BIOPROCESSING OF FOSSIL FUELS

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The overall objective of this research program is to develop novel technologies for processing fossil fuels using biocatalysis and bioprocessing concepts. As compared to current thermochemical technologies, the bio-based technologies operate at lower temperature and pressure and have the potential to be more energy-efficient. Processes based on oxidative as well as reductive reactions are being investigated for bioupgrading applications. One project is focused on sulfur removal and a second one on developing thermophillic enzymes for breakdown of polyaromatic molecules in an oil environment.

BIODESULFURIZATION OF PETROLEUM FEEDSTOCKS

The objective of this project is to develop improved enzymes for biodesulfurization of petroleum feedstocks. The goal was to improve the activity and broaden the selectivity of desulfurization enzymes using directed evolution as a tool as well as to explore the impact of ring-opening on biological desulfurization. This was the second year of the project and included work on genetic engineering of the desulfurization enzymes (particularly the desulfinase enzyme, which breaks the carbon-sulfur bonds in organosulfur compounds), investigation of the specificity of the desulfurization enzymes for removing diffeent organosulfur molecules from diesel, and a study of synergistic biodesulfurization-biocracking process. The project partners included ExxonMobil, ChevronTexaco, and University of Tennessee, Knoxville (UTK). ChevronTexaco was the lead in developing the biocracking catalysts and ExxonMobil assisted in the genetic engineering of the desulfinase enzyme. The first generation of mutagenesis of the desulfinase enzyme (cloned into an E. coli) produced about 800 colonies. A mutant with four-fold activity was isolated from this experiment. The specificity experiments were conducted with three different organisms and two different diesel fuels. The organisms studied were Rhodococcus sp. IGTS8, Pseudomonas putida, and E. coli. The IGTS8 was the parent strain (not genetically engineered) from which the desulfurization genes (dszABC) were cloned into the other organisms. The results indicated that the IGTS8 strain was able to achieve maximum desulfurization of the diesel fuels. The synergistic experiments with biocracking (ring-opening) catalysts conducted so far have not yielded any conclusive results and more research needs to be done to understand the effect of the synergistic process.

BIOLOGICAL HYDROGENATION OF ORGANOSULFUR COMPOUNDS

This project is aimed at investigating the potential of enzymatic and biomimetic catalysts for hydrogenation and hydrodesulfurization of oil compounds with the goal of upgrading crudes via sulfur removal and improving downstream processing. This was the second year of the project. During this year,

the enzyme hydrogenase was studied to determine the hydrogenation of dibenzothiophene. In its native state, it was unable to catalyse the reaction, although it was found to be able to perform the hydrogen activation even in 10% acetonitrile or 10% ethanol—water system. The need to bring the substrate near the active site (which has the active hydrogen bound to it) was realized and further work was conducted in isolating the enzyme active site. This was done by digestion of the enzyme followed by separation via size exclusion and ion exchange chromatography. An invention disclosure was submitted on this subject matter. The isolated active center was still able to activate hydrogen gas, however, the yield of the active center from the enzyme was very low, due to which large quantities could not be isolated. The active center potential seems to have potential to hydrogenate/reduce organosulfur compounds and these tests are in progress.

BIOUPGRADING OF HEAVY CRUDES USING THERMOPHILLIC ENZYMES

Heavy oils consist of a significant portion of asphaltenes, which are condensed polyaromatics that increase viscosity and melting point of the crudes. Enzymes which can degrade polyaromatic hydrocarbons can potentially be used to degrade the molecules via ring-opening. Cytochrome P450 enzymes are capable of introducing the first oxygen molecule into the PAHs. This project is focused on developing biocatalysts, which will be capable of ring-opening and operation at slightly higher temperatures in the oil phase. The cytochrome P450 class of enzyme also consist of some enzymes which have thermostability up to 90°C. We have initiated protein engineering of cytochrome P450 to produce hybrid enzymes which will be capable of PAH oxidation as well as operation at higher temperatures. Further optimization of these enzymes is planned using directed evolution and other genetic engineering techniques.

PUBLICATIONS AND PATENTS

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Invention disclosure: Borole, Abhijeet P., and S. R. Kulkarni, Development of Ni-Fe hydrogenation catalyst from *D. gigas* hydrogenase, submitted August 2002, approved for Patent application: November 2002.