterial growth medium. Biotreated samples resulting from experiments that employed pre-grown cells showed a greater retention of carbon, reduced nitrogen, and reduced ash content in comparison with biotreated samples resulting from growing-cell experiments. The chief differences between pre-grown and growing-cell experiments modified protein assay, described in Materials and Methods, was developed here and shown to be capable of reproducibly measuring the amount of biomass in mixtures of IBC-101(S) and biomass. (This modified protein assay can also be used to measure the amount of biomass present in solid coal samples [data not shown].) A standard curve demonstrating the relationship of absorbance at 562 nanometers to the biomass content of IBC-101(S) samples is shown in Figure 1. The direct measurement of the biomass/protein content of the biotreated IBC-101(S) samples listed in Table 4 was generally less than 10% and in no case did it exceed 12%. An IBC-101(S) sample that contains 10% biomass would have an elemental composition of 60.3% carbon, 4.1% hydrogen, 4.5% nitrogen, 2.3% sulfur, and a calorific value of 9,800.

At this time, then IGT is unable to account for all of the changes observed in biotreated samples, particularly as regards to increased nitrogen content; however, the data do indicate that biotreatment with IGTS8 results in the selective removal of organic sulfur that can not be explained by the mere dilution of IBC-101(S) with biomass. Looking only at sulfur values can be misleading, however, so perhaps the most important data are the carbon-to-sulfur and the Btu-to-sulfur ratios shown in Table 4. These values indicate that biotreatment with IGTS8 did indeed result in the selective removal of organic sulfur from IBC-101(S). Moreover, the percent desulfurization reported in Table 4 is calculated from the organic sulfur data but is also reported as the lowest value calculated either from carbon-to-sulfur ratio data or from the total sulfur data after correcting for the amount of biomass present in each sample. In general, it appears that samples treated with pre-grown cells yield results superior to those obtained from experiments involving growing-cell cultures. The average percent biodesulfurization according to the most conservative estimates, as determined from the lowest value calculated from carbon-to-sulfur ratios or total sulfur values corrected for the presence of biomass, was 41.8% for experiments employing pre-grown cells versus 29.1% for experiments employing growing-cell cultures. This point is more effectively demonstrated in Figure 2, which graphically presents the carbon-to-sulfur and the Btu-to-sulfur ratio data for biotreated and control IBC-101(S) samples. The data in Figure 2 clearly illustrate not only that organic sulfur was removed from IBC-101(S) by IGTS8 but also that carbon and calorific value (Btu/lb) was preferentially retained. The carbon-to-sulfur ratio data are presented in the left half of Figure 2, and the Btu-to-sulfur ratio data are presented in the right half of Figure 2. The untreated IBC-101(S) and the sterile control samples yielded carbon-to-sulfur ratios of about 25. Ratios that exceed 25 indicate that sulfur was preferentially removed in comparison with carbon. Similarly, the Btu-to-sulfur ratios

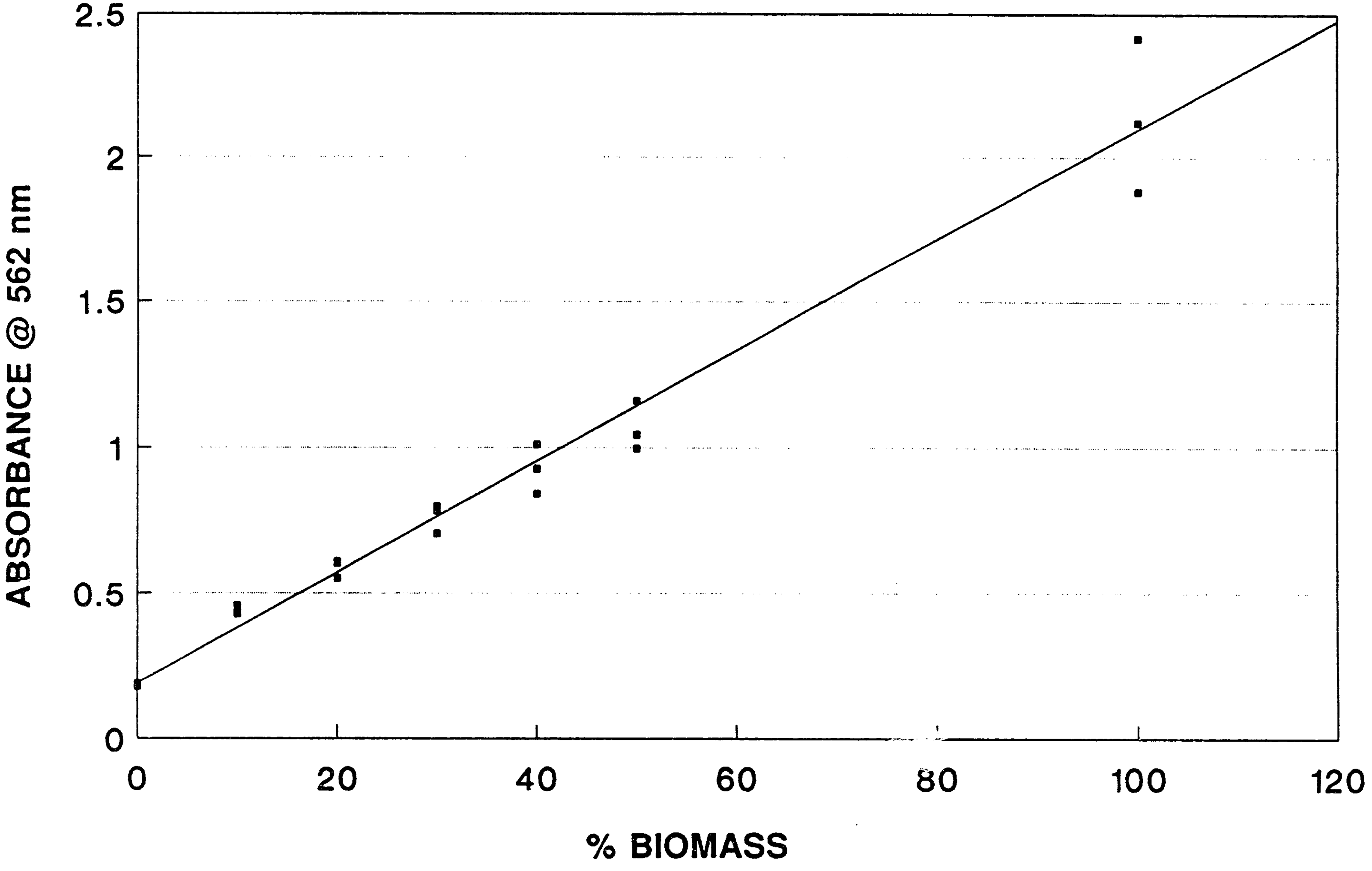
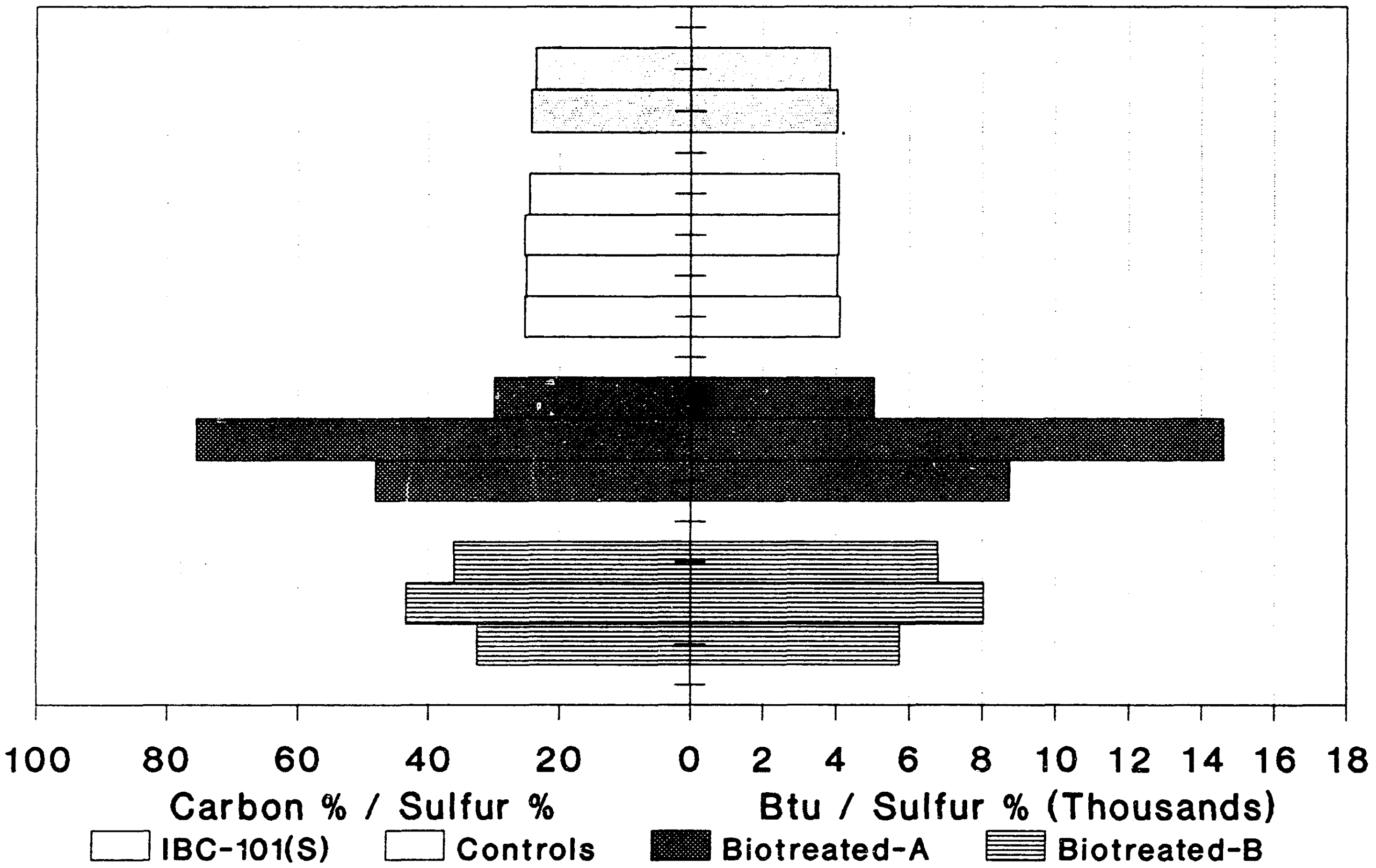


Figure 1. MEASUREMENT OF THE BIOMASS CONTENT OF COAL/BIOMASS MIXTURES



Biotreatment A involves pre-grown IGTS8 cells exposed to IBC-101(S) for 48 hours. Biotreatment B involves using IBC-101(S) as the sole sulfur source for the growth of IGTS8 in experiments of 3 to 6 weeks duration.

Figure 2. CARBON-TO-SULFUR AND BTU-TO-SULFUR RATIOS FOR UNTREATED AND BIOTREATED IBC-101(S)

for control samples are about 4000, and higher values indicate that sulfur was removed relative to the calorific value of the sample.

CONCLUSIONS

In summary, previous studies employing microbial cultures selected for their ability to co-metabolize the organosulfur compound DBT failed to demonstrate any selective removal of organic sulfur from water-soluble coal-derived material. The microbial culture *Rhodococcus rhodochrous* IGTS8 was selected because of its ability to use coal and organosulfur model compounds as sole sources of sulfur, but not as carbon sources. The results of this study indicate that IGTS8 can selectively remove organic sulfur from water-soluble coal-derived material. Moreover, pre-grown IGTS8 cells were shown to be capable of removing organic sulfur from water-soluble coal-derived material with better retention of carbon and calorific value, dramatically shorter treatment times, and 200-fold smaller reactor volumes as compared with the growth of IGTS8 in the presence of this material. *Rhodococcus rhodochrous* IGTS8 appears to be a highly promising microorganism for the development of a biodesulfurization process for the precombustion removal of organic sulfur from coal.

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