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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 was focused on biological hydrogenation and a second one on developing thermophillic enzymes for hydrocarbon oxidation in an oil environment.

Biological hydrogenation of organosulfur compounds (FEAC323)

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 final year of the project. During this year, an attempt was made to develop a catalyst from the D. gigas hydrogenase enzyme for reduction of organic molecules using hydrogen. The goal was to isolate the active center of the enzyme which is composed of Ni and Fe in a specific orientation. It was hypothesized that the Ni-Fe active center would be able to directly interact with organic molecules to carry out hydrogenation reactions. The structure of the catalyst is shown below. The active center was isolated via a slow digestion with protease followed by separation using column chromatography. The activity of the fractions were assayed using hydrogen uptake assay using methyl viologen. Based on these assays, the yields of the active center were quite poor. Due to this, sufficient catalyst could not be isolated for characterization purposes. Alternate means of generating/isolating the active center, such at that via genetic engineering, would be required for doing detailed characterization and further studies.

Bioupgrading of heavy crudes using thermophillic enzymes (FEAC326):

Heavy oils consist of a significant portion of asphaltenes, which are condensed polyaromatics that increase viscosity and melting point of the crudes. Enzymes capable of reducing viscosity via bioconversion or secondary chemical production were studied. Cytochrome P450 enzymes are capable of introducing an oxygen into alkanes and PAHs. This project was focused on developing biocatalysts which are capable of hydroxylation and ring-opening at temperatures up to 90^{o}C in the oil phase. The cytochrome P450 class of enzymes consists proteins which can hydroxylate alkanes or PAHs as well as some which have high thermostability. Both of these properties, however are not present in same enzyme. We conducted protein engineering to produce hybrid enzymes which have potential for hydrocarbon oxidation and can operate at higher temperatures. The project had to be ended prematurely, since the third year’s funding was not available. These enzymes can potentially have application in production of alcohols and phenols in the chemical industry as well.

Publications and Presentations:

Borole, A P and Hamilton, C Y; *'Developing hybrid cytochrome P450 enzyme from CYP101 and CYP119,* a poster presented at the 26^{h} Symposium on Biotechnology for Fuels and Chemicals, Chattanooga, May 2004.

Hamilton, C Y and Borole, A P; *'Improving biodesulfurization rates via the 4S pathway by mutagenesis of the desulfinase enzyme,* a poster presented at the 26^{h} Symposium on Biotechnology for Fuels and Chemicals, Chattanooga, May 2004.

Borole, A P; Dai S., Cheng C. L., Rodriguez, M. Jr. and Davison B. H., *Performance of Chloroperoxidase Stabilization in Mesoporous Sol-gel Glass Using in-situ GOX peroxide generation*, Applied Biochemistry and Biotechnology, 2004.

Borole, A P; C. L. Cheng, B. H. Davison, '*Substrate desolvation as a governing factor in enzymatic transformations of PAHs in aqueous-acetonitrile mixtures*,' Biotechnology Progress, 2004