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Task 4.6 - Biodesulfurization

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TASK 4.6 – BIODESULFURIZATION

1.0 INTRODUCTION

Conventional catalytic hydrodesulfurization involves high costs largely due to heavy metal deactivation of the catalysts. A potential lower-cost treatment is a microbiological or enzymatic desulfurization. Recent advances (1, 2) at the Energy & Environmental Research Center (EERC) at the University of North Dakota have improved our understanding of sulfur-specific microbial desulfurization pathways in *Rhodococcus* bacteria, but further work is needed to develop a technology based on biodesulfurization.

2.0 OBJECTIVES

The main focus in this task is the application of desulfurization enzymes to remove sulfur from crude oil, diesel fuel, or resid precursors for needle cokes. The most important question to be answered is how to utilize the *Rhodococcus* desulfurization complex in a nonaqueous solvent or medium, such as the oil itself. Successful application of nonaqueous enzymology to this problem will involve finding ways to stabilize the active conformations of the enzymes and provide for easy recovery of the enzymes, perhaps in an immobilized-enzyme packed-bed reactor. Understanding the desulfurization activity will require that we determine the regulatory and mechanistic properties of the enzymes.

The work will involve isolation and immobilization of the bacterial (*Rhodococcus*) enzyme systems that excise sulfur from thiophenic structures. Sulfur-specific microbial desulfurization has been shown to be an inducible trait that probably resides on the cell surface as a multienzyme cluster. Dead and broken cells exhibit the desulfurization activity, thus an immobilized cell-free system is plausible. Methods for dispersion and immobilization of enzyme clusters will be investigated. Immobilization is desired for ease in recovery and reuse and preparing packed-column reactors for continuous processing. The critical task is to develop a system that is active in a nonaqueous solvent so that the reaction is not limited by the diffusion of sulfur compounds from the oil phase into an aqueous phase and so that phase separations at the end of the processing are eliminated. Attachment of stabilizing groups to the support matrix or to the enzymes may be required to achieve activity in the nonaqueous solvents. Both nonpolar and polar solvents will be investigated. It is also essential that we determine whether cofactors or other regulatory species are needed for functioning of the cell-free enzymes. Further information will be obtained on the nature of the pathways and desulfurization mechanisms and the inducers and inhibitors of the microbial activity via experiments with alternative sulfur substrates and inducers and with labeled substrates.

3.0 WORK PERFORMED

In the previous period, methods were developed for recovering larger amounts of the metabolic intermediates that would at the same time preserve all the labeling from oxygen isotopes by preventing exchange and avoiding displacement during derivatization for the gas chromatography/mass spectrometry (GC/MS) analysis. Previously the analytical method involved cyclization of the sulfonate, and sulfonate intermediates resulted in the loss of one oxygen. The new

method allowed us to obtain better mass balances as well as more reproducible and more interpretable data. Strong anion exchange resins were added to the incubation mix during the metabolism experiments to trap the sulfinic and sulfonic acids, as well as sulfite and sulfate. After the incubation, the resins were removed from the aqueous suspension, washed to remove cells, and washed with acetonitrile to remove most of the water. The resin was treated with methyl iodide to derivatize the sulfonate and sulfite. The sulfonate was converted to the methyl sulfone derivative, but the sulfonate was not methylated under these conditions. Instead the sulfonate was converted to the sultone (loss of one oxygen) for GC/MS analysis.

In this period, methods were investigated for conversion of the sulfonate and the sulfite trapped on the resin to alkyl derivatives that could be analyzed by GC/MS and GC/Fourier transform infrared to determine the oxygen isotopic labeling. This information is needed to complete our understanding of the last steps occurring in the complex desulfurization system in the bacterium. The methods employed included treatment with diazomethane and trimethyloxonium tetrafluoroborate. Although these reagents have been previously used to prepare sulfonate esters from sulfonic acids, the reaction of sulfonates attached to a resin has not been attempted.

4.0 RESULTS/CONCLUSIONS

2'-Hydroxybiphenyl-2-sulfonate (synthetic) was stirred with the anion exchange resin to prepare a surrogate system for study. The reaction of the resin-bound sulfonate with diazomethane in ether-methanol gave a trace of the methyl ester. It is likely that the diazomethane will only react with the sulfonic acid form via the protonated diazomethane intermediate. We could not add acid to the reaction without converting the sulfonate to the sultone rather than the acid form. The reaction with trimethyloxonium tetrafluoroborate produced the methyl ester.

Addition of the anion exchange resins during incubation with the bacteria was successful in binding the sulfonate and sulfonate intermediates as they were released from the cells. However, treatment of the recovered resin, the trimethyloxonium reagent, did not produce any of the sulfonate ester. Further derivatization reactions need to be investigated. These may include highly reactive chloromethylethers, *tert*-butyldimethylsilyl chloride, or methylsulfate, and, perhaps, silver ion can be added to facilitate the reaction.

Of greater interest was the isolation of sulfite in a derivative form without loss or exchange of oxygens. Sodium sulfite was added to the anion exchange resin to prepare a surrogate for study of derivatization reactions. Diazoethane in ether methanol was added to the resin, but no methyl sulfite ester or methyl methylsulfite ester could be detected by GC. Similarly, the reaction with trimethyloxonium salt gave no ester product. Sulfite will react with methyl iodide to form the methylsulfonate ion, but this product did not react further to give the methyl methylsulfonate ester. Reaction of the methylsulfonate intermediate bound to the exchange resin with diazomethane gave a small amount of the desired methyl methylsulfonate ester. It is clear that a successful analysis of sulfite oxygen labels with GC/MS will require reaction of the resin-bound sulfite with methyl iodide or other reactive halide, followed by a second derivatization with a chloromethylether, *tert*-butyldimethylsilyl chloride, or methylsulfate, and perhaps silver ion. Oxygens on the sulfonate are reasonably stable to exchange, so none of these reactions should involve loss of labeled oxygen.

5.0 FUTURE WORK

The resin-trapping experiments can be further exploited to extend our knowledge of all kinds of bacterial cleavages of sulfur compounds. Label distribution in the products, including sulfite, will allow us to determine how the sulfur cleavage steps occur. Further research in obtaining cell-free systems is in progress.

6.0 REFERENCES

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