

BIOCATALYTIC UPGRADING OF PETROLEUM

Lisa J. Nash and Stephen R. Palmer
Energy BioSystems Corporation
4200 Research Forest Drive
The Woodlands, TX 77381

INTRODUCTION

Sulfur, nitrogen, metals and high viscosity in petroleum cause expensive processing problems in the refinery. Conventional technology does not exist to economically remove these contaminants from crude oil, so the problem is left for the refiners to handle downstream at a high cost.

Sulfur is the major concern for producers and refiners and has long been a key determinant of the value of crude oils for several reasons. First, sulfur presents a processing problem for refiners. Desulfurization offers refiners the opportunity to reduce the sulfur of their crude feedstocks before they ever enter the refinery system, minimizing downstream desulfurization costs. Secondly, the amount of sulfur in many finished products (i.e. diesel, gasoline) is limited by law. The regulations restricting allowable levels of sulfur in end products continues to become increasingly stringent. This creates an ever more challenging technical and economical situation for refiners as the sulfur levels in available crude oils continue to rise and creates a market disadvantage for producers of high-sulfur crudes. Lower-sulfur crudes continue to command a premium price in the market, while higher sulfur crude oils sell at a discount. Desulfurization would offer producers the opportunity to economically upgrade their resources.

Metals in petroleum lead to two major problems for the industry. Combustion of these fuels leads to the formation of ash with high concentrations of the metal oxides, leading to undesirable waste disposal issues. Also, when crude oil is refined, the metals are concentrated in the residual fraction. The residual fraction is often subjected to catalytic cracking where metals from the oil deposit on the cracking catalysts, resulting in the poisoning of the catalysts and decreasing their selectivity and activity. Like metals, nitrogen in oil also leads to the poisoning of the refinery catalysts and also results in increased nitrogen oxide emissions upon combustion in car engines.

High viscosity significantly hampers the pumping, transportation, refining and handling of petroleum. Common methods used to overcome problems associated with high viscosity include heating, dilution and chemical additives. All are expensive and require specialized equipment and/or safety procedures. Industry has long recognized the need for a safe, economical and effective method for reducing viscosity.

Biocatalytic processes for addressing these problems offers the petroleum industry potentially great rewards. Studies by Energy Biosystems Corporation (EBC) have focused on the removal of sulfur from crude oil and refinery streams by a microbial process, termed biocatalytic desulfurization (BDS). Furthermore, preliminary work has also been performed (and patented) on biocatalytic approaches to viscosity reduction and the removal of metals and nitrogen as additional approaches to fuel upgrading. Here, results of work performed on the biocatalytic desulfurization of crude oil will be presented.

MATERIAL AND METHODS

Materials and Equipment. 40 mL shake flasks and 500 mL batch stirred reactors (BSRs) were used to contact cells and crude oil. The reactor vessels are maintained at a constant temperature of 30°C by placing them in an incubator (shake flasks) or temperature controlled water jackets (BSRs).

Cells. Derivatives of the *Rhodococcus erythropolis* strain IGTS8 (ATCC 52986) used in these experiments are known for their ability to use organically-bound sulfur as the sole sulfur source. These cells catalyze the transformation of dibenzothiophene (DBT) to 2-hydroxybiphenyl (2HBP) and sulfate [1-3].

Oil. The crude oils used in the experiments were supplied by Texaco Exploration and Production Technology. Figure 1 shows chromatograms resulting from the sulfur chemiluminescence detector (SCD, sulfur-specific) of several crude oils. The sulfur contents range from 0.8 to 3.7 wt%. As a control, a model oil consisting of hexadecane (HD) and DBT dissolved to ~2600 ppm was utilized.

Procedures. A cell concentration of 12 to 50 g wet cell paste (WCP) per liter of total liquid volume contained in the reactor was utilized. Frozen cells were first added to sodium phosphate buffer (pH 7.5 at 0.156 mM) with 3% glucose. The cell slurry was placed in the reactor, agitated at 1000 rpm and sparged with 0.2 vvm air. The oil was added in the ratio of 1 part oil to 3 parts buffer. Shake flasks were taken down at specified times and BSRs were sampled at regular intervals to monitor sulfur concentrations. Samples were centrifuged at 39,000 x g for 10 minutes in order to separate the mixture of oil, water and cells. To ensure that any observed change was strictly cell-dependent, parallel experiments were performed without cells.

Analytical Methods. An HP 6890 gas chromatograph with electronic pressure control and detection by flame ionization detector (FID) and a Sievers model 350 flameless SCD was employed for crude oil analysis. The column was a Restek RTX-5, 15 meter, 0.25 mm ID with a 0.25 μm film thickness. The injection port was held at 340°C. The oven temperature program began at 50°C and was held for 2 minutes. The temperature was then increased by 15°C/minute to 320°C and was held for 10 minutes. A typical SCD chromatogram consists of a group of resolved peaks above a broad envelope of sulfur compounds (Figure 1).

A GC/MS SIM method for the quantitation of Cx-DBTs and Cx-benzonaphthothiophenes (Cx-BNTs) in crude oil was performed using a HP 5890 Series II plus gas chromatograph with electronic pressure control and mass spectrometric detection performed with an HP 5972 MSD. The column was a Restek RTX-1, 30 meter, 0.25 mm ID with a 0.5 μm film thickness. The injection port was held at 290°C. The oven temperature program began at 100°C, increased at a rate of 4°C/min to 315°C and held for 20 minutes.

Total sulfur quantitation of crude oils was performed with either a Horiba SLFA-1800H x-ray fluorescence analyzer or a Leco SC-444 Sulfur and Carbon Combustion Analyzer with infrared detection.

Sulfur XANES analyses were obtained at beam-line X-19A of the National Synchrotron Light source (NSLS), Brookhaven National Laboratory. Work was performed under contract with the University of Kentucky.

RESULTS AND DISCUSSION

Biocatalyst Development

The development of BDS for crude oil is complicated by the fact that the oil has a very wide boiling point range and that relatively little is known about the number and types of sulfur compounds and their concentrations present in crude oils. The state of EBC's analytical capabilities has greatly advanced to characterize crude oil including sulfur content, sulfur speciation and quantification, and physical properties. Many specialized techniques have been developed that have allowed us to gain valuable insight into the substrate specificity of the catalyst.

We have shown that the enzyme system in the *R. erythropolis* IGTS8 is extremely effective in transforming DBT, BNT, benzothiophene (BT) and their alkylated congeners in crude oil. Sulfur specific chromatograms of crude oil BDS samples reveal that the majority of these substrates have been removed (Figure 2). These results have been confirmed by GC/MS analysis developed to quantify the levels of the DBTs and BNTs in the crude oil (Figure 3). These methods have also revealed that the concentrations of these molecules were low in the crude oils tested and directly correlated to the amount of sulfur removed.

Attempts were made to characterize the sulfur species remaining after BDS (referred to as "Dsz recalcitrant material" or DRM). It was determined by XANES analysis that the majority of the sulfur in the DRM of this material is thiophenic (Figure 4) and, therefore, good targets for the IGTS8 catalyst.

A method to isolate and identify sulfur compounds from various oils and their DRMs was developed. The sulfur-containing species were selectively oxidized and converted to their corresponding sulfones. The sulfones were then separated from the hydrocarbon matrix by solid phase extraction (SPE). This powerful technique has allowed for the identification of the types of sulfur compounds remaining in the treated crude.

Other biocatalytic processes are under investigation that will result in crude upgrading. "Biocracking" has been investigated as a means to break down larger sulfur molecules so they will be small enough to enter the cell and to reduce the viscosity. Attempts were made to isolate organisms able to degrade compounds in the high molecular weight range. Proof-of-concept experiments were performed and validated with methylenebis-DBT (MBD). The goal was to isolate strains that could cleave the bond between the two DBTs. Soil samples were prepared and incubated with MBD. These enrichments were serially transferred, then plated to purify colonies. Individual isolates were obtained for further study.

In addition, if the sulfur bearing heterocycles contribute significantly to the viscosity of the oil, biocatalytic oxidative cleavage of at least one carbon-sulfur bond adjacent to the sulfur heteroatom(s) would result in the opening of the heterocyclic rings and sites of free rotation in the molecules formed effectively lowering the overall viscosity [4].

It has been shown that metals can be removed from crude by contacting the oil with an enzyme that degrades the metalloporphyrin molecules under conditions suitable for the removal and subsequent separation of the metals from the oil. The metals that were removed by the method [5] include nickel, vanadium, cobalt, copper, iron, magnesium and zinc.

Removal of nitrogen is also being investigated as an additional approach to fuel upgrading. This work is being performed at the University of Houston [6] and has been subsidized by EBC. Work has focused on the carbazole-degrading *Pseudomonad* LD2. Approaches in progress include isolation and characterization of the carbazole degradation enzymes, as well as the characterization and cloning of the genes encoding these enzymes.

The goal is to put all these catalytic activities together either in a single biocatalyst or a consortia to upgrade the petroleum by removing the sulfur, nitrogen, metals and reducing the viscosity in a single process step.

Process Development

Shake flask and BSR experiments were performed to address process concerns, such as reaction characteristics, separation characteristics, and catalyst stability and effectiveness. The effect of these parameters were determined in a series of assays designed to compare initial rates of desulfurization under a variety of process conditions. The assay results determined the optimum process conditions for BDS. The key parameters evaluated were water to oil ratio (WOR), catalyst to oil ratio (COR), mixing effects, oxygen demand, and temperature and pH optimum.

A process concept for the biodesulfurization of crude oil was developed based on the knowledge generated, a bench scale unit was constructed and proof-of-concept experiments were performed to develop a design basis specifying performance criteria, unit operations and process parameters for the biodesulfurization of crude oil. As part of the process flow diagram (PFD) development, a general description of the expected site conditions and product stream attributes was compiled. In addition, the process assumptions and equipment issues were delineated that were crucial to the design.

At this time, we envision a simple system capable of running with minimum operator intervention in the oilfield. The base case scenario for a field process is a batch reaction utilizing a pump and inductor for mixing and aeration. Separations will be performed with standard oilfield equipment, with desulfurized oil returning to storage and process water reinjected into a disposal well in the field. The spent catalyst will be inactivated and landfilled. The stored product oil would be tested for sulfur and other oil quality specifications prior to transport. Based on this process concept, the identified process parameters and assumptions, the attached PFD (Figure 5) was developed.

CONCLUSIONS

Significant progress has been made toward the commercialization of crude oil biodesulfurization. This progress includes the characterization of crude oil candidates for the BDS process; improved biocatalyst performance that directly relates to crude oil biodesulfurization; development of analytical methodology, which led to breakthroughs in the characterization of DRM; development of a process concept for crude oil BDS; and construction and testing of a prototype bench unit.

Technical hurdles still need to be overcome to achieve commercialization. The major obstacles to the economical biodesulfurization of crude oil include catalyst specificity and rate. Work continues to modify the catalyst to increase its effectiveness and to screen other organisms for additional desulfurization capabilities. In addition, mass transfer and separations hurdles must be overcome in crude oils with increased oil viscosity and density.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the National Institute of Standards and Technology (NIST) for support received through an Advanced Technology Program (ATP) grant for crude oil BDS, Phil Gibbs and Richard Willson of the University of Houston for their work on denitrogenation, Texaco Exploration and Production Technology for providing the crude oils and the many dedicated people at Energy BioSystems Corporation.

REFERENCES

- [1] Kilbane, J. J. and K. Jackowski. 1992. Biodesulfurization of water-soluble coal derived material by *Rhodococcus erythropolis* IGTS8. *Biotechnol. Bioeng.* **40**:1107-1114.
- [2] Monticello, D. J. 1993. Biocatalytic desulfurization of petroleum and middle distillates. *Environmental Progress.* **12**:1-4.
- [3] Gray, Kevin A., O. Pogrebinsky, G. Mrachko, L. Xi, D. J. Monticello and C. Squires. 1996. Molecular mechanisms of biocatalytic desulfurization of fossil fuels. *Nature Biotechnology.* **14**:1705-1709.
- [4] Monticello, D. J. and W. M. Haney. 1996. Biocatalytic process for reduction of petroleum viscosity. U. S. Patent #5,529,930.

[5] Xu, G., K. Mitchell and D. J. Monticello. 1997. Process for demetalizing a fossil fuel. U. S. Patent #5,624,844.

[6] Gibbs, P. R., R. R. Riddle, M. J. Benedik and R. C. Willson. Biochemistry of carbozole degradation. ACS Biotechnology Secretariat Symposium on Environmentally Benign Synthesis and Biocatalysis in Remediation. Boston, MA, USA, August 23-27, 1998.

Figure 1. Example SCD Chromatograms of Selected Crude Oils

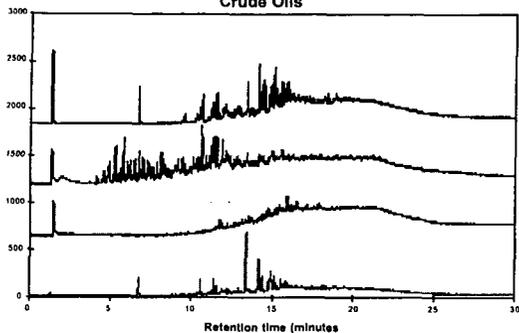


Figure 2. BDS of a Target Crude Oil

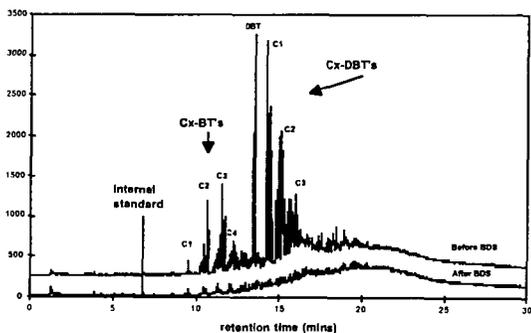


Figure 3. DBT Concentrations Before and After BDS for a Typical Crude Oil

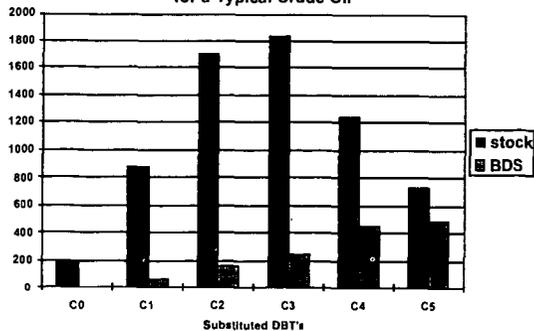


Figure 4. XANES Data for Various Petroleum

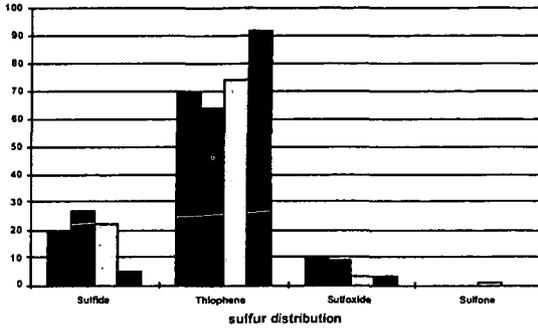


Figure 5: Proposed Process Flow Diagram for Crude Oil Biodesulfurization

