

## MICROBIAL HYDROGENATION OF COAL AND DIPHENYL METHANE

M.I.H. Aleem<sup>1</sup>, D. Bhattacharyya<sup>2</sup>, G.P. Huffman<sup>3</sup>, R.I. Kermode<sup>2</sup>, M.V.S. Murty<sup>1,2</sup>,  
H. Venkatachalam<sup>2</sup>, and M. Ashraf<sup>1</sup>

School of Biological Sciences<sup>1</sup>, Department of Chemical Engineering<sup>2</sup>, and  
The Consortium for Fossil Fuel Liquefaction Science<sup>3</sup>  
University of Kentucky, Lexington, Kentucky 40506

**Keywords:** Coal biohydrogenation, Microbial hydrogenation of diphenyl methane, H<sub>2</sub>-transferring hydrogenase in *Sulfolobus* and *Desulfovibrio*.

### ABSTRACT

The ability of *Desulfovibrio desulfuricans* and *Sulfolobus brierleyi* to catalyze the hydrogenation of coal and model compounds was investigated using Warburg-manometry in an atmosphere of H<sub>2</sub> without O<sub>2</sub>. *D. desulfuricans* catalyzed H<sub>2</sub> uptake at a rate of 48 μmol/h and 0.0701 μmol/h with methylene blue (MB) and diphenyl methane (DPM) respectively, while the uptake rate by KCERL #91182 coal was 4.63 μmol/g coal/h. *S. brierleyi* was 8-12 times more effective catalyst yielding hydrogenation values of 619 μmol H<sub>2</sub> taken up/μmol MB/h and 0.5357 μmol H<sub>2</sub> taken up/μmol DPM/h, while catalyzing the hydrogenation of coal at a rate of 44.1 μmol/g coal/h. The precipitated iron in the biodesulfurized coal by *S. brierleyi* was mainly in the form of super paramagnetic FeOOH as determined by the <sup>57</sup>Fe low temperature Mössbauer spectroscopy. These very fine paramagnetic particles (50-200 Å) exhibited good catalytic behaviour with higher liquefaction yield. Coals biotreated with *D. desulfuricans* also enhanced the chemical coal liquefaction yield by 5.67%.

### INTRODUCTION

Bioprocessing of coal is emerging as a new technology for coal cleaning and coal conversion processes<sup>1-7</sup>. Coal conversion under milder conditions continues to offer the potential for improved liquefaction of coal. The overall success of liquefaction may entail improved coal pretreatment, disposable catalysts, upgrading of coal liquids in terms of removal of nitrogen and organic sulfur, and novel hydrogen generation and utilization techniques. The development of these techniques as applied to the above objectives offers an alternative approach if appropriate microbial cultures and environmental conditions can be established. Our earlier work<sup>8-14</sup> has demonstrated that under appropriate culture conditions, over 90% of the pyritic sulfur from coals can be removed by the mesophilic sulfur oxidizing autotrophic bacteria *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, but these bacteria were incapable of removing organic sulfur. However, the thermophilic archaeobacterium *Sulfolobus brierleyi* was able to remove over 95% of the pyritic sulfur and over 30% of the organic sulfur from the untreated coal when the cells of *S. brierleyi* were acclimatized.

The general reported area of biological conversion of substrate materials to higher quality fuels and chemicals are bioconversion of coal, lignite, and peat; hydrogen production by anaerobic microorganisms; and desulfurization. The aerobic biosolubilization of low-rank coal to polar water soluble products has been demonstrated<sup>15-18</sup>. The microbial desulfurization of oil-water emulsions and metabolism of dibenzothiophene has been demonstrated in our recent work<sup>12</sup>. In addition, the in-situ formation of catalyst, FeOOH crystals on coal particles in the presence of *Sulfolobus brierleyi* for enhanced liquefaction has been observed in our laboratories<sup>10,14</sup>. To our knowledge the direct microbial hydrogenation of untreated coal or the model compounds has not so far been demonstrated. We report here the results of our preliminary experiments.

### MATERIALS AND METHODS

**Organisms:** Methods for growth and cell preparations for *sulfolobus brierleyi* have been described previously<sup>9</sup>. *Desulfovibrio desulfuricans* (ATCC strain No. 7757) was grown anaerobically in DSM medium #63 as described in the DSM catalogue (Deutsche Sammlung Von Mikroorganismen: German Collection of Organisms), Grisebachstrasse 8, D-3400 Göttingen, Germany. The cells were obtained by centrifugation (under anaerobic conditions) at 10,000 x g for 30 minutes and washed thrice with 0.05M phosphate buffer (pH 7.4). The cells were suspended in the same buffer and kept anaerobic at 4°C until use.

**Hydrogenase Assay:** The enzyme activity in *D. desulfuricans* was determined by Warburg manometry at 30°C and pH 7.4 and at 60°C and pH 2 in *S. brierleyi* with H<sub>2</sub> as the electron donor and methylene blue as the electron acceptor in an atmosphere of H<sub>2</sub> under anaerobic conditions. The reaction mixture had a total volume of 3 ml and contained 0.2 ml of 40% KOH in the center well of the reaction

flask to absorb any CO<sub>2</sub> released. Where applicable, the concentration of methylene blue or diphenyl methane was 4.2 and 6.5 mM respectively. Where indicated the concentration of untreated KCERL #91182 coal was 4.5%. The side arm of the Warburg flasks contained 0.3ml of the bacterial suspensions (approximately 3mg protein). The reaction flasks were shaken for 10 minutes under H<sub>2</sub> atmosphere for temperature equilibration before tipping-in the cells from the side arm. The H<sub>2</sub> uptake was recorded at 10-20 minute time intervals.

**Mössbauer Spectroscopy:** This technique provided a quantitative measurement of the reactions of pyrite and its transformations in Western Kentucky # 11 coal treated for biodesulfurization by *Sulfolobus brierleyi*. The techniques were essentially the same as described previously<sup>14</sup>. The coal liquefaction product yields (wt. %) determinations were performed by the University of Kentucky Center for Applied Energy Research using tetralin as a solvent, 800 psig H<sub>2</sub>, and 427°C.

**Samples for Mössbauer Spectroscopy:** The samples of a Western Kentucky #11 coal were desulfurized using *Sulfolobus brierleyi* in a coal-water slurry aerated with air containing 10% CO<sub>2</sub>.

## RESULTS AND DISCUSSION

### Comparative Rates of H<sub>2</sub>-Uptake Catalyzed by *Sulfolobus brierleyi* and *Desulfovibrio desulfuricans*

Several aerobic and anaerobic bacteria possess hydrogenases but the ability of these microorganisms to catalyze the hydrogenation of coal and/or model compounds has not so far been reported. We have used an aerobic sulfur-oxidising thermoacidophilic archaeobacterium *Sulfolobus brierleyi* and an anaerobic sulfate-reducing mesophilic bacterium *Desulfovibrio desulfuricans*. Since both organisms possess hydrogenase enzymes, the data in Table 1 show that both organisms transferred reducing equivalents, e.g., electrons from H<sub>2</sub> to methylene blue, a conventional method used for measuring the hydrogenase activity in microorganisms. The comparative H<sub>2</sub> uptake rates catalyzed by the cells of *D.desulfuricans* and *S.brierleyi* were 48 and 619 µmoles of methylene blue reduced per hour respectively. The respective specific activities of the cellular enzyme were about 16 and 206 µmoles/h/mg protein indicating that the hydrogenase from *S.brierleyi* was about 13 times more potent than the hydrogenase from *D.desulfuricans*. While the hydrogenase of *S.brierleyi* was oxygen insensitive, the enzyme from *D.desulfuricans* might have been inactivated during the preparation of cells since oxygen is extremely toxic to the growth of *D.desulfuricans*. Thus the much lower activity of hydrogenase might not reflect its real potential. It is indeed significant, however, that the cells from both organisms were able to transfer H<sub>2</sub> to both diphenyl methane and untreated coal. Here again the specific activity of H<sub>2</sub>-transferring enzyme e.g. hydrogenase was much lower in *D.desulfuricans* than that in *S.brierleyi*. The significant H<sub>2</sub> transfer rate catalyzed by *S.brierleyi* to the model compound diphenyl methane as well as to the untreated coal at 60°C is an important finding since the archaeobacterium is capable of not only removing sulfur from coal<sup>8-13</sup> but is also able to catalyze coal hydrogenation which should lead to higher liquefaction yields.

**Mössbauer Spectroscopy of the Biodesulfurized Coal:** The behaviour and transformation of pyrite in the bioprocessed coal can be demonstrated by <sup>57</sup>Fe Mössbauer spectroscopy. The data is shown in Figure 1 for two biodesulfurized Western Kentucky #11 coals from two different desulfurization tests (tests K87 and 22) in which the pyrite content was significantly reduced by pretreatment with *S.brierleyi*. In the upper spectrum (K87) the pyritic sulfur was decreased from 3.3% to 0.1%, while in the lower spectrum (K22) the pyritic sulfur content fell from 1.2 to 0.1%. In both cases significant amounts of iron-bearing oxidation products were precipitated from the solution. Ferric sulfate (Jarosite) was the main oxidation product of pyrite (upper spectrum). In the lower spectrum, iron precipitation occurred when the CO<sub>2</sub> was shut off, and was primarily in the form of superparamagnetic FeOOH which is easily distinguished from pyrite and Jarosite in low-temperature Mössbauer spectra. Such paramagnetic phases have very fine particle sizes (50-200 Å) and therefore, they exhibit good catalytic behaviour during liquefaction as indicated by data in Table 2 where the liquefaction conversion of the parent coal for run 22 was compared to that of biodesulfurized samples taken before and after the disruption of the CO<sub>2</sub> flow which caused the precipitation of superparamagnetic FeOOH. The higher conversion percentage of liquefaction for sample 22B compared to sample 22A indicates the catalytic nature of FeOOH which raised the conversion percentages of the desulfurized coal back to about that of the untreated pyrite-rich coal. Hence the knowledge of the forms of iron in bioprocessed coal provided by Mössbauer spectroscopy can provide strategies for optimization of sulfur reduction and other conversion technologies.

**Acknowledgements:** This research was supported by a US Department of Energy contract # DE-FC22-90PC90029.

1. Couch, G.R., *Biotechnology and Coal*, IEA Coal Research Report., 1987, KTIS/TR 38, London.
2. EPRI Report, First Annual Workshop on Biological processing of Coal, 1988, EPRI ER-5709-SR, Palo Alto, California.
3. Ehrlich, H.L., and Holmes, D.S. (Editors), *Workshop on Biotechnology for the Mining, Metal Refining, and Fossil Fuel Processing Industries*, Wiley, New York, 1986.
4. Atlia, Y.A.(Editor), *Processing and Utilization of High-Sulfur Coals*, Elsevier, Amsterdam, 1985.
5. Markuszewski, R., and Wheelock, T.D. (Editors). *Processing and Utilization of High-Sulfur Coals III*, Elsevier, Amsterdam, 1990.
6. Olson, G.I., Brinckman, F.E., and Iverson, W.P., *Processing of Coal with Microorganisms*. EPRI Final Report, EPRI AP-4472, 1986, Palo Alto, California.
7. Dugan, P.R., *Microbiological Desulfurization of Coal and its Increased Monetary Value*, *Biotechnology, Bioengineering Symposium*, 1986, 16, 185-204
8. Khalid, A.M. and Aleem, M.I.H., *Biological Desulfurization of Coal by *Sulfolobus* and *Thiobacillus* species*, Amer. Soc. Microbio. Annual Meeting., Miami Beach, Florida, Abstract 051, 1988.
9. Khalid, A.M., Bhattacharyya, D. and Aleem, M.I.H., *Sulfur Metabolism and Coal Desulfurization Potential of *Sulfolobus brierleyi* and *Thiobacilli**, S.Yunker (Editor), *Proc. Symposium on Biological Processing of Coal and Coal-Derived Substances*, EPRI ER-6572, 1989, pp. 2-19 to 2-36, Palo Alto, California.
10. Bhattacharyya, D., Hsieh, M., Francis, H., Kermode, R.I., and Aleem, M.I.H., *Biological Desulfurization of Coal by Mesophilic and Thermophilic Microorganisms*, *Resour., Conserv. Recycl.*, 1990,3,81-96.
11. Khalid, A.M., Bhattacharyya, D., and Aleem, M.I.H., *Coal Desulfurization and Electron Transport-Linked Oxidation of Sulfur Compounds by *Thiobacillus* and *Sulfolobus* species*, Pierce, G.E. (Editor), *Development in Industrial Microbiology (Soc. Ind. Microbiology)*, 1990, 31,115-126
12. Khalid, A.M., Aleem, M.I.H., Kermode, R.I., and Bhattacharyya, D., *Bioprocessing of Coal and Oil-Water Emulsions and Microbial Metabolism of Dibenzothiofene*, *Resour., Conserv., Recycl.*, 1991,5 (in press)
13. Khalid, A.M., Bhattacharyya, D., Hsieh, M., Kermode, R.I., and Aleem, M.I.H., *Biological Desulfurization of Coal*, Markuszewski, R., and Wheelock, T.D. (Editors), *Third International Conference on Processing and Utilization of High-Sulfur Coals III*, Elsevier, Amsterdam, 1990, pp. 469,480.
14. Huffman, G.P., Huggins, F.P., Francis, H.E., Mitra, S., and Shah, N., *Structural Characterization of Sulfur in Bioprocessed Coal*, Markuszewski, R., and Wheelock, T.D., (Editors), *Third International Conference on Processing and Utilization of High-Sulfur Coals III*, Elsevier, Amsterdam, 1990, pp. 23-32.
15. Cohen, M.S., Bowers, W.C., Aronson, H., Gray, E.T., *Cell-free Solubilization of Coal by *Polyporus versicolor**, *Appl. Environ. Microbio.*, 1987, 53, 2840-43.
16. Pyne, J.W., Stewart, D.L., Fredricson, J., and Wilson, B.W., *Solubilization of Leonardite by an Extracellular Fraction from *Coriolus versicolor**, *Appl. Environ. Microbio.*, 1987, 53, 2844-48.
17. Scot, C.D., Strandberg, G.W., and Lewis, N.S., *Micribial Solubilization of Coal*, *Biotechnol. Prog.*, 1986, 2, 131-139.
18. Wilson, B.W., Bean, R.M., Franz, J.A., Thomas, B.L., Cohen, M.S., Aronson, H., and Grey, E.T., *Microbial Conversion of Low Rank Coal: Characterization of Biodegraded Product*, *Energy Fuels*, 1987, 1, 80-84.

**Table 1**  
Comparative Rates of Hydrogen Uptake Catalyzed by  
*Sulfolobus brierleyi* and *Desulfotribrio desulfuricans*

Organism	Substrate	Hydrogen uptake $\mu\text{mol/g coal/h}$
<i>S. brierleyi</i>	Coal	44.10
<i>D. desulfuricans</i>	Coal	4.63
		$\mu\text{mol}/\mu\text{mol DPM}^*/\text{h}$
<i>S. brierleyi</i>	DPM	0.5357
<i>D. desulfuricans</i>	DPM	0.0701
		$\mu\text{mol}/\mu\text{mol MB}^{**}/\text{h}$
<i>S. brierleyi</i>	MB	619
<i>D. desulfuricans</i>	MB	48

See 'Materials and Methods' for experimental conditions.

\* - DPM - Diphenyl methane \*\* - MB - Methylene blue

**Table 2**  
Liquefaction product yields (wt.%) of biodesulfurized coal  
Tetralin, 800 psig H<sub>2</sub> (ambient)

	385°C			427°C		
	Parent Coal	22A <sup>a</sup>	22B <sup>b</sup>	Parent Coal	22A <sup>a</sup>	22B <sup>b</sup>
Gas	2	12 <sup>c</sup>	3	4	5	6
Oil	10		8	39	41	39
Asphaltene	22	24	20	32	29	33
Preasphaltene	39	21	36	13	10	12
IOM <sup>d</sup>	28					
Conversion	72	57	67	87	84	90

<sup>a</sup>Biotreated, before FeOOH precipitation. <sup>b</sup>Biotreated after FeOOH precipitation

<sup>c</sup>Gas and oil not determined separately for this sample. <sup>d</sup>Insoluble organic matter or residue.

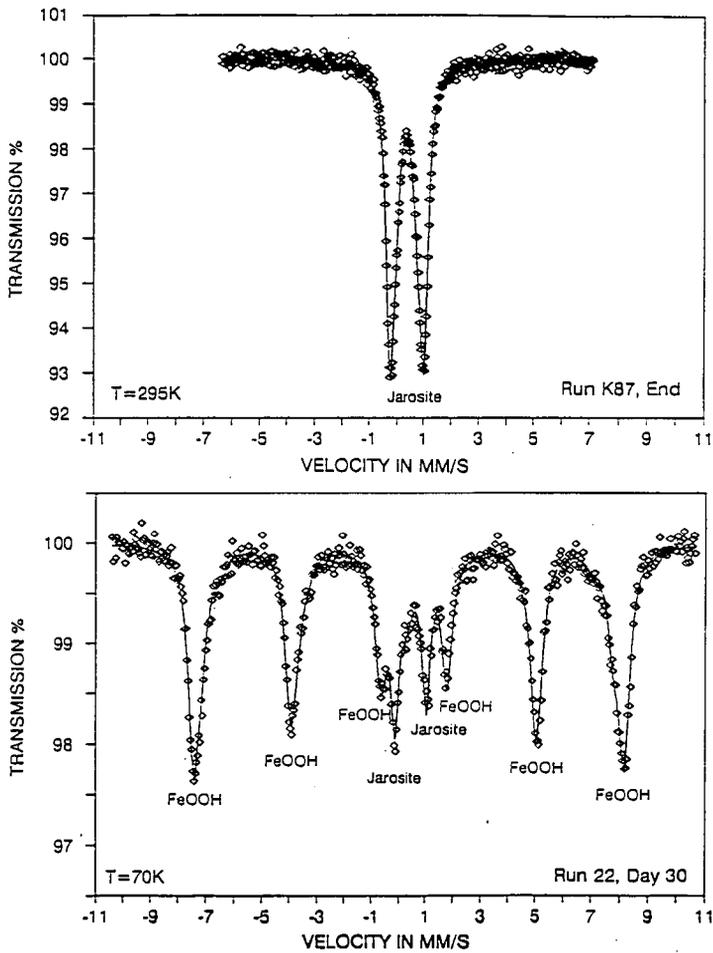


Fig. 1. Mössbauer spectra of biodesulfurized samples of Western Kentucky coals.