

BACTERIAL DESULFURIZATION STUDIES OF ORGANOSULFUR-ENRICHED MAYAN
CRUDE OIL EXTRACTS USING LIQUID CHROMATOGRAPHY/ATMOSPHERIC
PRESSURE CHEMICAL IONIZATION/ MASS SPECTROMETRY

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INTRODUCTION

The sulfur content in coal and petroleum products ranges from 0.025 to 11%, thus creating a potential problem through the production of sulfur dioxide which is a major component in acid rain.¹ As fossil fuel consumption increases, governments are forced to implement stricter controls on sulfur dioxide emissions in order to reduce the occurrence of acid rain. A regulatory burden will necessitate the development of methods which can be used to lower the sulfur content of fuels, both before and after processing. Inorganic sulfur can be successfully removed by a variety of physical separation methods. However, sulfur removal from organosulfur compounds is more difficult to achieve. The current methodology, hydrodesulfurization, involves the use of inorganic catalysts at high temperature and pressure to generate desulfurized hydrocarbon. The approach is expensive, produces hydrogen sulfide, and is ineffective for many classes of organosulfur compounds. Another approach, which has been well established by the work of Kilbane, involves bacterial systems, including *Rhodococcus* IGTS8, for the bacterial desulfurization of coal and petroleum²⁻⁷. These systems offer many advantages over hydrodesulfurization including the use of much milder conditions, and the generation of water-soluble sulfite or sulfate products as well as desulfurized hydrocarbon. Most importantly, bacteria are able to remove sulfur from a much broader range of organosulfur compounds.

In order to assess the efficacy of bacteria for the desulfurization of crude oil, we have developed a protocol which involves the isolation of organosulfur compounds from a Mayan crude oil, followed by the analysis of the extract using liquid chromatography/ atmospheric pressure chemical ionization/ mass spectrometry (LC/APCI/MS). Concurrently, the organosulfur isolate is used as a food source for the bacterium, *Rhodococcus* sp. IGTS8. By analyzing the mass spectrum before and after bacterial inoculation, we hope to delineate the specificity of the bacteria toward the range of organosulfur compounds that are found in petroleum products.

EXPERIMENTAL

The Maya crude oil (density = 0.91 g/mL) was provided by Mobil Oil Corporation from their Beaumont, TX refinery. The Maya crude was characterized by a series of distillations and was found to contain: 38% light distillate (the fraction that distills below 200°C under 1 atm pressure), 22% middle distillate (the remaining fraction that distills below 180°C under 20 torr external pressure) and 22% residue (the fraction that remains). The corresponding densities were 0.804 g/mL for the light distillate, 0.917 g/mL for the middle distillate and 1.013 g/mL for the residue.

A standard mixture of polyaromatic hydrocarbons (PAH: 7.51 mM fluorene, 6.78 mM fluoranthene, 6.57 mM phenanthrene, 4.94 mM pyrene, 5.39 mM chrysene, 4.28 mM benzo[k] fluoranthene) and 10.37 mM dibenzothiophene (DBT) was prepared in dichloromethane. The mix of PAH and DBT would be used in the evaluation of the ligand exchange protocol.

Alumina (Brockman Activity I, 80-200 mesh, Fisher, Fairlawn, N.J.) was dried at 200°C overnight. CuCl/silica was prepared by mixing approximately 100 g of silica gel (100-200 mesh, Fisher) with 5 g of cupric chloride (Aldrich) in distilled water, drying the mixture to a wet powder with a rotary evaporator, and then drying at 200°C in an oven for 24 h prior to use.⁹ PdCl/silica was prepared by mixing approximately 100 g of silica gel (100-200 mesh, Fisher) with 5 g of palladium (II) chloride (Aldrich) suspended in an aqueous solution, drying in an oven at 95°C overnight, then holding at 200°C for more than 24 h prior to use.¹⁰

Approximately 0.2-0.5 g of the Maya crude oil was dissolved in 5 mL of methylene chloride then adsorbed onto 3 g of neutral alumina. The solvent was removed from the alumina by vigorously stirring the mixture under a gentle stream of dry nitrogen gas. The alumina with the adsorbed sample was then packed on top of 6 g of neutral alumina in an 11 x 300 mm. The sample was eluted with the following chromatographic grade solvents: 20 mL of hexane which removes aliphatic hydrocarbons (designated as fraction A-1); 50 mL of benzene which removes polyaromatic hydrocarbons (designated as fraction A-2) Figure 1. illustrates the entire fractionation and isolation scheme for the sulfur compounds.¹¹

Fraction A-1 was adsorbed onto 0.5 g of the CuCl/silica gel then packed on top of 5 g of CuCl/silica gel in an 11 x 300 mm column. 50 mL of n-hexane were added in order to elute the aliphatic hydrocarbons (designated as fraction C-1)⁹. One hundred mL of chloroform/diethyl ether (9:1, v: v) were then used to elute the aliphatic sulfur hydrocarbons (designated as fraction C-2). See Figure 1.⁹

Fraction A-2 was adsorbed onto 0.5 g of the PdCl/silica gel then packed on top of 5 g PdCl/silica gel in an 11 x 300 mm column. Thirty mL of chloroform/n-hexane (1:1) were used to elute the polyaromatic hydrocarbons (PAH, designated as fraction P-1). A further fifty mL of the same eluent, chloroform/n-hexane (1:1), were used to elute the polyaromatic sulfur heterocycles (PASH, designated as fraction P-2). One hundred mL of chloroform/diethyl ether (9:1) were used to elute other sulfur polyaromatic compounds (S-PAC, designated as fraction P-3). Fractions P-2 and P-3 were reduced in volume to approximately 1 mL by rotary evaporation, after which 50 μ L of diethylamine were added, in order to break up the Pd complexes. Fraction P-3 was further cleaned by passing it through neutral alumina with 50 mL of benzene.¹² Fractions P-1, P-2, and P-3 were evaporated to dryness and redissolved in 5 mL of methylene chloride. See Figure 1.

The PAH and DBT mixture was analyzed using a Beckman 110 B solvent delivery system, an Altex 210A injection valve, and a Beckman Model 160 absorbance detector set at 254 nm. The HPLC system attached to the Finnegan LCQ included a Model P4000 pump and a Model AS3000 autosampler (Thermo Separation Products Inc., San Jose, CA.). The analytical column employed for both HPLC systems was a LiChrosorb Amino (4.6 x 150 mm, dp = 5 μ m) column (Phase Separations, Franklin, MA). The mobile phase employed for the separation of the standard mixture was 100% hexane while the mobile phase employed for the LC/APCI/MS analysis consisted of n-hexane and methylene chloride on a gradient from 100% n-hexane to (60:40) n-hexane/methylene chloride over a period of 6 minutes. For both HPLC systems, the flow rate was constant at 1.0 mL/minute, the column temperature was ambient, and the sample volume was 20 μ L.

Atmospheric pressure chemical ionization (APCI/MS) was performed on an LCQ ion-trap mass spectrometer equipped with an APCI source (Finnigan MAT, San Jose, CA). The APCI source parameters were as follows: discharge voltage, 5 kV; vaporizer temperature, 450 C; nitrogen sheath gas, 80 psi; nitrogen auxiliary gas, 10 psi; heated capillary, 150 C; capillary voltage, 5 V; and tube lens voltage, 5 V. NAVIGATOR Version 1.2 software (Finnigan MAT, San Jose, CA). was used for sample acquisition and data reduction.

The bacterial strain *Rhodococcus erythropolis* sp. IGTS8 was obtained from ATCC (ATCC 53968). Two different media were used for the growth of *Rhodococcus* IGTS8: a Difco nutrient media and a sulfur-free minimal media designated BSM2.⁸ *Rhodococcus* was grown to saturation in overnight tubes containing nutrient media. The cells were then concentrated by centrifugation and washed twice with BSM2 media, before suspension in 100 mL of BSM2 media. The sulfur source necessary for growth was excluded in the negative control, was 200 μ M dibenzothiophene (DBT) in the positive control, or was an aliquot of the sulfur-containing extract from crude oil. Growth was monitored by optical density measurements at 600 nm using a Beckman Model 7500 ultraviolet-visible spectrophotometer.

RESULTS AND DISCUSSION

The standard mixture of the PAH and DBT was fractionated according to the ligand exchange chromatography scheme illustrated in Figure 1. Fraction P-1 exhibited the PAH compounds as well as some of the DBT as previously noted.¹³

Fractions P-2 and P-3 did not exhibit any PAH compounds, thus validating the efficacy of the protocol for the isolation of sulfur compounds.

Both the Maya crude oil and the residue were also fractionated using ligand exchange chromatography. Fractions P-2 and P-3 from the residue were then added to *Rhodococcus erythropolis* sp. IGTS8. No bacterial growth was observed. Most of the polyaromatic sulfur heterocycles (PASH) and other sulfur polyaromatic (S-PAC) compounds are in the light and middle distillates and so this result was expected. The implication however is that *Rhodococcus erythropolis* sp. IGTS8 may not be very effective in the biodesulfurization of Maya crude oil residue.

Fractions P-2 and P-3 from the crude oil were investigated using LC/APCI/MS. Each fraction gave a single broad peak under the chromatographic conditions employed. Any attempt to use a

weaker mobile phase (e.g., 100% hexane) in order to better resolve the multi-component mixture results in the precipitation of some of the components from the mixture. A mass/charge (m/z) scan of the chromatographic peak for each fraction results in a broad mass spectral envelope. Fraction P-2 shows significant intensity extending from about 400 to 1600 m/z (the mass spectrum (MS) is shown in Figure 2). The first statistical moment of the distribution lies around 1000 m/z with local maxima at about 850, 1100 and 1220 m/z . The distribution can be regarded as a fingerprint for the polyaromatic sulfur heterocycles (PASH) in the Maya crude. The mass spectrum (MS) of fraction P-3 shows significant intensity extending from about 400 to 2000 m/z . The distribution features a maximum around 900 m/z . This distribution can be regarded as a fingerprint for the other sulfur polyaromatic compounds (S-PAC) in the Maya crude. Both m/z scans contain multiple peaks, possible fragments, and possible long-lived adducts from the nebulization and chemical ionization process; however, with further method developments in the fractionation of sulfur compounds, the selection of other sorbents and mobile phase eluents for optimal liquid chromatographic resolution, coupled with mass spectral optimization of the nebulization and ionization process, as well as the implementation of MS^n acquisitions, we hope to further delineate the distribution and composition of the P-2 and P-3 fractions.

Fractions P-2 and P-3 from the crude oil will also be added to the *Rhodococcus erythropolis* sp. IGTS8. Bacterial growth will be monitored for up to 30 days. An internal standard will be added to the aliquots and the loss of intensity at individual m/z values will be monitored. Those signals which exhibit a significant loss of intensity will be further explored via MS^n experiments in order to assess the chemical structure.

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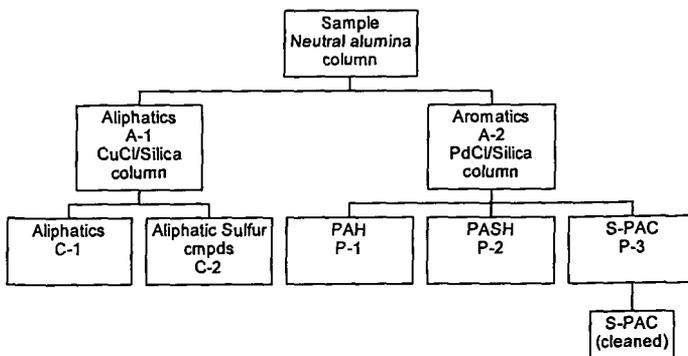


Figure 1. Fractionation and Isolation Scheme for Sulfur Compounds

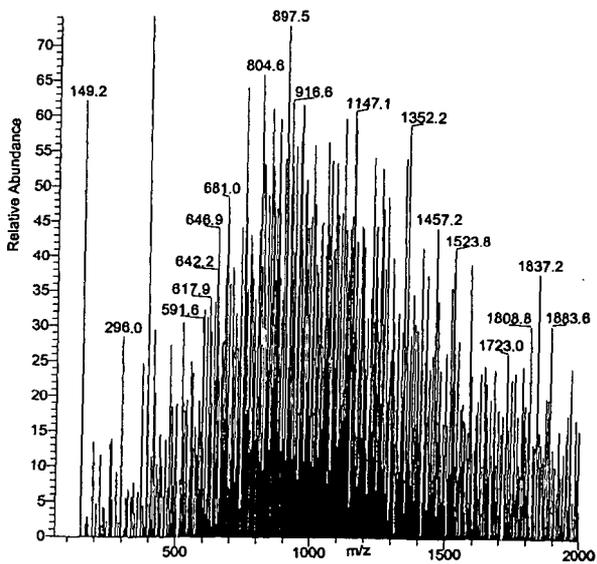


Figure 2. Mass Spectrum of the P-2 Crude Oil Fraction.