

MODIFICATION OF COAL-DERIVED MATERIALS BY *RHODOCOCCUS* SPP.

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INTRODUCTION

The isolation and identification of microorganisms which degrade organic sulfur compounds is often considered the first step in the development of a biological process for the removal of coal organic sulfur (1-4, 7-9). Organic sulfur compounds which contain thiophene, sulfide, disulfide, or thiol groups are used as analogs of the sulfur-containing functionalities and compounds found in coal and coal-derived materials. Because the organic sulfur moieties are assumed to be an integral part of the macromolecular structure, the preferred coal beneficiation process would selectively remove the sulfur and leave the carbon matrix intact with a minimum of oxidation. Microorganisms which degrade dibenzothiophene via the excision of the thiophenic sulfur (2, 3, 5, 6, 10) are of particular interest to the biological treatment of coals containing relatively high proportions of thiophenic sulfur.

Two bacteria, UMX3 and UMX9, isolated by Purdy et al (6) are able to desulfurize dibenzothiophene or dibenzothiophene sulfone. These bacteria, tentatively identified as members of the genus *Rhodococcus* are capable of sustained growth in media in which DBT or DBT-sulfone are the only added source of sulfur. Sulfur is selectively removed leaving 2 phenylphenol. Desulfurization activity is manifested during growth and is repressed by the presence of sulfate.

In order to be effective for coal desulfurization, microorganisms must be able to mediate the desired enzymatic transformation on coal, a chemically complex and heterogeneous material. This paper describes the evaluation of microbial strains UMX3 and UMX9 for the ability to modify water-soluble coal-derived materials. Soluble coal materials (Fig. 1) have been used as substrates to assess microbial desulfurization (7-9). The soluble coal materials are chemically representative but are without the physical limitations inherent with the use of particulate coal.

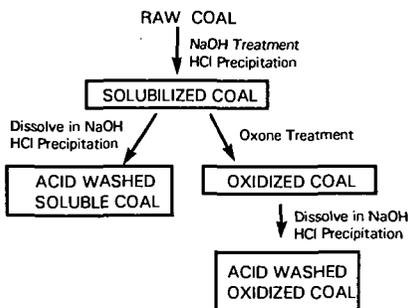


Figure 1: Schematic of the methods used to prepare water-soluble coal materials.

METHODS

Cultures UMX3 and UMX9 were provided by Bailey Ward, University of Mississippi, University, MS. Prior to their use in this study, cultures were transferred 3 times and confirmed for growth on sulfur-limited (SL) medium containing DBT-sulfone as the sole source of sulfur (6).

Cultures UMX3 and UMX9 were tested for the ability to remove sulfur from the soluble coal and oxidized soluble coal materials. Cells from starter cultures of UMX3 and UMX9 were harvested by centrifugation and washed twice with several volumes of sterile phosphate buffered saline (PBS) solution. The washed cells were resuspended in a small volume of PBS. Approximately one-half of the cells were used to inoculate the soluble coal medium and the remaining cells used to inoculate the oxidized soluble coal media. Cultures were shaken at 30°C for 7 days.

In an experiment which utilized a relatively large amount of biomass, one liter cultures of UMX3

and UMX9 were cultivated on SL medium, harvested as noted previously, and washed twice with sterile PBS. Approximately one-half of these cells were used to inoculate 1 liter of medium amended with 100 mg of oxidized soluble coal material (from a filter-sterilized 2.5% solution) while the remaining cells were used to inoculate medium which was amended with 20 mM MgSO₄ to inhibit desulfurization activity. There were cell-free controls for each treatment. Cultures were analyzed at 3 days.

To prepare samples for analysis, cells were removed by centrifugation followed by filtration (0.2µm pore size membrane filters). The coal material was precipitated by acidification to pH 1.5 using 6 N HCl. The precipitate was collected by centrifugation and washed twice with pH 1.5 water (acidified with HCl), then freeze-dried. Elemental composition was determined using an elemental analyzer (Model EA 1108, CarloErba/Fisons Instruments, Valencia, CA). Fourier transform infrared (FTIR) spectra of coal and soluble coal materials formed into KBr pellets were obtained by using an FTIR spectrometer (model FTS-65; Digilab). For XPS spectra (PHI 5400 ESCA, Perkin Elmer, Inc.), energy corrections (downwards by 0.5 to 1 eV) were made to correct for sample charging based on the C(1s) peak position at 284.5 eV. Data in Tables 1 and 2 were obtained using the services of Huffman Laboratories, Inc., (Golden, CO).

RESULTS AND DISCUSSION

Water-soluble materials derived by alkali treatment of weathered or low-grade coals have been used to examine the mechanisms by which bacteria modify coal organic sulfur (7-9). The water-soluble characteristic of the coal products makes them an excellent and easy-to-use substrate for biodesulfurization studies. The accessibility of the organic functional groups to microbial attack may be enhanced by the water solubility, while the absence of inorganic sulfur facilitates the interpretation of data. In this study, the sulfur (Table 1) in water-soluble materials derived from Ugljevik coal was almost entirely organic sulfur (Table 2).

TABLE 1. COMPOSITION DATA FOR MATERIALS DERIVED FROM UGLJEVIK COAL

ANALYSIS %	RAW	SOLUBLE	OXIDIZED SOLUBLE	ACID WASH	ACID WASH OXIDIZED
MOISTURE*	7.1	6.5	4.7	7.2	9.6
CARBON	56.5	57.0	57.8	59.2	57.9
HYDROGEN	4.9	4.1	4.5	4.4	4.3
OXYGEN	24.1	27.5	30.6	29.9	31.1
NITROGEN	1.5	1.8	1.8	1.7	1.7
SULFUR	6.8	6.4	5.6	5.9	5.0
ASH	15.0	3.9	0.4	0.3	0.2

*Moisture is reported on an 'as received' basis. All others are reported on a 'dry weight' basis.

TABLE 2. SULFUR FORMS DATA FOR RAW, SOLUBLE AND OXIDIZED SOLUBLE COAL MATERIALS DERIVED FROM UGLJEVIK COAL.

ANALYSIS (%)	RAW	SOLUBLE	OXIDIZED SOLUBLE	ACID WASH SOLUBLE	ACID WASH OXIDIZED
TOTAL SULFUR	6.8	6.2	5.6	5.9	5.0
SULFATE	0.3	0.7	1.3	0.1	0.1
SULFUR					
PYRITIC	1.1	<2	<0.2	0.0	0.04
SULFUR					
ORGANIC	5.4	5.6	4.3	5.7	4.9
SULFUR					

The cultures grew in medium amended with the water-soluble Ugljevik coal material. UMX3 increased from 4.1×10^8 cells/ml to 3.8×10^9 cells/ml, while UMX9 increased from 8.4×10^7 cells to 3.6×10^9 cells/ml. After 7 days of incubation, the cultures appeared to do little to the elemental composition of soluble coal material (Table 3).

TABLE 3. ELEMENTAL ANALYSIS OF WATER-SOLUBLE UGLJEVIK COAL MATERIAL TREATED WITH BACTERIAL CULTURES UMX3 AND UMX9

Sample	C (%)	H (%)	N (%)	S (%)	C/S	S (% control)
Day 0						
Control	57.7 ± 0.2	4.6 ± 0.2	1.9 ± 0.0	5.7 ± 0.2	10.1 ± 0.4	
UMX3	58.2 ± 0.1	4.8 ± 0.0	1.9 ± 0.0	5.9 ± 0.0	9.9 ± 0.1	103.2 ± 0.7
UMX9	57.9 ± 0.2	4.8 ± 0.1	1.9 ± 0.0	5.9 ± 0.0	9.8 ± 0.1	103.9 ± 0.3
Day 7						
Control	58.2 ± 0.2	4.9 ± 0.1	2.0 ± 0.0	5.7 ± 0.1	10.2 ± 0.1	
UMX3	58.7 ± 0.3	4.9 ± 0.2	2.1 ± 0.0	5.7 ± 0.1	10.3 ± 0.2	99.5 ± 1.8
UMX9	59.1 ± 0.2	4.7 ± 0.1	2.1 ± 0.0	5.8 ± 0.2	10.2 ± 0.4	101.8 ± 3.1

When cultivated on oxidized water-soluble coal material UMX3 increased from 7.1×10^8 cells/ml on day 0 to 4×10^9 cells/ml on day 7. UMX9 increased from 6.6×10^7 cells/ml to 2.6×10^9 cells/ml. No desulfurization activity was detected with the oxidized coal (Table 4).

TABLE 4. ELEMENTAL ANALYSIS OF OXIDIZED WATER-SOLUBLE UGLJEVIK COAL MATERIAL TREATED WITH BACTERIAL CULTURES UMX3 AND UMX9

Sample	C (%)	H (%)	N (%)	S (%)	C/S	S (% control)
Day 0						
Control	56.4 ± 0.3	4.7 ± 0.0	1.8 ± 0.0	5.2 ± 0.0	10.9 ± 0.1	
UMX3	55.2 ± 1.0	4.5 ± 0.2	1.8 ± 0.1	5.1 ± 0.0	10.9 ± 0.2	98.3 ± 0.7
UMX9	56.3 ± 0.1	4.6 ± 0.1	1.8 ± 0.0	5.3 ± 0.1	10.6 ± 0.2	102.5 ± 1.9
Day 7						
Control	57.8 ± 0.2	4.8 ± 0.1	2.0 ± 0.0	5.2 ± 0.2	11.2 ± 0.5	
UMX3	57.9 ± 0.1	4.9 ± 0.1	2.1 ± 0.0	5.1 ± 0.1	11.5 ± 0.2	97.7 ± 1.5
UMX9	56.3 ± 0.2	4.7 ± 0.1	2.0 ± 0.0	5.2 ± 0.1	10.9 ± 0.1	100.2 ± 1.2

The inability to detect changes in the sulfur content of bacterially-treated coal material may have been due to the extremely small amounts required for cell growth. The sulfur requirement for growth is only 0.2% of the wet weight of the biomass. Table 5 reports the data for an experiment in which a relatively high amount of biomass was used. Again, the bacterial cultures did not appear to desulfurize the coal material.

TABLE 5. ELEMENTAL ANALYSIS OF OXIDIZED WATER-SOLUBLE UGLJEVIK COAL MATERIAL TREATED WITH HIGH BIOMASS

	C (%)	H (%)	N (%)	S (%)	C/S	S (% CONT)
Starting Material	54.9 ± 0.6	4.3 ± 0.1	1.7 ± 0.0	5.3 ± 0.1	10.3 ± 0.1	
Sulfur "Limited"						
Control	54.6 ± 0.1	4.2 ± 0.1	1.9 ± 0.0	5.2 ± 0.1	10.4 ± 0.2	
UMX3	54.7 ± 0.1	4.3 ± 0.1	2.2 ± 0.0	5.3 ± 0.2	10.5 ± 0.4	98.3 ± 3.8
UMX9	54.5 ± 0.2	4.4 ± 0.1	2.3 ± 0.0	5.0 ± 0.3	10.9 ± 0.6	96.6 ± 5.3
Sulfate Amended						
Control	53.7 ± 0.2	4.3 ± 0.0	1.8 ± 0.0	5.3 ± 0.1	10.8 ± 0.2	
UMX3	54.5 ± 0.4	4.7 ± 0.0	2.4 ± 0.0	4.9 ± 0.1	11.2 ± 0.2	92.7 ± 2.4
UMX9	54.7 ± 0.2	4.5 ± 0.1	2.4 ± 0.0	5.0 ± 0.1	10.9 ± 0.1	95.1 ± 1.0

As determined by FTIR analysis, the treatment of the soluble coal materials with cultures UMX3 and UMX9 had little effect on the organic constituents of the materials (Data not shown). The microbially-treated oxidized coal material exhibited a slight depletion in the region of 3330 to 3400 cm^{-1} while the microbially-treated soluble coal material exhibited an enhanced signal in this region. This region was assigned to water and coal-OH and coal-NH functionalities. Both UMX3 and UMX9 treated oxidized coal material exhibited greater absorbances in the regions of 1780 cm^{-1} , 1630 cm^{-1} , and 1540 cm^{-1} . The increased absorbances at 1780 and 1630 cm^{-1} were attributed to highly conjugated carbonyl functionalities. The absorbance at 1630 cm^{-1} may also

have been due to primary amines or water. The peak at 1540 cm^{-1} was attributed to carboxylate anion functionalities, aromatic groups or conjugated and aromatic nitro groups.

Both UMX3 and UMX9-treated soluble coal had slight increases in absorbances in the regions of 3400 , 1630 , and 1030 cm^{-1} . An increase in absorbance at 1780 and 1540 cm^{-1} was observed for UMX3-treated soluble coal material while these same regions exhibited a decrease for soluble coal material treated with UMX9.

In view of the data presented here and in earlier publications (Stoner et al., 1990, 1991) the use of model compounds to select for bacteria that can desulfurize coal may be problematic. The effectiveness of such microorganisms may be dependent on the relative abundance of that organosulfur form in the coal. In this case, the ability of UMX3 and UMX9 to desulfurize the water-soluble coal materials may have been dependent on the amount of thiophenic moieties that were present. Coal and coal-derived materials are complex substrates, whose chemical structures are still undefined. The coal materials behave as complex polymers which would be quite different from the model compounds used to select the bacteria. Therefore, there is the possibility of steric hindrance interfering with enzymatic activity.

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