

ANAEROBIC BIOPROCESSING OF SUBBITUMINOUS COAL

Mahendra K. Jain*, Deborah Burgdorf, and Ramani Narayan
Michigan Biotechnology Institute, P.O. Box 27609, Lansing, MI 48909

Keywords: Anaerobic bioprocessing, coal decarboxylation, biocoal product

ABSTRACT

Subbituminous and lignite coals contain high levels of oxygen. Extensive structure reactivity studies of Wyodak coal done by us revealed that carboxy groups, along with ether linkages, are the predominant oxygen functionalities of this coal. Chemical decarboxylation to remove oxygen and upgrade the coal can be achieved at high temperatures and under such conditions the low rank coals undergo retrogressive reactions. We are developing a anaerobic microbial process to decarboxylate coal that would operate at ambient conditions. The anaerobic microbial consortia developed has resulted in decarboxylation of coal and anaerobically bioprocessed coal has exhibited an increase in H/C ratio in comparison to unprocessed coal. In this paper, we show our new results and their implications in microbial processing of coal in relation to the current bioprocessing schemes.

INTRODUCTION

The most common biological processes applied to coal conversion have been focussed on oxidative biosolubilization. The mechanism of such coal solubilization under aerobic conditions is probably dependent on the aromatic ring hydroxylations followed by ring scission. Such reaction results in oxygenated coal product which, in turn, makes the coal poorer fuel than the starting material. In addition, requirement of aeration makes the process highly energy intensive and less practical.

Anaerobic bioconversion of coal provides alternative technology for biological processing of coal. The major advantages of this approach is that it can: 1) remove oxygen from coal by decarboxylation; 2) cleave ether linkages of coal; and 3) reduce the contaminants such as sulfur from the coal. Moreover, the process is less energy intensive and requires simple engineering design. The approach to use anaerobic bacteria/enzyme promises to be most rewarding and exciting, since this involves depolymerization/solubilization of coal reductively, that is, the reduction of aromatic rings and promotion of reductive cleavages to produce hydrogenated products (Fig. 1). In other words, hydrogenation of coal via an anaerobic microbial process results in a more desirable fuel; oxidation via an aerobic microbial process results in a less desirable fuel form. Indeed, coal scientists throughout the ages have been trying to achieve this objective, that is, to find an inexpensive approach to producing a deoxygenated, hydrogen-rich coal fuel. In work done by us (Narayan, 1985; 1986 a,b; 1987; 1988; 1989) on the structure of Wyodak subbituminous coal (Figure 2) we have shown that carboxyl groups are a major oxygen functionality. They exist predominantly as carboxylate anions strongly chelating metal cations like Ca^{2+} and forming strong macromolecular crosslinks which contribute in large measure to the network polymer structure. Furthermore, the coal oligomer chains are not very long i.e. molecular weights of the coal clusters comprising the coal macromolecule have $M_n = 800-1000$ and $M_w = 2500-3500$.

Unfortunately, chemical or thermal decarboxylation can be performed only at elevated temperatures. This results in retrogressive crosslinking reactions. Indeed, Suuberg et al., (1985, 1987) have documented that low temperature crosslinking associated with low rank coal correlates well with evolution of CO₂. This correlation has been confirmed in recent work done by Solomon and co-workers (Deshpande et al., 1988; Solomon et al., 1988, 1990). In a structural sense, what these results imply is that at temperatures needed to remove the carboxyl group, the chains are still in close proximity, and the carboxylate and hydrogen bonded crosslinks are replaced by much stronger carbon-carbon covalent crosslinks. This results in a much more intractable coal macromolecule.

In principle then, removal of the carboxyl groups at room temperature (reductive decarboxylation) would unravel the macromolecular network, resulting in a very low molecular weight coal macromolecule with increased H/C ratio. With a increased hydrophobic character, this coal could be easily cleaned and serve directly as a solid fuel source. Since the coal macromolecular network has been dismantled, this coal could be easily processed in a subsequent liquefaction step. In summary, decarboxylation of coal at ambient temperatures has the potential for developing a coal product which has better fuel value and better processing prospects.

EXPERIMENTAL

All the experiments were carried out in anaerobic pressure tubes (27 mL, Bellco Glass, Inc., Vineland, NJ) and the manipulations were performed anaerobically using sterile syringes and needles. All chemicals and gases were of analytical grade. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Gases (N₂, H₂) and gas mixture N₂-CO₂ (95:5) and H₂-CO₂ (80:20) were obtained from Union Carbide Corp., Linde Division (Warren, MI) and passed over heated copper fillings to remove traces of O₂.

Liquid media and solutions were prepared and sterilized under a strictly anaerobic N₂ atmosphere by methods previously described (Zeikus, et al., 1980). The phosphate buffered basal (PBB) medium (Kenealy and Zeikus, 1981) was used for all the experiments. This medium was supplemented with (per 100 mL): 1.0 mL phosphate buffer, 1.0 mL vitamin solution (Wolin et al., 1963), 0.05 g yeast extract, and 2.5 mL of 2.5% Na₂S₉H₂O as well as, where added, 0.25 g supplemental carbon and energy source. Coal (Subbituminous, Wyodak) was added @ 0.15 g/10 mL media. The pH of the medium was about 7.0. Inoculum used in the present studies was obtained from a waste treatment site in Michigan and was collected and stored anaerobically at 4°C.

Initially, appropriate tubes were inoculated with mixed microbial consortia of anaerobic bacteria @ 5% and the tubes were then incubated at 37°C. Periodically the gas phase of the tubes was analyzed for CO₂ and CH₄. Gas samples were withdrawn from the tubes with a 1.0 mL glass syringe (Container Corp., Sioux City, Iowa) equipped with a gas tight mininert syringe valve (Alltech Associates, Inc., Deerfield, MI) and a 23 gauge needle. Coal was separated from medium by centrifugation at 4,500 rpm for 10 minutes, suspended in 3 N HCl and washed with distilled water until free of acid. The coal was then vacuum dried at 70°C for 24 hours before used for elemental analysis and subjected to FT-IR analyses. Carbon dioxide and methane gas was analyzed using a Gow-Mac series 580 gas chromatograph (GOW-MAC Instrument Co., Bridgewater, NJ) equipped with a thermal conductivity detector (TCD) and cabosttperre SS column with helium as carrier gas.

RESULTS AND DISCUSSION

Microbial Non-oxidative Decarboxylation of Coal:

Subbituminous Wyodak coal was used in the present studies since this coal contains much higher levels of oxygen than bituminous coals. It has been reported that the carboxyl groups account for an estimated two thirds of this oxygen (Sandreal and Wiltsee, 1984). Microorganisms capable of reductive decarboxylation of organic compounds may have the potential of removing carboxyl oxygen from coal. We, therefore, developed appropriate enrichments for developing anaerobic mixed cultures capable of decarboxylating coal under batch conditions. Various supplemental carbon and energy sources were used to support the growth of bacteria. The tubes containing coal and an additional carbon source were inoculated with a mixed microbial consortia and incubated at 37°C. The gas phase of these tubes was analyzed for CO₂. The coal was also analyzed for carbon and hydrogen and the change in H/C ratio was calculated.

The preliminary results presented in Table 1 indicate that CO₂ was produced in all the tubes and that level of CO₂ increased over the period of incubation. The increase in H/C ratio can be attributed to loss of carbon along with oxygen. Lactate, succinate and malonate supplemented tubes showed higher levels of CO₂ from coal than those supplemented with vanillate and glutamate. It seems that these substrates supported better growth of the microbial population that has decarboxylating enzymes. Crawford and Olson (1978) used vanillate as a model compound to examine microbial decarboxylation of complex aromatic compounds. They reported non-oxidative decarboxylation of vanillate by a single enzymatic transformation and also showed, using FT-IR, removal of carboxyl groups of coal when it was incubated with *Bacillus megaterium*. Decarboxylation of succinate to propionate under anaerobic conditions has also been observed using *Selenomonas ruminantium* (Scheifinger and Wolin, 1973), *Propionibacterium pentosaceum*, *Veillonella alcalescens* (Yousten and Delwiche, 1961; Samuelov et al., 1990) and *Propionigenium modestum* (Schink and Pfenning, 1982). Decarboxylation reaction also occurs when L-glutamate is anaerobically metabolized by *Acidaminococcus fermentans*, *Peptostreptococcus asaccharolyticus*, and *Clostridium symbiosum* (Dimroth, 1987). Thus, the supplemental carbon sources used in the present study have been shown by other groups to support growth of anaerobic cultures having decarboxylases.

Based on the preliminary experiment, succinate and lactate were selected to be used as supplemental carbon sources in conducting further experiments on coal decarboxylation. From the previous experiment it was not possible to conclude whether all or any CO₂ was produced from coal. Therefore, another experiment was designed to include controls without coal to determine CO₂ production from supplemental carbon sources as well. Also, since the tubes were inoculated from an anaerobic mixed microbial consortia that contained methanogenic population, it was likely that some of CO₂ will have converted to methane especially under the long-term incubation conditions. No attempts were made to inhibit methanogenesis since the adverse effects of inhibitors of methanogens on the organisms responsible for coal decarboxylation is not known. Therefore, gas phase of these tubes were analyzed for both CO₂ and CH₄. The results obtained are summarized in Table 2 and Figures 3 and 4.

The data presented in Table 2 show that CO₂ was produced from succinate as well as lactate. However, it is important to note that levels of CO₂ produced from succinate

supplemented coal and lactate supplemented coal were higher than succinate or lactate alone indicating that additional CO₂ was produced as a result of coal decarboxylation. In addition, methane was also produced and the increased levels of methane were observed in tubes containing coal and supplemental carbon source than in tubes that contained no coal. Figures 3 and 4 show decrease in CO₂ and increase in CH₄ levels after day 9. Also the levels of CO₂ and CH₄ produced from succinate or lactate were always lower than those obtained from coal supplemented with succinate or lactate. Acetate, methanol, methylamines, formate, H₂-CO₂, and CO are known to serve as methanogenic substrates to different methanogenic bacteria (Jain et al., 1988). Neither succinate nor lactate is a substrate for methanogens; however, under these experimental conditions methane is expected to be produced from acetate as well as CO₂. Succinate upon decarboxylation would be converted to propionate which in turn will be degraded to acetate by syntrophic propionate degraders. Acetate and CO₂ so produced will then be converted to methane by methanogenic bacteria. Syntrophic propionate degraders are very slow growing organisms and as a result propionate conversion to acetate is a slow reaction. It is likely that sudden increase in methane at 4 weeks time in coal supplemented with succinate may be the result of establishment of such a consortia (Figure 3). These results, however, clearly show decarboxylation of coal under anaerobic conditions. Since these experiments were carried out with mixed microbial consortia, it is not possible to hypothesize the number or type of organisms responsible for coal decarboxylation.

Energetics and Process Considerations:

One of the major problems plaguing coal bioprocesses is the consumption of coal carbon for microbial growth and maintenance. In the present decarboxylation scheme, the decarboxylation reaction is coupled to generation of an electrochemical gradient of sodium ions. This gradient can be transformed into a pH gradient that can be taken advantage of by the ATP synthase. The decarboxylation of oxaloacetate, for example, is associated with a free energy change of $\Delta G_o' = -30 \text{ kJ } (-7.2 \text{ kcal}) \text{ mol}^{-1}$ and one could expect synthesis of 1/3 ATP per 1 CO₂ formed (Gottschalk, 1986). The uptake of 3H⁺ per ATP synthesized would be in agreement with this assumption. Based on this assumption, a hypothetical model showing sodium dependent coal decarboxylation is proposed (Figure 5).

Another problem confronting coal bioprocesses particularly anaerobic is the reaction rate. In work done by us on succinate decarboxylation to propionate we used *Veillonella alcalescens* (Samuelov et al., 1990). Kinetic analysis of our results indicate that under steady-state conditions (D=0.02 h⁻¹) the optimal specific rate of propionate formation from succinate was 0.252 g propionate/g cells/h. The non-growth related production coefficient was 0.246 g propionate/g cells/h. The high ratio between these two kinetic parameters indicates that the decarboxylation energy was used mainly for culture maintenance. From the steady-state rate of propionate formation the apparent in-vivo specific activity of decarboxylation was calculated to be 90-100 m moles/mg protein/min (Samuelov, et al., 1990).

In conclusion, preliminary work reported in this paper demonstrates that decarboxylation of coal can be achieved at ambient temperature and pressure using anaerobic microbial catalyst. The ability to eliminate carboxyl groups and thereby break up of the macromolecular cross-links without the thermal retrogressive cross-linking reactions has major implications for processing of this low-rank subbituminous Wyodak coal and understanding its reactivity.

ACKNOWLEDGEMENTS

We thank Amit Lathia for assistance in elemental and FT-IR analyses of coal samples. This work was supported by funds from the Michigan Strategic Fund and the Kellogg Foundation to Michigan Biotechnology Institute.

REFERENCES

1. Crawford, R.L. and P.P. Olson, *Appl. Environ. Microbiol.* 36:539, (1978).
2. Dimroth, P., *Microbiol. Rev.* 51:320-340, (1987).
3. Deshpande, G.V., P.R. Solomon and M.A. Serio, *ACS Fuel Chem. Preprints* 33(2):310 (1988).
4. Gottschalk, G., *Bacterial Metabolism*, pp. 359, Springer-Verlag, New York, (1986).
5. Jain, M.K., L. Bhatnagar and J.G. Zeikus, *Indian J. Microbiol.*, 28:143-177 (1988).
6. Kenealy, W. and J.G. Zeikus, *J. Bacteriol.* 146:133-140 (1981).
7. Narayan, R., Abstracts of Papers, 189th National Meeting of the Amer. Chem. Soc., Miami, FL, INDE, 24, (1985).
8. Narayan, R., EPRI AP-4441, Final Report, Feb. 1986a.
9. Narayan, R., Proc. 10th Annual EPRI Contractors Conf., EPRI AP-4253-SR, p. 7, 1986b.
10. Narayan, R., Proc. 11th Annual Conf. on Clean Liquids and Solid Fuels, EPRI AP-5043-SR, 5-53, April, (1987).
11. Narayan, R., Proc. First Annual Workshop on Biological Processing of Coal, EPRI, EPRI ER-5709-SR, March, (1988).
12. Narayan, R., Proc. 1987 Workshop on Coal Structure, EPRI ER-6099-SR, November (1988).
13. Narayan, R., Final Report, EPRI GS-6473 Research Project 8003-1, July, (1989).
14. Samuelov, N., R. Datta, M.K. Jain, and J.G. Zeikus, *Ann. NY Acad. Biochem. Engg.* (in press) (1990).
15. Sandreal, E.A. and G.A. Wiltsee, *Ann. Rev. Energy*, 9:473 (1984).
16. Scheifinger, C.C. and M.J. Wolin, *Appl. Microbiol.* 26:789-795, (1973).
17. Schink, B. and N. Pfenning, *Arch. Microbiol.* 133:209-216, (1982b).
18. Solomon, P.R., D.G. Hamblen, R.M. Carangelo, M.A. Serio and G.V. Deshpande, *Energy and Fuels*, 2:405 (1988).
19. Solomon, P.R., M.A. Serio, G.V. Deshpande, and E. Kroo, *Energy and Fuels* (in press), (1990).
20. Suuberg, E.M., D. Lee and J.W. Larsen, *Fuel* 64:1668, (1985).
21. Suuberg, E.M., P.E. Unger and J.W. Larsen, *Energy and Fuels*, 1:305, (1987).
22. Wolin, E.A., M.R.J. Wolin and R.S. Wolfe, *J. Biol. Chem.*, 238:2882-2886 (1963).
23. Yousten, A.A., and E.A. Delwiche, *Bacteriol. Proc.* 61:175 (1961).
24. Zeikus, J.G., A. Ben-Bassat and P.W. Hegge, *J. Bacteriol.*, 143:432-440 (1980).

Table 1. Coal Decarboxylation by an Anaerobic Microbial Consortium in Presence of Other Carbon Sources.

	% CO ₂ in Gas Phase After		*H/C Ratio in decarboxylated coal at 100 days
	15 days	100 days	
Coal + Succinate	9.86	16.17	1.10
Coal + Lactate	5.98	19.04	1.37
Coal + Malonate	12.72	14.65	1.22
Coal + Vanillate	1.58	9.47	1.08
Coal + Glutamate	5.38	9.69	1.14

*H/C ratio of original control = 0.92

Table 2. Decarboxylation of coal in presence of succinate or lactate as supplemental carbon source.

Substrate	Gases after 9 days	
	CO ₂ (%)	CH ₄ (mM)
Succinate	8.74	1.04
Coal + Succinate	14.63	1.99
Lactate	8.47	1.83
Coal + Lactate	16.34	6.09

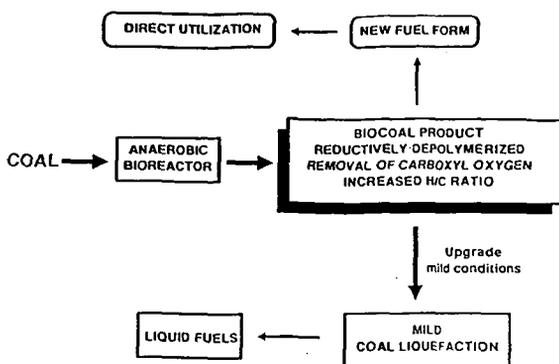
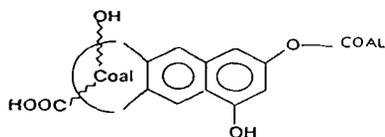
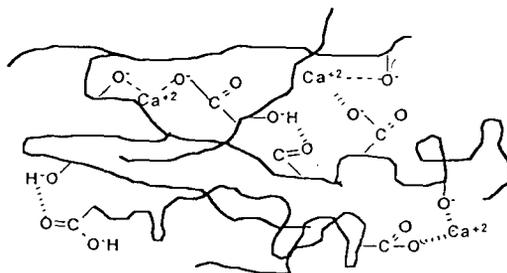


Figure 1. Schematic anaerobic bioprocess technology for conversion of coal to biocoal product and its further use.



Carboxy and dioxy groups are major oxygen functionalities



Network polymer structure due to secondary forces - hydrogen bonding and chelate crosslinks

Figure 2. Salient structural features of Wyodak subbituminous coal (Narayan, 1989).

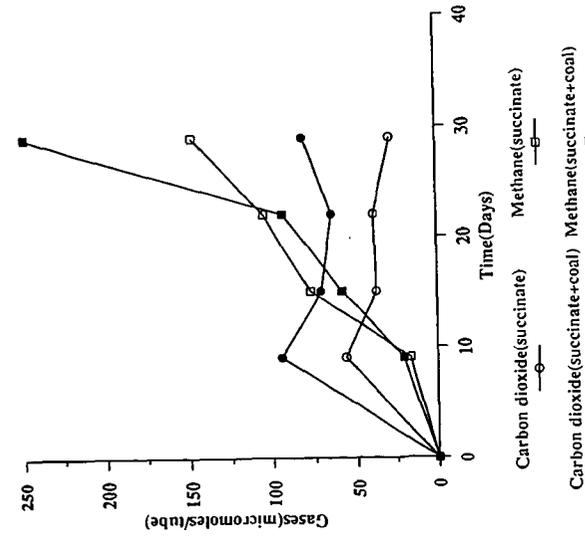


Fig.3. Production of carbon dioxide and methane from Succinate, and Coal + Succinate using anaerobic microbial consortium

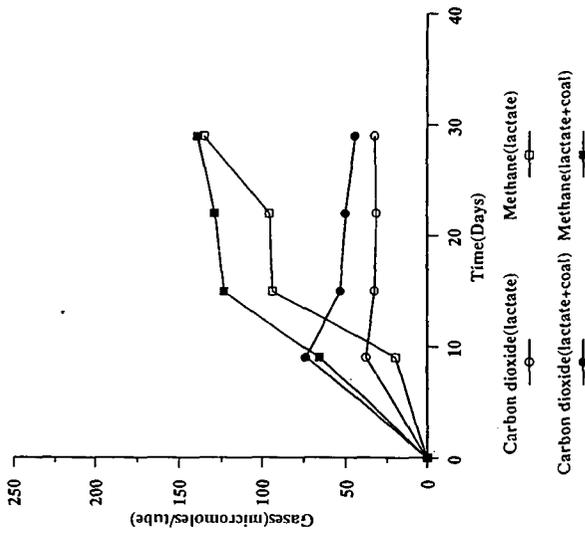


Fig.4. Production of carbon dioxide and methane from Lactate, and Coal + Lactate using anaerobic microbial consortium

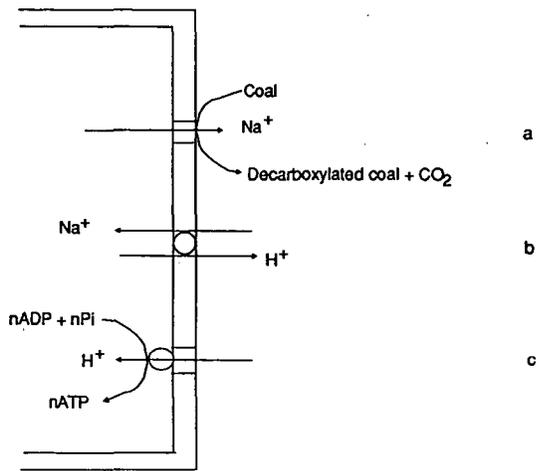


Figure 5. Hypothetical model showing sodium-dependent coal decarboxylation a: sodium translocation as coupled to the decarboxylation reaction. b: Na^+ - H^+ antiporter. c. Proton-translocating ATP synthase; n may be in the order of 1/3.

HYDROGEN PEROXIDE, PEROXIDASES AND LOW RANK COAL

D. R. Quigley, C. R. Breckenridge, J. K. Polman and P. R. Dugan
Biotechnology Unit and Center for Bioprocessing Technology
Idaho National Engineering Laboratory
Idaho Falls, ID 83415-2203

Keywords: peroxidases, biosolubilization, hydrogen peroxide

ABSTRACT

Horseradish peroxidase was tested to determine if it catalyzed any reaction between coal and hydrogen peroxide. Experiments were performed in aqueous buffers of either pH 5.0, 6.5 or 8.0 and used either particulate Wyodak, Beulah Zap, Texas lignite, Mississippi Wilcox lignite or solubilized Mississippi Wilcox lignite. Reactions were monitored by determining amounts of hydrogen peroxide consumed at various points in time. All coals reacted rapidly with hydrogen peroxide in the absence of peroxidase and these reaction rates increased as pH increased. Horseradish peroxidase did not measurably increase these reaction rates even when present in large concentrations. These data suggest that coal is not a substrate for horseradish peroxidase.

INTRODUCTION

Reports in the early 1980's that microorganisms were able to transform low rank coals into a liquid or water soluble form (1,2) generated interest in using bioprocessing as a possible route to convert coal into liquid fuels. Many organisms and enzyme systems have been examined since then for their ability to solubilize and/or depolymerize coal. Those enzyme systems that have received the most attention are those that catalyze the oxidative cleavage of carbon-carbon bonds in model compounds. These enzymes include laccases and peroxidases.

Laccase from *Trametes versicolor* (a.k.a. *Coriolus versicolor* and *Polyporus versicolor*) has been extensively studied (3,4). This enzyme utilizes molecular oxygen to cleave carbon-carbon bonds adjacent to aromatic nuclei (5) and has been implicated as the agent produced by *T. versicolor* responsible for the organism's coal solubilizing ability (1,3,4). Subsequent work indicated that the majority of coal solubilizing activity present in culture fluids was low in molecular weight (<1,000) and did not appear to be a laccase. A higher molecular weight material that had some coal solubilizing activity was found, but this was later identified as an esterase (6,7,8). It now appears that laccases are not significantly involved in coal biosolubilization.

Peroxidases are enzymes that utilize hydrogen peroxide and include horseradish peroxidase (HRP) and lignin peroxidases. When these enzymes are incubated with hydrogen peroxide (HP) and coal in either aqueous or nonaqueous solutions, coal biosolubilities increase (9,10). These increases have been attributed to the action of the peroxidases, but, since HP is quite reactive with coal, it is not known whether the rate of the enzyme catalyzed reaction is significant as compared to the nonenzymatic chemical reaction. In this manuscript we report on the relative rates of HRP catalyzed reactions as compared to the nonenzyme catalyzed chemical reaction.

EXPERIMENTAL

Mississippi Wilcox, Texas lignites were obtained as described and ground to -100 mesh (11). Beulah Zap (-100 mesh) and Wyodak (-100 mesh) were obtained from the Argonne Premium Coal Bank, Argonne, IL. Horseradish peroxidase type II (HRP) was obtained from Sigma Chemical Company, St. Louis, MO.

Solubilized Wilcox was obtained by placing 5g of coal in 1l of 50mM Tris buffer, pH 8.0. After mixing for several days, the suspension was centrifuged at 10,000xg for 20 minutes and the supernatant obtained. Solubilized coal was precipitated by acidifying the supernatant to pH 2 with HCl and allowing the suspension to stand for several hours. Precipitated coal was collected by centrifugation, washed several times with 1mM HCl and redissolved in 50mM Tris buffer. This solution was then filtered (Gelman type GA, 0.2 μ m pore dia.) to remove any insoluble material. The final concentration was approximately 2g coal/liter. Iron concentrations present in the solubilized coal was determined using an ARL Model 3520 atomic absorption spectrophotometer in the ICP mode.

Assays to determine relative rates of enzyme and nonenzyme catalyzed reactions were performed at 30°C and used either 1g of particulate coal or 5ml of solubilized coal. Reactions were initiated by the addition of 50ml of the appropriate buffer containing approximately 1mM HP. Actual concentrations of HP present were determined iodometrically (12). Buffers used were 500mM acetate, pH 5.0, 500mM phosphate, pH 6.5 and 500mM Tris, pH 8.0. At indicated time points, 50 μ l aliquots were removed from the reaction mixture and concentrations of HP present determined using the leucocrystal violet assay (13). Controls included HP with no additions, HP with 5mM resorcinol (positive control) and HRP, resorcinol with no other additions and HP with coal, resorcinol and HRP (positive control).

RESULTS AND DISCUSSION

Hydrogen peroxide, in the absence of HRP, reacted rapidly with all coals at each pH tested (Figure 1). Reaction profiles were similar in every case. Initial reactions were rapid and slowed as the reaction proceeded. This reaction appeared to be second order with respect to peroxide since a plot of 1/peroxide vs. time was linear (Figure 2). Assuming that this reaction was second order, values for the reaction constants could be obtained by determining slopes of lines generated by these plots (Table 1). Typically, these plots yielded lines with correlation coefficients 0.975 or better. As a general rule reactions were slowest at lower pH values and increased as pH increased. This is consistent with the literature indicating that hydrogen peroxide becomes a more powerful oxidizing agent as pH increases (14).

The reaction of HP with resorcinol as catalyzed by HRP was used as a positive control. This reaction not only demonstrated that HRP was active, but also gave an indication as to the activity of the enzyme with a preferred substrate and yielded some insight into the peroxidase reaction. Once again, a plot of 1/peroxide vs time yielded a straight line indicating that this reaction was second order with respect to HP (Figure 2). This result was obtained every time the experiment was performed and is consistent with earlier reports in the literature (15,16).

Because rates were determined by measuring HP disappearance, it was not known if the disappearance of HP was due to coal oxidation. Coals were not analyzed to determine if the coal itself was being oxidized because the amount of oxidation occurring would have been too small to measure using ultimate analyses. Other possible causes that could have accounted for the disappearance of HP were, therefore, investigated. An alternate material that might have been oxidized at the expense of HP would have been pyrite (17). The ability of HP to oxidize pyrite using the above conditions indicated that no significant rates of pyrite oxidation occurred at pH values of 5.0 and 6.5. At pH 8.0, significant amounts of HP were consumed in short periods of time indicating that pyrite was being oxidized (data not shown). Another possibility was that iron ions present in the coal could catalytically decompose HP (18). Solubilized Wilcox coal was analyzed and found to contain 1mM iron. Addition of 0.5mM and 1mM Fe⁺⁺⁺ to solubilized Wilcox did not increase rates of HP consumption and the addition of 2mM Fe⁺⁺⁺ resulted in only a slight increase in HP consumption (data not shown). These data suggest that the carbon present in the coal was being oxidized.

The addition of HRP to reaction mixtures containing both coal and HP generally had very little effect upon rates of HP consumption (e.g., Figure 1) and in most cases decreased rates of HP disappearance (Table 1). The lack of change in rates of HP consumption in the presence of coal was not due to the coal rendering the HRP inactive since HRP was able to oxidize resorcinol in the presence of coal. Also, the amount of enzyme in reaction mixtures containing coal was 10-fold greater than that present in positive controls containing resorcinol. Since no significant increases in rates of HP consumption were observed when large quantities of HRP were present in coal mixtures, then at least one of two possibilities must have existed. The first possibility was that HRP was unable to use coal as a substrate. A second possibility was that HRP was able to use coal as a substrate, but the number of sites where this could be done was extremely small which would have limited increases in HP consumption to below detection limits. Also, if there were such a small number of sites present, then increasing the amount of peroxidase present in reaction mixtures would not result in an increase in HP consumption.

Results from this study indicate that HRP is able to catalyze little or no reaction between coal and HP. Since peroxide reacts readily with coal and since HRP is an expensive enzyme, it would appear that continued use of HP in the presence of HRP would be of little value. This work does not preclude the possibility that other more powerful peroxidases (e.g., lignin peroxidases) might be able to catalyze the oxidative depolymerization of coal. A study involving the ability of lignin peroxidases to oxidatively depolymerize coal will be the subject of a subsequent manuscript.

ACKNOWLEDGEMENTS

This work was supported under contract no. DE-AC07-76ID01570 from the U.S. Department of Energy, Office of Advance Research and Technology Development, Office of Fossil Energy to the Idaho National Engineering Laboratory/ EG&G Idaho, Inc.

REFERENCES

1. Cohen, M. S. and P. D. Gabriele. 1982. Appl. Environ. Microbiol. 44:23.
2. Fakoussa, R. M. 1981. Ph.D. Thesis, Freidrich-Wilhelms University, Bonn, Federal Republic of Germany.
3. Cohen, M. S., W. C. Bowers, H. Aronson and E. T. Gray, Jr. 1987. Appl. Environ. Microbiol. 53:2840.

4. Pyne, J. W., D. L. Stewart, J. Fredrickson and B. W. Wilson. 1987. Appl. Environ. Microbiol. 53:2844.
5. Dodson, P. J., C. S. Evans, P. J. Harvey and J. M. Palmer. 1987. FEMS Microbiol. Lett. 42:17.
6. Campbell, J. A., D. L. Stewart, M. McCulloch, R. B. Lucke and R. M. Bean. 1988. Prep. Pap.-Am. Chem. Soc., Div. Fuel Chem. 33(4):515.
7. Pyne, J. W., D. L. Stewart, J. C. Linehan, R. M. Bean, M. A. Powell, R. B. Lucke, B. L. Thomas, J. A. Campbell and B. W. Wilson. 1987. Proceedings of the Biological Treatment of Coals Workshop, U.S. Department of Energy, pp. 174-194.
8. Bean, R. M., J. K. Fredrickson, J. A. Campbell, D. L. Stewart, J. A. Franz, B. L. Thomas, M. McCulloch, J. C. Linehan and B. W. Wilson. 1989. Proceedings: 1989 symposium on Biological Processing of Coal and Coal-Derived Substances, Electric Power Research Institute, pp. 3-1 - 3-25.
9. Scott, C. D. and S. N. Lewis. 1988. Appl Biochem. Biotech. 18:403.
10. Scott, C. D., C. A. Woodward, J. E. Thompson and S. L. Blankinship. 1990. Appl. Biochem. Biotech. In Press.
11. Quigley, D. R., C. R. Breckenridge, P. R. Dugan and B. Ward. 1989. Energy Fuel. 3:371.
12. Allen, A. O., C. J. Hochanadel, J. A. Gormley and T. W. Davis. 1952. J. Phys. Chem. 56:575.
13. Mottola, H. A., B. E. Simpson and G. Gorin. 1970. Anal. Chem. 42:410.
14. Sidgwick, N. V. 1950. Chemical Elements and Their Compounds, Vol II. Oxford University Press. pp. 868-874.
15. Chance, B. 1951. Advances in Enzymology. 12:153.
16. Chance, B. 1949. Arch. Biochem. 22:224.
17. Boron, D. J., A. G. Dietz and S. R. Taylor. 1981. Fuel. 60:991.
18. Walling, C. 1975. Acc. Chem. Res. 8:125.

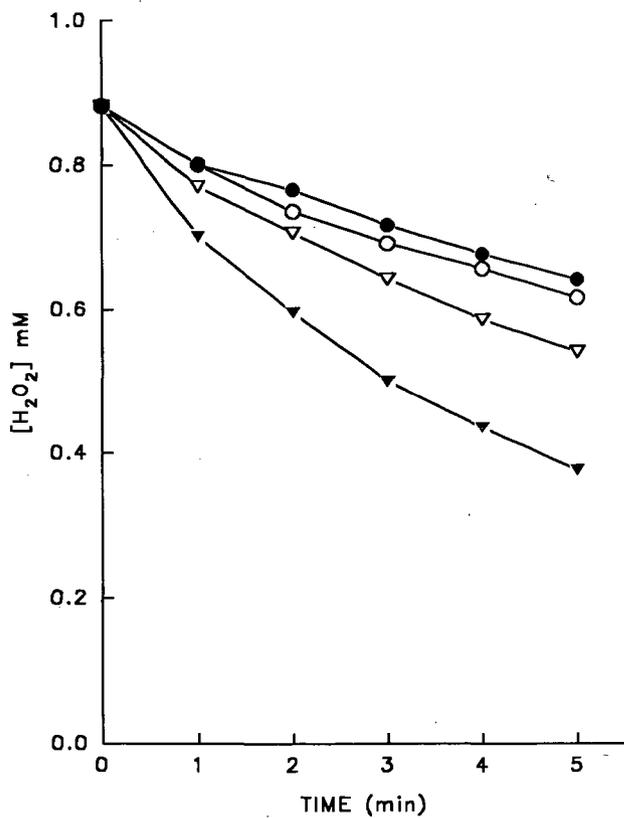


Figure 1. Disappearance of Hydrogen Peroxide in the Presence of Texas Lignite and Resorcinol at pH 8.0. Reaction mixtures are as described. Texas lignite = open circles; Texas lignite with HRP = closed circles; Resorcinol with HRP = open triangles; Texas lignite with Resorcinol and HRP = closed triangles.

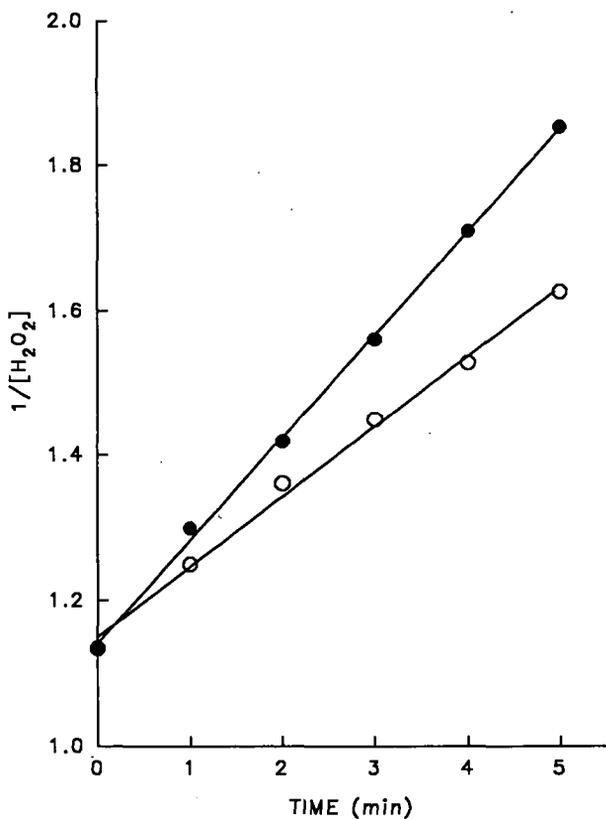


Figure 2. Plot of Inverse Hydrogen Peroxide Concentration vs. Time. Data taken from Figure 2. Correlation coefficients are 0.998 and 0.999 respectively for Texas lignite reacting with HP in the absence of HRP (open circles) and resorcinol reacting with HP in the presence of HRP (closed circles).

Coal		pH		
		5.0	6.5	8.0
Wilcox lignite	-HRP	0.078	0.146	0.269
	+HRP	0.053	0.112	0.319
Beulah Zap	-HRP	N.M.	0.029	0.064
	+HRP	N.M.	0.025	0.051
Texas lignite	-HRP	0.017	0.119	0.097
	+HRP	0.015	0.080	0.084
Wyodak	-HRP	0.073	0.058	0.109
	+HRP	0.045	0.039	0.099
Soluble Wilcox	-HRP	0.037	0.044	0.029
	+HRP	0.031	0.043	0.036

Table 1. Rate Constants for the Reaction of Various Coals and Hydrogen Peroxide in the Presence and Absence of Horseradish Peroxidase. -HRP indicates the absence of horseradish peroxidase; N.M. indicates not measured. Values are given as (mmoles/liter/min)⁻¹.

**CHARACTERIZATION OF EXTRACELLULAR BACTERIAL ENZYMES WHICH
DEPOLYMERIZE A SOLUBLE LIGNITE COAL POLYMER**

**Don L. Crawford
Rajinder K. Gupta**

**Department of Bacteriology and Biochemistry
Institute for Molecular and Agricultural
Genetic Engineering (IMAGE)
University of Idaho
Moscow, Idaho 84843**

KEYWORDS: Streptomyces
Depolymerization

ABSTRACT

Several Gram negative and positive soil bacteria, isolated by enrichment technique in a liquid minimal medium containing water soluble lignite coal polymer as a sole source of carbon and energy, were screened for their abilities to depolymerize coal when growing in peptone broth containing soluble coal. One Gram positive and 3 Gram negative strains significantly depolymerized the coal within 3-6 days. These strains were screened for the production of extracellular coal depolymerizing enzymes. Each bacterium, including Gram negative strains DLC-BB2, DLC-62 and DLC-63/9, and Gram positive strain DLC-21, produced lignite depolymerizing enzymes. Extracellular filtrates from 3-day cultures grown in peptone medium supplemented with an inducing level of soluble coal polymer, contained an enzymatic activity which caused significant depolymerization of the coal polymer after 4 hr of incubation of enzyme with coal (30°C), as shown by High Performance Liquid Chromatography. Each bacterium produced similarly acting enzymes which progressively converted the principal broad coal polymer peak of about 174,000 MW into a much sharper peak of about 113,000 MW. In some cases, even lower molecular weight products appeared upon prolonged incubation of the reaction mixtures. The activities were inactivated by boiling of culture supernatants. While chemical analyses of enzymatically depolymerized coal products indicated that the depolymerization was non-oxidative, the specific type(s) of enzymes involved in the depolymerization remain to be identified. The results show that nonoxidative, enzymatic depolymerization of coal is possible.

INTRODUCTION

Selected biotransformations of coal may aid in its conversion to liquid and/or gaseous fuels. Such transformations include depolymerizations (1), reductions (2), and/or solubilization (3-6). Both fungi and bacteria have been shown to solubilize coal. Some appear to excrete

enzymes that oxidize coal into water soluble polymeric products (7-9). However, coal solubilization more typically involves the excretion of basic, low molecular weight coal-solubilizing metabolites by the microbes, and coal solubilization is nonenzymatic (3-5, 10-12). Few papers have reported the microbiological depolymerization of coal. One paper (13) reported that lignin peroxidase from the white-rot fungus Phanerochaete chrysosporium oxidized water soluble coal polymers, converting them into smaller molecular weight polymers. Pseudomonas cepacia Strain DLC-07, when growing in liquid media at pH 5.5 depolymerized a water soluble Vermont lignite coal polymer (1). Depolymerization was found to be optimal when the bacterium was grown on coal in a mineral salts-peptone-soluble coal polymer medium (14). Here, we report on characterizations of 3 Gram negative and 1 Gram positive aerobic bacterial strains which metabolize lignite coals. These strains significantly depolymerize base-solubilized lignite when growing on the coal in liquid media and excrete enzymes which catalyze lignite depolymerization via a non-oxidative mechanism. This is the first report of such enzymes from both Gram negative and positive bacteria. Our results are indicative of the positive potential for using bacteria to biotransform coal into useful liquid fuels.

MATERIALS AND METHODS

Isolation of Aerobic Bacteria. The bacteria were isolated from soil associated with coal seams (1) and from other soils rich in decomposing plant residues. The enrichment medium (pH 5.5) was a mineral salts solution supplemented with 0.01% (w/v) of yeast extract (Difco, Detroit, MI) and 0.2% (w/v) of soluble, nitric acid pre-treated Alabama lignite coal polymer (1). The enrichment-isolation procedure was carried out at 30°C as described previously (1). Stock cultures of each bacterium were maintained on Sabouraud Dextrose Agar (SDA) slants at 4°C. Strains DLC-62, DLC-63/9, and DLC-BB2 are as yet unidentified aerobic Gram negative rods, while strain DLC-21 is a spore-forming, Gram positive Bacillus species.

Assay for Extracellular Coal Depolymerizing Enzyme Activity. Each bacterium was grown in shake flasks for three days (30°C) in 250 ml of mineral salts-peptone broth (14) also containing 2mg of water soluble coal polymer. Cultures were then harvested, and the cells removed by centrifugation. The supernatant was used as the source of enzyme. If desired, the supernatants can be concentrated 10-fold by ultrafiltration prior to use. For the assay, 100 ul of soluble coal polymer solution was added to 10 ml of crude or concentrated supernatant. After 0 and 4 hr incubation (30°C; pH 5.5) the reaction was stopped by acidifying 1.0 ml of the solution to pH 2.0. The precipitated coal was collected by centrifugation, washed, redissolved in 1.0 ml

of HPLC solvent, and analyzed by HPLC. Controls contained reaction mixtures treated similarly, but heat-inactivated supernatant was substituted for active enzyme. Coal depolymerization was monitored by observing the appearance of lower molecular weight HPLC peaks.

Coal Substrate Used and Growth of Bacteria on Coal. The coals used was a weathered Vermont lignite (1,2). A coal substrate water soluble at pH 5.5 or higher was prepared from the Vermont lignite as described by Gupta *et al.* (1). One hundred grams of powdered coal were soaked in water for 8 hr and then dissolved in 1 liter of 1N NaOH. The solution was centrifuged to remove undissolved coal, and the pH of the supernatant was adjusted to pH 7.0 with HCl. Coal that precipitated was collected, washed, dried, and powdered (yield: 40 g). The supernatant from the precipitation was acidified to pH 5.5, and the resulting precipitate was collected, washed (pH 5.5 water), dried, and powdered (yield: 15 g). The remaining coal in solution was precipitated at pH 1.5, collected, washed (pH 1.5), and powdered (yield: 10 g). This final coal polymer precipitate, which was soluble at pH's of 5.5 or higher, was used as the substrate in both growth and enzyme studies.

Molecular Weight Distribution of the Coal Polymers. Bacterial depolymerization of the soluble coal polymer was monitored by HPLC, as described previously (1), using a Hewlett Packard 1090A instrument equipped with an HP-1040 diode array detector and a Synchropak GPC-300 column (1000-500,000 MW separation capability) (Synchrom Inc., Lafayette, IN). The mobile phase consisted of phosphate buffer (0.02M KH_2PO_4) containing 0.5% (w/v) Tween 80, pH 7.1, set at a flow rate of 0.25 ml min^{-1} . The column was equilibrated with high and low molecular weight protein standards (1). Coal containing culture medium samples (0.5 ml) were centrifuged, the supernatants acidified to pH 2, and the precipitated coal recovered by centrifugation. The wet precipitate was redissolved in 0.5 ml of HPLC solvent and injected into the HPLC. Elution of the coal polymer was then monitored at 254 nm.

Elemental Analysis of the Coal Polymers. Elemental analysis of 1-3 mg samples of control and depolymerized coal polymers were performed by Desert Analytics, Inc. (Tucson, AZ) according to the procedure described by Gupta *et al.* (5).

RESULTS

Coal Depolymerization During Growth of the Bacteria. From among numerous bacteria isolated from the liquid enrichments, 4 were selected for their ability to depolymerize the soluble Vermont lignite coal in liquid mineral salts-peptone broth. Micrographs of each organism, including Gram negative strains DLC-62, DLC-63/9, and DLC-

BB2 and Gram positive strain DLC-21 are shown in Figure 1. DLC-21, the only Gram positive bacterium in the group, is a nonmotile, spore-forming rod (Fig 1,A). We have classified it as a Bacillus species. All of the Gram negative bacteria are strictly aerobic. DLC-62 and DLC-BB2 are both highly motile, short rods (Fig 1,B and D), while DLC-63/9 is a very long and thin nonmotile rod (Fig 1,C).

As shown in Figures 2 and 3, each bacterium significantly depolymerized soluble Vermont lignite coal polymer within 3 days when growing in mineral salts-peptone-coal polymer broth. Over the same period, the molecular weight of the coal polymer (130,000) remained unchanged in incubated, uninoculated, controls (Figs. 2A, 2B). In contrast, depolymerization resulting from bacterial metabolism (Figs. 3A-D) was significant, and greater than that previously seen with Pseudomonas cepacia DLC-07 after growth for 2 weeks on coal in the same medium (1).

Detection of Extracellular Coal Depolymerizing Enzymes.

Preliminary assays for the presence of extracellular enzymes catalyzing coal depolymerization were carried out by incubating cell free culture filtrates from 3-day cultures grown in mineral salts-peptone broth supplemented with an inducing level of coal polymer (2mg/250ml). Each bacterium produced lignite depolymerases. Cell free culture filtrates from each of the cultures enzymatically transformed the coal polymer within a period of 1-4 hr. (data not shown). HPLC elution profiles shifted to lower molecular weight peaks in a pattern like that observed when the bacteria were grown on the coal polymer in peptone broth. In contrast, elution profiles of reaction mixtures incubated with heat-inactivated (boiled; 5 min) culture filtrates replacing untreated filtrate (=inactivated controls) did not change over the incubation period. In active filtrates HPLC elution profiles of the coal polymer shifted from a single major peak with a retention time corresponding 167,000-174,000 MW to a sharper peak averaging about 113,000 MW, and having a higher molecular weight shoulder of 144,000-170,000 MW. With several of the reaction mixtures, a shoulder peak corresponding to about 87,000 MW also appeared after extended incubation. All of the bacteria produced similarly acting enzymes. The time of harvesting of the filtrates was critical to their activity. If harvested too early or too late, activities were low.

Elemental Composition of the Coal Polymers.

Elemental analyses of the depolymerized coal polymers showed no major changes in C, H, O, N, or S content relative to the starting coal (Table 1). The ash contents increased slightly. These data show that the depolymerizations were non-oxidative, although additional data will be needed to refine this conclusion.

DISCUSSION

This is the first report of coal depolymerizing enzymes in Gram positive and negative bacteria. Previously, Wondrack et al. (13) reported that the lignin peroxidase of the fungus Phanerochaete chrysosporium would depolymerize soluble base-solubilized coal polymers. The enzyme was oxidative, utilizing H_2O_2 to oxidize the coal. The enzymes produced by our bacterial isolates, in contrast, act non-oxidatively, perhaps hydrolytically.

We have found that careful timing of culture harvest is critical to obtaining optimally active enzyme preparations. Thus, we need to carry out a study that relates coal depolymerizing enzyme production to the growth curves of each of these bacteria. Then, we can establish the optimal incubation times for production of the enzymes by each strain. The rapid rate of depolymerization we observe (1-4 hr), and the appearance of a sharp product peak in HPLC chromatograms lead to some intriguing hypotheses concerning the nature of these enzymes. The enzymes may act to cleave structurally important internal linkages such as ether or ester bonds within the lignite macromolecule, thereby significantly depolymerizing the polymer. The remaining undepolymerized polymer, accumulating as the 113,000 MW peak in HPLC chromatograms, may represent a macromolecular structure more resistant to the enzymes and/or not containing susceptible linkages. Additional research will hopefully determine if this is the case. Regardless, the overall data show clearly that bacterial enzymes may be useful in breaking down the macromolecular structure of coal to produce lower molecular weight products potentially useful in the microbiological production of liquid or gaseous fuels.

ACKNOWLEDGMENTS

This research was supported by grant DE-FG22-88PC88919 from the U.S. Department of Energy, Pittsburgh Energy Technology Center, by a subcontract from EG&G Idaho, Inc. funded by the U.S. Department of Energy under contract DE-AC07076ID01570, and by the Idaho Agricultural Experiment Station.

REFERENCES

- 1) Gupta, R.K., L.A. Deobald, and D.L. Crawford. 1990. Depolymerization and Chemical Modification of Lignite Coal by Pseudomonas cepacia Strain DLC-07. Appl. Biochem. Biotechnol., 24/25: In press.
- 2) Deobald, L.A. and D.L. Crawford. "Isolation of Microorganisms Able to Reductively Transform Aromatic Compounds and Their Relevance to Coal Liquefaction. In Proceedings of the Second International, Institute of Gas Technology Symposium on Gas, Oil, and Coal Biotechnology. December 11-13, 1989. New Orleans, LA.
- 3) Scott, C.D., G.W. Standberg, and S.N. Lewis. "Microbial Solubilization of Coal," Biotech. Progress, 2: 131-139 (1986).
- 4) Quigley, D.R., B. Ward, D.L. Crawford, H.J. Hatcher, and P.R. Dugan. "Evidence that Microbially Produced Alkaline Materials are Involved in Coal Biosolubilization." Appl. Biochem. Biotechnol., 20/21: 753-763 (1989).
- 5) Gupta, R.K., J.K. Spiker, and D.L. Crawford. "Bio-transformation of Coal by Ligninolytic Streptomyces," Can. J. Microbiol., 34: 667-674 (1988).
- 6) Cohen, M.S. and P.D. Gabriele. "Degradation of Coal by the fungi Polyporus versicolor and Poria monticola," Appl. Environ. Microbiol., 44: 23-27 (1982).
- 7) Cohen, M.S., W.C. Bowers, H. Aronson, and E.T. Gray, Jr. "Cell-free Solubilization of Coal by Polyporus versicolor." Appl. Environ. Microbiol., 53: 2840-2843 (1987)/
- 8) Pyne, J.W., D.L. Stewert, J. Fredrickson, and B.W. Wilson. "Solubilization of Leonardite by an Extracellular fraction from Coriolus versicolor," Appl. Environ. Microbiol., 53: 2844-2848 (1987).
- 9) Moolik, R.T., J.C. Linden, and M.N. Karim. "Bio-solubilization of Lignite," Appl. Biochem. Biotechnol., 20/21: 731-742 (1989).
- 10) Maka, A., V.J. Srivastava, J.J. Kilbane II, and C. Akin. "Biogeochemical Solubilization of Untreated North Dakota Lignite by a Mixed Bacterial and a Mixed Bacterial-Fungal Culture," Appl. Biochem. Biotechnol., 20/21: 715-729 (1989).

- 11) Strandberg, G.W. and S.N. Lewis. "The Solubilization of Coal by an Extracellular Product of Streptomyces setonii," J. Ind. Microbiol., 1: 371-375 (1987).
- 12) Faison, B.D. and S.N. Lewis. "Production of Coal-Solubilizing Activity by Paecilomyces sp. During Submerged Growth in Defined Liquid Media," Appl. Biochem. Biotechnol., 20/21: 743-752 (1989).
- 13) Wondrack, L., M. Szanto, and W.A. Wood. "Depolymerization of Water Soluble Coal Polymer from Subbituminous Coal and Lignite by Lignin Peroxidase," Appl. Biochem. Biotechnol., 20/21: 765-780 (1989).
- 14) Crawford, D.L. and R.K. Gupta. "Influence of Cultural Parameters on the Depolymerization of a Soluble Lignite Coal Polymer by Pseudomonas cepacia DLC-07," Resources, Conservation, and Recycling, In Press.

Table 1. Elemental Analyses of Control and Bacterially Depolymerized Coal Polymer Samples after Incubation in the Peptone-Coal Polymer Broth for ? days.

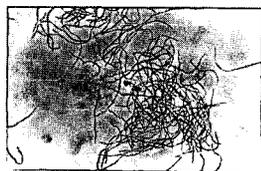
SAMPLE	%C	%H	%N	%O	%S	ASH
Control	49.1	4.1	3.3	35.6	1.0	3.8
DLC-21	49.9	4.0	3.1	33.8	1.5	4.8
DLC-62	50.7	4.0	3.1	35.0	1.2	4.2
DLC-63/9	51.0	4.0	3.1	35.4	1.3	5.1
DLC-BB2	50.6	4.0	3.2	35.5	1.2	5.1



A



B



C



D

Figure 1. Light micrographs of aerobic bacterial strains a) DLC-21, b) DLC-62, c) DLC-63/9, and d) DLC-BB2. All micrographs are from wet mounts photographed at 1000X magnification.

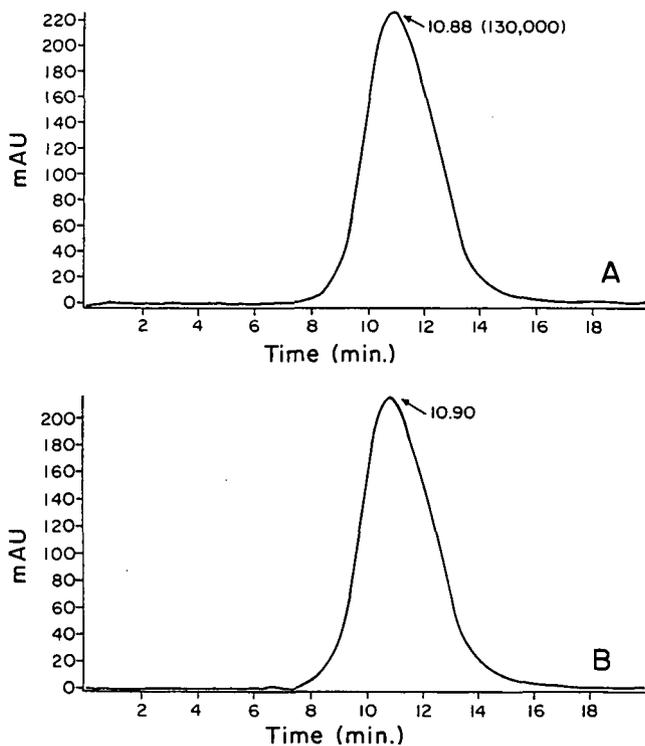


Figure 2. HPLC chromatograms showing the molecular weight distributions of the soluble lignite coal polymer in uninoculated controls at time 0 and after 3 days incubation in the mineral salts-peptone-coal broth at 30°C. A) Uninoculated control, time 0; B) Uninoculated control, 3 day. The retention time for the principal peak (10.9 min) represents an average molecular weight of 130,000 daltons.

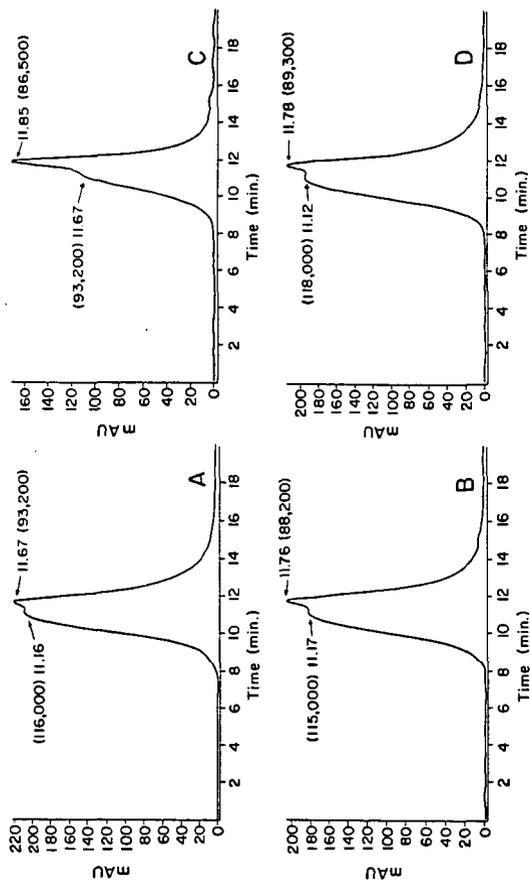


Figure 3. HPLC chromatograms showing the molecular weight distributions of the soluble lignite coal polymer in bacterially inoculated cultures after 3 days incubation in the mineral salts-peptone-coal polymer broth at 30°C. A) DLC-21; B) DLC-62; C) DLC-63/9; D) DLC-BB2. The retention times for the principal peaks are given in minutes, and the average molecular weights for each are presented beside the retention times in parentheses.

MASS AND ENERGY BALANCE CONSTRAINTS
ON THE BIOLOGICAL PRODUCTION OF CHEMICALS FROM COAL

Graham Andrews
Biochemical Engineering Unit
Idaho National Engineering Laboratory
P.O. Box 1625
Idaho Falls, ID 83415-2203

Keywords: Coal, Bioprocessing, Chemicals

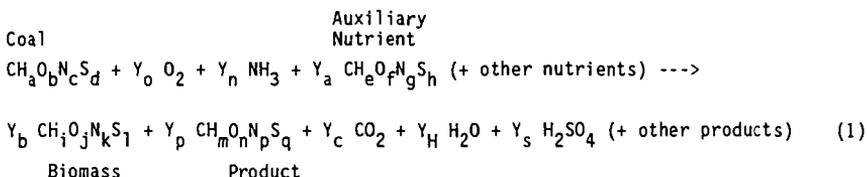
INTRODUCTION

Several proposals have been made for the bioprocessing of coal to produce useful liquid or gaseous chemicals. (1) They have included direct microbial production of methane, microbial production of chemicals from solubilized coal, (2) and a chemical gasification followed by a microbial product synthesis. (3) New microorganisms, biochemical pathways and potential products are reported at regular intervals. It is timely to ask where attention should be focused and which products and processes, if any, hold promise of commercial success. This is a complex economic balance, involving questions of market size, process rates, product separation costs, etc. An important factor in this overall equation is the yield of the process, that is the amount of product that can be obtained from unit mass of coal. The objective of this paper is to apply the standard theory of bioprocess yields to the particular problem of predicting the possible yields from coal bioprocesses.

The standard theory (4,5) is essentially a set of mass and energy balances applied to the particular conditions of a bioprocess. It provides insight into the main factors governing the yield of a product, fixes definite upper limits on the yield, and thus allows a preliminary economic analysis that will eliminate some products from consideration and allow a more rational choice between those that remain. The theory contains little information about metabolic pathways or the internal workings of microorganisms, and it can even be applied to hypothetical bioprocesses for which no microorganisms have yet been isolated. Experience seems to indicate that if a reaction involving naturally-occurring substances is stoichiometrically possible and thermodynamically advantageous in dilute aqueous solution then there exists a microorganism somewhere that will catalyze it.

THE MASS BALANCE

The simplest way to keep track of the substrates and products in a fuel bioprocessing operation is to write it as a pseudo chemical reaction. This reaction is best written in terms of carbon equivalents, that is the amount of organic matter that contains 1 mole of carbon. Nitrogen and sulfur will be included in the formulae because they are often important in fuel bioprocessing, and are significant microbial nutrients.



The Y values are yields expressed as carbon equivalents or moles of a compound produced or consumed per carbon equivalent of fuel. Converting these to a dry/mass basis requires correction for the inorganic (ash) constituents of the compound. For example

$$\text{Biomass yield from Fuel (wt/wt)} = \frac{Y_b M_b}{M_f} \frac{1 - r_f}{1 - r_b} \quad (2)$$

M is the mass of a carbon equivalent and r is the mass fraction of mineral matter ("ash") in the dry compound ($r=0.08$ for biomass).

Note that reaction (1) shows ammonia as a nutrient and water as a product. This is not necessarily so; the metabolism of coal and the auxiliary nutrients may involve more hydrolysis than dehydration steps and more deamination than amination. Water would then be a nutrient, ammonia a product and both Y_n and Y_H , as calculated here, would be negative.

If the possible other nutrients and products are ignored, Equation (1) contains eight unknown yields, and five element balances (C, H, N, O, S) can be written for it. Using these element balance equations to eliminate Y_N , Y_C , Y_H and Y_S gives the result

$$\gamma_p Y_p + \gamma_b Y_b + 4Y_o = \gamma_f + \gamma_a Y_a \quad (3)$$

This equation is essentially an oxidation/reduction balance over reaction (1). The γ coefficients represent the oxidation/reduction state of a compound, specifically the number of available electrons per carbon equivalent of each compound. Thus the definition for the fuel is:

$$\gamma_f = 4 + a - 2b - 3c + 6d \quad (4)$$

Typical values of γ for different ranks of coal, different types of (dried) biomass and several chemicals that could be produced by bioprocessing are shown in Table 1. Several points should be noted from this table.

First, this type of analysis is useful only because the γ values for biomass are surprisingly constant between species. This is confirmed by larger compilations of data on the elemental compositions of living material. (6) The data for the yeast *Candida utilis* shows that the carbon source used to grow a microorganism also makes little difference to its elemental composition. A rapidly growing microbe does have a lower value of γ (mainly due to increased RNA production) but the difference is not of major significance.

Table 1 also shows that the γ values for coal are not only fairly consistent between ranks, but also very close to the values for biomass. This may seem surprising in view of the very different elemental composition of the coal, yet it reflects the fact that coal is made from living matter. The coalification process consists mainly of natural reactions (dehydration, deamination) that do not alter the oxidation/reduction state of the starting material.

Most of the chemicals that we want to produce by liquefaction or gasification of coal are fuels which are, by definition, reduced compounds such as methane ($\gamma = 8$), methanol ($\gamma = 6$) and others shown in Table 1. The oxidation/reduction state of the biomass (and coal) is seen to be more comparable to that of carbohydrate ($\gamma = 4$).

Among bulk chemicals that could be produced from coal only acetic acid has a comparable γ value.

THE GENERAL PRODUCT YIELD

Equation (3) can easily be generalized for the common situation where several products are made. Each product adds an additional term to the left hand side of the equation. The auxiliary nutrients may be specific product precursors (e.g., phenyl-acetic acid in penicillin production) or less well defined compounds like yeast extract that provide a mixture of precursors for biomass growth. It is assumed that each additional nutrient is associated with the formation of a specific product, and that the ratio of nutrient consumed to product (or biomass) produced is a constant (e.g., for biomass $Y_{ab} = Y_a/Y_b$). Equation (3) becomes

$$\sum Y_p (\gamma_p - \gamma_a Y_{ap}) = \gamma_f - 4Y_o \quad (5)$$

cells and
products

Note that, for the purposes of this yield analysis, biomass can be treated as just another product. In coal bioprocessing it is unlikely (on economic grounds) that chemical precursors would be added to direct the formation of specific products, so the biomass term will be the only one in the summation which involves an auxiliary nutrient. The effect of this nutrient is to reduce the amount of fuel required to make biomass, and thus to decrease the "cells" term in the summation. In the limit where the auxiliary nutrient (yeast extract?) has the same composition as the biomass ($\gamma_a = \gamma_b$) and provides all the precursors for cell growth ($Y_{ab} = 1$) the "cells" term is zero. This situation produces the highest possible, or "theoretical," product yields but, since yeast extract costs 5 \$/lb and coal 0.01 \$/lb it is unlikely to be a commercial strategy.

The most obvious consequence of equation (5) is that the more oxygen is consumed by a process (higher Y_o) the lower the total yield of products. The same applies to most other, externally supplied, terminal electron acceptors (NO_3^- , SO_4^{2-}) but not to CO_2 , which can be reduced to CH_4 , a useful product, by methanogenic bacteria metabolizing hydrogen. A consortium of fermentative and methanogenic organisms similar to that used in anaerobic digestion would not only produce the highest yields, but also avoid the costs and problems associated with aerating a coal slurry. (7)

THE SINGLE PRODUCT

The above discussion outlines an optimum commercial process. It would be based on anaerobic metabolism and make a single product, since the cost of separating multiple products can be prohibitive. The bioreactor would be continuous and contain a high concentration of biomass to offset the low specific rates of anaerobic metabolism. The biomass must be immobilized and slow-growing to maximize product yields and minimize the cost of providing auxiliary growth nutrients. The question is what product should be produced?

The first constraint is that an anaerobic process cannot produce a single product that is more oxidized than the substrate. However the values in Table 1 show that most products of interest are fuels or chemicals with $\gamma_p > \gamma_f$, so this is not a serious restriction. The maximum possible, or "theoretical," yield of the product is $Y'_p = \gamma_f/\gamma_p$ (equation (5) with $Y_b = Y_o = 0$) so it makes sense to look for a product with γ_p only slightly larger than γ_f . Some potential candidates are listed

in Table 2. They are all known end-products of fermentative metabolism, although microorganisms capable of producing them from coal have not necessarily been isolated. Also shown in Table 2 are their approximate current prices. Multiplying these prices by the theoretical yields gives the maximum possible financial return per pound of coal processed. This must be considerably larger than the price of coal (approximately 1 c/lb) to make a feasible process. On this basis ethanol and propionic acid appear very promising and methane less so. However it must not be forgotten that the manufacture of methane, a gas, does not involve the considerable costs involved in separating the other two products from the fermentation media.

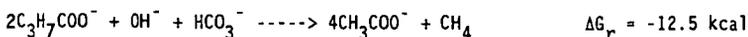
TWO PRODUCTS

Most fermentative metabolic pathways produce not a single product but a mixture of two or more. (5) For two products, the "theoretical yield" situation (equation (5) with $Y_{p1} = Y_{p2} = 0$) must be represented not by a single value but by a graph such as Figure 1 (for CH_4 and H_2 over the entire range of γ_f values for coal) or Figure 2 (for ethanol and acetic acid from a coal with $\gamma_f = 4.5$). These graphs are useful in several ways. They show clearly what combinations of yields are feasible (anything below the constant γ_f line) and what are ruled out by stoichiometry (anything above the line). They therefore provide a useful check on experimental data. For example the yields of methane and hydrogen from a Texas lignite ($\gamma_f = 4.52$) reported by Barik et al (2) are shown in Figure 1. They fall comfortably within the feasible region, and it is immediately apparent from the graph how much the yields could be improved either by improving the microbial culture or by using a more reduced lignite. Note however that a yield of $511 \text{ cm}^3 \text{ CH}_4$ per gram of coal ($Y_{p2} = 0.73$) claimed in the same report would be outside the feasible region, suggesting perhaps that some methane was generated from the auxiliary nutrient.

The "feasible" region on these graphs may be further constrained by biochemical hypotheses. For example there is no known mechanism by which non-photosynthetic anaerobic bacteria can produce molecular hydrogen from water. The dashed line in Figure 1 shows the maximum yield of H_2 that could be produced from the hydrogen in the lignite ($CH_{0.89} N_{0.016} S_{0.12} O_{0.26}$) and the data is seen to fall within this more constrained area.

When there are two products one of them can be more oxidized than the coal without violating the requirements of stoichiometry. This situation is illustrated for acetic acid ($\gamma_{p1} = 4$) and ethanol ($\gamma_{p2} = 6$) from a typical coal ($\gamma_f = 4.5$) in Figure 2. It creates the possibility of a truly optimal situation in which all the coal carbon is converted into products, with no net production of CO_2 . This happens along the section of the $(Y_{p1} + Y_{p2}) = 1$ line that is in the feasible region. Compare this with the methane/hydrogen situation (Figure 1) where $0.55 \geq Y_{p2} \geq 0$, implying that anywhere from 45% to 100% of the coal carbon must be converted to CO_2 .

In the area above the $(Y_{p1} + Y_{p2}) = 1$ line inside the feasible region in Figure 2, carbon dioxide is not a product but a reactant. This may seem unlikely, but is not excluded either by stoichiometry or by the energy balance which will be shown in the next section, to a first approximation, to be identical to the mass balance (greater precision requires knowledge of the free energy of formation of the coal's organic matter). Furthermore, if the reduced product is methane, there is no biochemical barrier to a net fixation of CO_2 . Besides the methanogenic bacteria that produce methane from CO_2 and hydrogen, there are several examples of exergonic carboxylation reactions catalyzed by the acetogenic bacteria. For example one step in the digestion of glucose to methane is the carboxylation of butyrate (8)



Long chain organic acids are known to be a major component of solubilized coal, and it is conceivable that a carefully controlled coal bioprocess continuously provided with CO₂ could result in a series of such reactions with a net fixation of carbon and production of acetate and methane with a total carbon yield greater than unity.

THE ENERGY BALANCE

The other main constraint on the formation of products is that they do not violate the conservation of energy. The energy balance for a bioprocess can be written in several different ways. For example an energy balance over the entire process can be written in terms of the heats of combustion (ΔH = heat of combustion per C equivalent of the substrates and products).

$$\sum Y_p (\Delta H_p + Y_{ap} \Delta H_a) = \Delta H_f - Q \quad (7)$$

cells and
products

Here Q is the heat generated per C- equivalent of fuel consumed. It is a common observation that the heat of combustion of a compound is approximately proportional to its degree of reduction; i.e., $\Delta H = Ky$. Substituting this into equation (7) and subtracting equation (5) gives a standard result for metabolic heat production.

$$Q = 4KY_0 \quad (8)$$

Note that for a fermentative process ($Y_0 = 0$) the approximation $\Delta H = Ky$ makes equations (5) and (7) identical, and thus the energy balance would produce no extra information. The implication that no metabolic heat would be generated ($Q = 0$) in this case is not correct but results from inaccuracies in the assumption that $\Delta H = Ky$ (K is in the range 26-31 kcal/mol electrons depending on the compound). Heat is generated during fermentative processes, although far less than in aerobic processes.

An alternative way of writing the conservation of energy for a bioprocess is to balance the production and consumption of ATP, the cell's main energy carrier, inside the cell. The general equation is (4)

$$\sum \alpha_p Y_p = \alpha_f - m \quad (9)$$

cells and
products

$\alpha_f = N + \gamma_f(P/O)/2$ is the amount of ATP that would be generated by substrate-level (1st term) and oxidative (2nd term) phosphorylation during the complete catabolism of one C-equivalent of fuel.

$\alpha_p = (1/Y_{ATP}) + (N - N_p)/Y_c + (\gamma_p - \gamma_a Y_{ap})(P/O)/2$ is the total ATP cost to the cell of making one C-equivalent of product. The first term gives the actual consumption of ATP in the anabolic pathways. This extension of the Y_{ATP} concept from biomass to any product has been discussed by Andrews. (4) For a catabolic product Y_{ATP} is infinite by definition. The second and third terms account for the ATP and reducing power (in the form of NADH etc.) that the cell can not produce due to the diversion of intermediates from the catabolic pathway to the anabolic reactions that form the product. The contribution of substrate-level phosphorylation (second term) is usually small enough to be ignored in respiratory processes. Adding an auxiliary

nutrient that provides better precursors reduces the oxidative phosphorylation contribution (third term) by reducing the amount of catabolic intermediates that must be diverted to product formation. When the auxiliary nutrient provides all the precursors and electrons needed for product formation (or growth in the case of biomass) then this term is zero. Note that the auxiliary nutrient may also increase the value of Y_{ATP} . In the case of biomass growth for example, it is obviously easier (less energy consuming) for the cell to make new biomass from pre-formed nucleotides and amino-acids than if it must synthesize these compounds from intermediates in the catabolic pathway. (9)

Equation (9) is general and it can usually be greatly simplified, for example in aerobic processes (substrate-level phosphorylation negligible), fermentative processes ($P/O = 0$) or in cases where no metabolic products are produced. It can be very useful for processes involving well-studied metabolic pathways giving, for example, quite accurate predictions for aerobic cell yield on carbohydrates ($\gamma_f = 4$) using the Embden-Meyerhof pathway ($N = 1/3$), the common cytochrome chain ($P/O = 2.5$) and the usual estimate of $Y_{ATP} \sim 10$ gm/mol. Unfortunately, in coal bioprocessing neither the substrate or the metabolic pathways leading to the products of interest are well characterized. Values of the energy parameters N , (P/O) etc. are not known, so application of equation (9) would be premature. Research in this direction should be encouraged.

CONCLUSIONS

The amounts and types of products that can be produced by any type of coal bioprocessing are constrained by the requirements of stoichiometry and energy conservation. The critical parameter is the number of available electrons per carbon equivalent in the organic fraction of the coal. This value shows no systematic variation with coal rank, but is always close to the value for carbohydrate and biomass ($\gamma \sim 4$). This reflects its origin as living matter and implies that, in this respect at least, coal is a reasonable substrate for biological activity.

Anaerobic (fermentative) processes will give higher product yields than processes based on respiratory metabolic pathways where an external electron acceptor is provided. The only possible exception is methanogenic metabolism in which CO_2 acts as the electron acceptor, being reduced to CH_4 . With certain combinations of products from coal, it may be possible to use these organisms to "fix" externally supplied CO_2 . For fermentative metabolism, the maximum "theoretical" yields of various combinations of products can be calculated directly from the mass balances. These provide an excellent yardstick with which to judge experimental data.

In order to obtain useful extra information from the energy balance equation several metabolic parameters, including the production of ATP by substrate-level and oxidative phosphorylation, must be known. This requires further study of coal bioprocessing organisms.

ACKNOWLEDGMENTS

This work was supported under Contract No. DE-AC07-76ID01570 from the U.S. Department of Energy, Office of Advanced Research and Technology Development, Office of Fossil Energy to the Idaho National Engineering Laboratory/EG&G Idaho, Inc.

NOMENCLATURE

ΔH Heat of combustion per C-equivalent
 M Weight of a C equivalent

m	Maintenance requirement for ATP
N	Moles ATP produced by substrate level phosphorylation per C-equivalent of compound
(P/O)	Oxidative phosphorylation ratio
Q	Metabolic heat release per C-equivalent of fuel
r	Mass fraction of mineral matter
Y	Yield; moles of C-equiv of compound per C equivalent of fuel
α	Total moles ATP involved in breakdown or production of a C-equivalent of compound
γ	Available electrons per C-equivalent of compound

Subscripts

a	Auxiliary nutrient
b	Biomass
f	Fuel
o	Oxygen
p	Product

LITERATURE CITED

- (1) Srivastava R. D., Campbell I. M., and Blaustein B. D. Coal Bioprocessing: A Research Needs Assessment, Chemical Engr. Progress, 85, #12, 45 (1989).
- (2) Barik S., Wyza R., Isbister J. D. Biological Conversions of Low Rank Coals, Proceedings: Symposium on the Biological Processing of Coal and Coal-derived Substances, EPRI #ER6572, (1989).
- (3) Vega J. L., Elmore B. B., Ackerson M. D., Clausen E. C., Gaddy J. L. Biological Production of Liquid Fuels from Coal, Proceedings: Symposium on the Biological Processing of Coal and Coal Derived Substances, EPRI #ER6572, p. 3-69 (1989).
- (4) Andrews G. F. Estimating Cell and Product Yields, Biotechnology Bioengineering, 33, 256 (1988).
- (5) Papoutsakis E. T., Meyer C. L. The Fermentation Equation. Biotechnology Bioengineering, 27, 67 (1985).
- (6) Atkinson B., Mavituna F. Biotechnology and Biochemical Engineering Handbook, Nature Press (1983).
- (7) Andrews G. F., Quintana J. Mixing and Mass Transfer in the Aerated Trough Bioreactor, 1st International Symposium on the Biological Processing of Coal. Orlando, FL. May (1990).

- (8) McCarty P. L. The Energetics of Organic Matter Degradation, In, Water Pollution Microbiology, Mitchell R. (ed) p. 91, Wiley (1972).
- (9) Forrest W. W., Walker D. J. The Generation and Utilization of Energy During Growth, Advances in Microbial Physiology, 5, 213 (1971).

TABLE 1. VALUES OF THE γ PARAMETER

<u>Substance</u>	<u>Composition of Organic Fraction</u>	<u>γ</u>
<u>Coals (Typical)</u>		
Anthracite	CH _{0.5} O _{0.03} N _{0.02} S _{0.01}	4.44
Bituminous	CH _{0.7} O _{0.06} N _{0.02} S _{0.01}	4.58
Sub-bituminous	CH _{0.8} O _{0.15} N _{0.02} S _{0.01}	4.50
Lignite	CH _{0.8} O _{0.22} N _{0.02} S _{0.01}	4.36
<u>Biomass (Dried)</u>		
Yeast (<i>C utilis</i>)		
Glucose $\mu = 0.08 \text{ hr}^{-1}$	CH _{1.82} O _{0.47} N _{0.19}	4.32
$\mu = 0.45 \text{ hr}^{-1}$	CH _{1.84} O _{0.56} N _{0.20}	4.12
Ethanol $\mu = 0.06 \text{ hr}^{-1}$	CH _{1.82} O _{0.46} N _{0.19}	4.33
$\mu = 0.43 \text{ hr}^{-1}$	CH _{1.84} O _{0.55} N _{0.20}	4.13
Bacteria (<i>A aerogenes</i>)	CH _{1.78} O _{0.33} N _{0.24}	4.40
<u>Possible Products</u>		
Activated sludge	CH _{1.4} O _{0.4} N _{0.2}	3.99
Carbohydrate	CH ₂ O	4.0
Acetic acid	CH ₂ O	4.0
Ethanol	CH ₃ O _{0.5}	6.0
Octane	CH _{2.25}	6.25
Methane	CH ₄	8.0

TABLE 2. POSSIBLE PRODUCTS FROM SUB-BITUMINOUS COAL

<u>Product</u>	γ_p <u>Electrons/Equiv</u>	Y'_p <u>Equiv/Equiv</u>	<u>Price</u> <u>c/lb</u>	<u>Possible Return</u> <u>c/lb coal</u>
Propionic acid	4.67	0.96	34	43
Butanediol	5.5	0.82	16	16
Ethanol	6.0	0.75	30	28
Methane	8.0	0.56	7.3	3.5

FIG 1 ; THEORETICAL YIELDS OF METHANE AND HYDROGEN

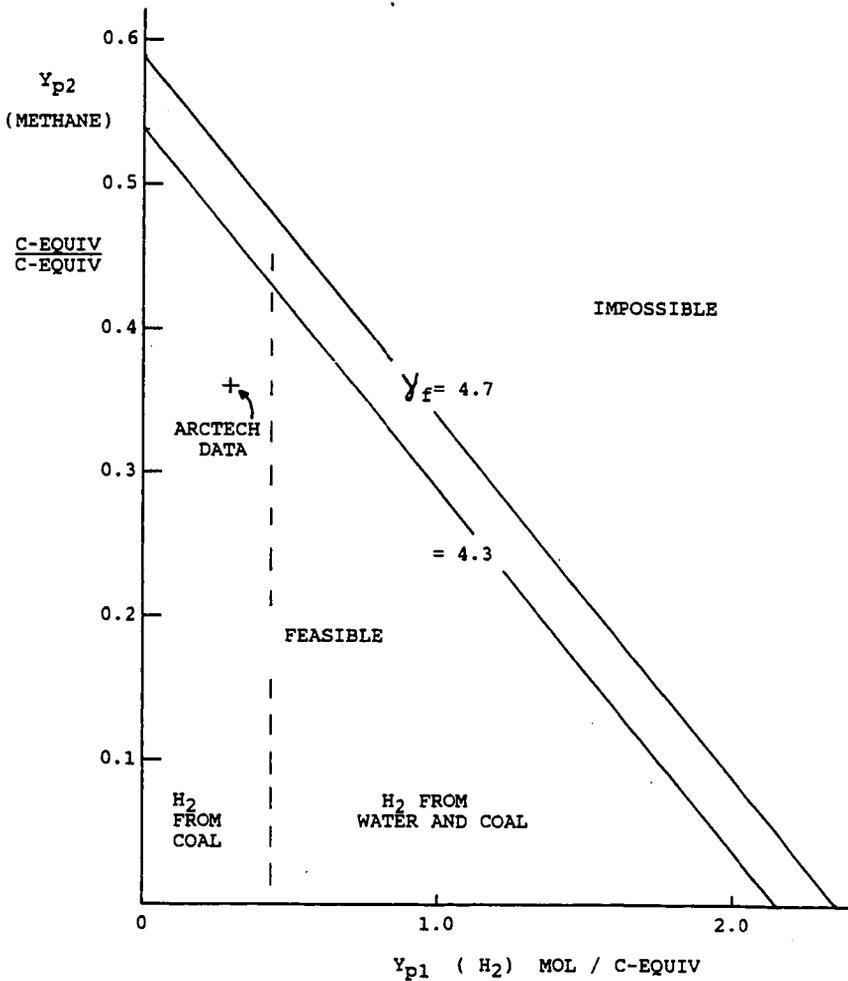
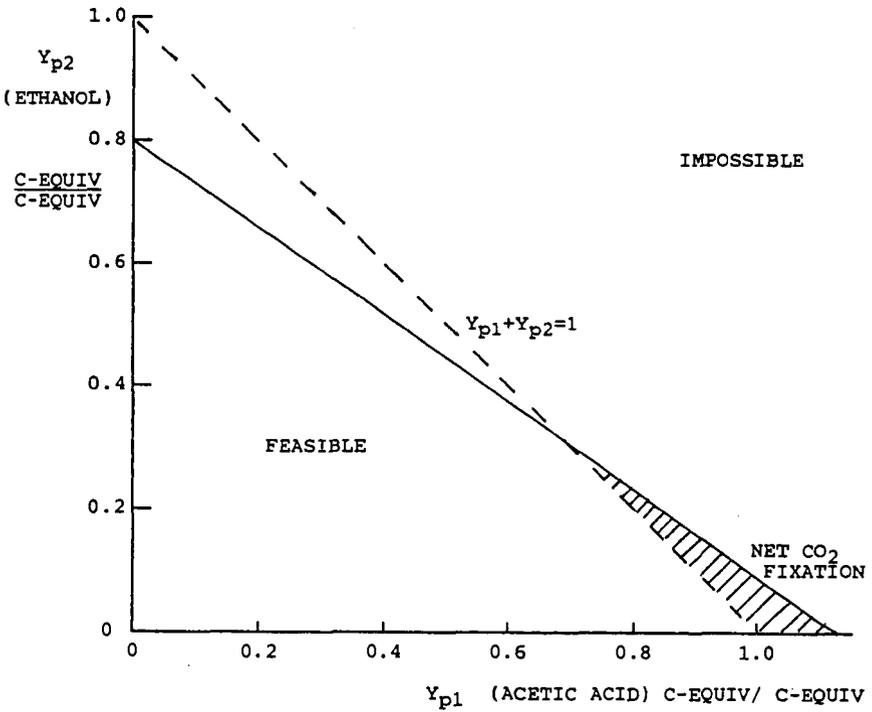


FIG 2 : THEORETICAL YIELDS OF ETHANOL AND ACAETIC ACID FROM A TYPICAL COAL ($\gamma_f = 4.5$)



GENETIC MANIPULATION OF ACIDOPHILIC BACTERIA WHICH ARE POTENTIALLY APPLICABLE IN COAL BENEFICIATION

F.F. Roberto, A.W. Glenn, D. Bulmer, D.F. Bruhn and T.E. Ward
Biotechnology, Mailstop 2203
Idaho National Engineering Laboratory
Idaho Falls, ID 83415

Keywords: acidophilic bacteria; conjugation; coal desulfurization

INTRODUCTION

The economic and practical aspects of a biological coal desulfurization process are the subject of increasing study (1-6). Depyritization of coal by the bacterium, *Thiobacillus ferrooxidans* has been known for some time (7-9), and pilot scale experiments are underway (3,6). A number of limitations have already been recognized for this process, foremost of which is the speed with which the microorganisms grow and attack the pyritic sulfur. Metal toxicity and mass transfer dynamics also present formidable hurdles.

Removal of organic sulfur substituents poses even more difficult problems at this time, not least of which is the lack of efficient candidate organisms. Potential candidates at this time resemble members of the *Pseudomonadaceae*, common environmental bacteria. These organisms enjoy moderate temperatures (28-37 °C) and neutral pH, while the organisms investigated for depyritization processes are typically acidophiles, some of which also happen to be thermophiles (*Sulfolobus*, for example).

These various limitations in the microorganisms being examined for a viable desulfurization process have led us to initiate studies on the extension of molecular genetic techniques to acidophilic bacteria, with an ultimate goal of introducing desirable characteristics for desulfurization (enhanced growth rate, metal resistance, biochemical capacity to degrade organic sulfur) either directly into *T. ferrooxidans*, or, alternatively, into a heterotrophic acidophile which can coexist in the same environment as *T. ferrooxidans*. We are focusing on members of the genus *Acidiphilium*, one such acidophilic heterotroph.

EXPERIMENTAL

Bacterial strains and plasmids. Strains and plasmids used in this study are described in Table 1. *Acidiphilium* strains were grown in Modified Acidophile Salts (MAS) media, which is a modification of that used by Wichlacz and Unz (10). MAS medium contained 1 mM (NH₄)₂SO₄, 2 mM KCl, 0.86 mM K₂HPO₄, 10 mM MgSO₄, 6.6 mM CaCl₂, 2.6 mM FeSO₄, 0.01% yeast extract and 0.1% glycerol, pH 3.5. Solidified media were prepared with the addition of 0.4 % GelRite gellan gum (Kelco). Where indicated, tetracycline (Tc) was added to the media at 40 µg/ml.

Electroporation. Cells were electroporated as described previously (14-16). A BTX Transfactor 100 electroporation device was used. Late log phase cells were washed and concentrated to cell densities of between 10⁹-10¹¹ cells/ml in 1 mM HEPES, pH 7.0. After electroporation, cells were diluted 20-fold into MAS medium to allow expression of the antibiotic resistance phenotype. Cells were

plated on 40 µg/ml Tc in MAS medium, and transformed colonies were clearly visible after three days. The experiments were performed at 32 °C unless otherwise indicated.

Conjugation. Spot matings were performed essentially as described by Miller (11). One milliliter exponential cultures of donor and recipient cells were centrifuged, washed twice and resuspended in 1 ml of 1 mM HEPES, pH 7.0. Donor cells were diluted 50-fold and a 25 µl aliquot was spotted onto a dry nutrient agar (Difco) plate. The liquid was allowed to absorb into the agar, at which time a 25 µl aliquot of the undiluted recipient cells was spotted directly onto the dried donor cells. Approximately 1×10^8 recipient cells were used. Matings were allowed to proceed for three hours, after which the cells were recovered and resuspended in 200 µl MAS medium. The cells were then plated on solid MAS medium containing 40 µg/ml Tc. Transconjugants usually appeared within 3-4 days.

Selection of rifampicin-resistant acidophiles. Mutants resistant to rifampicin were selected by plating cells on MAS plates on which a sterile filter disk (1/2" diameter) impregnated with 200 µg/ml rifampicin was placed. Colonies growing up to the filter disk, within the zone of inhibition were picked and checked for stable resistance by repeated subculturing on liquid and solid media. Mutants were typically resistant to 50-200 µg/ml rifampicin.

Conjugal transfer between acidophiles. Spot matings between acidophiles were performed as described above, with the exception that matings were allowed to proceed overnight, and all recipients used were resistant to rifampicin. All selective plates contained 50 µg/ml each of tetracycline and rifampicin.

Plasmid isolation, restriction digestion gel electrophoresis and ligation.

Plasmids were isolated using the alkaline lysis method of Birnboim and Doly (12), as described for small scale isolations in Maniatis (13).

Restriction enzymes and T4 DNA ligase were purchased from Boehringer Mannheim and Promega, and digestions and ligations were carried out in appropriate buffers according to the manufacturers' instructions. Agarose gel electrophoresis was typically performed using 0.4% agarose (FMC, LE grade) gels prepared in 0.5X TBE and run in the same buffer (13). After running, gels were stained in a 2µg/ml ethidium bromide solution for 30 minutes and DNA was visualized with a UV transilluminator (Spectroliner) at 310 nm.

RESULTS AND DISCUSSION

RP4-based plasmids have previously been introduced into *Acidiphilium facitlis* by conjugation and electroporation (14-16). Plasmids based on RP4 belong to the incompatibility group, IncP1. In order to determine whether plasmids from other incompatibility groups were stably maintained in *A. facitlis*, we obtained the plasmids pSUP104 (IncQ) and pUCD615 (IncW). These plasmids were introduced into the mobilizing *Escherichia coli* strain, S17.1 (17). When compared with the mobilization of pRK415 (ca. 1×10^{-5} transfers/recipient), transfer of pSUP104 and pUCD615 are much less efficient, with frequencies of transfer on the order of 10^{-9} and 10^{-8} per recipient, respectively.

Acidiphilium spp. contain numerous plasmids of varying sizes, whose functions are unknown (14-16). Conjugation functions could be readily assayed for by monitoring the ability of these strains to mobilize broad-host range plasmids to other acidophiles. The identification of such plasmid(s) would be of great interest, since it would be expected that such transfer could occur at acid pH,

allowing the horizontal transfer of genetic information, not only between acidiphilia, but between other acidophiles as well, including *Thiobacillus ferrooxidans*, which has been demonstrated to possess mobilizable, broad-host range plasmids (18, 19). The discovery of such a genetic transfer mechanism in acidophilic bacteria might allow the exploitation of such a process for genetic manipulation of these bacteria.

Acidiphilium strains PW2, CM9, and CM9A harboring the mobilizable plasmid, pRK415 (20, 14-16) were mated with rifampicin-resistant mutants PW1, PW2 and AWB. Very low frequencies of plasmid mobilization were observed, although PW2(pRK415) and CM9A(pRK415) donors gave rise to some 50 colonies in two instances (Table 2). Owing to the number of spontaneous Rf/Tc double mutants arising from CM9A (data not shown), there is some question as to whether these are legitimate transconjugants. However, in the case of the PW2 donor, no spontaneous Rf/Tc mutants were observed, and subsequent analysis of the putative transconjugants revealed the presence of the mobilized plasmid. Genomic fingerprinting experiments are underway to further verify that the transconjugants are derived from the recipient strains used, and not some other class of donor mutant.

As part of our goal to establish techniques for genetic manipulation of *T. ferrooxidans* and *Acidiphilium*, we are constructing vector plasmids using native plasmids. A 2.1 kilobase (kb) plasmid was isolated from *T. ferrooxidans* strain A6. This plasmid, designated pTYA6, was originally cloned in pBR322, and subsequently, subcloned into pUC128, taking advantage of a unique Hind III site. The orientation of restriction sites in this plasmid on a circular map is shown in Figure 1. The Hind III fragment containing the A6 plasmid DNA was clone *in toto* into pLVC18, a mobilizable pBR322 derivative (G. Warren, unpublished results). This construct is being used to examine the ability of a *Thiobacillus* origin of replication to function in other acidophiles, namely, *Acidiphilium*. The chimeric plasmid, pIRC4, was introduced into *E. coli* strain S17.1 and subsequently mobilized into *A. facilis* PW2. 19 putative transconjugants arose from this mating. Since pLVC18 does not possess a broad-host range origin of replication, we must assume that the origin of replication resident on the A6 plasmid is functioning in *Acidiphilium*. It should be noted that several *T. ferrooxidans* plasmids have previously been shown to replicate in *E. coli*, *Pseudomonas aeruginosa* and *T. novellus* (18, 19).

CONCLUSIONS

In our continuing efforts to develop genetic methodologies for manipulating acidophilic bacteria which are useful in the biological desulfurization of coal, we have discovered evidence for conjugative plasmids in *Acidiphilium*. The presence of such plasmids in acidophiles suggests that horizontal transfer of genetic information occurs naturally, and these plasmids may provide a suitable vehicle for the introduction of desirable traits into these bacteria.

In addition, an examination of the relative transfer frequencies of various plasmids of different incompatibility groups appears to indicate that IncP1-based vectors are the vehicles of choice when introducing exogenous genetic material into *Acidiphilium*.

ACKNOWLEDGEMENTS

This work was supported under contract No. DE-AC07-76IDO1570 from the U.S. Department of Energy to the Idaho National Engineering Laboratory/EG&G Idaho, Inc.

REFERENCES

1. Dugan, P. 1986. *Proceedings, Biological Treatment of Coal*, Herndon, VA, pp65-82, U.S. Department of Energy
2. Dugan, P. 1985. *Processing and Utilization of High Sulfur Coals*, Y.A. Attia, ed., pp717-726, Elsevier, New York, NY
3. Bos, P., T.F. Huber, C.H. Kos, C. Ras and J.G. Kuenen. 1986. *Fundamental and Applied Biohydrometallurgy*, R.W. Lawrence, R.M.R. Branion and H.G. Hubner, eds. Vancouver, BC, Canada, pp129-150, Elsevier, Amsterdam
4. Uhl, W., H.-J. Hone, M. Beyer and J. Klein. 1989. *Biotechnol. Bioengineer.* **34**, 1341-1356
5. Detz, C.M. and G. Barvinchak. 1979. *Mining Congr. J.* **65**, 75-86
6. Orsi, N., G. Rossi, P. Trois, P.D. Valenti and A. Zecchin. 1989. *Bioprocessing of Fossil Fuels Workshop*, Tyson's Corner, VA, CONF-890884, P.E. Bayer, ed., pp182-207, U.S. Department of Energy
7. Zarubina, A.M., N.N. Lyalikova and E.I. Shmuk. 1959. *Izvest. Akad. Nauk. SSSR Otdel. Tekh. Nauk. Me i Toplivo* **1**, 117-119
8. Silverman, M.P., M.H. Rogoff and I. Wender. 1963. *Fuel* **42**, 113-124
9. Beier, E. 1985. *First International Conference on Processing and Utilization of High Sulfur Coals*, Columbus, OH, pp653-672, Elsevier, Amsterdam
10. Wichlacz, P.L. and R.F. Unz. 1981. *Appl. Environ. Microbiol.* **41**, 1254-1261
11. Miller, J. H. 1972. *Experiments in Molecular Genetics*, pp82-83, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
12. Birnboim, H.C. and J. Doly. 1979. *Nucl. Acids. Res.* **1**, 1513-1523
13. Maniatis, T., E.F. Fritsch and J. Sambrook. 1982. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
14. Roberto, F.F., A.W. Glenn, M.L. Rowland, C.S. Watkins, D.F. Bruhn, D. Bulmer and T.E. Ward. 1989. *Bioprocessing of fossil fuels workshop*, Tyson's Corner, VA, CONF-890884, pp267-287, U.S. Department of Energy

15. Roberto, F.F. , A.W. Glenn and T.E. Ward. 1989. *Biohydrometallurgy '89*, EMR CanMet, U.S. Bureau of Mines, U.S. Department of Energy, Jackson, WY
Submitted for publication
16. Ward, T.E. , A.W. Glenn, M.L. Rowland , C.S. Watkins, D.F. Bruhn, D. Bulmer and F.F. Roberto. 1989. Pittsburg Coal Conference, Pittsburgh, PA *Submitted for publication*
17. Simon, R., U. Priefer and A. Puhler. 1983. *Biotechnol.* **1**, 784-791
18. Rawlings, D.E. and D.R. Woods. 1988. *Recombinant DNA and Bacterial Fermentation*, J.A. Thomson, ed., pp.277-296, CRC Press, New York, NY
19. Woods, D.R., D.E. Rawlings, M E. Barros, I.M. Pretorius and R. Ramesar. 1986. *Biotechnol. Appl. Biochem.* **8**, 231-241
20. Keen, N.T., S. Tamaki, D. Kobayashi and D. Trollinger. 1988. *Gene* **70**, 191-197
21. Priefer, U.B., R. Simon and A. Puhler. 1985. *J. Bacteriol.* **163(1)**, 324-330
22. Rogowsky, P.M., T.J. Close, J.A. Chimera, J.J. Shaw and C.I. Kado. 1987. *J. Bacteriol.* **169(11)**, 5101-5112

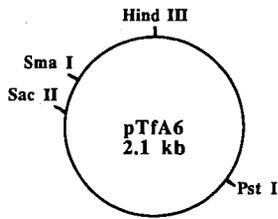
TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype	Source or reference
<i>Acidiphilium</i> strains		
PW2		10
PW2-Rf	Rf	This study
CM9		D. Thompson
CM9A		D. Thompson
AWB-Rf	Rf	10, This study
PW1-Rf	Rf	10, This study
<i>E. coli</i> strains		
S17.1	<i>pro res⁻ mod⁺ recA</i> RP4-2-Tc::Mu-Km::Tn7	17
Plasmids		
pRK415	<i>oriRK2 Tc^r 10.5 kb</i>	20
pLVC18	<i>oripBR322 RSF1010 mob bom</i> <i>Tc^r Ap^r 5.9 kb</i>	G. Warren
pSUP104	<i>ori15A oriRSF1010 Tc^r Cm^r 9.5 kb</i>	21
pUCD615	<i>lux promoter probe, oripSa</i>	22
pTfA6	<i>oripBR322 Ap^r Km^r 17.55 kb</i> Cryptic <i>T. ferrooxidans</i> plasmid, 2.1 kb	This study

TABLE 2. Transfer frequency of pRK415 between acidophilic heterotrophs (per recipient)

Donor (pRK415)	Recipient (Rf-resistant)		
	AWB	PW1	PW2
CM9	0	8.55×10^{-9}	1.57×10^{-8}
CM9A	3.65×10^{-7}	0	5.51×10^{-8}
PW2	6.41×10^{-9}	4.27×10^{-7}	1.57×10^{-8}

Figure 1. Restriction map of *T. ferrooxidans* plasmid TfA6



PRODUCTION OF BUTANOL AND ETHANOL
FROM SYNTHESIS GAS VIA FERMENTATION

R. M. Worden^{1,2}, A. J. Grethlein^{1,2}, M. K. Jain¹, and R. Datta¹

¹Michigan Biotechnology Institute
P.O. Box 27609
3900 Collins Road
Lansing, MI 48909

²Department of Chemical Engineering
Michigan State University
East Lansing, MI 48824

Keywords: Carbon monoxide, Fermentation, Butyribacterium methylotrophicum

INTRODUCTION

Development of alternative liquid fuels based on raw materials other than petroleum would benefit the U.S. both economically, by reducing the trade deficit, and strategically, by reducing U.S. dependence on politically unstable oil exporters. The 1987 D.O.E. report entitled "Energy Security--a Report to the President" concluded that if present trends continue, the U.S. is likely to become far more dependent on the Persian Gulf for oil and that "The most promising technological opportunities for further reductions in oil consumption rest in the development of alternative fuel systems." Indirect coal liquefaction could provide such alternative fuels. In this process coal is first thermally converted to synthesis gas, consisting primarily of carbon monoxide (CO) and hydrogen (H₂). The gas is then catalytically reacted to form liquid fuels, such as mixed alcohols.

The catalytic gas-conversion step is subject to several processing problems. First, sulfur contaminants found in the synthesis gas, primarily hydrogen sulfide (H₂S) and carbonyl sulfide (COS), are potent catalyst poisons. The sulfur-gas content of coal-derived gases varies considerably, but is typically in the range of a few percent (TRW, 1980). These gases must be removed in energy-intensive purification steps that add significantly to the product costs, particularly for coals having a high sulfur content (Wilson et al., 1988). Second, catalytic processing of synthesis gas often requires strict CO/H₂ ratios to maintain a particular product mix, necessitating gas recompression and shift reaction conversion operations. Third, the gas-phase reactors operate at high temperatures and pressures. Operation under extreme conditions increases reactor costs and presents potential safety issues. Fourth, the product specificity of the catalysts is often poor, resulting in a broad product spectrum, decreased product yield, and increased separations difficulties. In some cases, hazardous or environmentally unacceptable by-products may also be produced.

Biological conversion of synthesis gas offers some key advantages over catalytic routes. Sulfur-gas removal will not be required. With sulfur-tolerant microorganisms, the H₂S and COS gases will pass through the process as inert components. Thus, the capital and operating costs associated with conventional gas separation and upgrading equipment could be avoided. Because the CO/H₂ ratio is not a key factor in biological conversion, gas-shift operations would be unnecessary. Also, biological reactions have evolved to operate efficiently at relatively low temperatures and pressures--at least an order of magnitude less than in conventional gas-phase catalysis. The milder processing conditions may reduce both operating and capital costs. Finally, biological catalysts are typically more specific than their inorganic counterparts. Consequently, few by-products result from synthesis gas fermentations, and no hazardous or xenobiotic fermentation by-products are expected.

The recent discovery of microbial pathways to directly convert CO to useful products such as butanol, ethanol, butyric acid and acetic acid provides significant new capabilities for biological processing of synthesis gas. The purpose of this paper is to summarize the novel pathways and fermentation patterns we have discovered in the anaerobe Butyribacterium methylotrophicum and to discuss their impact on the feasibility of producing butanol and ethanol by fermentation of synthesis gas.

ANAEROBIC BIOCONVERSION OF 1-CARBON COMPOUNDS

Numerous microorganisms are capable of metabolizing 1-carbon compounds such as CO, methanol, and CO₂. Those able to grow on 1-carbon compounds as the sole carbon and energy source are referred to as unicarbonotrophs. This trait is desirable for commercial synthesis gas bioconversions, because no additional organic feedstock would be required as a co-substrate. Anaerobic bioconversion is also preferable to aerobic, because in anaerobic fermentation no electrons are lost to molecular oxygen. Thus, the chemical energy content of the gas is efficiently conserved in the products. Two groups of unicarbonotrophic, anaerobic bacteria, acidogens and methanogens, can use 1-carbon compounds or acetate as energy sources. Acidogens convert 1-carbon compounds to fatty acids such as acetate and butyrate, while the methanogens produce methane (Zeikus, 1983).

The metabolism of homo-acetogens is characterized by synthesis of the intermediate acetyl-CoA from either heterotrophic or unicarbonotrophic modes of growth (Zeikus, 1983). Clostridium thermoaceticum generally ferments hexose or pentose to acetate (Fontaine, et al., 1942), but C. thermoaceticum type strain Fontaine can also grow readily on H₂/CO₂. It can also be adapted to grow on CO as an energy source. However, this organism is not able to utilize methanol (Kerby and Zeikus, 1983). Pentostreptococcus productus strain U-1 is also capable of growth and production of acetate on either CO or H₂/CO₂ (Lorowitz and Bryant, 1984). Similarly, Acetobacterium woodii ferments H₂/CO₂, glucose, lactate, formate (Balch, et al., 1977) and can grow on CO after adaptation (Kerby et al., 1983). This species also ferments methanol or cleaves and ferments the methoxyl moieties from a variety of aromatic acids (Balch, et al., 1977). Clostridium ljungdahli is able to co-metabolize CO and H₂ to form acetate and ethanol (Clausen and Gaddy, 1988).

Butyribacterium methylotrophicum is a unique microorganism capable of growing on multicarbon compounds (glucose, lactate, or pyruvate) as well as 1-carbon compounds (H₂/CO₂, formate or methanol) (Zeikus, et al., 1980; Lynd and Zeikus, 1983). A strain of B. methylotrophicum (designated the CO strain) was adapted to grow with a 12 h doubling time on 100% CO, producing acetate and minor amounts of butyrate (Datta, 1982; Lynd et al., 1982). Eubacterium limosum appears to be similar to B. methylotrophicum in general fermentation properties, including the ability to ferment methanol or CO (Sharak-Genthner and Bryant, 1982; Sharak-Genthner, et al., 1981). However, E. limosum requires adaptation and utilization of methanol and needs rumen fluid, yeast extract or acetate in the medium (Sharak-Genthner and Bryant, 1987). Moreover, it produces copious amounts of slime (Sharak-Genthner et al., 1981).

Recently, we have discovered unique metabolic properties of the CO strain of B. methylotrophicum that greatly enhance the prospect of commercially feasible synthesis gas fermentations. In extended batch fermentations, where 100% CO was continuously sparged as the sole carbon and energy source, pH was found to strongly influence the relative amounts of acetate and butyrate produced from CO. By decreasing the pH from 6.8 to 6.0, the fraction of electrons from CO going into butyrate was increased from 6% to 70% at the expense of acetate production (Worden et al., 1989). This finding was significant in that it demonstrated the synthesis of a 4-carbon organic acid from a 1-carbon, inorganic substrate. High levels of butyrate are desirable for the production of butanol in a two-stage

synthesis gas fermentation, as discussed below. In continuous-culture experiments using a 100% CO gas sparge, small quantities of butanol and ethanol were produced in addition to butyrate and acetate (Grethlein et al., in press). This discovery represented the first evidence of a direct microbial pathway for butanol production from CO.

In both batch and continuous-culture experiments with B. methylotrophicum, we have consistently observed a trend toward production of more reduced products (acids with longer chain lengths and alcohols) as the fermentation pH is reduced. This trend is evident in the steady-state fermentation carbon balances shown in Table 1 (Grethlein et al., in press). This pH effect provides a potential mechanism by which the product spectrum of CO fermentations may be manipulated to give either high acid or alcohol yields, depending on the fermentation objectives.

Although the pathways and stoichiometries obtained from the batch and continuous CO fermentations were promising, both the volumetric reactor productivities and the product concentrations were low. To increase these parameters, additional continuous-culture experiments were conducted using a microfiltration-based, cell recycle system. Details of the experimental system have been described elsewhere (Grethlein et al., manuscript submitted). Results to date have been encouraging. Five- to 20-fold increases in cell and product concentrations have been observed relative to continuous culture without cell recycle, and the system has been operated for more than 5 weeks at a time without significant membrane fouling.

SOLVENT PRODUCTION BY CLOSTRIDIUM ACETOBUTYLICUM

Acetone, butanol, and ethanol (ABE) were produced commercially via fermentation of glucose by Clostridium acetobutylicum from World War I until the 1950's. This batch fermentation follows a biphasic pattern (Weizmann, 1918). During the initial, acidogenic phase, growth is exponential, and organic acids (acetic and butyric) and H₂ are the primary products. After accumulation of a certain concentration of acids, the pH decreases to approximately 4.5, and the fermentation switches to the solventogenic phase, where the organic acids are reduced to solvents, the rate of H₂ gas production decreases, and cell growth ceases. Early attempts to induce C. acetobutylicum to take up acids for alcohol production indicated that a concentration of butyric acid higher than 2 g/L inhibited the solvent production (Soni et al., 1982). More recently, it has been shown that acids concentration in the first stage is a determinant factor for solvent production in the second stage of a two-stage continuous fermentation (Godin and Engasser, 1989).

Continuous cultures of C. acetobutylicum may be maintained in either the acidogenic or the solventogenic phase by manipulating the fermentation conditions. A generally observed trend is that higher pH values are associated with acid production, and lower pH values favor solvent production (Kim and Zeikus, 1985). Meyer et al. (1986) found that CO gasing led to continuous butyrate uptake and solvent production without the production of acetone. Extremely high specific butyrate uptake rates and butanol production rates were measured during this CO challenge. These trends were attributed to altered electron flow arising from CO inhibition of production hydrogenase activity. During acidogenesis, excess electrons are typically eliminated by H₂ formation. Because this reaction was inhibited by CO, electron flow to other products had to be increased. Acetone formation does not consume electrons, so the cellular control mechanisms shut off carbon flow to acetone, and increased carbon flow to electron-consuming alcohol production.

ENERGETICS OF ACID AND ALCOHOL PRODUCTION FROM CO

The ΔG° values for production of acids and alcohols from CO, shown in Table 2, were calculated using published free energy of combustion data (Roels, 1983).

The values for butyrate and acetate production are approximately the same, and only slightly higher than those for butanol and ethanol production. All four reactions are sufficiently exergonic to drive ATP synthesis. However, it is not currently known whether metabolic mechanisms exist in B. methylotrophicum for net ATP synthesis during direct conversion of CO to alcohols. Acetyl CoA and butyryl CoA are thought to be intermediate branch points from which either acids or alcohols may be produced. Acid production generates ATP but consumes no electrons, whereas alcohol production consumes electrons but produces no ATP. Consequently, the cells can replenish energy reserves via acid production and eliminate excess electrons via alcohol production. If no net ATP is generated via alcohol formation, then alcohols must be produced either in the absence of growth or concurrently with acid formation. Further research is needed to elucidate the mechanisms for energy flow within B. methylotrophicum under these fermentation conditions.

PROPOSED PROCESSES FOR SYNTHESIS GAS BIOCONVERSION TO BUTANOL AND ETHANOL

Single-stage Fermentation Process

A proposed single-stage fermentation process is shown in Figure 1. In this process, CO and H₂ would be fed to a bioreactor containing B. methylotrophicum, in which the fermentation conditions were optimized for solventogenesis. Our previous results indicate that growth and alcohol production are metabolically uncoupled, and that a reduction in pH may be used to trigger solventogenesis. Thus, the fermenter could be operated batchwise, with a pH shift at the onset of the stationary phase (Worden et al., 1989). Alternatively, the fermenter could be operated continuously, using pH oscillations to allow alternate periods of alcohol production and cell regeneration. In either case, the product stream would be sent to a separation unit for selective removal of the alcohols, and the acids would be recycled to the reactor for further conversion. Either cell recycle or cell immobilization would be used to maintain high reactor cell densities.

This single-stage process appears to be technologically feasible, based on preliminary fermentations using 100% CO as the gaseous feedstock. At a pH of 5.5, transient butanol concentrations as high as 2.7 g/L have been achieved in continuous culture using cell recycle (Grethlein et al., manuscript submitted). By comparison, butanol concentrations from commercial ABE fermentations were approximately 16 g/L. Thus, even before process and strain optimization, CO fermentation yields are within an order of magnitude of the maximum butanol concentration produced by C. acetobutylicum from glucose.

Ideally, the reducing equivalents needed to convert acetic and butyric acids to the respective alcohols would come from H₂ in the synthesis gas. B. methylotrophicum is known to take up and metabolize H₂ and CO₂ to form primarily acetate (Lynd and Zeikus, 1983). However, the effects of factors such as the presence of CO and fermentation pH on this reaction are unknown. Carbon monoxide is a potent inhibitor of production hydrogenase in C. acetobutylicum (Kim et al., 1984), but its effect on the uptake hydrogenase of B. methylotrophicum is not known. Fermentation experiments using a mixture of H₂ and CO are currently underway in our laboratory to determine these effects. Even if hydrogen uptake is inhibited by CO, it may be possible to substitute other metabolizable electron donors, such as glucose. This substitution should not greatly affect the process economics, because only a small amount of the electron donor would be required. On a combustion energy basis, 83% of the butanol energy content would come from CO, and only 17% would come from the secondary electron donor.

Two-Stage Fermentation Process

The flowsheet of a proposed 2-stage fermentation process is shown in Figure 2. In the first, acidogenic stage, CO would be converted to primarily butyric

and acetic acids using an acidogenic culture of B. methylotrophicum. Results to date suggest that B. methylotrophicum is quite well suited for this application. Using fermentation conditions appropriate for acidogenesis, butyrate and acetate concentrations of 4 g/L and 8 g/L, respectively, have been achieved in steady-state, continuous operation with cell-recycle (Grethlein et al., manuscript submitted). Future strain development efforts should increase these values even further. The acids produced in the first stage would then be combined with H₂ from the synthesis gas (or some other electron donor) in the second stage to yield butanol and ethanol. Two alternative biocatalysts are currently under investigation for use in the second stage: C. acetobutylicum and B. methylotrophicum. C. acetobutylicum has been studied much more extensively and thus has better characterized enzymes and metabolic-regulation mechanisms. It is capable of producing high concentrations of mixed solvents when grown on glucose, and can also take up externally added acids and H₂ for solvent production. When grown in a glucose-limited chemostat in the presence of CO, H₂ and acetone formation are inhibited, while butyrate uptake and butanol production are increased to extremely high levels (Meyer et al., 1986). These effects are beneficial, since production of H₂ and acetone reduces alcohol yields. Thus, it may be possible to use residual CO from the first stage to improve alcohol yields in the second stage.

The solventogenic capability of B. methylotrophicum was discovered in our laboratory only recently (Grethlein et al., in press), and it has not yet been well characterized. Results to date have included transient butanol concentrations as high as 2.7 g/L in continuous operation using cell recycle. Ongoing investigations include characterization of enzyme-regulation mechanisms, and capacity for H₂ and acid assimilation.

As indicated in Figure 2, bioreactors for both stages will utilize either cell recycle or immobilization to maintain high cell concentrations. The product stream from the solventogenic bioreactor will be stripped of alcohols in a separation unit and then recycled to the first stage.

FUTURE PROSPECTS FOR BIOCONVERSION OF SYNTHESIS GAS TO BUTANOL AND ETHANOL

Two basic problems with the classic ABE fermentation using C. acetobutylicum were identified in a report to the Office of Technology Assessment entitled "Biological Production of Liquid Fuels and Chemical Feedstocks" (Humphrey and Nolan, 1979): the formation of multiple products, requiring additional recovery steps, and the low solvent yields (34 g/100 g fermentable sugar). Our results to date indicate that these problems may not be as severe for the unique synthesis gas fermentation processes proposed here, as described below.

Acetone production by B. methylotrophicum has never been observed. It is currently unknown whether the enzymes necessary for acetone production from acetoacetyl CoA (e.g., phosphate acetoacetyltransferase, acetoacetate kinase and acetoacetate decarboxylase) are lacking in B. methylotrophicum, or whether one or more of these enzymes may be under strong regulation by CO. In the proposed two-stage process, where C. acetobutylicum is used in the solventogenic stage, the presence of CO has been shown to block acetone production while enhancing butanol production to the highest levels ever reported (Meyer et al., 1986). Thus, it appears possible to avoid undesirable acetone formation.

Despite extensive strain-development and optimization efforts, ABE fermentations using C. acetobutylicum are presently unable to achieve high electron recovery in butanol and ethanol. An improved process for ABE production from corn starch using an efficient, asporogenous mutant and stillage recycle was recently described by Marlatt and Datta (1986). In this process, only 63% of the electrons from the glucose ends up in the alcohols, and 31% ends up in H₂ and acetone. For our proposed process using C. acetobutylicum in the solventogenic bioreactor, the presence of CO may block or reduce unwanted production of H₂ and

acetone, maximizing electron capture in alcohols. Less is known about the electron-capture efficiency of B. methylotrophicum. Using carbon and electron balances (Erickson and Oner, 1983), we have estimated that in recent continuous fermentation experiments using cell recycle, as much as 44% of the electrons from CO has been transferred to butanol (Grethlein et al., manuscript submitted). The butanol concentration measured during this time was 2.7 g/L. However, because these data were not measured at steady-state, they should only be taken as suggestive that high electron capture efficiency in alcohols appears possible with B. methylotrophicum.

The unique metabolic capabilities for CO conversion found in B. methylotrophicum indicate its potential for production of butanol and ethanol from synthesis gas in either a 1- or 2-stage fermentation system. In addition, the CO-induced enhancement of acid uptake and butanol production in C. acetobutylicum make this species well-suited for assimilation of H₂ and acids in the second, solventogenic stage. Research to better understand the complex metabolic regulation patterns of these two species is continuing in our laboratory. Successful manipulation of carbon and electron flow in these cultures could make bioconversion of synthesis gas to butanol and ethanol a commercial reality.

ACKNOWLEDGMENT

This research was supported by the U.S. Department of Energy, under Contract No. 22-88PC79815.000. Fellowship support for A. J. Grethlein was provided by the Michigan Biotechnology Institute.

REFERENCES

- Balch, W. E., S. Schoberth, R. S. Tanner and R. S. Wolfe. (1977) *Int. J. of Syst. Bacteriol.* 27:355.
- Clausen, E. C. and J. L. Gaddy (1988) Proc. of Indirect Liquefaction Contractors Review Meeting for U.S. D.O.E., Pittsburgh Energy Technology Center, 639-670.
- Datta, R. (1982) *Biotech. and Bioeng. Symp.* 11:521-532.
- Erickson, L. E. and M. D. Oner. (1983) *Ann. N.Y. Acad. Sci.* 413:99.
- Fontaine, F. E., W. H. Peterson, E. McCoy, E. Johnson and M. T. Titter. (1942) *J. Bacteriol.* 43:704.
- Godin, C. and J. M. Engasser. (1989) *Biotechnol. Lett.* 12:903-906.
- Grethlein, A. J. (1989) M. S. Thesis, Dept. of Chemical Engineering, Michigan State University, East Lansing, Michigan.
- Grethlein, A. J., R. M. Worden, M. K. Jain and R. Datta. 1990. *Appl. Biochem. Biotechnol.* 24/25 (in press).
- Humphrey, A. E. and E. J. Nolan (1979) Biological Production of Liquid Fuels and Chemical Feedstocks, Report to U.S. Office of Technology Assessment, 5-7.
- Kerby, R., W. Niemczura and J. G. Zeikus. (1983) *J. Bacteriol.* 155:1208-1218.
- Kerby, R. and J. G. Zeikus. (1983) *Curr. Microbiol.* 8:27-30.
- Kim, B. H., P. Bellows, R. Datta and J. G. Zeikus. (1984) *Appl. Environ. Microbiol.* 48, 4,764-770.
- Kim, B. H. and J. G. Zeikus. (1985) *Dev. Ind. Microbiol.* 26:549-556.
- Lorowitz, W. H. and M. P. Bryant. (1984) *Appl. Environ. Microbiol.* 47:961-964.
- Lynd, L. H., R. Kerby and J. G. Zeikus. (1982) *J. Bacteriol.* 149:255-263.
- Lynd, L. H. and J. G. Zeikus. (1983) *J. Bacteriol.* 153:1415-1423.
- Marlatt, J. A. and R. Datta. (1986) *Biotechnol. Progress*, 2,1, 23-28.
- Meyer, C. L., J. W. Roos and E. T. Papoutsakis. (1986) *Appl. Microbiol. Biotechnol.* 24:159-167.
- Roels, J.A. (1983) Energetics and Kinetics in Biotechnology, Elsevier, New York, 40.
- Sharak-Genthner, B. R., C. L. Davis and M. P. Bryant. (1981) *Appl. Environ. Microbiol.* 42:12-19.
- Sharak-Genthner, B. R. and M. P. Bryant. (1982). *Appl. Environ. Microbiol.*, 43:70-74.
- Sharak-Genthner, B. R. and M. P. Bryant. (1987) *Appl. Environ. Microbiol.*, 53:471.
- Soni, B. K., K. Das and T. K. Ghose. 1982. *Biotechnol. Lett.* 4:19-22.

TRW (1980) Development Status of Key Emerging Gasification Systems, TRW Report, Sept., 1980.

Weizmann, C. (1918) U. S. Patent #1,315,585.

Wilson, J. S., J. Halow and M. R. Ghate. (1988) Chemtech. Feb. 1988, 123-128.

Worden, R. M., A. J. Grethlein, J. G. Zeikus and R. Datta. (1989) Appl. Biochem. Biotech. 20/21:687-698.

Zeikus, G. Z. (1983) Adv. Microb. Physiol. 24:215-297.

Zeikus, J. G. (1980) Ann. Rev. Microbiol. 34:423-464.

Zeikus, J. G., L. H. Lynd, T. E. Thompson, J. A. Krzycki, P. J. Weimer and P. Hegge. 1980. Curr. Microbiol. 3:381-386.

Table 1. Influence of pH on Steady-State Fermentation Stoichiometry of Butyribacterium methylotrophicum (Source: Grethlein et al., in press)

pH	Fermentation Stoichiometry*
6.8	$4\text{CO} \longrightarrow 2.09 \text{CO}_2 + 0.63 \text{Ac} + 0.043 \text{Bu} + 0.027 \text{EtOH} + 0.43 \text{Cells}$
6.5	$4\text{CO} \longrightarrow 2.13 \text{CO}_2 + 0.56 \text{Ac} + 0.082 \text{Bu} + 0.026 \text{EtOH} + 0.37 \text{Cells}$
6.0	$4\text{CO} \longrightarrow 2.27 \text{CO}_2 + 0.30 \text{Ac} + 0.161 \text{Bu} + 0.032 \text{EtOH} + 0.029 \text{BuOH} + 0.31 \text{Cells}$

Table 2. Standard Free Energies of Reaction for CO Bioconversion

Reaction Stoichiometry*	ΔG° (kcal/gmole CO)
$10 \text{CO} + 4 \text{H}_2\text{O} \longrightarrow \text{Bu} + 6 \text{CO}_2$	- 9.7
$4 \text{CO} + 2 \text{H}_2\text{O} \longrightarrow \text{Ac} + 2 \text{CO}_2$	- 9.8
$12 \text{CO} + 5 \text{H}_2\text{O} \longrightarrow \text{BuOH} + 8 \text{CO}_2$	- 9.0
$6 \text{CO} + 3 \text{H}_2\text{O} \longrightarrow \text{EtOH} + 4 \text{CO}_2$	- 8.1

*Ac, Bu, EtOH, and BuOH stand for acetic acid, butyric acid, ethanol, and butanol, respectively. The coefficient on cells represents the number of moles of carbon contained in the cells mass.

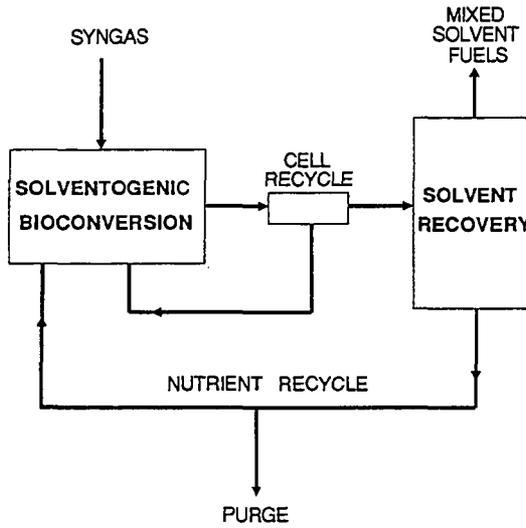


Figure 1. One-Stage Fermentation Process for Synthesis-Gas Bioconversion

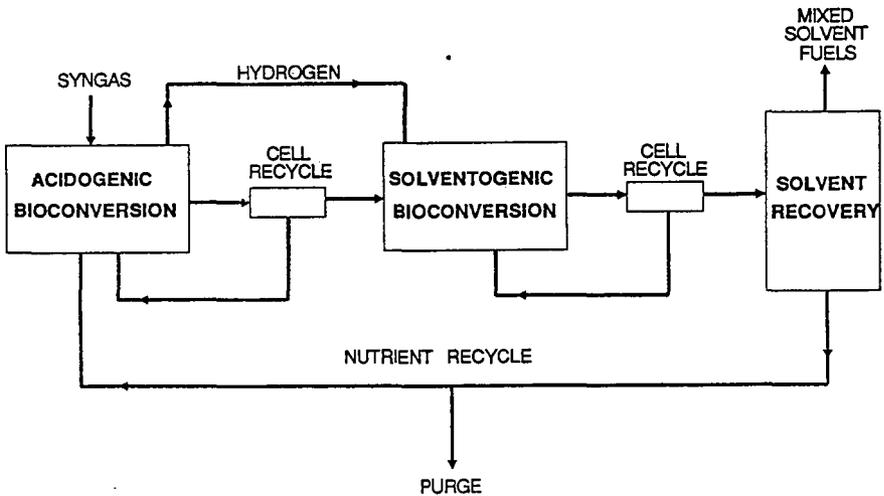


Figure 2. Two-Stage Fermentation Process for Synthesis-Gas Bioconversion

BIOREACTOR DESIGN FOR SYNTHESIS GAS FERMENTATIONS

K. T. Klasson, M. D. Ackerson, E. C. Clausen, and J. L. Gaddy
Department of Chemical Engineering
University of Arkansas
Fayetteville, AR 72701

ABSTRACT

Fermentation of slightly soluble gaseous substrates, such as CO and H₂, requires the transport of the substrate from the gas phase, through the liquid phase, and into the solid phase for conversion. These reactions are generally mass transport limited, and bioreactor designs must achieve high mass transfer coefficients, as well as high cell concentrations, to minimize reactor volume. Immobilized cell systems are ideal for these fermentations, and operation at high pressure facilitates gas solubility and faster mass transfer. This paper compares the performance of a continuous stirred tank reactor, a bubble column reactor, and a trickle-bed reactor for the conversion of CO, CO₂, and H₂ in coal synthesis gas into methane using a tri-culture of *Rhodospirillum rubrum*, *Methanobacterium formicicum*, and *Methanosarcina barkeri*. *R. rubrum* is a photosynthetic bacterium, and special provisions for supplying light for growth of this organism are necessary. Mass transfer coefficients are compared and intrinsic kinetics presented. Gas retention times of a few minutes have been achieved for complete conversion of the gaseous substrate.

INTRODUCTION

Synthesis gas, a mixture of primarily CO, H₂ and CO₂, is a major building block in the production of fuels and chemicals. The gas may be produced from several sources, including coal, oil shale, tar sands, heavy residues, biomass or natural gas. Most synthesis gas is produced today by catalytic reforming of natural gas, although the partial oxidation of heavy liquids is also practiced (Graboski, 1984). Only a small percentage of the synthesis gas currently produced is by gasification of solid fuel. However, because of the large reserves of coal in the United States (300 year supply at the current consumption rate (Specks and Klussman, 1982)), synthesis gas production from coal will become an important technology in the future.

Coal gasification, which is a combination of pyrolysis and combustion reactions, (Simbeck *et al.* 1982), produces a gas consisting of more than 50 percent H₂ and CO, the balance being a mixture of CO₂, CH₄, H₂S, COS and nitrogen compounds. The actual composition depends upon process conditions and the coal that is employed. The raw gas has a low to medium Btu content, with a heating value of 160-450 Btu/SCF, depending on whether air or oxygen is used during gasification (Coffin, 1984). Following quenching and purification, the synthesis gas contains 25-35 percent H₂, 40-65 percent CO, 1-20 percent CO₂, 0-7 percent CH₄ and other compounds in small quantities.

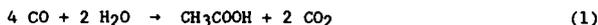
Catalytic processes may be used to convert syngas into a variety of fuels and chemicals, such as, methane, methanol, formaldehyde, acetic acid, etc. (Courty and Chaumette, 1978). Microorganisms may also be used to convert synthesis gas components into fuels and chemicals. Biological processes, although generally slower than chemical reactions, have several advantages over catalytic processes, such as higher specificity, higher yields, lower energy costs and generally greater resistance to poisoning. Furthermore, the irreversible character of biological reactions allows complete conversion and avoids thermodynamic equilibrium relationships.

The purpose of this paper is to present data for the development of optimal bioreactor concepts for syngas fermentations. Laboratory data for continuous culture experiments for the conversion of synthesis gas components into methane and ethanol are presented. Various bioreactor schemes for synthesis gas fermentations have been investigated and mathematical models that define intrinsic kinetics and mass transfer relationships are developed. Methods to predict reactor performance and gas retention times for the CSTR and immobilized cell reactor are presented.

SYNTHESIS GAS FERMENTATIONS

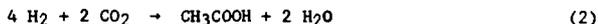
Methane Production

Methane may be produced by methanogenic bacteria from either acetate or H₂ and CO₂, both of which may be produced from syngas components. Acetate may be produced by several anaerobic bacteria, including Peptostreptococcus productus, (Barik *et al.* 1987; Lorowitz and Bryant, 1984), Acetobacterium woodii (Kerby *et al.* 1983) Clostridium thermoaceticum (Wood *et al.* 1982; and Kerby and Zeikus, 1982) and Eubacterium woodii (Genthner and Bryant, 1982), which produce acetate by the reaction:



Among these bacterial species, P. productus utilizes CO very rapidly with a doubling time of less than two hours, and can grow with as much as 90 percent CO in the gas phase (Barik *et al.* 1987).

Many anaerobic bacteria, including P. productus, are known to produce acetate from H₂ and CO₂, (Mayer *et al.* 1977; Sleat *et al.* 1985; and Balch *et al.* 1977), which produces a homoacetic fermentation by anaerobically oxidizing hydrogen and reducing CO₂ according to the equation:



Two species of purple non-sulfur bacteria, Rhodospseudomonas gelatinosa (Uffen, 1976; and Dashkevich and Uffen, 1979) and Rhodospirillum rubrum (Breed *et al.* 1977) are known to perform the water gas shift reaction to produce H₂ as follows:



R. gelatinosa grows under strict anaerobic conditions on the dark with CO as the only carbon and energy source, although growth is stimulated by the addition of trypticase. R. rubrum requires tungsten light and the presence of a carbon source other than CO (sugars, acetate, yeast extract etc.) for growth. In comparing these two species, R. rubrum grows faster and reaches higher cell concentrations that uptake CO more rapidly. R. rubrum has also been found to tolerate small amounts of oxygen and sulfur compounds often present in synthesis gas.

Almost all methanogenic bacteria, including Methanospirillum hungatii, Methanobacterium formicicum, Methanobrevibacter smithii, Methanosarcina barkeri, utilize CO₂ and H₂ to produce CH₄ according to (Thauer *et al.* 1977; Balch *et al.* 1979; and Zehnder *et al.* 1981:



Methane may also be produced from acetate by Methanosarcinaceae sp., such as Methanosarcina barkeri, as well as Methanothrix soehngenii (Jones et al. 1987). While Methanosarcina barkeri will utilize acetate only in the absence of other preferred substrates (such as H₂ and CO₂), Methanothrix sp. does not utilize H₂ and CO₂ and growth and methane formation is observed exclusively in the presence of acetate (Huser et al. 1982). Both microorganisms show comparable specific growth rates at low acetate concentrations (< 3mM). However, from the Monod saturation constants (K_s = 0.7 mmol/l for Methanothrix and 5 mmol/l for M. barkeri), it is expected that at low acetate concentrations Methanothrix will give faster rates and predominate.

From the above, it can be seen that the production of methane from syngas is a two-step process; formation of the methane precursors (acetate or hydrogen) and the biomethanation of the precursor. These reactions may be carried out in separate stages or as a CO culture in the same reactor. Compatibility of the cultures with substrates and products is essential for an efficient process.

Methane Production from Acetate. In order to produce methane from synthesis gas through acetate, CO (and possibly CO₂ and H₂) is first converted to acetate using the bacterium P. productus. The acetate is then reacted to methane using either Methanothrix sp. or M. barkeri.

In order to develop a successful co-culture, both the acetogenic and methanogenic bacteria must have resistance to CO toxicity. Studies of the CO uptake rate with time for P. productus using various initial CO partial pressures showed an increase in the rate of reaction with increasing partial pressure up to a partial pressure of 1.6 atm. At a partial pressure of 2.5 atm, however, the culture failed to utilize the gas after a short initial period of uptake. At 2.5 atm, the dissolved CO concentration reached toxic levels due to insufficient cell mass to keep the reaction mass transfer-limited. Studies have shown that both growth and CO uptake by P. productus are inhibited at dissolved CO tensions above 0.16-0.8 atm (Vega et al. 1989). Higher gas phase CO partial pressures may be employed as long as a sufficient number of cells are present to keep the dissolved CO tension low.

Figure 1 illustrates a gradual stepwise procedure where CO partial pressures as high as 10 atm are successfully employed. The pressure was gradually increased in this study only after the cell concentration increased in order to keep the process mass transfer-limited. The effects of CO on methanogens may be illustrated using the bacterium M. barkeri. The consumption of H₂ with time for M. barkeri at various initial CO partial pressures is shown in Figure 2. As noted, the time for consumption essentially doubled when increasing the CO partial pressure from 0 to 0.59 atm. This result is expected due to the well-known inhibitory effect of CO on hydrogenases. Similar results were obtained for Methanothrix sp. It is thus essential for hydrogen-utilizing organisms such as methanogenic bacteria that a low dissolved CO concentration be maintained.

A second factor in evaluating methane production from synthesis gas through acetate is the potential inhibitory effects of acetate on both P. productus by product inhibition and methanogens by substrate inhibition. It has been found that acetate concentrations of 20-25 g/L may be successfully employed with P. productus without appreciable product inhibition. Figure 3 shows the effect of acetate concentration on methane production by M. barkeri in batch culture. As noted, the methane production increased with increasing acetate concentration up to 6 g/L acetate, with some inhibition noted at the 6 g/L level. At acetate concentrations above 6 g/L, however, methane production was severely inhibited. Similar inhibitory effects were seen with Methanotherix sp. where methane production was slowed at 9 g/L acetate and stopped at 12 g/L.

Figure 4 shows the methane productivity in an immobilized cell reactor (ICR) employing Methanotherix sp. at various feed acetate concentrations. The column was operated over a period of nearly 250 days by gradually increasing the inlet acetate concentration and flow rate as cell growth allowed. As noted in the figure, a maximum inlet acetate concentration of 10 g/L was successfully employed at a methane productivity of 5 VVD. Further increases in the acetate concentration were not possible, even with the simultaneous addition of high concentrations of yeast extract. Thus, comparatively low productivities, even in an ICR, result for methane production from acetate.

Co-culturing of P. productus and methanogens utilizing acetate was not found feasible due to the slow rate of growth and low acetate tolerance of methanogens. P. productus totally dominated the co-cultures, producing acetate much faster and in higher concentrations than the methanogens were capable of utilizing. The methanogens were thus inhibited by acetate and the co-culture could not be sustained. The production of methane through acetate will, therefore, require separate reaction vessels.

Methane Production from H₂ and CO₂. An alternative route involves the conversion of CO and H₂ by R. rubrum, followed by conversion of all the H₂ and CO₂ to methane using either M. formicicum or M. barkeri. It has been found that H₂ production by R. rubrum is essentially unaffected by CO partial pressures up to 2.0 atm. Therefore, as with P. productus, the limiting factor in CO utilization by R. rubrum is the ability to maintain a high cell concentration and, consequently, a low dissolved CO tension in the liquid phase. M. formicicum has been shown to be able to uptake H₂ and CO₂ to produce methane much faster than M. barkeri. However, in order to form a successful co-culture, M. formicicum must be able to tolerate low levels of dissolved CO. The utilization of H₂ by M. formicicum in the presence of various CO partial pressures is shown in Figure 5. As noted, nearly complete inhibition of H₂ uptake was found at a CO partial pressure of only 0.76 atm. The inhibition of CO on M. barkeri might enable a rapid rate of uptake of H₂.

R. rubrum is a photosynthetic bacteria requiring tungsten light for growth, but not for CO uptake. Figure 6 shows the growth and consumption of CO with time at various light intensities for R. rubrum. As shown, the cell growth rate increased with light intensity up to 1490 lux, however, no further enhancement was found at higher intensities. CO consumption was essentially

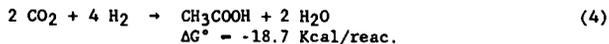
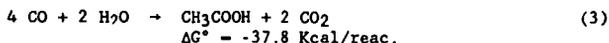
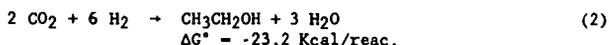
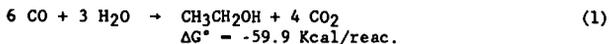
unaffected by the presence of light. Methanogens have been found to be unaffected by the presence of light.

An experiment was performed in a continuous stirred-tank reactor to study the simultaneous conversion of CO₂ and H₂ directly to CH₄ employing a co-culture of *R. rubrum* and *M. formicicum*. Since the organisms have different optimum temperatures, the lower temperature, 30°C, was chosen for study. Figure 7 shows the CH₄ and CO production with time since start-up in the CSTR. Following a significant period of methanogen acclimation, almost complete conversion of both CO and H₂ occurred after 300 hours of operation. The methane production rate shown in Figure 7 reached a steady-state level after 350 hours of operation of about 1.6 mmole CH₄/hr, which represents a methane yield from CO, and H₂ and CO₂ of about 96 percent of theoretical. The system was operated with a retention time of one hour and stable operation was monitored for several weeks.

Ethanol Production

While many anaerobic, facultatively anaerobic and even some strictly aerobic microorganisms form various amounts of ethanol from glucose (Wiegel, 1980), no organisms were known to form ethanol autotrophically from synthesis gas components. In 1987, a strict anaerobic mesophilic bacterium was isolated that was capable of converting CO, H₂ and CO₂ to a mixture of acetate and ethanol (Barik *et al.* 1987). Identification and characterization studies have shown that the bacterium is a new clostridial species, named *Clostridium ljungdahlii*, Strain PETC, in honor of Dr. Lars G. Ljungdahl for his work on clostridia and acetogens (Clausen and Gaddy, 1988). *C. ljungdahlii* is a gram-positive, motile, rod-shaped anaerobic bacterium which sporulates infrequently. In addition to synthesis gas components, it is capable of growing on xylose, arabinose and fructose. As with other class I clostridia, it is expected that ethanol and acetate are formed through acetyl-CoA as the central intermediate (Rogers, 1986).

The overall stoichiometry for the formation of ethanol and acetate from CO and H₂/CO₂ has been established as (Vega *et al.* 1989):



Under usual laboratory conditions, *C. ljungdahlii* produces acetate as the major product, with only small quantities of ethanol present in the product stream. Figures 8 and 9 show the acetate and ethanol production profiles as a function of yeast extract concentration. The data show an ethanol/acetate

ratio of only 0.5. It is also noted that yeast extract has an influence on the product ratio, which leads to the hypothesis that high ethanol production is non-growth related. An examination of the acetyl-CoA pathway shows that production of acetate is balanced in ATP, while ethanol production results in a net consumption of ATP which would not support growth of the bacteria. Therefore, studies to minimize acetate production have concentrated upon factors which would not support growth of the bacteria. Therefore, studies to minimize acetate production have concentrated upon factors which regulate the growth of the organism.

Control of Growth Rate Parameters. *C. ljungdahlii* grows well and produces ethanol and acetate within a pH range of 4-6 with typical anaerobic media. Figure 10 shows the product distribution for *Clostridium* sp. with various initial yeast extract concentrations in batch culture. As noted, a molar ethanol to acetate ratio of 1:0 was obtained for yeast extract concentrations between 0.005 and 0.05 percent. The normal ratio of 1:22 results under more favorable growth conditions when employing 0.1 and 0.2 percent yeast extract. Studies with a defined medium of only vitamins, minerals and salts showed similar results in increasing the product ratio to about .1.

Recent research has shown that the presence of reducing agents in the liquid media of *Clostridium* fermentations has brought about an increase in solvent formation (Rao and Muetharsan, 1987; 1988). Reducing agents apparently cause altered electron flow, which direct carbon flow and acid to alcohol production. Reducing equivalents are directed to the formation of NADH which, in turn, resulted in increased alcohol production. Batch experiments were carried out with *C. ljungdahlii* by adding small quantities of reducing agents (30, 50 and 100 ppm) to assess the feasibility of increasing the ethanol to acetate ratio. The experiment carried out with 100 ppm of reducing agents resulted in very limited growth in all cases. On the other hand, 50 ppm and 30 ppm concentrations were successful in improving the ethanol to acetate ratio in some cases, as is shown in Table 1. The experiment with benzyl-viologen at a concentration of 30 ppm produced 3.7 mmol of ethanol with a ratio of 1.1, the highest ratio observed in batch experiments. It is interesting to mention that those reducing agents that improved the product ratio always resulted in slower growth rates of the bacteria, as could be expected from decreased ATP formation.

Table 1. Peak Levels for Ethanol Production and the Molar Ratio
(ETOH/ACH at 30 and 50 ppm Reducing Agent Concentrations)

Reducing agent	(50 ppm)		(30 ppm)	
	EtOH(mmol)	ETOH/ACH	ETOH(mmol)	ETOH/ACH
Control	0.60	0.12	1.40	0.24
Sodium thioglycolate	1.30	0.20	1.30	0.25
Ascorbic acid	1.50	0.24	1.50	0.25
Menthyl Viologen	1.90	0.20	2.50	0.40
Benzyl Viologen	1.25	0.21	3.70	1.10

Recently, the connection between sporulation and increased solventogenesis has been identified (Jones *et al.* 1982; Long *et al.* 1984; Gattschal and Morris, 1981). Under certain conditions, which are strain-dependent, a shift of the bacteria into a sporulation phase is accompanied by morphological changes (elongation of the cells) and the production of solvents rather than acids. A batch experiment with *C. ljungdahlii* was conducted on the premise that by forcing the culture to grow at a reduced rate, sporulation could be induced with an accompanying improvement in ethanol production. Synthesis gas was used as the primary carbon substrate. However, the complex nutrient yeast extract was replaced by various sugars and starches which, in previous studies, promoted sporulation of *Clostridium thermosaccharolyticum* (Pheil and Ordal, 1967). Table 2 summarizes the results obtained for each of the nutrients studies, along with the maximum values obtained for cell concentration, ethanol concentration and molar product ratios. As noted, the highest product ratios were obtained for cellobiose and rhamnose, with product ratios over 3 times the ratio obtained in the presence of yeast extract. Ethanol and cell concentrations were highest in the presence of cellobiose and galactose, where the ethanol concentrations were over 4 times the value obtained in the presence of yeast extract and the cell concentrations were 20 percent greater. Thus, cellobiose as a nutrient produces not only higher ratios of ethanol to acetate, but also higher concentrations of ethanol and cells.

Table 2. Summary of Results with Nutrient Sources Bringing About Sporulation

Nutrient	Maximum		ETOH/ACH molar ratio
	Cell Conc (mg/L)	ETOH (mmol)	
Yeast Extract	140	0.13	0.13
Cellobiose	170	0.56	0.45
Rhamnose	135	0.31	0.44
Galactose	168	0.53	0.36
Starch	130	0.27	0.36

Continuous Stirred-Tank Reactor Performance. An obvious method to produce high ethanol ratios is to operate two continuous reactors in series, with the first used to promote cell growth, while the second reactor is used for increased ethanol production. A pH shift between the reactors from 4.5 to 4.0, as well as a dilution rate shift, are used to cause the onset of ethanol production while, at the same time, causing growth to cease. Media constituents to promote growth can be added to the first reactor, and constituents to promote ethanol production at the expense of acetate can be added to the second reactor.

Figure 11 shows the molar product ratios for both stirred-tank reactors. yeast extract (0.02 percent) was added to the liquid medium of Reactor A (first in the series initially and cellobiose later). Ethanol concentrations in Reactor B increased to nearly 3 g/L and seemed to be stimulated somewhat by the use of cellobiose as the nutrient for cell growth. Substrate CO and H₂ conversions were essentially 100 percent in Reactor A, and fluctuated somewhat in Reactor B. The product ratio increased with time in both reactors, reaching a value of about 1.0 in Reactor A and a value of about 1.5 in Reactor B. The addition of cellobiose seemed to improve the product ratio over yeast extract. By subtracting the product concentrations produced in Reactor A, an ethanol ratio of 4 moles is obtained in Reactor B.

The specific productivity steadily improved to levels of 250-300 mmole ethanol/gcell·day throughout the experiment, which is a 30-fold improvement over specific productivities in a single CSTR.

BIOREACTOR DESIGN

The choice of a suitable bioreactor for synthesis gas fermentations will be a matter of matching reaction kinetics with the capabilities of the various reactors. It has been found that for these slightly soluble gases, the rate of mass transfer usually controls the reactor size (Vega *et al.* 1989a, 1989b). Mass transfer capabilities of the reactor must be balanced with the cell

density achieved. The proper reactors for these systems will likely be ones that achieve high mass transfer rates and high cell densities. These concepts will be expanded in this and the following section.

Gas-Liquid Mass Transfer Concepts

The transfer of gas phase substrates in fermentation systems involves three heterogeneous phases: the bulk gas phase, the culture medium (liquid) and microbial cells (solid) suspended in the medium. The reactants, present in the gas phase, must be transported across the gas-liquid interface and diffuse through the culture medium to the cell surface to be consumed by the microbes. In general, a combination of the following resistances can be expected (Bailey and Ollis, 1986)

1. Diffusion through the bulk gas to the gas-liquid interface.
2. Movement across the gas-liquid interface.
3. Diffusion of the solute through the relatively unmixed liquid region (film) adjacent to the bubble and into the well-mixed bulk liquid.
4. Transport of the solute through the bulk liquid to the stagnant film surrounding the microbial species.
5. Transport through the second unmixed liquid film associated with the microbes.
6. Diffusive transport across the liquid/solid boundary and into the microbial floc, mycelia, or particle, if appropriate. When the microbes take the form of individual cells, this resistance disappears.
7. Transport across the cell envelope to the intracellular reaction site.

As with the conventional chemical engineering analysis of absorption processes, mass transfer through the bulk gas phase is assumed to be instantaneous. Also, when individual cells are suspended in a medium, the liquid film resistance around the cells is usually neglected with respect to other resistances, because of the minute size and the enormous total surface of the cells (Finn, 1954). Thus, for the transfer of sparingly soluble gases, such as CO, the primary resistance to transport may be assumed to be in the liquid film at the gas-liquid interface.

It can be shown that the substrate rate per unit of reactor volume, $\frac{dN_S^G}{V_L dE}$, is given in terms of the gas phase partial pressures as:

$$\frac{d N_S^G}{V_L dt} = \frac{K_L a}{H} (P_S^G - P_S^L) \quad (1)$$

where N_S^G - moles substrate transferred from the gas phase, V_L is the volume of the liquid phase, t is time, K_L is the overall mass transfer coefficient, a is the gas-liquid interfacial area per unit volume, H is Henry's law constant, P_S^G is the partial pressure of the substrate in the bulk gas phase, and P_S^L is the partial pressure (dissolved tension) of the substrate in the liquid phase ($P_S^L = HC_L$). The rate of transport from the gas phase must be equal to the rate of consumption in the liquid phase, given by a Monod relationship:

$$\frac{d N_S^G}{V_L dt} = \frac{X q_m P_S^L}{K'_p + P_S^L + (P_S^L)^2/W'} = \frac{K_L a}{H} (P_S^G - P_S^L) \quad (2)$$

where X is cell concentration and q_m , K'_p , and W' are Monod constants.

Equation (2) shows that a bioreactor for these gaseous systems must operate in either of two regimes. In one case, sufficient cells are present to react more solute, but the mass-transfer rate cannot keep pace. Therefore, the liquid phase concentration goes to zero and the reactor is mass transport limited. The cell concentration and rate of consumption are limited by the ability of that particular reactor to transfer substrate. In the other case, sufficient substrate can be supplied, but the cell concentration does not allow consumption at an equal rate. Then the liquid phase concentration is not zero (with possible inhibitory effects) and the rate is limited by the cell concentrations in that particular bioreactor. Obviously, the best bioreactor is one that will achieve high cell concentrations and high mass transfer rates.

Bioreactors for Synthesis Gas Fermentations

Since large volumes of syngas must be processed, continuous reactors are dictated. Stirred-tank reactors achieve high mass transfer rates, but require substantial energy input for agitation. Immobilized cell reactors achieve high cell concentrations, without agitation, and are promising for these applications. Trickle-bed columns, where the gas is the continuous phase and the liquid flows over packed internals, is a unique means of increasing the mass transfer for these systems.

Stirred-Tank Reactor. The traditional CSTR assumes complete mixing and uniform concentrations throughout the bulk liquid phase. For syngas fermentations, the gas must be sparged into the liquid phase, be consumed, with any excess and product gases leaving the top of the liquid and eventually the reactor. High gas flow rates are required and near complete conversion of substrate is necessary. Conversely, only small liquid flow rates, essential to supply nutrients and remove liquid products, are necessary. Consequently, high cell concentrations should be possible. In most cases, the reactor volume will be controlled by the necessary gas retention time to achieve the desired conversion of substrate. Relatively high agitation rates will be required to promote transfer of the slightly soluble gas substrate.

Mass transfer coefficients, necessary for prediction of CSTR performance and scale-up, may be obtained from an analysis of the operation under mass-transfer limited conditions. A material balance around the CSTR with perfect mixing gives the relationship defining concentrations:

$$\frac{1}{Y_0} = \frac{1}{Y_1} + \frac{V_L}{Y_1} \frac{K_L a}{H} \frac{P_I^G}{n_I} \quad (3)$$

Equation (3) is expressed in terms of an inert component, whose quantity and partial pressure does not change through the system. Therefore, to simplify the model, concentrations are in the ratio of substrate to inert (Y), with Y_0 at the outlet and Y_1 at the inlet. P_I^G is the partial pressure of inert in the gas stream and n_I is the molar flow rate of inert. The agreement of this model with experimental data for $P_{\text{productus}}$ is shown in Figure 12. Good agreement is achieved with a linear relationship. The slope of this line gives the mass transfer coefficient, $K_L a/H = 30$. A model including Equation (3), as well as material balances for the gases flowing into the reactor and equilibrium relationships for the gas phase CO_2 with the bicarbonate and the pH level in the liquid, has been developed (Vega *et al.* 1989b). Solutions of the model for various volumetric mass transfer coefficients and various total operating pressures are shown in Figures 13 and 14, respectively. Experimental data at 1 atm and a mass transfer coefficient of 30 are also included in the figures. As observed, increases in the mass transfer coefficient or in total operating pressure leads to higher reactor productivities. Due to the perfect mixing in a CSTR, complete conversion is only possible when the gas flow rate is very low.

Figure 13 shows that with a mass transfer coefficient of 100, a pseudo retention time of one hour would result in a conversion of 80 percent. From Figure 14, the retention time could be reduced to 6 minutes at 10 atm for the same amount of CO converted. The use of the model allows the extrapolation of performance of the CSTR system and will permit preliminary economic evaluation of an industrial scale process when coupled with suitable equations for scale-up of properties such as the mass transfer coefficient.

Immobilized Cell Column (ICR). Column fermenters, with immobilized or suspended cells, offer the advantages of high cell densities and plug flow operation. These systems do not require mechanical agitation, with mixing provided by counter flow of gas and liquid. Energy for mixing is supplied by gas pressure drop and such systems are potentially more economical than the CSTR. Packed columns also offer the advantages of high surface to volume ratios and high mass transfer rates with reduced back-mixing.

Whole cell immobilization techniques can be classified into two major groups, entrapment and carrier binding (Vega *et al.* 1988). Entrapment includes both enclosure of a catalyst being a membrane or within a gel structure. Carrier binding includes all methods where there is a direct binding of cells to water-soluble carriers by physical adsorption or by ionic

and/or covalent bonds. Potential mass transfer limitations are always present with entrapment systems, either across the gel matrix or gel occlusion, or across the system membrane. On the other hand, the carrier-binding methods allow direct contact between the fermentation broth and the biocatalyst, with potentially enhanced mass transfer rates.

Microorganisms can be immobilized to insoluble biosupport materials by two methods: crosslinking and adsorption. Crosslinking, or covalent bonding, involves the use of a chemical agent, like glutaraldehyde or cyanuric chloride, to link the cells to the support. The chemical reaction is between the hydroxyl or lipid groups in the cell wall and a durable coating, like gelatin or agar, applied to the packing. Adsorption is the physical (occasionally ionic) attachment of the cell to the support. This method has been found to be effective for some small bacteria that can adhere to crevices in a support like wood chips.

In these reactors, the microorganisms are in direct contact with the substrate, minimizing diffusional resistance. These packed columns operate close to plug flow and, thereby, offer kinetic advantages for these reactions. Cells attached to the support grow and multiply into a film, which may be several layers of cells in thickness. In fact, cell overgrowth can result in completely filling the interstitial spaces, such that channeling may be a problem. Therefore, high cell densities and low retention times are possible.

By combining a material balance along the column with the rate expression for gas transport into the liquid phase, the following expression for the ratio of partial pressures of gaseous reactant entering and leaving the reactor is obtained:

$$\ln \frac{P_S^0}{P_S^1} = \frac{K_L a}{H} \frac{\epsilon_L h R T S}{G} \quad (4)$$

where ϵ_L is the fraction of liquid in the column, h is the height of the column, S is the cross-sectional area of the column, R is the ideal gas constant, T is the absolute temperature, and G is the gas flow rate. A plot of $\ln Y_1/Y_0$ vs $ShRT/G$ yields a straight line with slope $K_L a \epsilon_L / H$. The numerical solution (Runge-Kutts) of the differential equations that describe the system were solved for other operating conditions and are shown in Figure 15. Experimental data are given for $K_L a \epsilon_L / H$ of 13.5.

The immobilized cell column achieves higher rates of specific CO conversion than the stirred tank reactor without the need for more expensive mechanical agitation. More importantly, at the same mass transfer coefficients as in the CSTR, conversions are substantially higher. For example, at $K_L a / H = 100$, the conversion at a one hour retention time is 95 percent, compared to 80 percent for the CSTR. Alternatively, 80 percent conversion could be achieved in a retention time only 3 min in the ICR. The major disadvantage of the ICR is the lack of flexibility in operating conditions since the contacting capabilities are mainly fixed with the design of the column dimensions and packing.

CONCLUSIONS

The fermentation of coal synthesis gas has been demonstrated to methane and ethanol. Two pathways for the indirect production of methane from synthesis gas have been evaluated. Production through acetate as an intermediate is limited by acetate inhibition of methanogens. Production through H_2 with a co-culture of R. rubrum with methanogens gives faster rates without inhibition.

Ethanol can be produced from synthesis gas with a new species of Clostridium isolated from animal waste. The ratio of ethanol to acetate in the product stream is affected by many variables including pH, nutrient composition and the introduction of reducing agents to alter electron flow. High ethanol ratios are favored by non-growth conditions. Product ratios of 4:1 (ethanol to acetate) are achieved in a two-stage continuous culture with pH and dilution rate shift.

Bioreactors that achieve high mass transfer rates and high cell concentrations are desirable for synthesis gas fermentations. Methods to determine mass transfer coefficients for CSTR and ICR reactors have been developed. High pressure has been found to increase the reaction rate proportionately. Models for these bioreactors show high conversion of gaseous substrate can be achieved in a retention time of a few minutes.

LIST OF REFERENCES

- Bailey, J. E. and D. F. Ollis, Biochemical Engineering Fundamentals, McGraw-Hill, New York (1986).
- Balch, W. E., S. Schoberth, R. S. Tanner, and R. S. Wolfe, "Acetobacterium, New Genus of Hydrogen-Oxidizing, Carbon-Dioxide-Reducing Anaerobic Bacteria," Int'l. J. Syst. Bacteriology, 27, 355-361 (1977).
- Barik, S., E. R. Johnson, E. C. Clausen, and J. L. Gaddy, "Conversion of Coal Synthesis Gas to Methane," Energy Progress, 7, 157 (1987).
- Barik, S., J. L. Vega, E. R. Johnson, E. C. Clausen, and J. L. Gaddy, "Methanation of Synthesis Gas Using Biological Processes," CRC Series, (1987).
- Breed, R. S., E. G. D. Murray, and N. R. Smith, Bergey's Manual of Determinative Bacteriology, The Williams and Wilkins Company, Baltimore, MD (8th ed.) (1987).
- Clausen, E. C. and J. L. Gaddy, "Advanced Studies of the Biological Conversion of Synthesis Gas to Methane," Topical Report 1: Reactor Optimization, Performed on METC Contract DE-AC21-86MC23281, U. S. Department of Energy, March 1988.
- Coffin, J. M., "Industrial Coal Gasification: Applications and Economy," Energy Progress, 4, 131-137 (1984).
- Courty, Ph. and P. Chaumette, "Syngas: A Promising Feedstock in the Near Future," Energy Progress, 7, 23-30 (1987).
- Dashekovicz, M. P. and R. L. Uffen, "Identification of a Carbon Monoxide-Metabolizing Bacterium as a Strain of Rhodopseudomonas gelatinosa," International Journal of Systematic Bacteriology, 29, 145-148 (1979).
- Finn, R. K., "Agitation-Aeration in the Laboratory and in Industry," Bacteriol. Rev. 18, 154-274 (1954).
- Genthner, B. R. S. and M. P. Bryant, "Growth of Eubacterium limosum with Carbon Monoxide as the Energy Source," Appl. Environ. Microbiol. 43, 70-74 (1982).
- Gottschal, J. C. and J. G. Morris, Biotechnology Letters, 3, 525-530 (1981).
- Graboski, M. S., "The Production of Synthesis Gas from Methane, Coal, and Biomass. In Catalytic Conversion of Synthesis Gas and Alcohols to Chemicals, R. G. Herman (ed.) Plenum Press, New York, pp. 37-50 (1984).
- Huser, B. A., K. Wuhmann, and A. J. B. Zehnder, "Methanotherix soehgenii gen. nov. sp. nov., a New Acetotrophic Non-hydrogen-oxidizing Methane Bacterium," Arch. Microbiol. 132, 1-9 (1982).

- Jones, D. T. et al. "Solvent Production and Morphological Changes in Clostridium acetobutylicum Induced by Viologen Dyes," Applied and Environmental Microbiology, 53, No. 6, 1232-1235 (June 1987).
- Jones, W. J. D. P. Nagle, Jr., and W. B. Whitman, "Methanogens and the Diversity of Archaeobacteria," Microbiol. Rev. 51, 135-177 (1978).
- Kerby, R., W. Niemczura, and J. G. Ziekus, "Single Carbon Catabolism in Acetobacterium woodii and Butyribacterium methylotrophicum by Fermentation and ¹³C Nuclear Magnetic Resonance Measurement," J. Bacteriol. 155, 1208-1218 (1983).
- Kerby, R. and J. G. Ziekus, "Growth of Clostridium thermoaceticum on H₂/CO₂ or CP as Energy Source," Curr. Microbiol. 132, 1-9 (1982).
- Long, S et al. "Isolation of Solvent Production, Clostridial Stage and Endospore Formation in Clostridium acetobutylicum." Applied Microbiology and Biotechnology, 20, 256-261 (1984).
- Lorowitz, W. H. and M. P. Bryant, "Peptostreptococcus productus Strain that Grows Rapidly with CO as the Energy Source," Appl. Environ. Microbiol. 47, 961-964 (1984).
- Mayer, F., R. Lurz, and S. Schoberth, "Electron Microscopic Investigation of the Hydrogen-Oxidizing Acetate-Forming Anaerobic Bacterium Acetobacterium woodii," Arch. Microbiol. 112, 207-214 (1977).
- Pheil, C. G. and Z. G. Ordal, "Sporulation of the Thermophilic Anaerobes," Applied Microbiology, 51, No. 4, 893-898 (1967).
- Rao, G. and R. Murtharasan, "Altered Electron Flow in a Reducing Environment in Clostridium acetobutylicum," Biotechnology Letters, 10, No. 2, 129-132 (1988).
- Rao, G. and R. Mutharasan, "Altered Electron Flow in Continuous Cultures of Clostridium acetobutylicum Induced by Viologen Dyes," Applied and Environmental Microbiology, 53, No. 6, 1232-1235 (June 1987).
- Rogers, P., "Genetics and Biochemistry of Clostridium Relevant to Development of Fermentation Processes." Advances in Applied Microbiology, 31, 1-60 (1986).
- Simbeck, D. R., R. L. Dickenson, A. J. Moll, "Coal Gasification, An Overview," Energy Progress, 2, 42-46 (1982).
- Sleat, R., A. Mah, and R. Robinson, "Acetoanaerobium noterae: New Genus, New Species, An Anaerobic Bacterium that Forms Acetate from Hydrogen and Carbon Dioxide," Intl. J. Syst. Bacteriol. 35, 10-15 (1985).
- Specks, R. and A. Klussmann, "German Hard Coal Conversion Projects," Energy Progress, 2, 60-65 (1982).
- Thauer, R. K., K. Jungnermann, and K. Decker, "Energy Conservation in Chemotrophic Anaerobic Bacteria," Bacteriol. Rev. 41, 100-180 (1977).

Uffen, R. L., "Anaerobic Growth of a Rhodospseudomonas Species in the Dark with Carbon Monoxide as Sole Carbon and Energy Substrate," Proc. Natl. Acad. Sci. U.S.A. **73**, 3298-3302 (1976).

Vega, J. L., S. Prieto, B. B. Elmore, E. C. Clausen, and J. L. Gaddy, "The Biological Production of Ethanol from Synthesis Gas," Appl. Biochem and Biotech. **20**, 781 (1989).

Vega, J. L., E. C. Clausen, and J. L. Gaddy, "Biofilm Reactors for Ethanol Production," Enzyme Microb. Technol. **10**, 403 (1988).

Vega, J. L., E. C. Clausen, and J. L. Gaddy, "Study of Gaseous Substrate Fermentations: Carbon Monoxide to Acetate. 1. Batch Culture," Biotechnol. Bioeng. (accepted for publication) (1989a).

Vega, J. L., G. M. Antorrena, E. C. Clausen, and J. L. Gaddy, "Study of Gaseous Substrate Fermentations: Carbon Monoxide to Acetate. 2. Continuous Culture," Biotechnol. Bioeng. (accepted for publication) (1989b).

Wiegel, J., "Formation of Ethanol by Bacteria. A Pledge for the Use of Extreme Thermophilic Anaerobic Bacteria in Industrial Ethanol Fermentation Processes." Experientia, **36**, 1434-1446 (1980).

Wood, H. G., H. L. Drake, and S. Hu, "Studies with Clostridium thermoaceticum and the Resolution of the Pathway Used by Acetogenic Bacteria that Grow on Carbon Monoxide or Carbon Dioxide and Hydrogen." Proc. Biochem. Sym. 29-56 (1982).

BIODESULFURIZATION SYSTEMS FOR REMOVAL OF ORGANIC SULFUR FROM COAL: A CRITICAL REVIEW

Abdel El Sawy and David Gray
The MITRE Corporation
7525 Colshire Dr.
McLean, VA 22102

KEYWORDS: Biodesulfurization

ABSTRACT

This study evaluates approaches for the biological removal of organic sulfur from coal. In this area, Atlantic Research Corporation's (ARCTECH's) biodesulfurization system is the only one that has been demonstrated on coal with mixed success on a continuous bench-scale unit of 10 lb/day capacity. Other biocatalytic systems developed by the Institute of Gas Technology (IGT) or Southern Illinois University (SIU) are still in the laboratory-scale microbial selection and screening stage. Yet, the successful use by IGT of a sulfur-limited, continuous chemostat for the selection of bacterial strains with appropriate desulfurization activities has provided a convenient and powerful strain selection technique. The IGT work has also established the possibility of changing the metabolic pathway by proper modification of the bacterial growth medium and introduced the sulfur bioassay technique to help compare the effectiveness of different microorganisms grown with different substrates under different conditions on a comparable basis. SIU was successful in mapping the desulfurization genes in a mutant *E. coli*.

There is still a need for faster growing, stable, and more active biodesulfurization microorganisms than those which have been developed so far. This calls for a program of strain selection and improvements through molecular genetics, a thorough understanding of coal biodesulfurization metabolism and its associated metabolic pathways, investigation of extracellular enzymatic removal of organic sulfur from coal, and identification of new acidophilic heterotrophs that have broad organic sulfur removal capabilities and that can coexist with other bacterial strains currently used for inorganic sulfur removal.

BACKGROUND

Historically, most of the research on the biological processing of coal was directed to pyrite removal; very few studies have been explicitly devoted to organic sulfur removal although there are three studies that are particularly interesting. One study conducted in 1979 by Chandra et al.⁽¹⁾ indicated that a heterotrophic bacterium, enriched on dibenzothiophene (DBT), can remove up to 20 percent of the organic sulfur present in Indian coal after 10 days of incubation in a laboratory rotary shaker at 30°C. Another study conducted by Gokcay and Yurteri⁽²⁾ in 1983 on Turkish lignite showed that 50 to 57 percent of the organic sulfur and 90 to 95 percent of the pyritic sulfur were removed over a 25-day incubation period. The third study was conducted by Kargi and Robinson⁽³⁾ at Lehigh University using bituminous coal suspended in a growth medium inoculated with *Sulfolobus acidocaldarius* organism. After 28 days of incubation with the DBT-adapted culture at 70°C, nearly 19 percent of the initial organic sulfur in the pretreated coal was removed. Further studies on the ability of the *Sulfolobus* species to desulfurize coal continued at Lehigh University in 1983 under the sponsorship of the U.S. Department of Energy's (DOE) Pittsburgh Energy Technology Center (PETC).

CURRENT STATE OF THE ART

Since 1983, DOE has continued to sponsor the investigation of various biological approaches for organic sulfur removal from coal at various U.S. institutions. Thus, ARCTECH Inc. (formerly Atlantic Research Corporation) was funded to continue their work on the development of *Pseudomonas* and other "Coal Bugs" that were able to release organically-bound sulfur from DBT and from selected coals. The Institute of Gas Technology (IGT) was funded to investigate the general feasibility of the microbial removal of organic sulfur from coal. Southern Illinois University (SIU) was also funded under DOE's University Coal Research Program in support of the overall coal biodesulfurization mission. All these DOE-supported research efforts are critically reviewed in this report.

ARCTECH Inc. has isolated a mutant *Pseudomonas* microorganism designated CB1 ("coal bug 1") that has shown the ability to remove sulfur, both from model sulfur compounds and from various coals.⁽⁴⁾ Studies using dibenzothiophene (DBT) indicate that CB1 appears to be most effective in removing thiophenic sulfur. In laboratory-scale studies of coal desulfurization, CB1 reduced the percent organic sulfur by 18 and 47 percent at residence times of 9 - 18 hours depending on the coal, particle size, initial organic sulfur content and other, as yet unidentified, parameters. Various coals have also been treated with CB1 in a continuous bench-scale unit that can process 10 pounds/day of coal. Percent organic sulfur reductions varied from 10 to 29 weight percent, depending apparently on the coal and other unidentified parameters.

ARCTECH Inc. has also isolated another microorganism designated CB2 that has shown the ability to oxidize aryl sulfide model compounds like diphenyl sulfide (DPS) and benzyl phenyl sulfide (BPS). This microorganism was also tested on various coals with mixed success. Between 14 and 34 weight percent organic sulfur reduction was achieved for three coals. Again, the variation in effectiveness seemed to be coal dependent, but other factors were obviously present. Like CB1, CB2 has a negligible effect on the pyritic sulfur.⁽⁴⁾

Since experimental evidence from model compound studies indicated that CB1 and CB2 were metabolizing different sulfur functionalities, coal desulfurization experiments were performed by ARCTECH using a mixed culture of CB1 and CB2 in an attempt to improve the overall sulfur removal. The results indicated that coal desulfurization using mixed cultures was generally less effective than that achieved using the pure culture.⁽⁴⁾ Furthermore, it appears that a metabolic by-product of CB2 inhibits the growth of CB1, thus allowing CB2 to become predominant in the total biomass.⁽⁴⁾ This indicates that sequential desulfurization using the two cultures independently may be the preferred and only solution.

The Institute of Gas Technology has developed a sulfur bioavailability assay to identify microorganisms capable of degrading model sulfur compounds.⁽³⁾ Recently (1989) efforts in this area have resulted in the successful adaptation of the IGT Sulfur Bioavailability Assay to microtiter plates.⁽⁶⁾ This allows large numbers of mutagenized colonies to be conveniently screened to detect desulfurization-deficient mutations. Using this bioassay, IGT has identified a strain of microorganism designated IGTS7 that, when grown on several carbon substrates, is capable of degrading a wide variety of model sulfur compounds, including DBT. Using a sulfur-limited continuous chemostat, IGT further tested this microorganism on Illinois #6 coal. The chemostat effluent was monitored for the presence of metabolizable sulfur, and two peaks were found corresponding to 30 and 70 days of operation. It was surmised that the second peak represented the metabolism of organic sulfur in the coal by the IGTS7 strain that had survived the continuous operation of the chemostat during the sulfur-free period following the decline of the first peak. To test this, samples of coal were analyzed initially, at day 53 and at day 91. The last sample indicated a decrease in organic sulfur content for the coal of about 24 weight percent. Subsequent to this work, IGT has reported an organic sulfur removal of 90 percent using this microorganism after 212 days of chemostat operations. The coal apparently experienced a carbon loss of 39 percent during this procedure. Such an extraordinarily high sulfur removal needs to be replicated in further experiments at considerably shorter residence times before general scientific acceptance is forthcoming. The major thrust of current experimental efforts at IGT is the isolation of pure cultures out of the IGTS7 mixed culture.⁽⁷⁾ A pure culture capable of desulfurization would greatly aid future research in genetics. Two pure cultures of bacteria

that are each capable of utilizing dibenzothiophene (DBT) as their sole source of sulfur were isolated from the mixed IGTS7. These cultures have been identified as *Rhodococcus rodochroas* and *Bacillus sphaericus* species, and have been designated IGTS8 and IGTS9, respectively.⁽⁷⁾ None of these cultures alone was found capable of sulfur-specific metabolism. However, the pairwise combinations of any of these cultures with *Enterobacter* species can reproducibly perform well in the Sulfur Bioavailability Assay. At this point, it is believed that the *Enterobacter* species is only a nutritional component needed for the growth of the *Rhodococcus* and *Bacillus* species, with no contribution to desulfurization.⁽⁸⁾

Research at SIU at Carbondale has utilized two approaches for isolating and developing bacteria capable of removing organic sulfur from coal: enrichment culture and genetic manipulation. In the enrichment culture approach, which incidentally is the approach used by ARCTECH, the organism is isolated from naturally-occurring bacteria and adapted for growth on model sulfur compounds. The adapted organism is then subjected to a repetitive selection and mutation cycle to provide the enriched culture with the desired traits. The desulfurization potential of the isolated strains was determined by measuring sulfate and/or hydrogen sulfide released during bacterial degradation of model sulfur compounds. Several isolates were selected that could degrade dibenzothiophene sulfane (DBTS), dibenzothiophene (DBT), benzene sulfonic acid (BSA) and cystine (CYL).⁽⁹⁾ The isolates that degraded the latter compound were particularly active in their growth and were selected as potential candidates for future coal desulfurization studies.

The genetic manipulation approach of SIU involves mutation of *E. coli*, a genetically well-understood organism for metabolizing sulfur containing amino acids. *E. coli* NAR3 is a bacterial strain produced after successive cycles of mutation and selection. This strain can degrade thiophenes, furans, and other sulfur-containing aromatic compounds.⁽¹⁰⁾ Genetic analysis of NAR3 has been undertaken at SIU, and since then other mutants showing improved degradation of thiophene and other sulfur containing aromatic substrates have been isolated. One of these, designated NAR41, has shown increased affinity for thiophene rings and decreased affinity for other non-sulfur containing rings.⁽¹⁰⁾ This is clearly in the right direction since the goal is to remove sulfur with as little loss of coal carbon as possible.

CRITICAL REVIEW

The basic goal of all biodesulfurization processes is to remove the organically-bound sulfur from coal while retaining the fuel value of the coal. This means that biodesulfurization should follow a metabolic pathway that eliminates sulfur with little destruction of the coal carbon. Investigations to date have focused on the metabolic pathway analysis for DBT and not for coal. In the desirable pathway, the so-called 4-S, the DBT is successively oxidized to sulfate and 2,2'-dihydroxybiphenyl.^(11,12) The other pathway results in destruction of the aromatic ring structure of DBT with no liberation of sulfur. CB1 apparently releases sulfur from DBT as sulfate, and 2,2'-dihydroxybiphenol has been identified as the organic product. Thus, CB1 seems to operate via the 4-S pathway. IGT finds monohydroxybiphenyl as a product, so it is likely that IGTS7 metabolizes DBT using a variant of the 4-S pathway. The other microorganisms under development appear to involve some participation of carbon-destructive metabolic pathways.

All coal desulfurization organisms developed so far have been recovered from microbial populations isolated from soil near coal mines or petroleum refineries. These microorganisms are single cell (prokaryotes), rod-like aerobic bacteria that remain active only in neutral or alkaline medium at temperatures between 25 and 35°C. Because of this, they are unable to coexist with those acidophilic heterotrophs currently used for inorganic sulfur removal. Furthermore, the metabolism of most of these organisms is poorly understood at present, and therefore, their growth media have generally not been optimized.

Despite its attractive potential, the biodesulfurization of coal has its problems and limitations. One major problem is the heterogeneity of coal, which means that the same microorganism may not be effectively used with all types of coal. In other words, the biological removal of organic sulfur from coal may have to be tailored to each coal separately. Possible instability of genetically-engineered microbial cultures is another problem. Over

extended periods of usage, an originally effective desulfurization organism may give rise to spontaneous derivatives that lack desulfurization ability. The long residence time required for bioprocessing is also a major obstacle to the usefulness of this technology. Low biodesulfurization rate, slow bacterial growth, and low process yield may all contribute to limit future application of biodesulfurization. The other potential limiter is the surface availability of the organic sulfur for microbial attack.

For any desulfurization process to be viable from a utility boiler aspect, it should be capable of reducing the sulfur content of coal to produce compliance fuels. For typical U.S. coals with 3 percent total sulfur and assuming 90 percent physical removal of inorganic sulfur, the removal of 50 percent of the organic sulfur is required. Thus, about 60 percent of the total coal sulfur must be accessible for microbial metabolism at the coal surface. This can be achieved theoretically with finely-ground coal having an average particle size of 38 microns (200-400 mesh), assuming that the thickness of the outer coal surface layer accessible for microbial action is 5 microns. Fine grinding of coal can be both an economic and a technical penalty since dewatering of coal slimes is a difficult problem. Whether microbial action can penetrate below the coal surface for non-extracellular enzymatic processes remains to be investigated.

An important economic criterion is the growth rate of the microorganism. This determines the capacity of the fermenters and therefore impacts capital cost. Economically attractive growth rates of 0.66 hour^{-1} have been quoted in the literature,⁽¹³⁾ and this corresponds to a bacterial generation time of 1.05 hours, compared to 3.8 hours for CB1 and 4.0 hours for CB2. Other microorganisms isolated so far in the biodesulfurization program have much longer generation times of two days or more. Another consideration is the biomass yield. This determines the necessary growth media, oxygen demand, productivity, plant size and thus operating and capital costs. The typical economic biomass yield for *Pseudomonas* grown on benzoic acid is 0.60 grams of dry cell biomass per gram of benzoic acid consumed. This is 15 percent higher than that achieved by CB1 under current growth conditions on benzoic acid. The kinetics of the process also determine economic viability. Assuming first-order biodesulfurization kinetics and removal of 50 percent organic sulfur at the best currently achieved residence time of 9 hours, the rate constant is 0.08 hour^{-1} . The desulfurization rate constant for CB1 currently ranges between 0.01 and 0.05, depending on conditions and the coal type.

CONCLUSIONS AND RECOMMENDATIONS

This analysis of the various techniques being investigated for the chemical cleaning of coals has not been able to positively identify the most promising approaches to this problem. One reason is lack of overall process data in much of the current research effort. Emphasis has been on overall sulfur removal efficiency. The relative proportion of organic vs inorganic sulfur has often not been identified. The issues of carbon losses and product characteristics have essentially been neglected. Other aspects generally not addressed in current research are the potential costs and process energy requirements.

If, as conventional wisdom suggests, the organic sulfur in coal is an integral part of the coal matrix, then disruption of the coal matrix must occur before organic sulfur can be removed. This disruption implies that the product may have properties and characteristics different from the parent coal, this difference being a function of the severity of the desulfurization process.

These considerations point to major recommendations for research priorities in the area of coal desulfurization. Of prime importance is to attempt to develop analytical techniques for identifying organic sulfur speciation. Potential techniques are already in existence, and very likely several of them will have to be used in combination to unequivocally assign sulfur functionalities in the coal matrix. Once we know what compounds we are dealing with and have a reliable way to measure them, we stand a better chance of developing chemistry and biochemistry that will remove them.

In addition, research in coal desulfurization must constantly be concerned not only with desulfurization efficiency, but also with the characteristics and potential uses of the desulfurized product. Superclean coals may well be superclean from the standpoint of low sulfur and mineral matter content, but they may also have limited utility as a fuel form.

The use of biological means for the removal of organic sulfur from coal must be looked upon as a potential long-term development. Assuming that stable microorganisms can be developed that degrade organic coal sulfur, there are still many uncertainties yet to be resolved before biodesulfurization of coal becomes a realistic commercial option. These uncertainties include (1) a realistic estimate of the accessible organic sulfur available for microbial metabolism at the coal particle surface, (2) reliable determination of the various sulfur species in coal, (3) scale-up considerations for bioreactors, and (4) reasons for the variable response of different coal types to bioprocessing.

The ARCTECH biodesulfurization system is currently the only one that has been demonstrated on coal, although with mixed success, using a continuous bench-scale unit of 10 lb/day capacity. Other biocatalytic systems developed by IGT or SIU are still in the laboratory-scale microbial selection and screening stage. The research work of IGT and SIU has, nevertheless, contributed significantly to the coal biodesulfurization mission. IGT's successful use of a sulfur-limited, continuous chemostat for the selection of bacterial strains with appropriate desulfurization activities has provided a convenient and powerful strain selection technique. The IGT work has also established the possibility of changing the metabolic pathway by proper modification of the bacterial growth medium, and has introduced the sulfur bioassay technique to help compare on a common basis the effectiveness of different microorganisms grown with different substrates under different conditions. SIU was successful in mapping the desulfurization genes in a mutant *E. coli*. This is an important step towards the application of molecular genetics for the development of improved bacterial strains with enhanced desulfurization capabilities.

The conclusion is that there is a need for faster growing, stable, and more active biodesulfurization microorganisms than those that have been developed so far. To this end, there are several major research needs that can be identified. There is a need for a structured and systematic program of strain selection and improvements through molecular genetics, and a need for a thorough understanding of coal biodesulfurization metabolism and its associated metabolic pathways. A similar approach was successfully applied in penicillin production and resulted in a thousandfold increase in yields. There are also needs to investigate extracellular enzymatic removal of organic sulfur from coal, and to identify new acidophilic heterotrophs that have broad organic sulfur removal capabilities and that can coexist with other bacterial strains currently used for inorganic sulfur removal.

ACKNOWLEDGEMENT

This work was supported at the MITRE Corporation by Sandia National Laboratories under contract to the U.S. Department of Energy (DE-AC04-76DP00789).

REFERENCES

1. Chandra, D., P. Roy, A. K. Mishra, J. N. Chakrabarti, and B. Sengupta. "Microbial Removal of Organic Sulfur From Coal". *Fuel*, **58**, 549, 1979.
2. Gokcay, C. F. and R. N. Yurteri. "Microbial Desulfurization of Lignites by a Thermophilic Bacterium". *Fuel*, **62**, 1223, 1983.

3. Kargi, F. and J. M. Robinson. "Removal of Organic Sulfur from Bituminous Coal". *Fuel*, 65, 397, 1986.
4. *Microbially Mediated Removal of Organic Sulfur from Coal*. A report prepared by Atlantic Research Corporation under DOE/PETC Contract No. DE-AC22-85PC81207, November 6, 1987.
5. Kilbane II, J. and Andrea Maka. *Microbial Removal of Organic Sulfur from Coal*. Tenth Quarter Report, prepared for DOE/PETC, DOE Contract No. DE-AC22-85PC81201, IGT Project No. 61078, March 1988.
6. Bielage, Barbara and John J. Kilbane. *Molecular Biological Enhancement of Coal Biodesulfurization: Third Quarter Report*. Prepared for DOE/PETC under DOE Contract No. DE-AC22-88PC8891, IGT Project #61095, July 1989.
7. Bielaga, Barbara and John J. Kilbane. *Molecular Biological Enhancement of Coal Biodesulfurization: Second Quarter Report*. Prepared for DOE/PETC under DOE Contract No. DE-AC22-88PC91, IGT Project #61095, April 1989.
8. Bielaga, Barbara and John J. Kilbane. *Molecular Biological Enhancement of Coal Biodesulfurization: First Quarter Report*. Prepared for DOE/PETC under DOE Contract No. DE-AC22-88PC91, IGT Project #61095, December 1988.
9. Klubek, B. and D. Clark. *Microbial Removal of Organic Sulfur from Coal (Bacterial Degradations of Sulfur-Containing Heterocyclic Compounds)*. Final Report Submitted to the U. S. Department of Energy under Contract No. DE-FC22-86PC91272, 1987.
10. Abdulrashid, N. and D. Clark. "Isolation and Genetic Analysis of Mutations Allowing the Degradation of Furans and Thiophene by *E. Coll.*" *Journal of Bacteriology*, 169, 1267-1271, 1987.
11. Kadama, K. S. Nakatani, K. Umehara, K. Shimizu, Y. Minoda, and K. Yamada, "Microbial Conversion of Petrosulfur Compounds: Isolation and Identification of Products from Dibenzothlophene", *Agr. Biol. Chem.*, 34, 1320-1324, 1970.
12. Kilbane II, J. *Microbial Removal of Organic Sulfur from Coal: Current Status and Research Needs*. A preprint of a chapter to appear in *Biotechnology Applied to Fossil Fuels*, Donald L. Wise, Editor, Boca Raton, Florida: CRC Press, 1988.
13. Atkinson, B. and F. Mavituna. *Biochemical Engineering and Biotechnology Handbook*, New York: The Nature Press, 1983.

COAL SULFUR TRANSFORMATIONS MONITORED BY HYPERTHERMOPHILIC ARCHAEABACTERIA

T. L. Peeples, S. Hirose, V. Muralidharan, R. M. Kelly

Department of Chemical Engineering
The Johns Hopkins University
Baltimore, MD 21218

Gregory J. Olson

Polymers Division
National Institute of Standards and Technology
Gaithersburg, MD

Introduction

Microbial processes have been used as the basis for significant chemical transformations in several industries including but not limited to pharmaceuticals, foods processing and waste management. However, biotransformations are often limited by the metabolic rate of the organism and the stability of its constituent enzymes and other biomolecules. Industries which have successfully applied microorganisms for commercial purposes have developed processes that are consistent with whatever biological limitations prevail.

The spread of biotechnology has resulted in considerable attention to the use of biological systems in mineral and fossil fuel processing. For example, the acidophilic bacterium *Thiobacillus ferrooxidans* has been used in copper and uranium leaching as well as in small scale studies of pyrite removal from coal (1,2). Other potential uses for microbes in coal processing include desulfurization, denitrification, oxygen removal, solubilization and gasification of coals (3). The wide range of metabolic characteristics available in the global pool of microorganisms may enable researchers to discern more about the functionalities in coal structure as well as to build cultures in which the desired conversions can be engineered.

Hyperthermophiles

During the past decade, several bacteria have been isolated that thrive at temperatures at or above 100 °C (4). These hyperthermophiles may have potential for a variety of significant biotransformations in the field of biotechnology. The high temperature optima of hyperthermophiles and their associated biomolecules may be useful in industrial processes by carrying out transformations at faster rates and with high levels of stability. Recently, we have been evaluating the potential for using hyperthermophilic archaeobacteria in coal upgrading (5). In doing so, both the ecology and physiology of these organisms must be considered.

Hyperthermophiles are associated with geothermally heated areas, which often are sulfur- and metal-rich environments. Several sulfur-metabolizing species have been isolated from these areas. The ability of these organisms to transform sulfur compounds at high temperatures is the interesting metabolic trait that could be capitalized upon for sulfur removal from coal. However, the nature of the various sulfur metabolisms differ and are not completely understood (4).

In preliminary studies, *Pyrococcus furiosus*, an anaerobic heterotroph isolated from geothermally heated marine sediments off of the coast of Vulcano, Italy (6), has been used as a representative hyperthermophilic strain. In the absence of S^0 , *P. furiosus* produces H_2 , which is inhibitory for growth, and CO_2 (7,8). In the presence of S^0 , *P. furiosus* produces H_2S , either respiring sulfur or utilizing it to remove H_2 from the environment (8). *P. furiosus* grows to higher maximum cell densities (10^8 cells/ml) with faster doubling times (1 hour) than most of the hyperthermophilic

archaeobacteria (6,9). Because of the easier cultivation of the organism, *P. furiosus* appears to be more suitable for coal upgrading. In addition to sulfur removal capability, thermophilic heterotrophy may make *P. furiosus* suitable for breaking down complex coal constituents.

Hyperthermophiles and coal-sulfur

Previously, we examined the feasibility of coal desulfurization with *P. furiosus* (5,10,11). Various coals and model compounds were screened for sulfur removal activity. Success in sulfur removal was measured by the production of H_2S . Only highly weathered gob coals showed the production of H_2S upon exposure to *P. furiosus* (5,11). Continuous culture of *P. furiosus* with coal containing elemental sulfur showed that *P. furiosus* can remove sulfur from coal at rates comparable and in most cases higher than mesophilic organisms studied for sulfur removal (10). The drawback is that the sulfur within coal must be in the form useful to the microorganism in order to be converted. Very few of the more pristine coals tested showed H_2S production upon exposure to *P. furiosus*, suggesting that sulfur metabolizable by *P. furiosus* is not present in these coals (5,11).

Model compound studies showed that *P. furiosus* metabolizes only those sulfur compounds which are polysulfidic or form polysulfides near culture temperatures (98°C). Compounds with $-(S-S-S)-_x$ bonds are believed to be only a small fraction of coal sulfur. The specificity of *P. furiosus* for these compounds may be useful in settling a few disputes about polysulfidic compound generation in coal. (7)

Coal Weathering and Sulfur Speciation

Elemental sulfur in coal is believed to be a product of pyrite oxidation (12-14). During coal weathering under varying reaction conditions, oxidation products from FeS_2 have been reported to include S_n as well as FeO , FeS , Fe_2O_3 , $FeSO_4$, $Fe_2(SO_4)_3$, SO_2 and SO_3 . The weathering products of the organic sulfur components of coal have not yet been determined, because the organic sulfur species themselves are not known. Postulated organic sulfur compounds in coal are thiols, sulfides, disulfides, and thiophenic residues. Oxidation experiments both with model sulfur species and with coal have resulted in the production of sulfones, sulfonic acids and sulfates (12,13).

The formation of elemental sulfur from organic sulfur compounds under mild oxidation is thought to be unlikely primarily because the oxidation of the organic moieties would require gasification of the carbon skeleton of the coal (14). Mild oxidation of coals is a surface phenomenon involving the addition of oxygen molecules and small-scale rearrangement. Break-down of the carbon backbone would require more extreme processing conditions (15).

By ASTM guidelines, sulfur speciation in coals requires experimental determination of sulfatic, pyritic, and total sulfur content. Organic sulfur is determined by difference. This calculational procedure tends to propagate experimental error of the analytical techniques and therefore leads to inaccuracy in organic sulfur values (12,13,16-18). In addition to technical error, the presence of elemental sulfur can lead to overestimation of organic sulfur content (19).

Artificial Weathering Experiments

Many mild oxidation experiments have been run on on both coal and mineral pyrite in attempts to discern the mechanisms of sulfur transformations during weathering. Although the reactivities differ between ore pyrite and coal pyrites and among pyrites from different coal sources (20-22), parameters that have been tested and shown to affect the distribution of sulfur oxidation products for all pyritic substrates include: temperature, humidity, oxygen content, acidity and the presence of chemical oxidants.

Water, both in vapor and liquid forms has been shown to have an accelerating effect on the weathering process (12,13,17,24). These results indicate the interaction of water with oxygen groups on the coal surface. The enhancement of oxygen uptake due to the presence of water agrees with the theory of peroxygen formation at the coal surface as the initiation step of weathering (25).

Results from several artificial weathering studies suggest that the mechanism of pyrite oxidation is strongly dependent upon temperature. Sulfate products have been found to prevail at low temperature conditions, while a complex range of oxidation products are generated at higher temperatures (13,17). Even within the range of 'realistic' weathering temperatures (25 to 80 °C), the products of coal pyrite oxidation vary considerably. (23)

In the pyrite crystal, iron is anchored in the lattice and sulfurs extend from the surface. Each of the two pyritic sulfurs is bound to another sulfur molecule yielding an FeS_4^{-4} configuration of pyrite. The extended sulfurs become oxygenated as a result of weathering. Oxygenation can occur as the result of peroxygen or hydrated Fe^{3+} producing dissociation conditions on the pyrite surface. (26) The Fe-S bond weakens and breaks before the S-S bond in the disulfide groups. This is confirmed by the appearance of thiosulfate as a specific intermediate (26,27). The production of a polysulfide could be consistent with this theory. At pH lower than 5 thiosulfate decomposes to elemental sulfur and sulfate.

There have been several studies of pyrite oxidation in aqueous suspension. Most of the work at low temperature 30 °C and pH greater than 6 has shown little or no production of elemental sulfur. McKay and Halpern weathered mineral pyrite in aqueous suspension under varying levels of acidity (27). Through mass balance it was suggested that elemental sulfur was formed at low levels. These authors assumed that no thiosulfates and thionates were formed from the oxidation. All of the oxidized sulfur that was not accounted for in the form of sulfate was assumed to be elemental sulfur. It was found that 'elemental' sulfur was produced at low pH.

Moses et. al. used ion chromatography to analyze pyrite oxidation solutions for sulfoxo anions (26). At higher pH, oxidation of pyrite to sulfate was rapid with little production of sulfoxo intermediates. At low pH, 10 - 25% of sulfur in solution was determined to be thiosulfate and polythionate. No analysis for elemental sulfur was performed.

Like Moses, Goldhaber predicts the production of elemental sulfur at low pH. However, neither of these researchers performed low pH oxidations (28). McKibben and Barnes oxidized pyrite at low temperature under acidic conditions and did not note the production of elemental sulfur (29).

Despite agreement on the theory of the formation of sulfoxo intermediates, there is some confusion as to the products of pyrite oxidation. Luther suggests that in the presence of excess Fe^{3+} , thiosulfate is oxidized to sulfate (26). Meyer used Fe^{3+} to oxidize pyrite and produced elemental sulfur as an oxidation product (30). Which parameters can be manipulated to increase the selectivity of the thiosulfate oxidation for elemental sulfur has yet to be determined.

The microbial role in weathering

The deposition of elemental sulfur in coals is thought to be largely the result of microbial action (31). Although most of the microbial generation of elemental sulfur is thought to be through sulfate reduction, several pyrite oxidizing organisms have been reported to produce S^0 . Numerous studies on the feasibility of microbial coal desulfurization have focused on the use of such organisms for leaching pyrite from coals. Much of the work has centered around acidophilic aerobic systems including *Thiobacillus ferrooxidans*, *Sulfolobus*, and mixed cultures of *T. ferrooxidans* and *T. thiooxidans* (1-3). These bacteria mediate the oxidation of FeS_2 to Fe^{3+} and SO_4^{2-} . Elemental sulfur has frequently been observed as an intermediate or end product of these oxidations (32).

Due to the aerobic nature of microbial pyrite leaching systems, it is difficult to discern whether the distribution of oxidation products is a direct result of microbial metabolism or of simultaneous abiotic weathering. It has been suggested that the initial dissolution of FeS_2 to Fe^{2+} is a result of chemical weathering (33). Microbial action mediates the oxidation of Fe^{2+} to Fe^{3+} at rates higher than those in the absence of microbial catalysts (34) The accelerated accumulation of Fe^{3+} in the biological systems could account for the production of more elemental sulfur than in chemical weathering. In this scenario, elemental sulfur is not the result of direct microbial action, but of the precipitation of sulfur in the acidic culture media as affected by Fe^{3+} levels in solution. However, it is plausible that elemental sulfur is generated by the microbiological utilization of

pyritic sulfur.

Pyrite leaching experiments with a *Leptospirillum*-like bacterium in pure culture produced higher levels of elemental sulfur than in abiotic weathering (34). In mixed culture with *T. ferrooxidans* sulfur yields were comparable if not lower than abiotic controls. These results suggest that the *Leptospirillum*-like organism "prefers" the presence of S^0 whereas S^0 is oxidized by *T. ferrooxidans*. The discussion becomes more confusing as the severity of the weathering conditions are considered. The *Leptospirillum*-like bacterium carries out pyrite leaching reactions at pH values lower than *T. ferrooxidans*. The higher acidity of the culture environment may be the determining factor in S^0 generation. Again, the interaction between biological and abiotic sulfur production is unclear and may vary from organism to organism.

Experimental

Currently, we are investigating the effects of varying conditions on the products of pyrite weathering. Motivations for this study are two-fold: 1.) to achieve a chemical/microbial mechanism that would enable us to capitalize on the high rates of S^0 or polysulfide utilization of *P. furiosus* 2.) to determine the involvement of both organic and inorganic sulfur species in the deposition of polysulfidic compounds in coal.

Using approaches outlined in previous literature on artificial weathering, we are examining the activity of *P. furiosus* to sulfur species in coal exposed to various levels of temperature, acidity, ferric iron concentrations among other parameters. Weathering conditions have been chosen in attempt to optimize the selectivity for reducible sulfur generation rather than sulfate formation. In conjunction with the *P. furiosus* bioassay, ASTM speciation of coal sulfur, as well as standard chemical analysis for sulfoxo intermediates in solution are being used to follow the transformation of sulfur species during weathering.

Figure 1 shows the results of a long-term coal weathering experiment in which *P. furiosus* is used to determine the levels of available reducible sulfur. The results illustrate the interrelationship between iron and sulfur species and show that the reducible sulfur available to the bacteria varies through the process. The weathering conditions are relatively mild so that only inorganic sulfur in coal is likely to be affected. The bacteria in the bioassay are probably active towards elemental sulfur formed from pyrite. The sulfide generated at various time points suggest that there is no accumulation of S^0 but that it is an intermediate in the weathering process as inorganic sulfur is converted to sulfate.

The exposure of coals to elevated levels of oxygen and temperature under aqueous conditions has been considered as a means for both inorganic and organic sulfur removal from coal. Air/water oxydesulfurization of coal was evaluated by Warzinski et. al. (35) They showed that while inorganic sulfur could be converted to sulfate under conditions that minimized the loss in heating value of coals, organic sulfur removal lead to significant heating value losses. For example, for an Indiana No.5 coal, they showed that the percent heating value loss in the coal was approximately the same as the apparent sulfur removal. However it may be possible that more subtle changes in the coal matrix related to certain sulfur moieties could lead to effective biological treatment.

Table 1 shows the results of a mild weathering experiment using two coals: a pristine Illinois No. 6 containing 0.7% (w/w) pyritic sulfur and 3.4% (w/w) organic sulfur; and an Australian brown coal containing 0.03% (w/w) pyritic sulfur and 3.7% (w/w) organic sulfur. After 21 days of exposure to the conditions listed, coal samples were incubated with *P. furiosus* at 98°C to determine to what extent the coal sulfur had become available to this bacterium. Only an end point sample of weathered coal was taken in this weathering experiment. Whether the distribution of sulfur endproducts is the result of one day of weathering versus 21 days of weathering cannot be ascertained. Note the unweathered coals yielded little if any sulfide when exposed to *P. furiosus*. In several of the experiments with the Australian coal, we detected significantly larger amounts of sulfide than in the experiments with the Illinois coal. Although only about 1% of the organic sulfur in the Australian coal was apparently converted to sulfide, the fact that less sulfide was generated from the Illinois coal was surprising. The pyritic sulfur in the Illinois coal was expected to be converted to elemental sulfur and sulfate with the result that significant amounts of sulfide would be

generated from S^0 by *P. furiosus*. The differences between the sulfide generated from the two coals may or may not reflect differences in the forms of organic sulfur in the two coals. As mentioned earlier, *P. furiosus* metabolizes aliphatic sulfur compounds such as cystine (8) but not aromatic sulfur in compounds such as dibenzothiophene. Further work is needed in the development of the bioassay, but it is clear that biological activity may prove to be a sensitive and illuminating probe for sulfur speciation in coals.

Summary

In developing biologically-based desulfurization processes to determine not only the organic sulfur content of the coal, but how it occurs. For example, certain microbial systems will be more active to aliphatic sulfur than aromatic sulfur. While analytical techniques for determining the distribution of organic sulfur in coal are emerging, they are not readily available. Nonetheless, microbial treatment processes based on better chance of succeeding. Secondly, organic sulfur removal in coal may be best approached by a combination of biological and chemical steps. The chemical steps need not severely reduce the heating value of the coal but rather be directed at modifying the sulfur bound in the coal matrix to improve its biological availability. For the case of the weathering experiments described here, it would be interesting to see if microbially mediated weathering of coal would produce similar changes in sulfur availability to sulfur reducers such as *P. furiosus*. This would suggest that the microbial portion of a chemical/microbial treatment system not be limited to a particular species, but may encompass several microbial metabolisms. The weathering experiment described here is preliminary, but closer examination to combining the oxydesulfurization process with subsequent biological treatment will be pursued.

References

1. C. L. Brierly, "Microbiological Mining" *Scientific American* 247 44-53, 1982
2. D. J. Monticello and W. R. Finnerty "Microbial Desulfurization of Fossil Fuels" *Ann. Rev. of Microbiol.* 39 371-389 1985
3. R. Srivastava, I. M. Campbell and B. D. Blaustien "Coal Bioprocessing: A Research-Needs Assessment" *Chemical Engineering Progress* 85 45-53 1989
4. K.O. Stetter. "Diversity of Extremely Thermophilic Archaeobacteria." in *Thermophiles: General, Molecular, and Applied Microbiology.*, T.D. Brock, ed., John Wiley and Sons, New York, 1986.
5. R. N. Schicho, S. H. Brown, G. J. Olson, E. J. Parks, and R. M. Kelly "Probing coals for non-pyritic sulfur using sulfur-metabolizing mesophilic and hyperthermophilic bacteria." *Fuel* 68 1368-1375 1989
6. G. Fiala and K.O. Stetter. "*Pyrococcus furiosus* sp. nov. represents a novel genus of marine heterotrophic archaeobacteria growing optimally at 100 °C." *Arch. Microbiol.*, 145, 56-61, 1986.
7. B. Malik, W-w. Su, H.L. Wald, I.I. Blumentals and R.M. Kelly. "Growth and Gas Production for the Hyperthermophilic Archaeobacterium, *Pyrococcus furiosus*." *Biotechnol. Bioeng.*, 34, 1050-1057, 1989.
8. I.I. Blumentals, M. Itoh, G.J. Olson and R.M. Kelly. "Role of Polysulfides in the Reduction of Elemental Sulfur by the Hyperthermophilic Archaeobacterium *Pyrococcus furiosus*." *Appl. Environ. Microbiol.*, in press.
9. K.O. Stetter, H. König and E. Stackebrandt. "Pyrodicticum gen. nov., a New Genus of Submarine Disc-Shaped Sulfur-Reducing Archaeobacteria Growing Optimally at 105 °C." *Sys. Appl. Microbiol.*, 4, 535-551, 1983.
10. T. L. Peeples, R.N. Schicho, R. M. Kelly and G. J. Olson "Bioprocessing of Fossil Fuels Using Hyperthermophilic Archaeobacteria" EPRI symposium paper *Biological Processing of Coal and Coal-Derived Substances* May, 1989
11. D. Wise. *Bioprocessing and Biotreatment of Coals.*, Marcel Dekker, New York, in press.
12. D. C. Frost, W. R. Leeder and R. L. Tapping. "X-ray photoelectron Spectroscopic investigation of coal." *Fuel* 53 206-211, 1974
13. D. C. Frost, W. R. Leeder, R. L. Tapping and B. Wallbank. "An XPS study of the oxidation of pyrite and pyrites in coal." *Fuel* 56 277-280, 1977
14. R. K. Sinha and P. L. Walker "Removal of sulphur from coal by air oxidation at 350-450 °C" *Fuel* 51 125-129 1972
15. H. H. Lowry, ed. *Chemistry of Coal Utilization* vol II John Wiley and Sons 1945 New York
16. American Society for Testing and Materials. Designation D 2492-84 "Standard Test Method for Forms of Sulfur in coal
17. V. Calemma, R. Rausa, R. Margarit and E. Girardi. "FT-i.r. Study of coal oxidation at low temperature." *Fuel* 67 764-770, 1988
18. D. L. Perry and A. Grint, "Application of XPS to coal characterization" *Fuel* 62 1024-1033 1983
19. R. Markuszewski, "Some thoughts on the difficulties in the analysis of sulfur forms in coal." *Journal of Coal Quality*, Jan. 1988, vol 7. no. 1 pp 1-4
20. F. G. Smith "Variation in the properties of pyrite" *The American Mineralogist* 27 1-19

21. R. M. Garrels and M. E. Thompson "Oxidation of pyrite by iron sulfate solutions" *American Journal of Science* **258-A** 57-67 1960
22. M. C. Esposito, S. Chander and F. F. Aplan "Characterization of pyrite from coal sources" in: *Process Mineralogy VII* Vassilou ed. 475-493 The Metallurgical Soc., Warrendale, PA 1987
23. M. M. Wu, G. A. Robbins, R. A. Winschel and F. P. Burke "The effects of weathering on floatation and thermoplastic properties of coal" *Abstracts of Papers of the American Chemical Society Division of Fuel Chemistry*, **193** 73 1986
24. D. J. Maloney, R. G. Jenkins, and P. L. Walker Jr. "Low-Temperature air oxidation of caking coals. 2. Effect on swelling and softening properties." *Fuel* **61** 175-181, 1982
25. G. R. Ingram and J. D. Rimstidt. "Natural weathering of coal." *Fuel* **63** 292-296, 1984
26. C. O. Moses, D. Nordstrom, J. S. Herman and A. L. Mills. "Aqueous pyrite oxidation by dissolved oxygen and by ferric iron" *Geochimica et Cosmochimica Acta.*, **51** 1561-1571, 1987
27. G. W. Luther. "Pyrite oxidation and reduction: Molecular orbital considerations" *Geochimica et Cosmochimica Acta* **51** 3193-3199, 1987
28. D. R. McKay and J. Halpern. "A kinetic study of the oxidation of pyrite in aqueous suspension." *Transactions of the Metallurgical Society of AIME* **210** 301-309, 1958
29. M. B. Goldhaber. "Experimental study of Metastable sulfur oxyanion formation during pyrite oxidation at pH 6-9 and 30 °C" *American Journal of Science* **283** 193-217, 1983
30. M. A. McKibben and H. L. Barnes. "Oxidation of pyrite in low temperature acidic solutions: Rate laws and surface textures", *Geochimica et Cosmochimica Acta* **50** 1509-1520, 1986
31. P. R. Dugan. "Microbial Conversions of Sulfur and their Potential for Bioprocessing of Fossil Fuels", Proceedings: Bioprocessing of Fossil Fuels Workshop, Tysons Corner, VA , August 1989
32. W. Hazeu, R. Steudel, W. H. Batenburg-van de Vegete, P. Bos, and J. G. Kuenen, "Elemental sulfur as an intermediate in the oxidation of reduced sulphur compounds by *Thiobacillus ferrooxidans* Localization and characterization" Proceedings: Bioprocessing of Fossil Fuels Workshop, Tysons Corner, Va., August, 1989
33. W. Strumm and J. J. Morgan ed. *Aquatic Chemistry* 2nd ed. John Wiley and Sons, New York 1981, 469-471
34. U. Merrettig, P. Wlotzka and U. Onken, "The removal of Pyritic Sulfur from coal by *Leptospirillum*-like bacteria." *Applied Microbiology and Biotechnology* **31** 626-628, 1989
35. R. P. Warzinski, S. Friedman, J. A. Ruether and R. B. LaCount. "Air/Water Oxydesulfurization of Coal - Laboratory investigation." Department of Energy Report DOE/PETC/TR-80-6, Pittsburgh Energy Technology Center, Pittsburgh, PA, August, 1980.

Table 1. Artificial Weathering Experiments with Illinois/Australian Coals

Sulfide Generation by *Pyrococcus furiosus*

Coal	Temp (°C)	pH	Air (atm)	Sulfate (ppm)	Sulfide (nmol/ml)	Cell Density (cells/ml)
AUS	80	1.6	1	953	21	9.8E6
	80	0.6	1	1480	16	1.5E7
	80	1.6	3	280	160	1.4E7
	80	0.6	3	275	263	1.1E7
	100	1.6	1	262	133	9.9E6
	100	0.6	1	967	158	1.3E7
	100	1.6	3	250	89	9.0E6
	100	0.6	3	358	152	1.0E7
ILL	80	1.6	1	738	22	8.8E6
	80	1.6	1	870	25	8.8E6
	80	1.6	1	798	20	9.4E6
				±54	±2	±0.2E6
	80	0.6	1	1015	63	1.5E7
	80	1.6	3	798	93	1.1E7
	80	0.6	3	1743	67	8.3E6
	100	1.6	1	228	74	1.5E7
	100	0.6	1	645	18	1.2E7
	100	1.6	3	905	45	1.0E7
	100	0.6	3	824	51	1.1E7
<i>P. furiosus</i> Growth on S°					>2000	2.4E8
AUS (Unweathered)					30	8.4E6
ILL (Unweathered)					12	9.6E6

Weathering of Illinois #6
(70 C, pH 1.6, 1Atm Air)

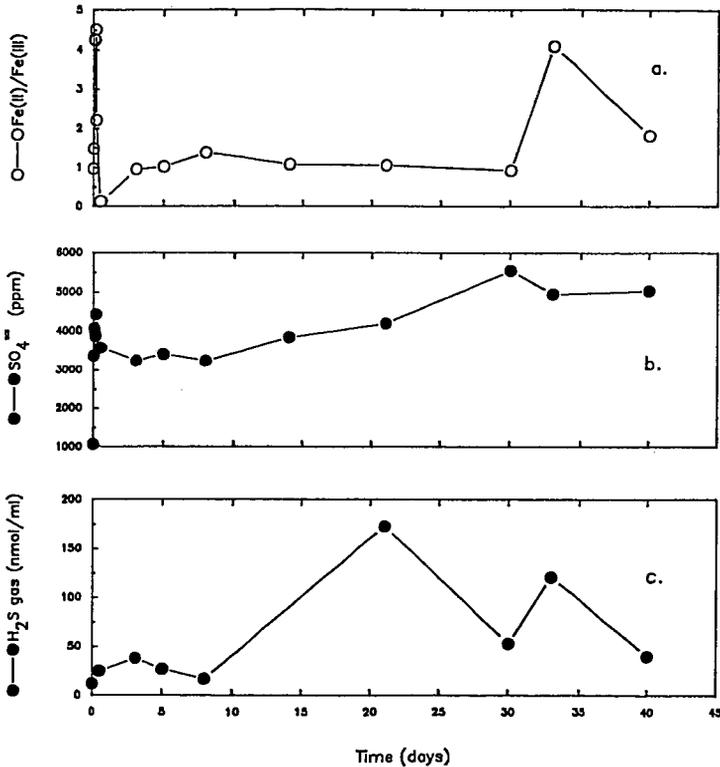


Figure 1. Illinois no. 6 coal (20 g) weathered in an acidic, aqueous suspension (1L, 0.04 M H₂SO₄) gave the above profiles for iron and sulfate concentrations in solution and H₂S generation upon bioassay. a) For the Fe(II)/Fe(III) ratio, total and ferrous iron concentrations were determined spectrophotometrically (1,10 phenanthroline reaction, absorbance 510nm). Ferric iron was determined by difference. b) Concentration of SO₄ was determined turbidimetrically (BaSO₄, absorbance 340nm). c) In the bioassay, gaseous H₂S was determined through gas chromatographic analysis of head gas from batch cultures of *P. furiosus* on timepoint samples of weathered coal (0.25g). Values of H₂S are normalized by gas injection volume.

Note: The amount of reducible sulfur available to the microorganisms changes during the course of weathering suggesting a change in the distribution of oxidation products.

Coal Desulfurization Studies: Inability of *Sulfolobus* spp. to oxidize sulfur compounds in coal

J. B. Risatti, Illinois State Geological Survey, 615 E. Peabody Dr., Champaign, IL 61820;
K.W. Miller, Dept. Biological Sciences, Illinois State Univ., Normal, IL ; S.
Broeren, Illinois State Geological Survey, 615 E. Peabody Dr., Champaign, IL
61820

Key Words: *Sulfolobus*, chemolithotrophy, desulfurization

Introduction

A major problem associated with direct combustion of high sulfur coal is the emission of sulfurous gases into the atmosphere. To address this problem, a number of pre- and post combustion processes, including bacterial leaching, have been proposed. Economically, microbial desulfurization requires low capital output and consequently, has the potential to be significantly less expensive than other methods.

A number of chemolithotrophic bacteria (eubacteria) readily obtain energy by oxidizing elemental sulfur and sulfide minerals. The sulfidic mineral, pyrite, is a major sulfur contaminant in many coals and the conditions governing rates of bacterial pyrite oxidation in coal have been extensively studied^(1,2,3). Although iron and sulfur oxidizing microorganisms may remove up to 97% of the pyrite from coal in approximately 8 days⁽⁴⁾ they are unable to remove the organic sulfur fraction⁽⁵⁾.

Recently, several coal leaching studies using archaea⁽⁶⁾ belonging to the genus *Sulfolobus* have reported the oxidation of both inorganic (pyritic)^(7,8) and organic sulfur^(9,10,11) in coal and also, the oxidation of dibenzothiophene⁽¹²⁾ by *Sulfolobus acidocaldarius*. Because of these studies, we compared *Sulfolobus acidocaldarius* strains 98-3 and DSM 639 and *S. solfataricus* ATCC 35091 with *Thiobacillus ferrooxidans* to determine how effectively *Sulfolobus* could remove organic and inorganic sulfur from a pyritic Illinois coal and a washed (low pyrite) Illinois coal. In addition, the oxidation of sulfur to sulfate by *Sulfolobus* spp. was determined for elemental sulfur, thiosulfate and dibenzothiophene.

Experimental Methods

Sulfolobus acidocaldarius strains DSM 639 and 98-3, were provided by Carl Woese (University of Illinois, Urbana-Champaign). *S. solfataricus* ATCC 35091 was purchased from the American Type Culture Collection. Cultures were maintained at 70 °C in Allen's⁽¹³⁾ mineral salts medium (SMS) as modified by Brock et al.⁽¹⁴⁾ and amended with sucrose (0.2%) and yeast extract (0.1%). To adapt cells to chemolithotrophic growth, yeast extract was replaced with either pyrite at 5 g L⁻¹ or with elemental sulfur at 10 g L⁻¹. Elemental sulfur was sterilized by tyndallisation and added separately to autoclaved medium. The type strain of *Thiobacillus ferrooxidans* was obtained from A. Harrison, University of Missouri, Columbia, MO, and maintained at 28°C on ATCC medium 64 with pyrite (0.5%) replacing FeSO₄·7H₂O as a growth substrate.

Coal sample IBC-104, obtained from the Illinois Basin Coal Bank Program, Champaign, IL, is a high-sulfur, run of mine Herrin coal which was deslimed to lower the ash yield to 15% and ground to -200 mesh. Coal sample FCC-103 was prepared from Illinois Bank Coal IBC-103 (a blend of 80% Springfield no.5 and 20% Herrin no.6) by froth flotation to sulfur contents of 1.76% total, 0.42% pyritic, and 1.26% organic. Forms of sulfur in coal were determined using ASTM standard methods⁽¹⁵⁾. Mineral pyrite (Sargent-Welch, Skokie, IL), approximately 85% pure, dibenzothiophene (DBT), elemental sulfur and sodium thiosulfate (analytical grade) were used as sulfur sources. Coal at 5%

pulp density, was added to 125 mL Erlenmeyer flasks containing 50 mL SMS medium without yeast extract for *Sulfolobus* spp. or to TMS medium without pyrite for *T. ferrooxidans*. Sulfate salts in the media were replaced with their chloride equivalents. The pH was adjusted to 2.5 with HCl and the flasks autoclaved at 121°C for 20 minutes. *T. ferrooxidans* inocula consisted of 0.5 ml of exponential phase culture. *Sulfolobus* inocula (10%) consisted of 48 h cultures washed with unamended SMS and with OD's adjusted to 0.5 at 620 nm. *T. ferrooxidans* cultures were incubated at 28°C with shaking at 150 rpm; *Sulfolobus* spp. were incubated at 70°C in a waterbath shaker. Uninoculated controls containing the sterile media and target substrates were incubated with all experiments and all experiments were performed in duplicate. Periodically, 1.0 ml samples were withdrawn, centrifuged to remove particulates, and analyzed turbidometrically for sulfate (16). At this time, flasks were weighed to determine evaporation, which was never more than 5% during the course of an experiment. At the conclusion of the experiments, coal was retrieved by vacuum filtration, rinsed with 0.1 N HCl, air dried, and analyzed for forms of sulfur.

Results

After 27 days of leaching by *T. ferrooxidans*, 90.9% of the inorganic sulfur in the IBC-104 coal was solubilized to sulfate, decreasing the total sulfur content of the coal from 4.78% to 2.58%. Assuming that all of the original sulfate in the coal (0.12%) was leached into the supernatant, *T. ferrooxidans* removed at least 89.6% of the pyritic sulfur at a maximum rate of about 12% day⁻¹. In the concomitant controls, pyrite decreased by approximately 24%. Most probable number (MPN) estimates of viable cells increased from 1.9×10^6 to 2.4×10^9 cells ml⁻¹ in the inoculated coal cultures; no cells were observed in the sterile controls.

After 22 days of leaching coal samples IBC-104 and FCC-103 with *S. acidocaldarius* 98.3 and DSM 639 and *S. solfataricus*, organic sulfur content (2.38% and 1.26% respectively) remained unchanged and pyrite decreased by approximately 80 to 83% in all cases (including controls) implying *Sulfolobus* spp. did not oxidize either the pyritic or the organic sulfur in these coals. The observed decreases in pyrite were not from microbial activity but are a result of the increased oxidation occurring at 70°C at a pH of 2 to 3. As determined from MPN estimates, viable cells on the order of 1.9×10^4 cells mL⁻¹ were present after 22 days both in cultures with coal and in inoculated controls without coal. Sterilized, uninoculated controls showed no cell growth after 22 days. These results demonstrate that neither the pyrite nor the organic sulfur in the coal supported growth of *Sulfolobus* spp. and also that the coals had no deleterious effects on the organisms.

In experiments with pyrite (-150 mesh), sulfate was produced at the same rate in both the inoculated and the uninoculated flasks and there was no evidence that any of the three strains of *Sulfolobus* oxidized pyrite. Additional experiments in which pyrite was amended with sucrose or yeast extract, gave similar results. *Sulfolobus* spp. were also unable to utilize elemental sulfur or thiosulfate. After 15 days of incubation, 4.6 - 5.6% of the elemental sulfur in the cultures was oxidized to sulfate and in experiments with thiosulfate as the sole energy source, only 3.0 - 3.6% of the thiosulfate (1 mg mL⁻¹) was oxidized to sulfate.

Experiments with dibenzothiophene (DBT) as sole substrate indicated that *S. solfataricus* and both strains of *S. acidocaldarius* converted approximately 10-15% of the sulfur in DBT to sulfate. However, based on protein analyses(17) and cell counts by light microscopy, DBT did not appear to be utilized as a growth substrate.

From our data, we question the ability of *S. acidocaldarius* 98-3 and DSM 639 and *S. solfataricus* ATCC 35901 to oxidize pyritic minerals or organic sulfur in coals at a demonstrable rate or to grow lithotrophically using elemental sulfur, pyrite or thiosulfate.

Acknowledgement

Research sponsored by the Illinois Coal Development Board through the Center for Research on Sulfur in Coal (CRSC) under contracts to the Illinois State Geological Survey (JBR) through the University of Illinois at Urbana-Champaign.

References

- (1) Silverman, M. P. and D. G. Lundgren, 1959 *Jour. Bacteriology* 78:326-331.
- (2) Silverman, M. P., M. H. Rogoff and T. Winder, 1961 *Applied. Microbiology*. 9:491-496.
- (3) Detz, C. M. and C. Barvinchak, 1979 *Mining Congress Journal* 66:75-86
- (4) Dugan, P.R. and W. A. Apel, 1978 *Microbial desulfurization of coal*. pp.223-250. In *Metalurgical applications of bacterial leaching and related microbiological phenomena*. L. E. Murr, A. E. Torma and J. Breirley (eds.), Academic Press, New York.
- (5) Bos, P., T. F. Huber, C. Kos, C. Ras and J. G. Kuenen, 1985 *International Symposium on Biohydrometallurgy*, Vancouver, British Columbia.
- (6) Woese, C., O. Kandler and M. Wheelis, 1990 *Proceed. Nat. Acad. of Science* (In Press).
- (7) Kargi, F. and J. M. Robinson, 1982 *Applied and Environmental Microbiology* 44:878-883
- (8) Kargi, F. and J. M. Robinson, 1982 *Bioeng. and Biotechnology* 24:2115-2121
- (9) Murphy, J., E. Riestenberg, R. Mohler, D. Marek, B. Beck and D. Skidmore, 1985 *Coal desulfurization by microbial processing*. pp.643-652. In *Processing and Utilization of High Sulfur Coals*, Y. A. Attia (ed.), Elsevier.
- (10) Kargi F. and J. M. Robinson, 1985 *Bioeng. and Biotechnology* 27:41-49
- (11) Kargi, F. and J. M. Robinson, 1986 *Fuel* 65:397-399
- (12) Kargi F. and J. M. Robinson, 1984 *Bioeng. and Biotechnology* 26:687-699
- (13) Allen, M. B., 1959 *Arch. Mikrobiol.* 32:270-277
- (14) Brock, T. D., K. M. Brock, R.T. Belly and R.L. Weiss, 1972 *Arch. Mikrobiol.* 84:54-68.
- (15) *Annual Book of American Society of Testing Materials Standards*, 1979 American Society of Testing Materials, Philadelphia, PA, USA.
- (16) Miller and Risatti, 1988, *Biooxidation of pyrrhotites in coal chars*. *Fuel* 67: 1150- 1154.
- (17) Lowry, O. H., N. J. Rosrbrough, A. L. Furr and R. J. Randall, 1951 *Jour. Biol. Chemistry* 193:265-275.

BIOLEACHING OF MOLYBDENUM FROM A COAL
LIQUEFACTION CATALYST RESIDUE

Bernard Blaustein, David Suhy and Eric Spana
U.S. Department of Energy
Pittsburgh Energy Technology Center
Pittsburgh, PA 15236

Keywords: bioleaching, molybdenum disulfide, catalyst residue

ABSTRACT

Molybdenum disulfide, MoS_2 , is used as a catalyst in coal-oil coprocessing. For most processes using molybdenum catalysts to be economical, the molybdenum must be recovered and recycled. Chemical recovery schemes have been devised, but typically recover only a fraction of the molybdenum. There are reports in the literature that bacteria can oxidatively solubilize MoS_2 (molybdenite) ore. This paper reports the initial results of experiments where Thiobacillus ferrooxidans are shown to microbially solubilize MoS_2 present in coal liquefaction catalyst residues. Small-scale shake-flask experiments show that after leaching of the catalyst residue by T. ferrooxidans at pH -2 for 6-7 weeks, as much as 66-71% of the Mo can be solubilized. Analyses of the microbially-leached products and mass-balance calculations can account for 78-103% of the Mo initially present in the coal liquefaction residue.

INTRODUCTION

Molybdenum disulfide is used as a catalyst in coal-oil coprocessing. It is effective at concentrations as low as 200 ppm Mo by weight, based on coal (ref. 1). Molybdenum is added to the coal in the form of aqueous solutions of ammonium heptamolybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, or ammonium tetrathiomolybdate $(\text{NH}_4)_2\text{MoS}_4$. The MoS_2 catalyst is then formed in situ at coal liquefaction conditions at about 350°C, in the presence of hydrogen.

Even though MoS_2 is used at low concentrations, it is necessary to recover the molybdenum so that it can be recycled. A chemical recovery scheme has been developed (ref. 2). The coal liquefaction residue is first roasted (calcined). Under these conditions, the mineral matter in the residue and the molybdenum from the MoS_2 form compounds that are only partially soluble in the aqueous NH_3 used to leach the roasted material. This results in only a partial recovery of the molybdenum from the MoS_2 present in the coal-oil coprocessing liquefaction residue.

There are reports in the literature (refs. 3-7) that bacteria can oxidatively solubilize MoS_2 (molybdenite) ore. This paper reports on the use of Thiobacillus ferrooxidans to aerobically solubilize MoS_2 present in a coal liquefaction (coprocessing) catalyst residue to facilitate recovery of the molybdenum.

EXPERIMENTAL

Catalyst Residue: Two gallons of liquids produced from reaction of Illinois No. 6 hvB bituminous coal and Maya atmospheric tower bottoms under coprocessing conditions (ref. 1) were processed as shown in Figure 1. Approximately 100 grams of catalyst residue that contained MoS_2 , obtained as the tetrahydrofuran-insolubles, were dried in a vacuum oven at 100°C for 4 hours and then ground to

pass a 60-mesh screen. Analysis of the catalyst residue (done by Huffman Laboratories, Inc., Golden, Colorado) is given in Table 1.

Source of Microbes: Several strains of Thiobacillus ferrooxidans were obtained from the Center for Bioprocessing Technology of the Idaho National Engineering Laboratory (INEL). The original source of two of the strains (23270 and 13598P) was the American Type Culture Collection. Two other strains, A6 and PH, were obtained from Doris Thompson at INEL. The Bureau of Mines, Bruceton Research Center, provided a sample of acid mine drainage muck, which was designated as culture 80M and assumed to contain, inter alia, T. ferrooxidans.

In general, the several strains of T. ferrooxidans were maintained on 9K or INEL medium (see below). After the preliminary bioleaching experiments with catalyst residue were completed, inocula were taken from these finished experiments to start new bioleaching experiments. Thus, in some cases, the bacteria now being used have been exposed to catalyst residue and/or molybdate leachate solutions for as long as 6 months.

Preparation of Media: T. ferrooxidans grow optimally at pH 2.0-2.5. After some preliminary experiments, it was decided to use the 9K medium as described by Silverman and Lundgren (ref. 8). However, due to large amounts of ferric iron precipitates on the glassware with the use of 9K, most additional work was done with the INEL medium. The recipe (shown below) was obtained through Idaho National Engineering Laboratories, via Dr. Paul Wichlacz (ref. 9).

Recipe for INEL Salts

To 1 liter of distilled water adjusted to pH 2.0 with H_2SO_4 , add:

$(NH_4)_2SO_4$	0.15 g
KCl.....	0.15 g
K_2HPO_4	0.15 g
$MgSO_4 \cdot 7H_2O$	3.36 g
$CaCl_2 \cdot 2H_2O$	1.28 g
$Al_2(SO_4)_3 \cdot 18H_2O$	2.25 g
$MnSO_4 \cdot H_2O$	0.12 g

200 mL of the solution is removed. Autoclave the remaining 800 mL portion in ten flasks (250 mL size) with 80 mL in each. Add 7.46 g of $FeSO_4 \cdot 7H_2O$ to the 200 mL portion while stirring. Filter sterilize the iron solution and add 20 mL aliquots to each 80 mL of sterile salts when cool.

Bioleaching experiments: The bioleaching experiments were carried out in 250 mL Erlenmeyer flasks on a shaker (125 rpm) in an incubator maintained at 29°C. Catalyst residue (0.5g) was added to 100 mL of medium (pH = 2). This mixture was then inoculated with 1 mL of the bacterial culture. After bacterial growth and leaching for 6-7 weeks, the microbially-treated sample was filtered and worked up as shown in Figure 2. The filtrates were analyzed for Mo by atomic absorption spectral analysis. The initial and final solid residues were analyzed for molybdenum as follows. First, the sample was ashed in a muffle furnace at 750°C. The ash was fluxed in lithium tetraborate and then dissolved in HCl. The HCl solution was analyzed by AA according to ASTM D3682-87.

RESULTS AND DISCUSSION

Results from small-scale shake-flask experiments are shown in Table 2. The dates that the experiments were run are given in parentheses, above the column headings. In these shake-flask experiments, which demonstrate the feasibility of bioleaching MoS_2 , as much as 71% of the Mo initially present in the catalyst

residue was solubilized. Results of the control experiments given in Table 2 show that the amounts of Mo solubilized in the controls are low compared to amounts of Mo solubilized in the microbial leaching experiments. In order to estimate the overall precision in these experiments, values calculated for the mass balance for molybdenum are also shown in the sixth row of the table. For the experiments described in Table 2, the amount of Mo found by analysis of solutions B and D and of residue E (see Figure 2) could account for 78 to 103% of the molybdenum present in the original catalyst residue A.

These initial experiments, which show that some strains of Thiobacillus ferrooxidans can solubilize MoS_2 when it is present in a residue from coal-oil coprocessing, also show that the other materials present in the residue do not stop the bioleaching of the molybdenum. It is interesting to note that molybdate concentrations in solution at the end of the experiments were as high as 51 ppm. Reports in the literature (refs. 4-7, 10-12) suggest that a concentration as low as 10 ppm molybdate is toxic to T. ferrooxidans. However, inocula taken from these high concentration molybdate flasks, and used in subsequent experiments, proved viable and capable of additional bioleaching. This would suggest that we have developed a strain of T. ferrooxidans that has adapted to high molybdate concentrations. But also, it is possible that the condition designated as "toxic" to the bacteria might be better described as "inhibitory."

Both media used (9K and INEL) contain ferrous iron, as does the liquefaction catalyst residue. After bio-oxidation, this results in relatively high concentrations of ferric iron in the leachate solutions. We hypothesize that the iron(III) species present in solution form insoluble iron(III)molybdate compounds, as depicted in Figure 2. After the microbially-leached solutions are filtered, the iron(III) molybdate present (mixed with the unreacted catalyst residue in mixtures) was dissolved in 3N HCl. After filtration, this HCl solution (acid extract D) contains most of the molybdate that was formed during the bioleaching. In the best result, solutions B and D together contain 71% of the Mo solubilized from the MoS_2 initially present in the catalyst residue.

ACKNOWLEDGEMENTS

David Suhy and Eric Spana held appointments as Professional Interns at the Pittsburgh Energy Technology Center. This intern program is administered for the U.S. Department of Energy by Oak Ridge Associated Universities, Oak Ridge, Tennessee.

DISCLAIMER

Reference in this paper to any specific commercial product, process, or service, is made to facilitate understanding and does not imply its endorsement or favoring by the U.S. Department of Energy.

REFERENCES

1. Lett, R.G., Cugini, A.V., Utz, B.R., Krastman, D., Cillo, D.L., and Jin, G.T. 1989. Dispersed-Phase Catalyst Approaches in Coal Liquefaction and Coprocessing. Sixth Joint U.S.-Korea Workshop on Coal Utilization Technology, October 16-18, 1989, Seoul, Korea.
2. Krastman, D., Utz, B.R., Cugini, A.V., and Lett, R.G. 1990. Dispersed Phase Molybdenum Catalyst Recovery in Coprocessing. Preprints, Div. Fuel Chem., Am. Chem. Soc., 35(2):570-576.
3. Bryner, L.C., and Anderson, R. 1957. Microorganisms in Leaching Sulfide Minerals. *Ind. Eng. Chem.* 49:1721-1724.
4. Bhappu, R.B., Reynolds, D.H., and Roman, R.J. 1965. Molybdenum Recovery from Sulfide and Oxide Ores. *J. of Metals.* 17:1199-1205.
5. Brierley, C.L. 1974. Molybdenite Leaching: Use of a High-Temperature Microbe. *J. Less Com. Met.* 36:237-247.
6. Lyalikova, N.N., and Lebedeva, E.V. 1984. Bacterial Oxidation of Molybdenum in Ore Deposits. *Geomicrobiology Journal.* 3:307-318.
7. Kelley, B.C. 1986. Biological Contributions to Mineral Cycling in Nature with Reference to Molybdenum. *Polyhedron.* 5:597-606.
8. Silverman, M.P., and Lundren, D.G. 1959. Studies of the Chemoautotrophic Iron Bacterium Ferrobacillus ferrooxidans I. An Improved Medium and Harvesting Procedure for Securing High Yields. *Journal of Bacteriology.* 77:642-647.
9. Wichlacz, P.L., and Unz, R.F. 1985. *Appl. Environmental Microbiology.* 50:460-467.
10. Tuovinen, O.H., Niemelä, S.I., and Gyllenberg, H.G. 1971. Tolerance of Thiobacillus ferrooxidans to Some Metals. *Antonie Van Leeuwenhoek.* 37:489-496.
11. Karavaikd, G.I., Dzhanugurova, R.S., and Pivovarova, T.A. 1989. Factors Increasing Resistance of Thiobacillus ferrooxidans to Molybdenum. *Mikrobiologiya.* 58:412-418.
12. Thompson, D.L., Wichlacz, P.L., and Bruhn, D.F. Heavy Metal Tolerance in Thiobacillus ferrooxidans and Genus Acidiphilium, In Press.

TABLE 1. Analysis of the THF-Insoluble MoS₂/Co-Processing Residue

<u>Element</u>	<u>Percent</u>
Carbon	24.5
Hydrogen	1.95
Oxygen (Direct)	7.2
Nitrogen	0.72
Sulfur	7.4
Iron	9.9
Molybdenum	2.80

TABLE 2. Results of Shake-Flask Bioleaching Experiments Using 0.5 Gram MoS₂ Catalyst Residue

Inoculum Medium	9K Experiments (7/26 - 9/8)				INEL Experiments (8/17 - 10/4)				Bioleaching Group #s			
	23270 + 9K	13598P + 9K	UNIMOC 9K	23270 + Salts (9K)	23270 + INEL	PH + INEL	A6 + 9K	BOM + 9K	UNIMOC + INEL	13598P + INEL	23270 + INEL	
Mo Conc., Filtrate B (ppm)	40	43	0.3	47	39	0.5	0.1	33	2.3	34	31	
Mo in Filtrate B (mg)	2.71	3.00	0.02	1.89	3.45	0.04	.01	2.28	0.15	2.46	2.25	
Mo in Acid-Extract D (mg)	3.36	4.13	1.13	3.26	5.82	1.44	1.38	3.36	1.26	6.15	6.11	
Mo in Acid-Extract Residue E (%)	1.65	1.70	3.16	2.19	1.40	2.80	2.65	2.25	3.06	1.20	1.31	
Mo in Acid-Extract Residue E (mg)	5.91	5.98	11.22	6.24	3.84	9.38	8.93	7.43	9.85	3.92	4.20	
Mo Recovery (%)	85	94	88	81	103	78	74	93	80	90	90	
Mo Solubilized (%)	43	51	8	37	71	11	10	40	10	62	60	

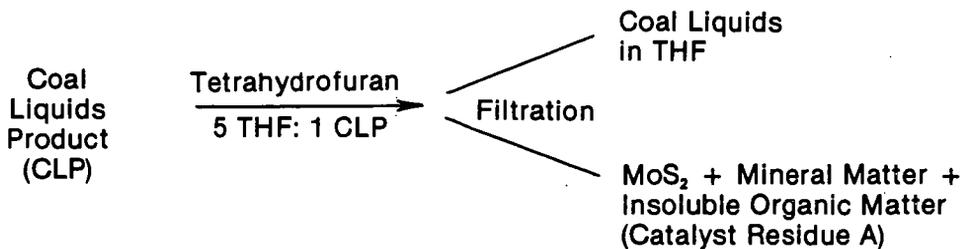


FIGURE 1. SEPARATION OF MoS_2 CATALYST RESIDUE FROM COAL-OIL CO-PROCESSING LIQUIDS.

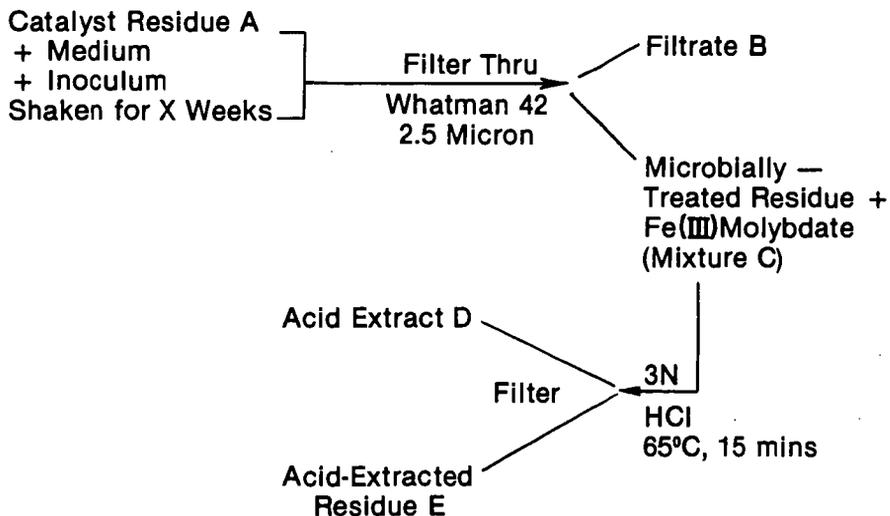


FIGURE 2. SHAKE-FLASK BIOLEACHING OF MoS_2 CATALYST RESIDUE: MASS BALANCE EXPERIMENTS.

BACTERIOELECTRIC DEASHING OF COALS

Norman Lazaroff
State University of New York, Binghamton, NY 13901

John E. Wey and Patrick R. Dugan
Idaho National Engineering Laboratory
EG&G Idaho, Inc.
Idaho Falls, ID 83415-2203

The premise that a combination of electric current and bacterial action would remove metals from coal more effectively than either of the factors acting alone, stemmed from earlier observations of enhanced pyritic ore leaching and iron corrosion by suspensions of *Thiobacillus ferrooxidans* in galvanic cells. Although sulfide minerals are oxidized more slowly than native metals under acid abiotic conditions, like metals their oxidation can be greatly accelerated by an imposed voltage in a galvanic cell. The consequent release of metal ions from pyritic substrates as the result of electrooxidations is analogous to the solubilization of minerals by the action of chemolithotrophic microorganisms which catalyze the transport of electrons from oxidizable cations or sulfide ligands to oxygen.

When metals are placed at the anode of a galvanic cell, the liberation of ionic species by electro-oxidation is dependent upon provision of a polarizing voltage and the presence of electron acceptors that promote current flow by cathode depolarization. While a metal in elementary form can be electrolytically oxidized to form a soluble cation, the insoluble metal in a sulfide mineral is already in cationic form complexed with a sulfur anion. If a sulfide mineral species conducts electricity, its cations can be released by electro-oxidation of the reduced sulfur ligands to produce elementary sulfur. The knowledge that pyritic minerals are good electrical conductors (Decker, R. F., 1986) as well as substrates for oxidation by thiobacilli, implied that the two phenomena could operate synergistically. It was visualized that the Fe(II) released electrolytically would be oxidized by chemolithotrophic iron oxidizing bacteria and that the soluble Fe(III) produced in an acid environment would oxidize pyritic sites insulated from electro-oxidation.

Conceptually, a suitable electrical potential, would liberate Fe(II) cations by oxidizing reduced sulfur anions of pyritic minerals. Electric current would flow from the source as the cathode is depolarized by hydrogen ions of the acid lixiviant and Fe(II) cations move into solution toward the cathode. In dilute sulfuric acid solution, as the Fe(II) is oxidized by iron oxidizing thiobacilli, the resultant Fe(III) sulfato complex would have the possibility of participating in several different reactions depending upon the conditions existing in the galvanic cell (Figure 1). These include: (1) aggregation (polymerization) to an insoluble amorphous hydrated Fe(III) sulfate; (2) reduction at the cathode forming additional substrate for bacterial iron oxidation in the bulk phase of solution; (3) reduction, after recirculation to non-anodic sites on pyritic surfaces, liberating oxidized sulfur species and Fe(II) from the pyrite; (4) alkaline precipitation; when proton consumption by cathode reactions occurs to the extent that the environment of the cathode becomes basic, the Fe(III) sulfato complex decomposes and iron is precipitated in the form of Fe(III) oxides and oxyhydroxides. The proton concentration in this system is also dependent upon the activities of thiobacilli which oxidize

sulfides, polythionates and elementary sulfur derived from the oxidation of pyritic minerals. Acidity produced from those activities counteracts proton consumption from cathode depolarization reactions and the reduction of O_2 by chemolithotrophic oxidation of Fe(II). At low pH the iron sediments formed are those typical of chemolithotrophic iron oxidation, amorphous hydrated Fe(III) sulfate and jarosites. Consequently, cleaning coal by ordinary bacterial leaching may leave iron sediments remaining on the coal that contain sulfur in iron sulfato complexes. However, in a galvanic cell the iron and sulfur of the sulfato-complexes appear to be transported together to the cathode and remain undissociated under acid conditions. Therefore, this means of sulfatic sulfur removal represents further benefit to be gained from cleaning coal by a combined bacterial and electrolytic leaching process.

The following account describes an exploratory investigation of the feasibility of using the bacterioelectric phenomenon for metal removal and recovery from different coals.

Materials and Methods

Bulk leaching was carried out in magnetically stirred glass beakers equipped with glass cathode and anode chambers fitted with platinum electrodes as shown in Figure 2. Initially, approximately 0.4 g of a powdered coal sample was placed at the anode either wrapped in ashless filter paper, such as Whatman 41, or placed within a cellulose extraction thimble. The powdered coal was compressed around the platinum foil of the electrode by inserting the wrapped combination into the tightly fitting perforated glass tube that served as anode chamber. The leaching system was filled with a lixiviant of pH 2.5 sulfuric acid or some experimental modification thereof. The bacteria that were used, consisted of suspensions of the Leathen strain of *Thiobacillus ferrooxidans* prepared as previously described (Lazaroff, et al. 1982). The electric current and voltage were monitored by provision of individual integrated circuits for each bacterioelectric unit. These employed National Semiconductor LM117 voltage regulators as shown in Figure 2. In subsequent experiments powdered coal was rapidly circulated around an anode with a magnetic stirrer and the cathode was inserted in a medium porosity "alundum" extraction thimble. Usually the cathode chamber sediments were collected in centrifuge tubes, then washed by sedimentation and decantation; first in pH 2.5 H_2SO_4 , then distilled water. Prior to analysis washed sediment or coal samples were dried overnight at 85°C in a forced draft oven. The pre-weighed coal samples were ashed in tared porcelain crucibles in an electric furnace kept at 850°C overnight.

Energy Dispersive X-Ray analysis were used to identify and measure relative amounts of the different metals in sediments collected from the cathode chamber, as coatings on the platinum cathodes and from drying the bulk lixiviant to recover solubilized metals. Conventional methods were used to examine the IR spectra of sediments prepared in KBr discs (Lazaroff et al. 1982).

A laboratory scale continuous bacterioelectric reactor was constructed that utilized a porous alundum cathode chamber contained in a horizontal air-lift device that impinges a rapidly stirred coal slurry on a platinum anode. The system shown in Figures 3 and 4 allows periodic recovery of processed coal and electrically separated metals, while adding the feedstock coal.

Results

The metals deposited on the cathode or in the cathode chamber following galvanic treatment of coal qualitatively reflect the metal composition of the heterogeneous starting material. While iron is most abundant in cathode sediments from pyritic coals, significant amounts of sodium, potassium, rubidium, magnesium, calcium, strontium, aluminum, titanium, copper, manganese, zinc, lead, nickel and chromium have also been recovered there, depending upon the coal sample studied. Although the univalent cations, sodium and potassium are found in the cathode sediments, often larger quantities are found in soluble form in the acid lixiviant along with much aluminum, calcium and magnesium as well as smaller amounts of heavier metals such as iron. In some coal samples, the rarer elements, lutecium, ytterbium, lanthanum and neodymium were found usually by inspection of localized deposits on the platinum cathodes with scanning electron microscopy and ED x-ray analysis (Lazaroff and Dugan, 1989). The non-metallic elements found at the cathode, presumably complexed with cations, include sulfur, phosphorous, silicon and less frequently chlorine. One coal sample yielded significant amounts of bromine. Not all of the metals leaching from coal samples deposited at the cathodes of the galvanic systems.

PSOC 667, a sub-bituminous Iowa coal containing approximately 6% pyrite, was found to be particularly suitable for studying bacterioelectric deashing. Earlier studies had shown that bacterial presence during galvanic treatment resulted in more complete removal of metals from the slurried coal compacted at a platinum anode (Lazaroff and Dugan, 1989). Table 1 compares the results of deashing in the presence of bacteria or by increasing the conductivity of the galvanic cell through addition of $10^{-3}M Li_2SO_4$. The enhancement of conductivity alone, resulted in removal of as much as 75% of the ash from the PSOC 667 coal in 20 hrs treatment. It was found that comparable results could be obtained with dispersed stirred coal particles at the anode if the lithium solute was present or with slower rates of deashing in the presence of bacteria in the absence of lithium sulfate. With protracted bacterioelectric leaching of stirred PSOC 667 coal slurries, over 90% of ash has been removed (Table 2).

Examination of the leachability of other coals by the bacterioelectric system indicated that most pyritic bituminous and subbituminous coals were susceptible to galvanic deashing but with considerable variation in efficiency of metal removal. The lignites investigated were not enhanced in deashing by the presence of bacteria which was possibly correlated with the absence of Pyrite (Table 1).

Although pyritic coals show the effect of bacterial enhancement of metal removal, there is no clear indication that the only metals removed from the coal are associated with pyritic inclusions. A good example of this is observed with PSOC 1322, an Illinois #6 high volatile bituminous coal. The presence of bacteria significantly contribute to metal removal. The cathode chamber deposit is mainly iron when the iron oxidizing bacteria are present but copper, calcium, silicon, manganese and aluminum are present as well. Without the additional iron oxidizing thiobacilli, the smaller cathode accumulation is qualitatively similar in metal composition but is predominantly calcium with comparatively little iron. The calcium is removed from the coal in large amounts without the added bacteria but it is found in

soluble form in the lixiviant rather than precipitated at the cathode (Figure 5). Figures 6 and 7 are ED-X-ray spectra of additional pyritic bituminous coals presented in Table 1 (PSOC 1316 and IBCSP #2) that further illustrate the removal of metals by the bacterioelectric effect.

An interesting aspect of this fractionation of metals in different phases of the system undergoing deashing is shown in Figure 8. As with the PSOC 1322 coal, the cathode chamber sediment from bacterioelectric leaching is largely iron while the comparable sediment from galvanic leaching without bacteria is mainly calcium. However, the calcium can be collected as a solid phase separate from the main cathode sediment or the dissolved solutes of the lixiviant by placing a membrane filter over the cathode chamber port. The calcium then deposits almost exclusively on the filter along with iron, and very little calcium enters through the porous membrane to deposit in the cathode chamber. This does not occur in the absence of added iron oxidizing bacteria suggesting that the Fe(III) sulfato-complex produced by chemolithotrophic iron oxidation is in some way responsible for co-precipitation of iron and calcium on the membrane.

The encouraging results obtained from deashing subbituminous coal galvanically in dispersed suspension, in the presence of bacteria or lithium sulfate led to construction of a system which allows re-use of the lixiviant and bacteria in a continuously fed laboratory scale reactor (Figures 3 and 4). In limited trials, so far, it appears possible to use the horizontal air-lift flowing system to remove 80 to 90% of ash from bacterially pre-treated Iowa subbituminous or Illinois #6 bituminous coal.

Summary

1. Metals can be removed from bituminous and sub-bituminous coals in galvanic cells at low voltages and currents. Originally this involved compressing powdered coal at an inert anode in a stirred dilute sulfuric lixiviant.
2. Pyritic coals may be simultaneously oxidized by acidic iron formed by iron oxidizing thiobacilli. This results in some coals yielding enhanced metal deposition at galvanic cathodes.
3. More complete deashing of a sub-bituminous coal was accomplished at high current densities in systems containing 10^{-3} - 10^{-2} M Li_2SO_4 in the sulfuric lixiviant. 74% of ash removal has been accomplished.
4. The galvanic deashing can be carried out with dispersed-suspended coal particles in the Li_2SO_4 lixiviant or in systems containing cells of *Thiobacillus ferrooxidans* or with coal slurries pre-treated with *Thiobacillus ferrooxidans*.
5. A continuous laboratory-scale system for bacterioelectric deashing of pyritic coals has been developed.

Acknowledgments

This work was supported under contract No. DE-AC07-7610D1570 from the U.S. Department of Energy, to the Idaho National Engineering Laboratory/EG&G Idaho, Inc.

References

Decker, R. F. 1986. Metall. Trans A, 17:5-30.

Lazaroff, N., Sigal, W., and Wassenman, A. ,1982. Appl. and Env. Microbiol. 43:924-938.

Lazaroff, N. and Dugan, P. R. 1989. Proceedings, Bioprocessing of Fossil Fuels Workshop. Tyson's Corner, VA. pp. 79-104 (CONF-890884) available from NTIS U.S. Dept. Commerce, Springfield, VA 22161.

TABLE 1. ELECTROBACTERIAL DEASHING OF COAL SAMPLES

Coal Sample	Treatment	Ash (% by wt.)
<u>HBV</u> PSOC 1322 1.98% pyrite S	None	12.13
	46 hrs 8V, 2mA + Bact.	9.92
	46 hrs 8V, 2mA - Bact.	10.92
	Bact. pre-treat 56 hrs. 10V, 5 mA	6.99
<u>HBCB</u> PSOC 1316 1.47% pyrite S	None	11.06
	91 hrs 16V, 5mA + Bact.	10.86
	91 hrs 16V, 5mA - Bact.	11.16
<u>PSOC 551</u> 4.68% pyrite S	None	14.74
	43 hrs 16V, 5mA + Bact.	11.10
	43 hrs 11V, 5mA - Bact.	4.24
<u>IBCSP 2</u> 2.34% pyrite S	None	5.70
	90 hrs 15V, 5mA + Bact.	4.17
	90 hrs 15V, 5mA - Bact.	4.19
<u>Sub-bit. A</u> PSOC 667 6.0% pyrite S	None (as rec.)	18.73
	Bact. pre-treat + 45 hrs 7V, 2mA ave	7.04
	None (dry basis)	21.23
	in thimble, 20 hrs 12V, 5mA	13.04
	in thimble, 20 hrs 12V, 35mA + Li ₂ SO ₄ dispersed, 14 hrs, 12V, 38mA	4.80 7.96
<u>Sub-bit. C</u> PSOC 637 0.64% pyrite S	None	10.41
	Bact. Pre-treat + 67V, 3mA ave	4.79
<u>Lignite</u> PSOC 1489 0.02% pyrite S	None	6.90
	41 hrs 17V, 5.0mA + Bact.	3.60
	41 hrs 15V, 5.0mA - Bact.	3.20
Spanish Lignite	None	12.48
	66 hrs 10V, 2mA - Bact.	9.46
<u>Lignite A</u> PSOC 245 0.22% pyrite S	None	8.89
	90 hrs 10V, 2.5mA + Bact.	7.14
	90 hrs 13V, 2.5mA - Bact.	6.69

TABLE 2. BACTERIOELECTRIC DEASHING	
	Ash %
Iowa 667 Subbituminous Coal H ₂ SO ₄ pH 2.5	
	10 volts, 8.75 ma
Untreated control	21.23%
72 hrs	4.72%
140 hrs	1.22%
	10 volts, 21.82 ma
24 hrs	12.72%
96 hrs	7.74%
	5 volts, 7.70 ma
24 hrs	15.92%
96 hrs	5.53%
Illinois #6 Coal Argonne ID 301	
	10 volts, 12.5 ma
Untreated control	25.5%
72 hrs	15.5%
96 hrs	10.68%
168 hrs	10.70%
With $1 \times 10^{-3}M$ Li ₂ SO ₄	
168 hrs	8.50%

SOLUBILIZATION OF PYRITE AND FORMATION OF OXIDIZED IRON SEDIMENTS IN A GALVANIC CELL WITH AN IMPOSED E.M.F., IRON OXIDIZING THIOBACILLI AND H_2SO_4 pH 2.5

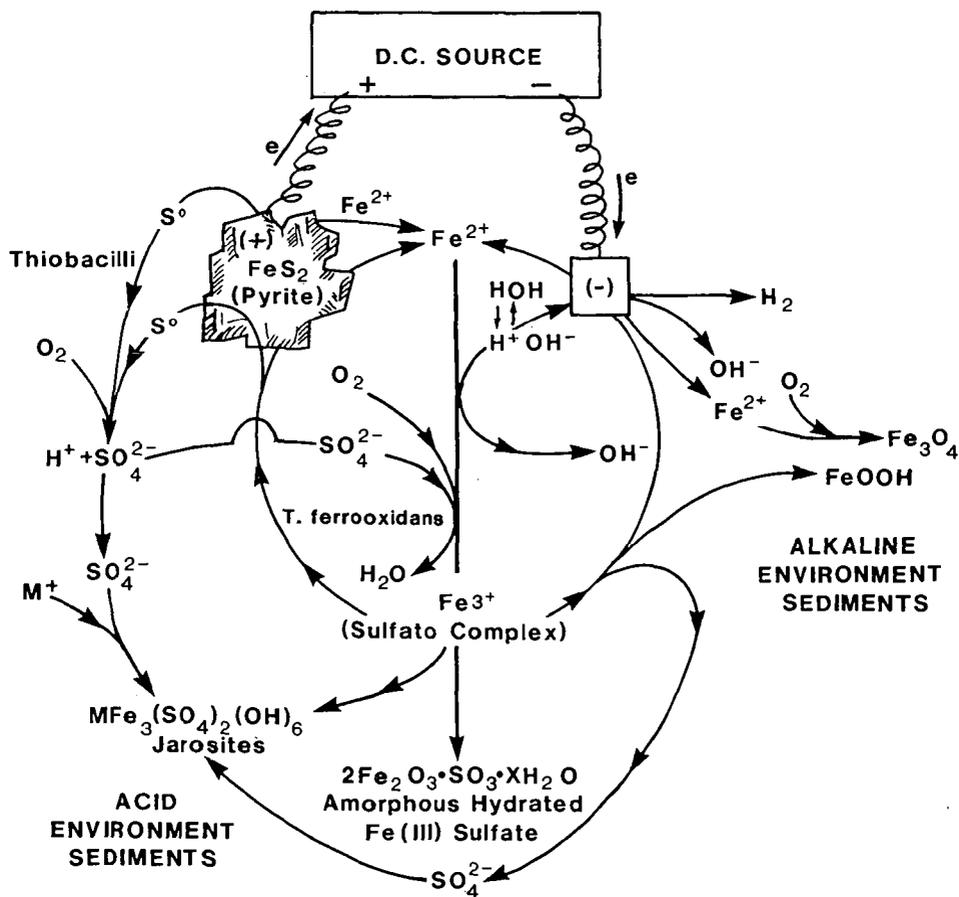


FIG. 1

LAB SET-UP of D.C. POWER SUPPLY WITH VOLTAGE REGULATOR
and VOLTAGE, CURRENT MEASUREMENT

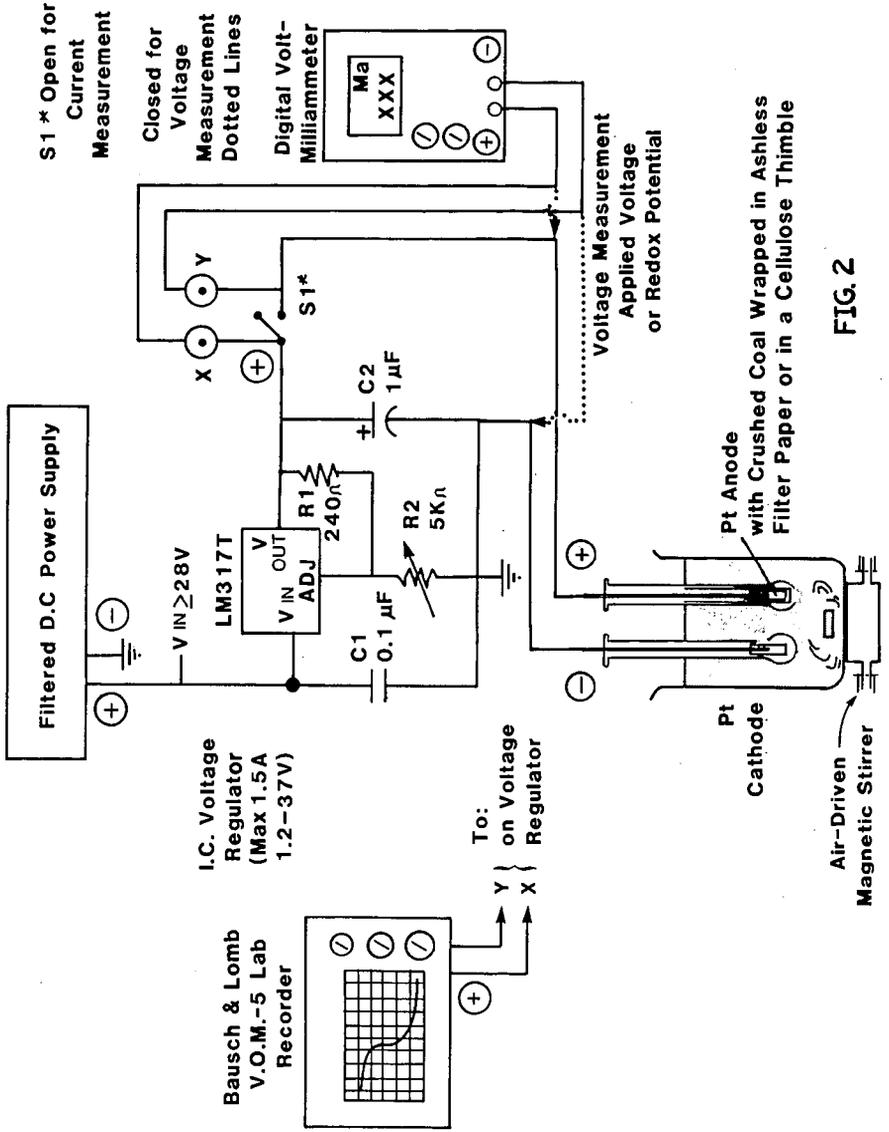


FIG. 2

LABORATORY SYSTEM for CONTINUOUS
BACTERIO-ELECTRIC REMOVAL of METALS FROM COAL

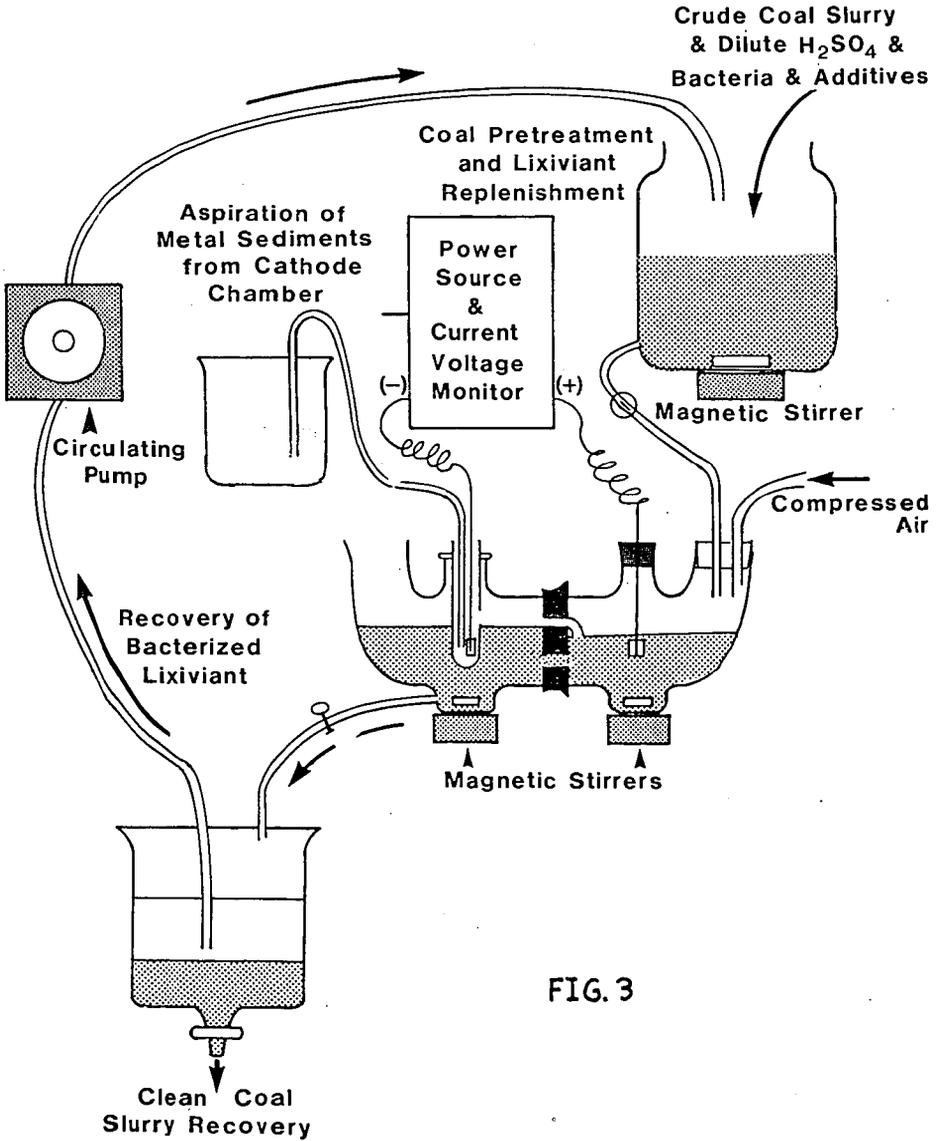


FIG. 3

HORIZONTAL AIRLIFT BACTERIO-ELECTRIC LEACHING CHAMBER

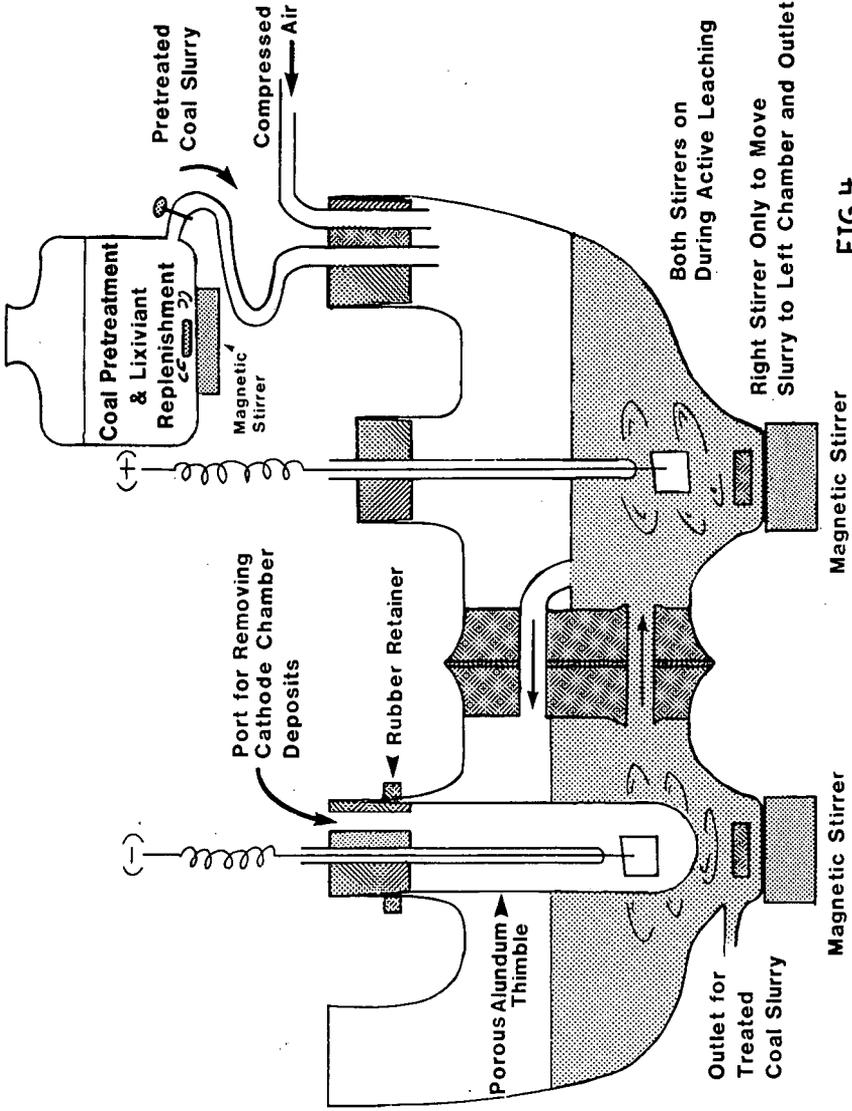


FIG. 4

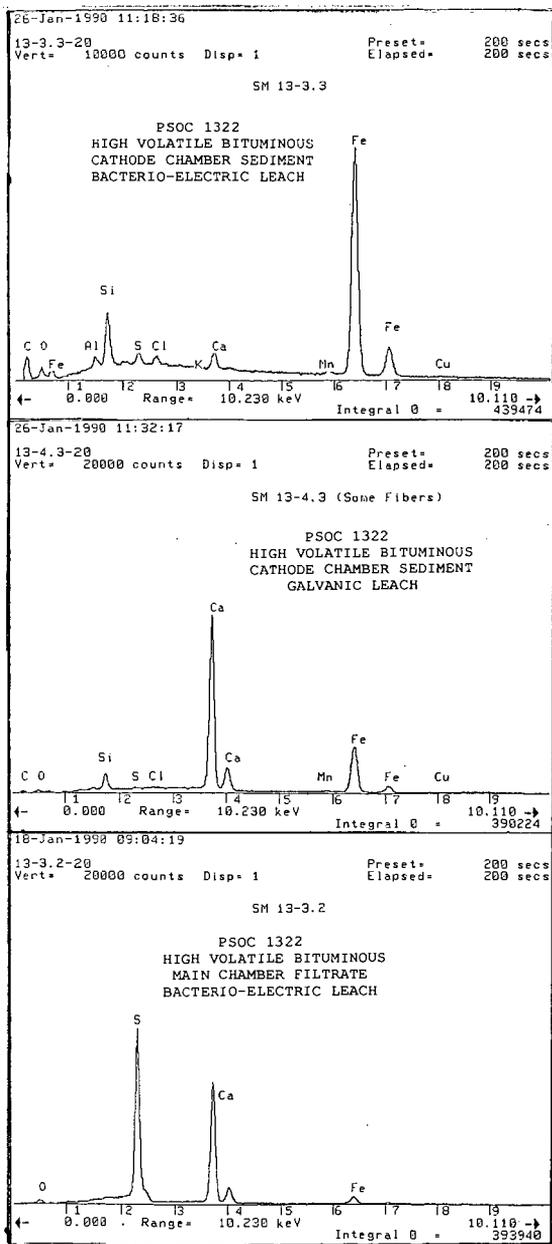


FIG. 5

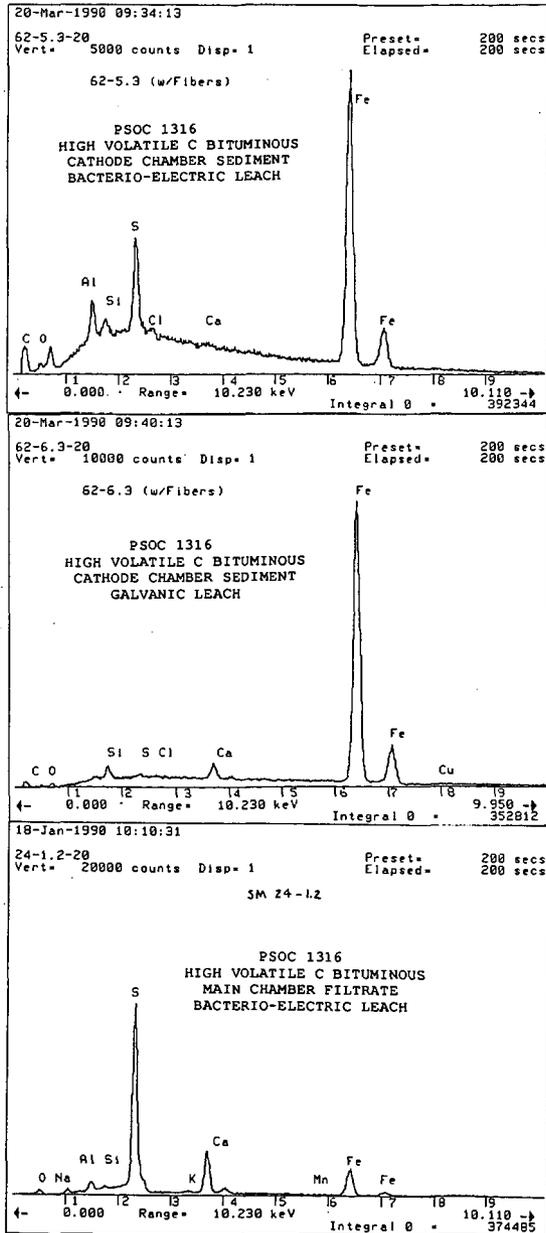


FIG. 6

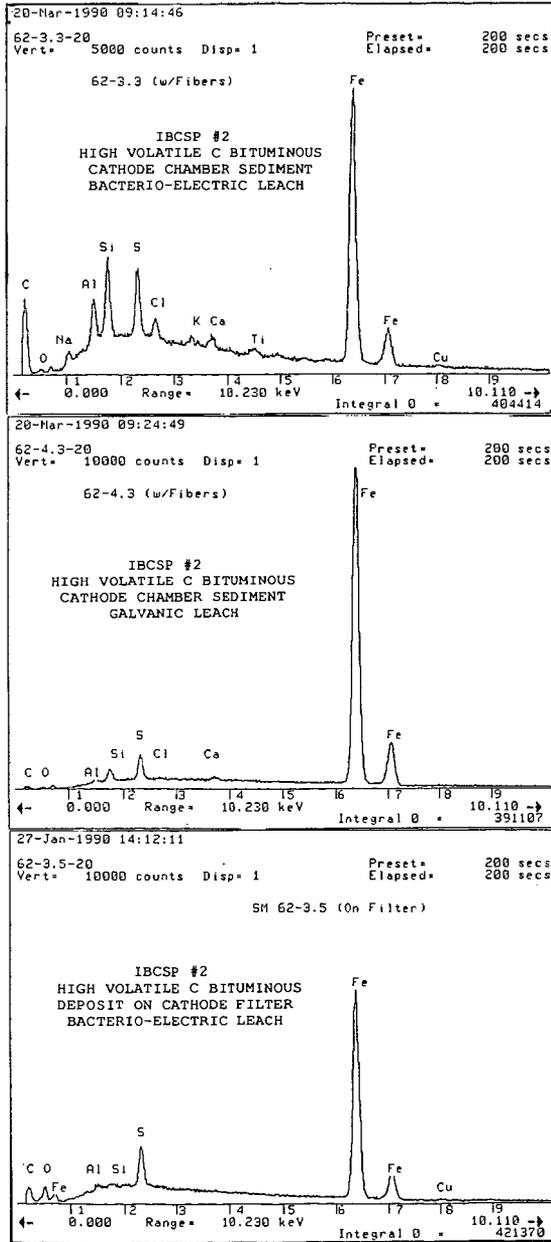


FIG. 7

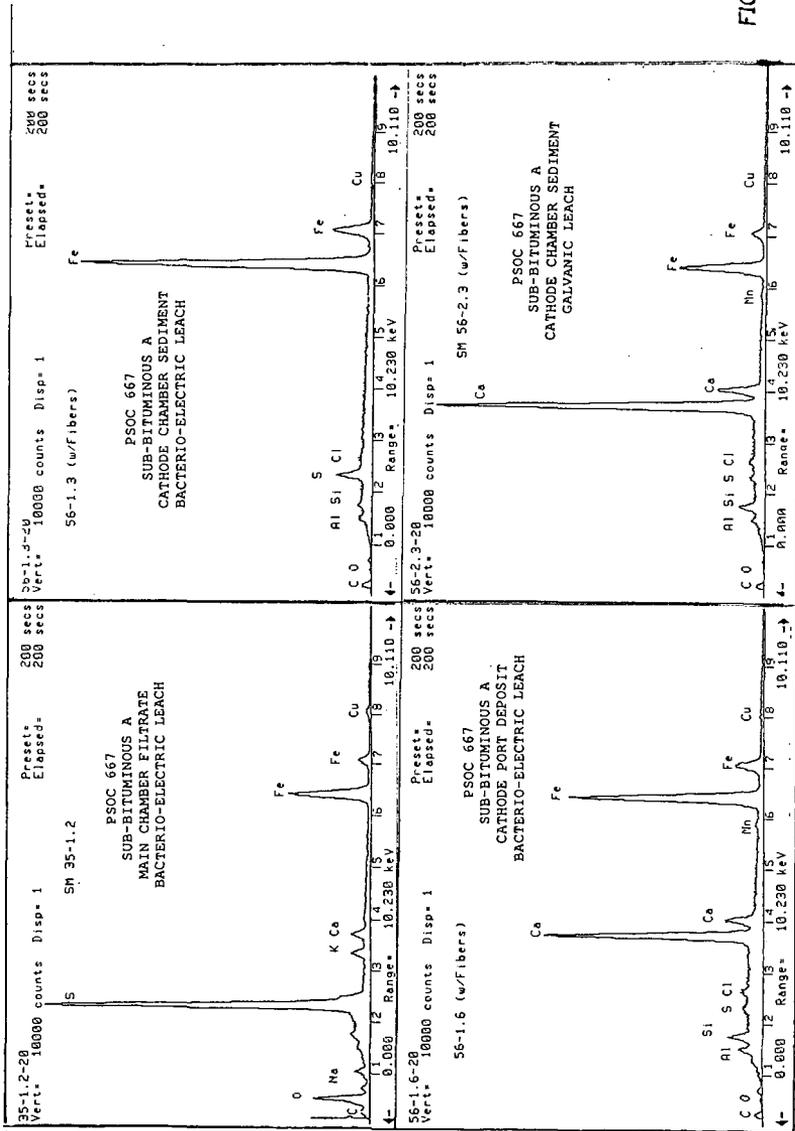


FIG. 8

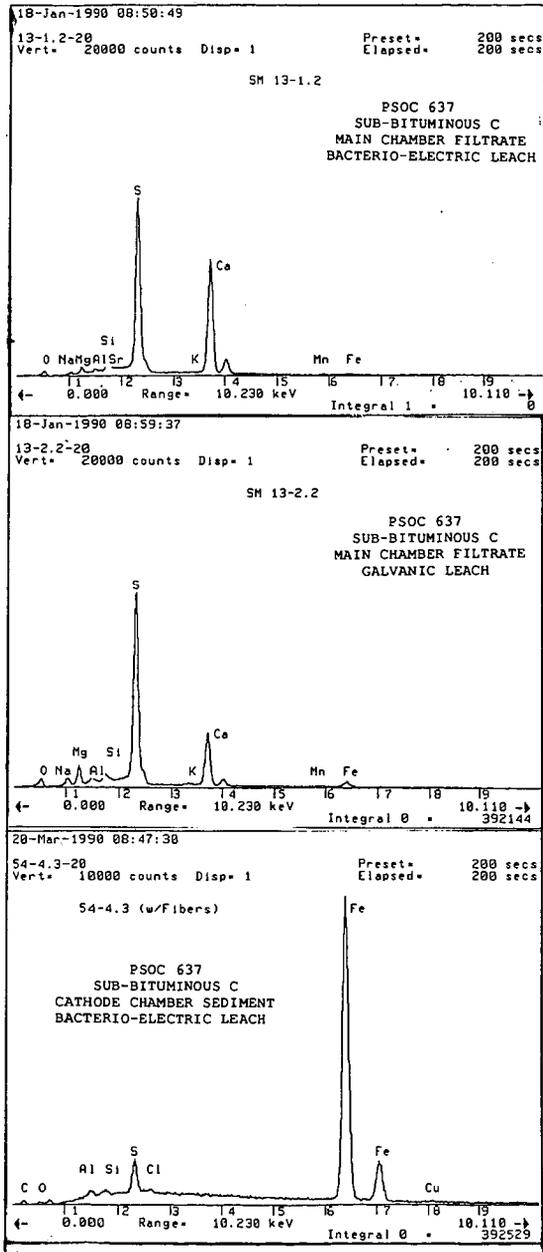


FIG. 9

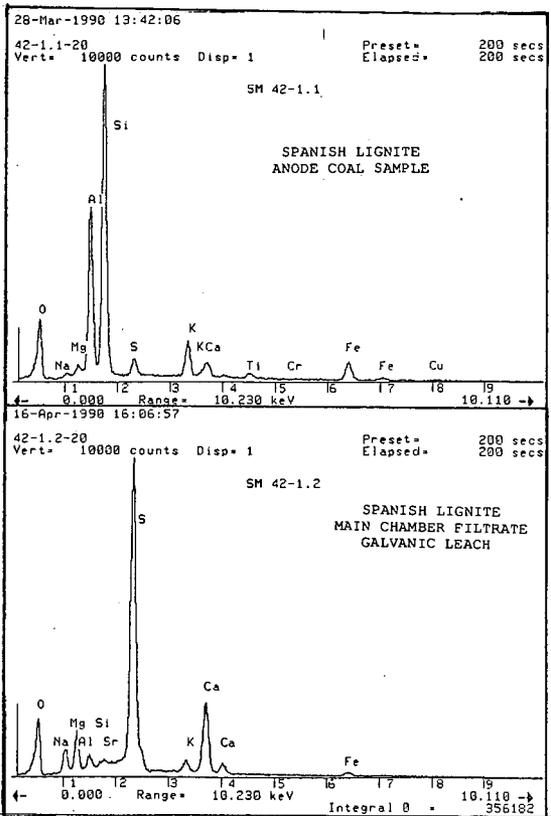


FIG 10

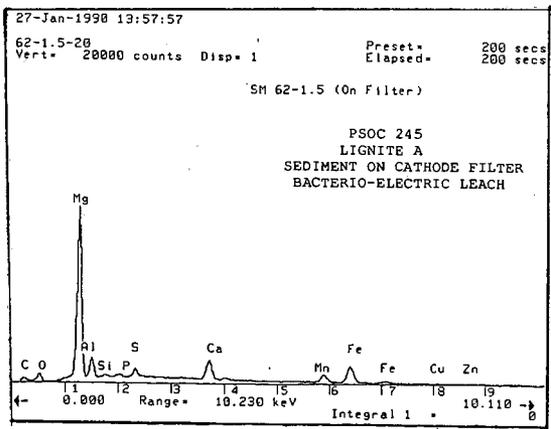


FIG 11

**USE OF METHANOTROPHIC BACTERIA IN GAS PHASE BIOREACTORS
TO ABATE METHANE IN COAL MINE ATMOSPHERES**

William A. Apel, Patrick R. Dugan, and Michelle R. Wiebe

The Idaho National Engineering Laboratory
EG&G Idaho, Inc.
P.O. Box 1625
Idaho Falls, Idaho 83415-2203

Keywords: Methanotrophic Bacteria, Methane Removal From Mine
Atmospheres, Gas Phase Bioreactors

Introduction

Coal mining activities often lead to the release of methane into the mine atmosphere from subterranean pockets that are disturbed during the normal course of mining. This methane can pose a distinct explosion hazard in the mine environment when combined with oxygen from air. It has been reported that the explosive range for methane in air is 5.53% to 14% with methane concentrations above 14% burning without explosion (10). In reality, many mine operations have safety requirements dictating evacuation if mine methane levels exceed 1-2%, since the accidental ignition of methane at concentrations below 5.53% may initiate coal dust explosions (3). Thus, the presence of methane in mines can result in economic loss. This is due to the need to either install ventilation systems and sustain air flow for maintaining methane at safe levels, or terminate operations and evacuate the mine if methane concentrations exceed those deemed safe.

Certain types of bacteria collectively known as methanotrophs are capable of utilizing methane as their sole source of cellular carbon and energy (9). The methanotrophs are nonpathogenic and taxonomically are assigned to several different genera. These bacteria are designated as type I or type II depending on the intracytoplasmic membrane arrangement displayed when grown on methane (1). Methanotrophic bacteria aerobically oxidize methane via a sequential pathway with biomass, carbon dioxide and water being the primary end products of the process (2). Some isolates under certain conditions also have the capability to grow on alternate carbon and energy sources such as alcohols, propane, short chained organic acids, hexadecane, etc. (4).

The methanotrophs are ubiquitous in nature and actively grow in environments where both methane and oxygen or alternate growth substrates are available. This type of environment is most typically found in rich soils, water, and upper layers of

sediments from lakes, harbors, estuaries, ponds, ditches, marshes, and other sites of active methanogenesis (5,8). As a result of their metabolic activities in these environments, methanotrophic bacteria are believed to play a key role in eutrophication by capturing and locking into their ecosystem the carbon from methane (6).

Due to their unique ability to utilize methane as a sole carbon and energy source, methanotrophic bacteria appear to be ideally suited for growth in gas phase bioreactors. In these reactors methane is readily available for cellular metabolism. As such, gas phase bioreactors offer an advantage over liquid phase bioreactors where under certain conditions methane can become limiting due to its relatively low solubility in water.

This paper reports the results from preliminary studies on the growth of a particular type I methanotrophic bacterium, Methylomonas methanica, in gas phase bioreactors. The ability of these bacteria to strip methane from methane-containing atmospheres such as those sometimes found in mine environments was also examined.

Experimental

Culture Maintenance

Methylomonas methanica isolate number O.S.U. 739 was obtained courtesy of the Ohio State University Department of Microbiology culture collection. The culture was maintained in 50 ml aliquots of CM mineral salts medium (7) contained in 125 ml serum bottles sealed with teflon coated rubber stoppers. The bottles were gassed with approximately 30% methane in air and incubated at 37° C on a rotary shaker. Gas levels in the culture vials were monitored using gas chromatographic analysis as described below. Culture bottles were regassed when either the methane or oxygen levels were depleted. Cultures were transferred to fresh medium at least every two weeks to maintain viability.

Gas Phase Bioreactor Design and Maintenance

The bioreactors were constructed from a 3 X 30 inch i.d. glass column sealed at the open end with a rubber stopper (Figure 1). Flexible 5/32 inch o.d. teflon tubing connected the upper end of the column to a stoppered 1 L Erlenmeyer flask that served as a gas volume reservoir. The flask in turn was connected via tubing to the lower end of the column so that a closed recirculation loop was formed. A peristaltic pump which allowed recirculation of gas through the closed system was situated in line between the gas reservoir flask and the lower end of the column. The column interior was filled with polypropylene bio-rings which acted as supports for the growth of the methanotrophs in the gas phase.

The bioreactors were prepared for growth of M. methanica by removing the stopper from the top of the column and pouring

approximately 50 ml of CM mineral salts medium into the upper end of the column. The medium was allowed to trickle over the bio-rings and collect in the bottom of the column. A 50 ml culture of stationary phase M. methanica grown in serum bottles as described above was then poured into the column in a manner similar to that described for the medium. Both the CM mineral salts medium and the inoculum were allowed to remain as a heel in the base of the column to help humidify the bioreactor. Following this, the stopper was tightly reinserted into the upper end of the column and further secured into place by wrapping with parafilm.

The inoculated bioreactor was incubated at $20 \pm 2^\circ \text{C}$ for a period of 3 weeks. During this period, methane levels were targeted to approximately 30% methane in air. The gas mixture was constantly recirculated through the column at a rate of 200 ml per minute and gas levels were monitored via gas chromatography. The bioreactors were regassed to the above target levels whenever the methane or oxygen levels fell below 5.0%. Growth of M. methanica was monitored visually via the appearance of the pink pigmented organism on the bio-rings.

Rates of gas depletion were determined by first flushing the bioreactors with air and then gassing the bioreactors with a known mixture of methane in air. The gas mixture was recirculated through the bioreactor at a rate of 200 ml per minute. Gas levels were monitored via gas chromatography.

Analytical Methods

Gas levels (methane, oxygen, and carbon dioxide) in the serum bottle cultures and the bioreactor were analyzed using a Gow-Mac Series 550P gas chromatograph equipped with a thermal conductivity detector and an Alltech CTR1 column. The gas chromatograph was connected to a Hewlett Packard model 3390A integrator. Samples consisted of 600 μl gas volumes manually injected into the gas chromatograph which was operated with helium as the carrier gas at a flow rate of 60 ml per minute under isocratic conditions at 30°C .

Results and Discussion

M. methanica was capable of growing to relatively high densities on the polypropylene bio-ring supports contained in the gas phase bioreactors. This growth was apparent visually in the form of highly pigmented pink biomass which adhered to the supports. The ability to directly visualize the growth of M. methanica throughout the bioreactor due to the organism's distinct pink pigmentation was of significant aid in easy, direct, nondestructive evaluation of growth patterns. Visual observation showed the growth to be distributed relatively evenly over the supports throughout the bioreactor with the exception of somewhat

heavier growth on the supports near the gas/liquid interface in the very bottom portion of the bioreactor.

The biomass in the bioreactor was quantitated by simple weighings which showed the average amount of biomass per support to be approximately 0.2 g (wet weight), with the total amount of biomass in the bioreactor being calculated to be 133.4 g (wet weight).

The *M. methanica* biomass in the bioreactors was assessed relative to its capability to strip methane from air. Figure 2 illustrates the results of experiments to strip a variety of methane levels from air over a 24 hour period. In these experiments the methane/air mixture inside the bioreactor was allowed to continuously recirculate. As can be seen in Figure 2, 35% methane in a total gas volume of 4.5 L was depleted by 90.4% in 24 hours. As would be anticipated, lower methane levels, e.g. 10.6%, were depleted to below the analytical detection limit in less than 24 hours.

In an effort to better simulate the methane levels likely to be encountered in mine environments, the same experiments were repeated using significantly lower starting methane levels measured at more frequent intervals. The results from these experiments are shown in Figure 3 using computed best fit curves. The data indicate that at levels up to 10% methane in air, the removal of methane by *M. methanica* is linear with the same rates of removal over the entire range under consideration. This is supported by the similar slopes on all three curves.

Under the conditions employed in the experiments illustrated by Figure 3, (i.e. methane < 12%), rates of methane removal for the 133.4 g (wet weight) of biomass contained in the bioreactor were calculated to be 22.9 mg of methane per hour. At higher methane levels such as 30%-45% methane in air, rates of removal were approximately 60% higher averaging 37.7 mg of methane removed per hour.

Further experimentation is necessary to ascertain the reason(s) for this difference in methane removal rates. One possible explanation could be increased transport of the higher concentrations of methane through the biofilm growing on the bio-rings. This increased transport could thus be making methane more available to cells deep within the biofilm and, as a result, greater rates of overall methane degradation could be observed.

Figure 4 illustrates the change in oxygen and carbon dioxide levels in the column during methane removal by the methanotropic bacteria. Since oxygen serves as a terminal electron acceptor for the methane oxidation pathway, oxygen levels decrease as methane decreases. Similarly as methane is oxidized, the carbon from methane is either incorporated into bacterial biomass or released from the oxidation process as carbon dioxide, thus.

explaining the gradual observed increase in carbon dioxide as methane is removed. In the specific example illustrated, with methane levels starting near 11%, a 50% decrease in methane led to an 11% decrease in oxygen. Concurrently, carbon dioxide increased from below the lower limit of detection to approximately 0.8%. These data indicate that methanotrophic bacterial bioreactors would also lower oxygen levels in coal mines. However, the amount of oxygen removed would be modest relative to the amount of methane eliminated, i.e. methane in a mine environment would usually be below 2% whereas oxygen would be approximately 20%.

Conclusions

Conclusions from these preliminary studies are as follows:

- ♦ Methanotrophic bacteria such as *M. methanica* are capable of growing to significant densities in gas phase bioreactors of the types used in this work.
- ♦ These organisms remove significant amounts of methane at significant rates from air/methane mixes, and as such may be of practical use in stripping methane from mine atmospheres.
- ♦ Additional work needs to be done to optimize reaction rates. This would include a more refined gas phase bioreactor design to (1) increase overall bacterial cell numbers, and (2) maximize bacteria/gas contact. Concurrently, methanotrophic culture optimization needs to be performed. Experiments already being initiated indicate the methane removal rate can be ultimately increased to at least 10 times those reported in this paper.

Acknowledgement

This work was supported under contract no. DE-AC07-76ID01570 from the U.S. Department of Energy, to the Idaho National Engineering Laboratory/EG&G Idaho, Inc.

References

- (1) Davies, S. L., and R. Whittenbury. 1970. Fine Structure of Methane- and Other Hydrocarbon Utilizing Bacteria. *J. Gen. Microbiol.* 61: 227-232.

- (2) Haber, C. L. , L. N. Allen, S. Zhao, and R. S. Hanson. 1983. Methylophilic Bacteria: Biochemical Diversity and Genetics. *Science* 221: 1147-1153.
- (3) Lambecki, K. T. 1988. Methane Explosions at High Volume and Low Concentration. Fourth International Mine Ventilation Congress, Brisbane, Queensland, Australia
- (4) Reed, W. M., and P. R. Dugan. 1987. Isolation and Characterization of the Facultative Methylophilic Mycobacterium ID-Y. *J. Gen. Microbiol.* 133: 1389-1395.
- (5) Reed, W. M., and P. R. Dugan. 1978. Distribution of Methylophilus methanica and Methylophilus trichosporium in Cleveland Harbor as Determined by an Indirect Fluorescent Antibody-Membrane Filter Technique. *Appl. Environ. Microbiol.* 35:(2) 422-430.
- (6) Weaver, T. L., and P. R. Dugan. 1972. The Eutrophication Implications of Interactions Between Naturally Occurring Particulates and Methane Oxidizing Bacteria. *Water Res.* 6: 817-828.
- (7) Weaver, T. L., and P. R. Dugan. 1975. Ultrastructure of Methylophilus trichosporium as revealed by freeze etching. *J. Bacteriol.* 121:704-710.
- (8) Whittenbury, R. and H. Dalton. 1981. The Methylophilic Bacteria. In: The Prokaryotes. A Handbook on the Habitats, Isolation, and Identification of Bacteria, Starr, H. P., H. Stulp, H. G. Trupper, A. Balows, and H. G. Schlegel (eds). Springer-Verlag, Berlin.
- (9) Whittenbury, R., K. C. Phillips, and J. F. Wilkinson. 1970. Enrichment, Isolation, and Some Properties of Methane-Utilizing Bacteria. *Gen. Microbiol.* 61:205-218.
- (10) Windholz, M., S. Budavari, R. F. Blumetti, and E. S. Otterbein. 1983. The Merck Index, 10th Edition, pp. 852-853, Merck and Co., Inc., Rahway, N.J.

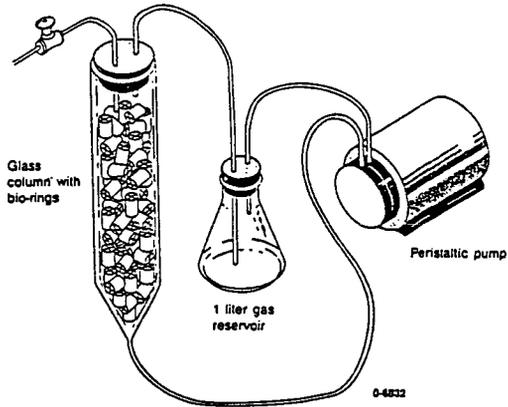


Figure 1
Schematic Diagram of Gas Phase Bioreactor

FIGURE 2
PER CENT METHANE REMOVED IN 24 HOURS
(4.5 L gas volume; 133.4 g (wet weight) *M. methanica*)

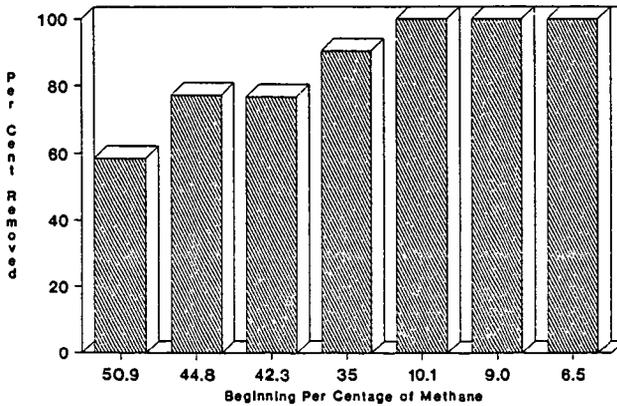


FIGURE 3
METHANE REMOVAL FROM AIR BY M. METHANICA

(4.5 L of gas; 133.4 g (wet weight) of bacteria)

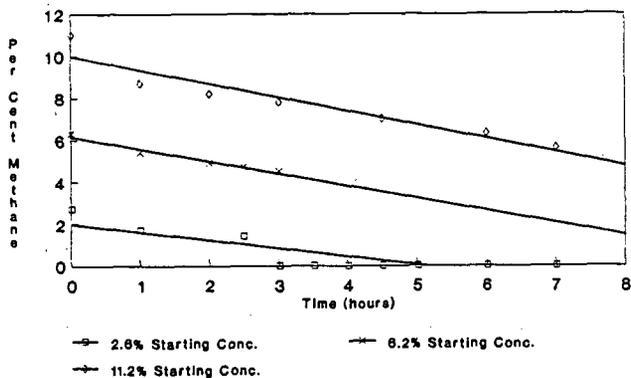
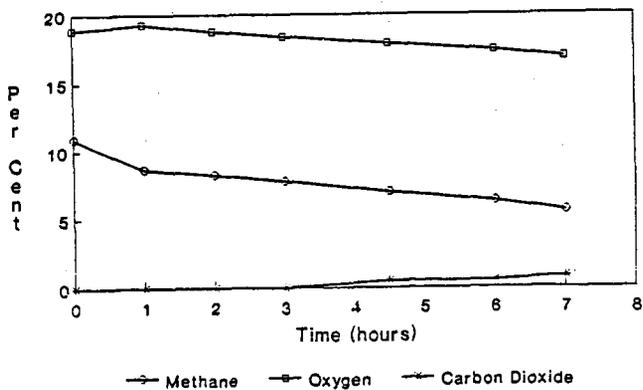


FIGURE 4
CH₄ REMOVAL RELATIVE TO O₂ AND CO₂

(4.5 L of Gas; 133.4 g (wet weight) M. methanica)



CELLS ON ROTATING FIBERS FOR CLEAN FUELS

Robert A. Clyde
P.O. Box 740644
New Orleans, LA 70174

Keywords: Zymomonas, yeast, coal, methane

INTRODUCTION

Rotary biological contactors have been made 10 feet in diameter but the discs are plastic. Fibers have more surface area. Half a pound of celite trapped in the fibers has the area of a football field so more cells can be immobilized. This concept is covered by a Clyde patent (1). Another patent (2) describes photo production of hydrogen and a third (3) cane fibers from the discs. Sulfur can be removed from coal and methane converted to methanol. In a recent 28 page booklet (4), several observations were made:

- pg. 6. At current rates, there will be 13,000 to 23,000 deaths in California from cancer.
- pg.18. About one billion gallons of gasoline would be displaced by clean fuel in 1997, increasing to about 11 billion by 2006.
- pg.21. The year 2000 marks the first year in which all vehicles for sale in California would either be LEVs (low emission vehicles) or ULEVs (ultra low emission vehicles).

ALCOHOL FROM SUGAR

Parekh and Wayman (5) describe fermentation of glucose to ethanol in 15 minutes as "remarkable", using Zymomonas in a 4 inch diameter unit of rotating fibers (Fig. 1). In a letter they say the CO₂ came off so fast it's like an "explosion". They did not use Celite entrapped in the fibers. Celite is not expensive and neither is polyester fiber. Eight inch diameter units have been run by Clyde using Reemay polyester (6) type 2033 which is only 70 cents per sq. yard. Typar style 3301 from the same company is only 42 cents per sq. yard. Larger units are now being constructed. To provide stiffness, the fiber can be stapled to a screen.

ALCOHOL FROM WOOD

Wyman et. al. (7) say that SSF (simultaneous saccharification and fermentation) has great potential for production of ethanol at competitive prices. The key to this process is the ability to rapidly convert the sugars because they inhibit the conversion. They do it in 7-12 days, but Chen and Wayman (ref. 8 and Fig. 1) do it in 2 days using rotating fiberglass discs.

HYDROGEN

Several investigators have described hydrogen production from algae. Laws (9) describes the advantages of a flashing light which can be done in a rotary biological contactor. Weetal (10) also has a method. Greenbaum (11) also has a method and Mitsui at the University of Miami has written several articles. Veziroglu, also at the University of Miami, is the

editor in chief of a hydrogen journal. Nelson (12) at Argonne Lab. said one problem was availability of CO₂ but large amounts of that are produced in an alcohol plant.

COAL

Thiobacillus ferrooxidans grows on fibers as in patent 4,530,763 and it removes pyrite from coal. Pseudomonas and Phanerochaete chrysosporium solubilize coal and the former removes organic sulfur (13). Loganback from Morgantown (14) describes biotreatment of syngas.

METHANE

Large amounts of methane are flared (wasted) from oil refineries, since it cannot be economically transported. Lipscomb from the University of Minnesota (15) has a bacterium which converts methane to methanol, but burning methanol in a car or turbine produces formaldehyde. Methanol can be converted to clean burning hydrogen and CO with a 30% increase in energy (because waste heat is utilized) as in patent 4,420,462 where the catalyst can easily be removed out the bottom (Fig. 2). In other designs, catalyst is inside the tubes, but when the tubes expand with heat, the pellets pack in, and when the tubes cool they crush the catalyst.

REFERENCES

1. 4,407,954
2. 4,446,236
3. 4,600,694
4. Low Emission Vehicles/Clean Fuels and New Gasoline Specifications, by Air Resources Board, 9528 Telstar Ave., El Monte, Cal.
5. Parekh, S., Parekh, R., and Wayman, M. Ethanolic Fermentation of Wood-derived Cellulose Hydrosylates by Zymomonas mobilis in a Continuous Dynamic Immobilized Biocatalyst Bioreactor. *Process Biochem.* June '89 p. 88-91.
6. Reemay Co. Industrial Road. P.O.Box 511, Old Hickory, TN 37138
7. Wyman, C. et.al. The Impact of Glucosidase in the Simultaneous Saccharification and Fermentation Process. Paper given at the AIChE 1990 meeting in Orlando in March.
8. Chen, S. and Wayman, M. Continuous Production of Ethanol from Aspen Cellulose by Co-immobilized Yeast and Enzymes. *Process Biochem.* Dec. '89 p. 204-207.
9. Laws. *Biotech and Bioeng.* 25, 2319-2335
10. Weetal. *Biotech and Bioeng.* 23, 605-614
11. Greenbaum, E. *Photochem. and Photobiol.* Oct. '89 p. 571-576
12. Nelson. Availability of CO₂ in the Southwest
13. Srivastava, R. et.al. Coal Bioprocessing: A Research-Needs Assessment. *Chem. Eng. Progress* Dec. '89 p.45-53
14. Talk 100A, Amer. Inst. Chem. Eng. Orlando, Mar. 1990
15. Talk given at Midwest Biotech. Symp. May 1990, St. Paul

SEPARATION OF PHENOLIC COMPOUNDS FROM COAL LIQUIDS

Yoichi Kodera, Koji Ukegawa, and Tetsuo Nakayama
National Research Institute for Pollution and Resources
16-3 Onogawa, Tsukuba, Ibaraki 305, Japan

Keywords: solvent extraction, phenolic compound, coal liquid

INTRODUCTION

Coal liquids contain considerable amounts of phenolic compounds which are industrially important chemicals. Upgrading of coal liquids have been performed by catalytic hydrogenation to produce fuel oils of good quality. Because of the existence of phenolic compounds, the upgrading requires hydrogen in quantity. Thus, an economical and effective method for separation of these compounds from coal liquids has been expected to develop. Here we report that a solvent extraction of phenolic compounds from naphtha distillates of coal liquids using methanol and water as solvents (1). The present presentation discusses the experimental conditions for the effective separation and the composition of the products.

EXPERIMENTAL

Materials. Feed oils, naphtha distillates of Battle River and Wandoan coal liquids, were supplied by a 1 t/d plant of Sumitomo Metal Ind. Ltd. The boiling range of each sample is IBP-185 °C. The distribution of acidic compounds in the feed oils is summarized in Table 1. Methanol (99.6 % pure) and dichloromethane (99.0 % pure) were from Wako Pure Chemical Ind. Ltd. and used without purification.

Procedure for solvent extraction. Scheme 1 shows the procedure of the present separation. To a mixture of a feed oil (5.0 mL) and methanol (5-20 mL) was added water (5-40 mL) at 30 °C. The resulting mixture immediately separated into two layers of a methanol-water layer and an oil layer. Phenolic compounds in a feed oil were extracted into the methanol-water layer. After 10 min, the methanol-water layer was taken out and evaporated to remove methanol. The aqueous phase was extracted with dichloromethane (30 mL x 3). The extracts were dried over sodium sulfate. Filtration and removal of the solvent gave a mixture of phenolic compounds as a brown oil. The compound distribution and selectivities of phenolic compounds in the products were determined with a gas chromatograph using a Shimadzu 50-m HR-101 (corresponding to OV-101) capillary column and a Shimadzu FAP-S 3.1-m x 3 mm packed column.

RESULTS AND DISCUSSION

Effect of solvents on the percent extraction

Methanol. When the feed oil was treated with water, only 20 % of phenolic compounds were extracted. However, extraction of a mixture of the feed oil (5 mL of a Battle River naphtha) and methanol (5-20 mL) with water (5 mL) gave better results in the extraction percents as shown in Fig. 1. The percent extraction was determined by the comparison of phenolic compounds separated

as a mixture with those contained in the feed oil. The yields increased significantly with the amount of methanol. There must be an appreciable interaction between phenolic compounds and methanol in the mixture of the feed oil and methanol. Addition of water caused phase separation to give an oil layer and a methanol-water layer rich in phenolic compounds. The methanol-water layer contained larger amounts of phenolic compounds with the increase of methanol. Removal of methanol and subsequent extraction with dichloromethane of the resulting aqueous solution gave a mixture of phenolic compounds as a brown oily product. It is noteworthy that the extraction of phenolic compounds from an aqueous solution with dichloromethane gave these compounds in 90 % or above under the present conditions.

Water. Fig. 2 shows the results of the extraction using the feed oil (5 mL), methanol (5 mL), and water in the range of 5 to 40 mL. The percent extraction of phenolic compounds increased with the amount of water. However, the yield was reached to the limiting value of about 40 %. Thus, methanol gave a stronger effect than water on the yields of the phenolic compounds.

Effect of solvents on the compound distribution

The effects of the amounts of the solvents on the distribution of the phenolic compounds in the separated products was examined. The results of the distribution of the representative compounds, phenol and o-cresol were shown in Fig. 3 and 4. These results indicate that the changes of the amounts of methanol and water gave little influence on the distribution. These values of the distribution (about 36 % for phenol and about 10 % for o-cresol) correspond with those of the distribution of acidic components in the feed oil. Similarly, other compounds, m, p-cresol, o, m, and p-ethylphenol were extracted efficiently, corresponding with the distribution of the acidic components as shown in Table 2.

Application

The present method can be applied to the separation of nitrogen compounds in middle distillates of coal liquids (2) and in coal tar (3). Nitrogen compounds such as quinoline and indole can be extracted efficiently.

In summary, the solvent extraction using methanol and water is easy to perform. All solvents can be recovered. The present method provides a new effective method for the industrial separation of phenolic compounds from coal liquids.

References

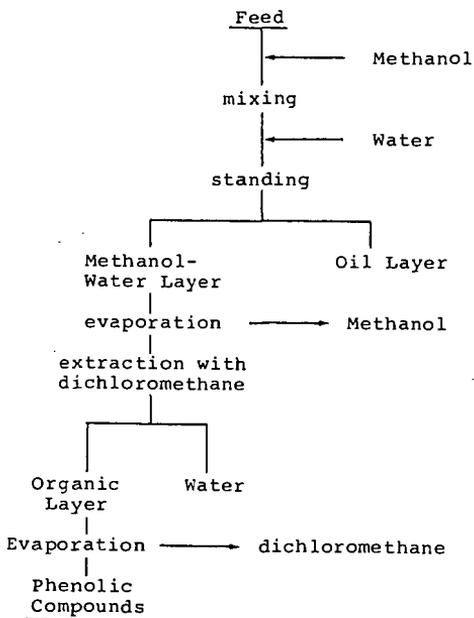
- 1) Y. Kodera, K. Ukegawa, A. Matsumura, T. Kondo, and T. Nakayama, Japanese Patent, Serial No. Toku-gan hei-1-225967 (1989).
- 2) Y. Kodera, K. Ukegawa, and T. Takahashi, 1989 International Conference on Coal Science, Tokyo, Oct. 24, 1989.
- 3) K. Ukegawa, A. Matura, K. Yazu, and T. Kondo, 1989 International Conference on Coal Science, Tokyo, Oct. 24, 1989.

Table 1 Characteristics of naphtha distillates

	Battle River	Wandoan
Acidic components, wt%	22.5	13.8
Compounds distribution of acidic components, wt% a)		
phenol	39.4	36.6
o-cresol	12.5	13.3
m-cresol	19.8	14.2
p-cresol	17.3	15.6
o-ethylphenol	1.2	1.5
m-ethylphenol	3.5	6.0
p-ethylphenol	1.7	3.7
others	4.6	4.1

a) The distribution was determined by GC.

Scheme 1 Procedure for the solvent extraction



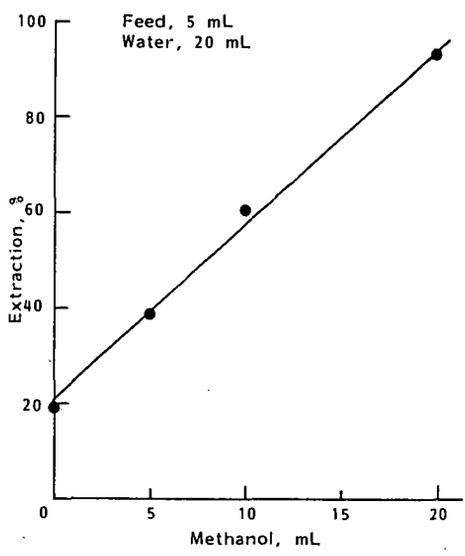


Figure 1 Effect of methanol on the percent extraction

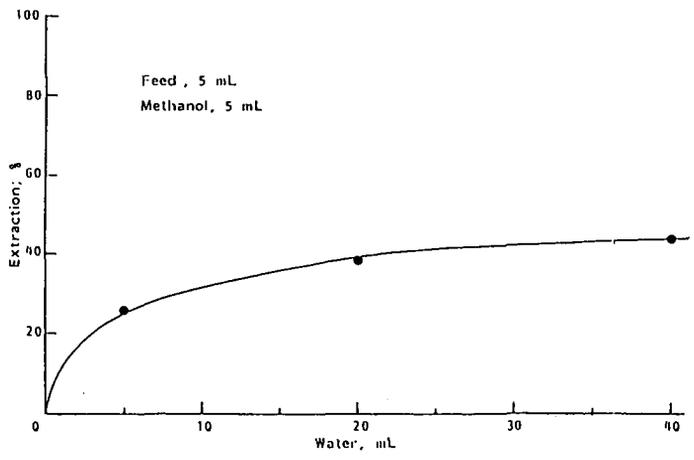


Figure 2 Effect of water on the percent extraction

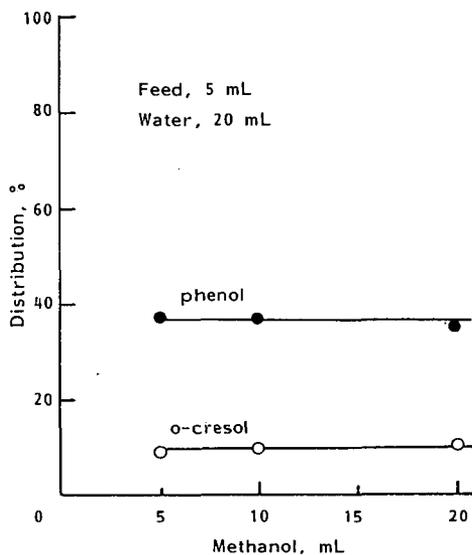


Figure 3 Effect of methanol on the compound distribution

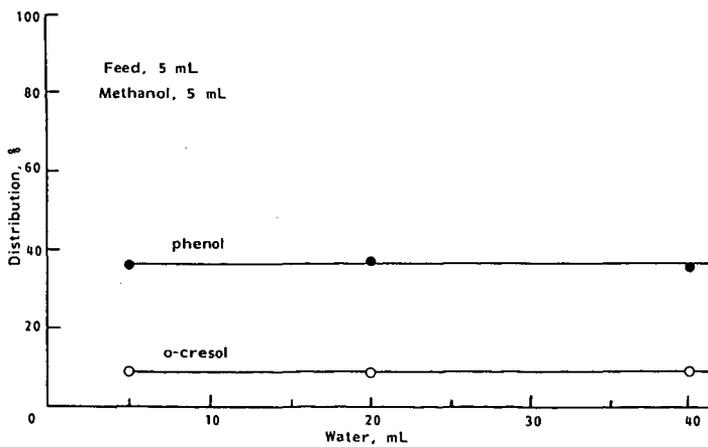


Figure 4 Effect of water on the compound distribution

Table 2 Compound distribution in the separated products

Entry	Methanol mL	Water mL	cresol (m,p)	Distribution, % ethylphenol(o,m,p)
1	5	5	16.9, 12.1 /	- , 2.9, 1.7
2	5	20	15.9, 11.4 /	- , 2.2, 1.2
3	5	40	16.8, 11.8 /	1.0, 2.3, 1.5
4	10	20	17.5, 12.7 /	1.4, 2.9, 1.4
5	20	20	18.3, 12.7 /	1.5, 3.2, 1.2

CORRELATION OF THE pH OF A COAL-METHANOL/WATER SOAK WITH COKE STRENGTH AFTER REACTION WITH CO₂ (CSR)

David H. Buchanan and Kenneth J. Coombs

Chemistry Department
Eastern Illinois University
Charleston, IL 61920

Hardarshan S. Valia

Inland Steel Flat Products Co., Research Laboratories,
East Chicago, IN 46312

Keywords: Coke Strength After Reaction (CSR), Weathering Index, pH Coal Slurry

ABSTRACT

Weathering of coals during storage at coke plants leads to a decrease in coke quality and an increase in operating costs for iron making. Coke Strength After Reaction with CO₂ (CSR) is an important measure of coke quality for blast furnace operation. This study was undertaken to predict changes in CSR values of coke caused by weathering of coals during storage. CSR values of coke were compared with a variety of feed coal properties, including pH of a methanol/water soak. The results indicated that an increase in coal oxidation resulted in a drop in pH of methanol/water soak. CSR generally dropped with a drop in pH for all the coals. However, good correlation existed between CSR and pH for lower rank (high volatile) coals.

INTRODUCTION

At Inland Steel Flat Products Company, the improvement in CSR had a major stabilizing influence on blast furnace operation.(1) The CSR is primarily dependent on the plastic properties of coal which are known to deteriorate with oxidation of coal.(2-4) Hence, a research program was initiated to decipher how coal oxidation affects coke quality, especially CSR, and cokemaking operations. The primary objectives were to develop means for measuring coal oxidation and to learn how to interpret these measurements in ways useful to the coke plant operators.

Although documentation exists detailing the relationship of coal oxidation to coke properties, little information has been published regarding the effect of coal oxidation on hot strength properties of coke. Crelling, et al. (5) correlated coke reactivity to the amount of weathered coal in the mix. The reactivity increased with an increase of weathered coal in the mix; however, the reactivity was measured through the Bethlehem method. Huffman, et al. (6) reported loss in coke reactivity for the most highly weathered Pittsburgh seam (VM = 36.2%, db) coal; the coke reactivity was measured as percent of coke reacted after 2 hours at 1000 °C in CO₂. Pis, et al. (7) reported an increase in coke reactivity with increase in coal oxidation under accelerated oxidation conditions; the reactivity was measured through the ECE

method. Price, et al. (8) indicated a decrease in CSR for a western Canadian coal after storage in barrels for 20 weeks.

Because an appreciable portion of coal used in the coking industry is stored in large piles for various periods of time, it was appropriate to study the deterioration in coal properties due to natural weathering and assess its effect on coke properties, especially CSR, and cokemaking. In this paper, only the statistically significant correlations between CSR and pH of methanol/water soak are discussed. The effect of weathering on other properties of coke and cokemaking operations are discussed more fully elsewhere.(9)

EXPERIMENTAL

Six piles, 3 tons each, of each of the coals that were in use at Inland, were made in the open yard at the Research pilot facility. The coals were Coal A (High Volatile), Coal B (High Volatile), Coal C (High Volatile), and Coal D (Medium Volatile). The analytical data for the fresh coals are given in Table I. Pile No. 1 was the base fresh coal and was subjected to carbonization in Inland's 565 kg movable-wall pilot oven with interior dimensions of 1,143 mm high x 1,219 mm long x 457 mm wide.(10) The operational data summary for the carbonization tests is given elsewhere.(2) Also, a wet charge of 30% Coal A, 30% Coal B, and 40% Coal D was carbonized in the pilot oven for coals from the respective piles. CSR and other coke quality parameters were measured. The CSR was determined through the NSC method. Coke quality data, from the pilot oven carbonization of fresh coals, are also included in Table I. Pile Nos. 2 to 6 were carbonized after 35 days, 70 days, 105 days, 180 days, and 420 days of natural oxidation, respectively. The coals from each pile were subjected to the following analyses: rheological, proximate, ultimate, alkali solubility, petrography, pH (methanol/water soak), FTIR-PAS, and sole-heated oven (SHO) analysis. For the pH measurements, HPLC grade methanol and Milli-QTM purified water (resistance > 16 M Ω) were used. A 25 mL aliquot of 20% (v/v) methanol/water was pipetted into a 50 mL Erlenmeyer flask containing 2.000g coal. The flask was closed with a rubber septum cap fitted with gas inlet and outlet needles and nitrogen gas bubbled through the slurry for 20 minutes. Placing the flask in an ultrasonic bath maintained at 25 Deg.C improved coal wetting. A septum cap with an all-glass pH electrode inserted through a hole was placed on the flask and the pH measured after equilibrating the electrode for 10 minutes in the slurry. The Orion Research Model 710 pH meter was calibrated with buffers prepared in 20% methanol/water at pH 4 and 7.

RESULTS AND DISCUSSION

1) Change in pH with Time

Figure 1 shows plots of pH measurement of methanol/water soak for individual coals; measurements were not made for the blend. For all coals, there is a rapid drop in pH for the first 2-3 months. Beyond this time, the drop is generally insignificant. A drop in pH with oxidation has been reported in literature.(11-13) It is also apparent that the pH of methanol/water soak from fresh Coal A is distinctly acidic (possibly due to the combination of lower rank, higher microporosity, and higher sulfur content) and with oxidation, it becomes more acidic due to the release of sulfur and acids produced from coal oxidation reactions. It is known that lower

rank coals produce more acidic products during weathering.(13) The pH of methanol/water soak from the fresh Coal D, Coal C, and Coal B is distinctly basic, and the pH value drops with an increase in oxidation. Thus, both the absolute value and the change in pH are coal dependent.

The reproducibility of the pH measurements on triplicate samples was = 0.07 units. Because particle size has an significant effect on the pH of the slurry it is important to standardize the grinding of coal samples for these measurements for sample to sample consistency. Data in Table 2 are for a -8 mesh, weathered Illinois No. 6 coal (River King Mine) which was ground in a nitrogen-flushed ball mill and separated into size fractions by sieving. The pH of the larger particles is lower than that of the smaller particles. A sample of the same River King coal which had been ground to -100 mesh prior to weathering had a pH of 5.02 compared to the pH of 5.36 found for the -100 mesh fraction of the coal weathered as larger particles. These results indicate that the surfaces exposed in grinding had been protected from oxidation and had developed fewer acidic groups than surfaces on the un-ground larger particles. The observed differences in pH values of the unweathered coals may be a function of the mineral matter composition of each coal.

2) Change in CSR with Time

Figure 2 shows variation in CSR with weathering time for all individual coals and blends.(9) CSR in all cases decreases with an increase in weathering time. The drop in CSR was most dramatic during the first few months of summer exposure; thereafter, the CSR generally decreased with time or there was little change in CSR.

Using the highest and lowest CSR values, and not the trend lines, it can be deduced that the magnitude of CSR drop is highest for the lowest rank Coal A, followed by the blend, Coal B, Coal D, and Coal C. The CSR dropped by about 24 points for Coal A, 19 points for the blend, 13 points for Coal B and D, and 8 points for Coal C. The large drop in CSR for the blend may be due to a combination of higher amount of Coal A and higher amount of oxyvitrite from Coal A, Coal C, and Coal D. It is interesting to note that Coal D, a medium volatile rank, undergoes oxidation-induced loss in CSR by the same amount as the high volatile Coal B. The Coal C is least susceptible to weathering-induced CSR loss.

3) Correlation of Change in pH to CSR

The changes observed in this study were for a small 3-ton pile. Different natural conditions exist in large coal piles, hence, the time period may not be applicable when the results are applied to the large commercial piles. Hence, it is important to come up with a coal oxidation monitoring device that directly relates to coke properties and can be monitored constantly in the pile. With this premise, the changes in coal quality were correlated to coke quality. The increase in coal weathering resulted in deterioration in CSR and was accompanied by a drop in pH of methanol/water soak.

Figure 3 shows the correlation between the pH of coal-methanol/water soak and the CSR for all the coals. CSR generally drops with a drop in pH for all the coals. However, good correlation exists between CSR and pH for Coal A and Coal B (the lower rank high volatile coals). It was reported elsewhere that for Coal C and Coal

D (the borderline high volatile/medium volatile coal, and medium volatile coal) the fluid temperature range, (as determined through Gieseler plastometer) correlated well with CSR.(9) This relationship can be used to predict CSR of lower rank (high volatile) coals by monitoring pH.

APPLICATION

On the basis of results from this study, a coal oxidation monitoring plan for the lower rank (high volatile) coal has been devised as follows:

- 1) Obtain the pH of the incoming coals from the respective mines.
- 2) Identify the placement of incoming coals in the coke plant yard.
- 3) Monitor the drop in the pH of coal in the coal piles.
- 4) Estimate the loss in CSR by using a set of graphs that depict a drop in pH versus a drop in CSR. Figures 4 shows one such example.
- 5) Once the coal oxidation has affected CSR in such a way that the target CSR is not met, as indicated through the drop in pH, then the usage of oxidized coal in the blend should be redefined.
- 6) If new high volatile coals are brought in, the graphs of a drop in pH versus CSR could be developed while monitoring the new coal pile and verifying the results through pilot oven carbonization.

CONCLUSIONS

On the basis of this study, the following conclusions can be made:

- 1) An increase in coal weathering resulted in a drop in pH of methanol/water soak.
- 2) An increase in coal weathering resulted in a drop in CSR and the drop in CSR can be correlated to a drop in pH of coal methanol/water soak.
- 3) pH measurement can be used as a quality control tool for monitoring weathering of low rank (high volatile) coals that are characterized by low fluid properties.

ACKNOWLEDGEMENT

Partial support of this work by the Council on Faculty Research at Eastern Illinois University is gratefully acknowledged. H.S.V. would also like to acknowledge the assistance of several colleagues at the Inland Steel Research Laboratory for their assistance with various aspects of this work.

REFERENCES

- 1) Valia, H.S., et al., "Production and Use of High CSR Coke at Inland Steel Company," ISS-AIME Proc., Vol 48, 1989, pp. 133-146.

- 2) Valia, H.S., "Prediction of Coke Strength After Reaction with CO₂," Iron and Steelmaker, May 1989, pp. 77-87; also to be published in Iron & Steel Soc. Trans., Vol 11, 1990.
- 3) Schmidt, L.D., "Changes in Coal During Storage," in "Chemistry of Coal Utilization," Lowery, H.H., (Ed), Vol. I, 1945, John Wiley & Sons, N.Y., pp. 627-676.
- 4) Gray, R.J. and Lowenhaupt, D.E., "Aging and Weathering," in "Sample, Selection, Aging, and Reactivity of Coal," Klein, R. and Wellek, R. (ED.), 1989, John Wiley & Sons, N.Y., pp. 255-334.
- 5) Crelling, J.C., et al., "Effects of Weathered Coal on Coking Properties and Coke Quality," Fuel, Vol. 58, 1979, pp. 542-546.
- 6) Huffman, G.P., et al., "Comparative Sensitivity of Various Analytical Techniques to the Low Temperature Oxidation of Coal," Fuel, 1985, Vol. 64, pp. 849-856.
- 7) Pis, J.J., et al., "Effect of Aerial Oxidation of Coking Coals on the Technological Properties of the Resulting Cokes," Fuel Processing Technology, 1988, Vol. 20, pp. 307-316.
- 8) Price, J.T., et al., "Effect of the Properties of Western Canadian Coals on Their Coking Behavior," ISS-AIME Proc., 1988, Vol. 47, pp. 39-55.
- 9) Valia, H.S., "Effects of Coal Oxidation on Cokemaking," ISS-AIME Proc., 1990, Vol. 49.
- 10) Kaegi, D.D. and Osterman, C.A., "The Use of Illinois Coal for the Production of High Quality Coke," ISS-AIME Proc., 1980, Vol. 39, pp. 239-248.
- 11) Gray, R.J., et al., "Detection of Oxidized Coal and the Effect of Oxidation on the Technological Properties," SME-AIME Trans., Vol. 260, 1976, pp. 334-341.
- 12) Mikula, R.J. and Mikhail, M.W., "A Delta P Technique for the Prediction and Monitoring of Coal Oxidation," Coal Preparation, 1987, Vol. 5, pp. 57-69.
- 13) Yun, Y., et al., "Attempted Development of a 'Weathering Index' for Argonne PCSP Coals," Am. Chem. Soc. Div. Fuel Chem. Preprints, 1987, Vol. 32, No. 4, pp. 301-308.

Table 1. Analyses of coals and their cokes

	Coal A	Coal B	Coal C	Coal D	Blend 30%A 30%C 40%D
Petrographic Analysis					
Total Inerts (%)	7.61	16.78	20.62	20.29	14.55
Oxytrinite (%)	0.0	0.0	0.60	0.20	0.20
Pseudovitrinite (%)	0.60	0.40	0.20	0.60	0.40
Mean Max. Vitrinite Reflectance (%)	0.67	0.90	1.07	1.35	1.04
Alkali Extraction (% Transm.)	97.0	96.0	99.0	97.0	98.0
Proximate Analysis (% db)					
Volatile Matter	35.7	31.2	27.1	23.2	27.7
Fixed Carbon	58.7	62.3	67.0	71.7	66.9
Ash	5.6	6.5	5.9	5.1	5.4
Sulfur	1.11	0.56	0.82	0.8	0.87
ABall Index	1.98	0.81	1.82	1.89	1.56
Free Swelling Index	3.0	7.5	8.0	7.5	5.0
Ultimate Analysis (% db)					
Carbon	76.64	79.07	81.90	83.94	81.46
Hydrogen	5.09	4.96	5.01	4.86	5.03
Nitrogen	1.74	1.52	1.67	1.55	1.64
Oxygen (by difference)	9.83	6.88	5.00	3.13	5.43
Gieseler Max Fluidity (log dppm)	1.25	2.78	4.43	3.50	3.03
Gieseler Fluid Range (Deg.C)	66.0	71.0	116.0	106.0	106
pH (methanol/water)	4.12	8.50	6.99	7.57	NA
Area under the absorbance peak, carbonyl band (1850-1638 cm ⁻¹), arbitrary unit	20.7	21.03	17.74	16.83	NA
Sole-Heated Oven Analysis (at normalized conditions 52 pct, 2% moisture)	+1.0	-8.0	-5.4	+0.3	NA
Heating Value (cal/gm)	7618	7874	8140	8084	8084
Pilot Oven Carbonization Test					
Coal Moisture (% db)	8.5	2.9	1.7	1.9	3.7
Coal Grind (% -3.35 mm)	82.2	86.5	89.1	91.5	85.6
Dry Oven Bulk Density (kg/m ³)	801.0	885.0	894.0	866.0	906.0
Max. Oven Wall Pressure (kPa)	3.7	6.7	12.9	16.3	5.9
Coking Time (h)18.4	18.4	17.3	16.2	17.1	17.0
ASTM Stability	23.0	50.0	55.0	65.0	63.0
NSC Coke CSR	36.0	62.0	69.0	66.0	60.0
NSC Coke CRI	57.0	24.0	22.0	27.0	31.0
Coke Hardness	71.0	67.0	68.0	71.0	72.0
Coke Size (mm)	50.7	81.5	72.7	69.6	66.8
Coke Yield (%)	67.9	69.2	76.2	79.3	72.4
Coke Volatile Matter (%)	2.2	0.9	0.9	0.9	0.6

TABLE 2 pH of Size Fractions of Ground +8 mesh Illinois No.6 Coal

Mesh size	-8 + 24	-24 + 100	-100
Wt% of total	47.6	35.4	17.0
pH	2.88	3.00	5.36

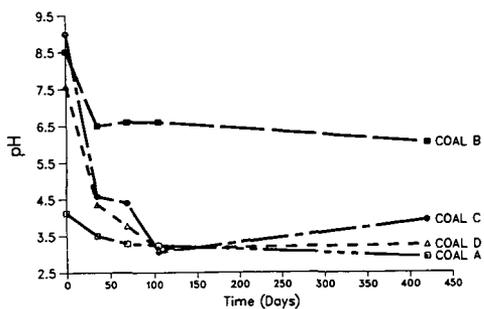


Fig. 1 pH (methanol/water) as a function of coal oxidation time

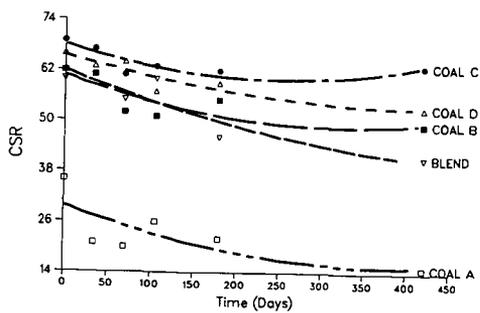


Fig. 2 Effect of coal oxidation time on CSR

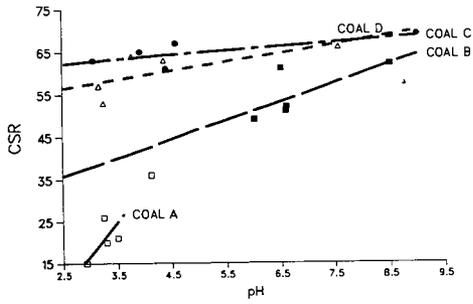


Fig. 3 Oxidation effects indicated through pH (methanol/water) and CSR

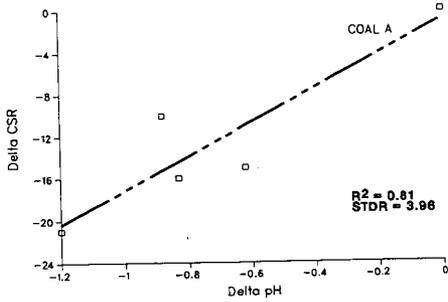


Fig. 4 Use of pH (methanol/water) for assessing CSR loss

EFFECT OF ELECTROREDUCTION PRETREATMENT IN AQUEOUS MEDIA
ON HYDROPYROLYSIS OF A BITUMINOUS COAL

Li Baoqing

Service de Chimie Générale et Carbochimie, Université Libre de
Bruxelles, Av. F.D. Roosevelt, 50(CP 165), B-1050, Bruxelles, Belgium

Keywords: electrochemical reduction, hydropyrolysis, pretreatment

Abstract

Electroreduction of coal (ER) is studied in aqueous tetrabutylammonium hydroxide (TBAOH) solution and tetrabutylammonium tetrafluoroborate (TBABF₄)-THF-H₂O solution by using platinum as anode and Hg pool as cathode. The electrolysis in aqueous TBAOH solution seems to have little effect on the following hydropyrolysis (HyPy) although the pyridine extractable yield of the reduced coal increases. In an undivided cell the re-oxidation of the reduced coal occurs. Cathodic reduction in TBABF₄-THF-H₂O solution gives a pronounced effect on HyPy. Higher conversion (60.9%) and total oil yield (50.6%) (THF soluble + oil formed in HyPy) are obtained in a divided cell as compared with 42.1% and 28.4% for untreated coal, respectively. The electroreduction pretreatment of coal enhances the rate of oil formation and oil yield in HyPy, indicating that the reduced coal can be depolymerized more easily due to the solvolysis and hydrogenation.

Introduction

The conversion of coal to oil needs undoubtedly to increase the H/C ratio in coal. Therefore, gaseous hydrogen is always used in coal liquefaction, HyPy and other hydrogenation processes. Considering the high activation energy for the dissociation of gaseous hydrogen, the addition of hydrogen to coal using gaseous hydrogen is so difficult that the elevated pressure has to be used. As a result, the high cost makes these processes hardly competitive with those using petroleum as raw materials. To reduce the hydrogen consumption and even to avoid the use of gaseous hydrogen and/or elevated pressure are very interesting in coal hydrogenation processes.

Previous studies¹⁻³ showed the possibility to improve the quantity and quality of oil and the efficiency of hydrogen utilization in HyPy by pretreatment of coal using catalyst and various gases. Although these pretreatments are effective, the improvements are still not optimistic even the use of impregnated sulphided Mo catalyst because of the cost of catalyst and an increasing sulphur content in char.

Earlier studies^{4,5} indicated that hydrogen can be added to coal by electroreduction using LiCl as electrolyte in an organic solvent. Recently, It is reported⁶ that hydrogenation of coal can be carried out by electroreduction in an aqueous solution. They used a mediator (CrCl₃)/Ni powder/LiCl/aqueous THF solution/ultrasonic irradiation system, which is too complicated to be performed practically. It is known⁷⁻⁹ that the effective electroreduction of benzene and related compounds can be performed in tetraalkylammonium aqueous solution. Reduction in water is economically attractive because of the cost and of the high conductivity of the medium. Kariv-Miller⁷ has mentioned that a practical application of this method could be in hydrogenation processes which are important for coal conversion. However, the studies are still limited to the model compounds.

This research is aimed to investigate the cathodic reduction of coal in aqueous solution using TBAOH and TBABF₄ as electrolyte. The effects of various parameters on electroreduction of coal are studied in TBAOH solution system, while the cathodic reduction in TBABF₄-THF-H₂O solution is investigated as comparison.

Experimental

A cylindrical cell, 15 cm high, with a hot water jacket and a 44,18 cm² mercury pool cathode at the bottom is used as a electrolysis cell. With a fritted glass-cup dipped in the solution as an anode potential it is a divided cell. A coiled platinum wire is used as anode (7,85 cm²). The reference electrode is a calomel electrode. The cell is fitted a thermometer, a gas exit with a reflux condenser and a gas inlet for a nitrogen flow during the electrolysis. The cell is filled with 45 ml Hg, 150 ml electrolyte solution and 7,5 g coal (as received). The stirring is carried out during electrolysis by means of a magnetic bar placed on the mercury surface.

Mercury is purified before use. All the electrolyses are performed by using constant current. During electrolysis the cathode potential and the cell voltage are recorded. Gas formed during electrolysis is totally collected in a tyre. Hydrogen in gas is analysed by G.C. and oxygen in gas is determined by paramagnetic Magnos 3 analyser.

After electrolysis mercury is separated and the treated coal is filtered out. When TBAOH is used as electrolyte, the treated coal is washed with water, 10% HCl and again with water to neutral state. In the case of as electrolyte, the treated coal is washed by THF, dried and then extracted with THF in a Soxhlet for 6 hr. The washed coal is dried at reduced pressure for one night. Although more or less grey 'TBA-Hg' compound which is difficult to be separated from the reduced coal, it can be evaporated and/or decomposed before 600 K, i.e. before coal thermal decomposition occurs. Thus, the content of 'TBA-Hg' in the reduced coal can be estimated by thermogravimetric study.

The effect of electroreduction pretreatment on HyPy is studied in a thermobalance under 3 MPa and heating rate of 5 K/min upto 1073 K. The oil yield is determined by carbon balance. Pyridine extraction is carried out in a Soxhlet with a sample of 1 g under nitrogen for 24 hr. The residue is dried at 60 °C and reduced pressure for 6 hr.

A bituminous Beringen Belgian coal with a granulometry of 45-90 μm is used in this study. Its characteristics are: proximate analysis (wt%, as received): moisture, 1.5; ash, 4.7; volatile matter, 34.5; ultimate analysis (wt%, daf): C, 84.7; H, 4.9; N, 1.7; S, 0.9; O (by difference), 7.8.

Results and Discussion

1. Electroreduction in TBAOH Aqueous Solution

The effects of various parameters on pyridine extractable yield, current efficiency for H₂ production and oil yield in HyPy of the reduced coal are listed in Table 1.

The electrolyte concentration remarkably influences the electro-chemical reactions. When TBAOH concentration decreases from 40% to 30%,

pyridine extractable yield is reduced from 17.0% to 7.6%. With increasing concentration, the current efficiency for hydrogen production decreases from 68.7% with 30% TBAOH solution to 46.6% with 55% TBAOH solution.

The influence of current density ranging from 1 to 8 A/dm² is shown in Table 1. The higher pyridine extractable yield is given with a current density of 4 A/dm² in a 40% TBAOH solution. Similar results were obtained by other authors in the study on effect of current density on current efficiency in electroreduction of benzene in TBAOH solution⁷.

It is suggested that the first step for the electroreduction in TBAOH solution is the formation of TBA-metal which could transfer an electron to the aromatic hydrocarbon to initiate the reduction^{7,11}. The formation of colloidal Hg on the cathode surface is visually apparent during electrolysis. The slow formation of the colloidal Hg might be related to the current density and time.

It is also found that CO₂ yield obtained in HyPy enhances with increasing current densities. Re-oxidation of the reduced coal appears to occur, which will be discussed later.

Figures 1 and 2 show the influence of current density on conversion and oil yield formed in HyPy of the electroreduced coal. About 4% higher conversion and 3% more oil yield are obtained from the coal reduced in a 55% TBAOH solution than from the unreduced coal.

With increasing charge the current efficiency for H₂ production increases, while the pyridine extractables decreases at 0.088 F/g(daf) of charge transferred.

Table 2 shows the comparison of elemental analysis between raw coal and reduced coal. After electrolysis the H/C atomic ratio slightly increases from 0.69 to 0.70, while the O/C ratio enhances from 0.07 to 0.10. 1 hydrogen and 3 oxygen atoms per 100 carbon atoms are added to coal after electrolysis in these conditions, indicating the re-oxidation of cathodic reduced coal in an undivided cell.

Table 1 also compares the results obtained by electrolysis of coal at 60 °C and 80 °C using a 55 % TBAOH solution in an undivided cell. An increase in temperature decreases the current efficiency for the production of hydrogen. The little higher pyridine extractable yield and less current efficiency for hydrogen production obtained in a 40% TBAOH solution at 80°C also demonstrates the positive effect of the temperature on the reduction. However, the oil yields formed in HyPy of the coal reduced at 60 °C and 80 °C are the same.

The comparison of electrolysis in divided and undivided cell shows that lower pyridine extractable yield and higher current efficiency for hydrogen production are obtained in electrolysis with the divided cell. These might result from the re-oxidation in undivided cell as described before.

The comparison of elemental analysis of reduced coal in divided and undivided cells are also given in Table 2. The O/C ratio of the reduced coal in the divided cell is unchanged as compared to the raw coal, while it increases in the undivided cell. This further indicates the re-oxidation of the reduced coal in the undivided cell. The comparison of CO₂ yield (Figure 3) formed in HyPy of coal reduced in the same

conditions gives another prove. The higher CO₂ yield is produced in HyPy of the coal pretreated in the undivided cell.

The oil yield obtained in HyPy of the reduced coal in various conditions is 2-4% higher than that of the raw coal. The electrolysis in aqueous TBAOH solution seems to have little effect on the following HyPy although the pyridine extractable yield increase. Kariv-Miller et al.⁸ concluded that the solubilization of the reactant in water by TBAOH could be an important factor. Coal can be easily dispersed but is hardly dissolved in a TBAOH solution (See Table 1). The less efficiency in improving the oil yield may be related to the low solubility of coal and its reduction products in TBAOH solution⁸. Therefore, the electro-reduction of coal in a TBAOH solution is not sensible to the various parameters and little or not efficient for subsequent HyPy.

2. Electroreduction in a TBABF₄-THF-H₂O Solution

Electroreduction of coal in 0.5 M TBABF₄-THF-H₂O (5 M) solution is performed at 33 °C, a current density of 0.6 A/dm² and a charge amount of 0.16 F in divided and undivided cells. The results are shown in Table 3. As a comparison, the results obtained in 55% TBAOH solution and catalytic HyPy using impregnated sulphided Mo catalyst are also listed.

The THF soluble yield for raw coal is 4.5%, indicating the solubility of coal in THF. After electrolysis in the divided cell the THF solubles markedly increase to 21.7%, showing the notable depolymerization of coal during electrolysis. The less THF solubles obtained in electrolysis in the undivided cell (12.4%) demonstrates that electro-reduction in the divided cell is much more effective than in the undivided cell. This may be due to the re-oxidation of reduced coal in the undivided cell as described in electrolysis in TBAOH solution.

The dramatic increase in THF solubles also indicates that the electron and proton produced in electrolysis can attack to not only the weak bonds but also macromolecular structure in coal, leading to the depolymerization of coal. The products thus formed can be dissolved in THF and be further reduced in solution. Due to the remove of the products by disolvation, more surface are exposed to electron and proton and thus, more THF solubles are produced. This may explain the importance of solubility of products.

The conversion and oil yield produced in HyPy of reduced coal are shown in Table 3 and Figures 4 and 5. Cathodic reduction in TBABF₄-THF-H₂O solution gives a pronounced effect on HyPy. Higher conversion (60.9%) and total oil yield (50.6%) are obtained in a divided cell as compared with 42.1% and 28.4% for unreduced coal respectively. The cathodic reduction of coal is found to be a better method for coal pretreatment even compared to catalytic HyPy using impregnated sulphided Mo catalyst (conversion: 47.7%; oil yield: 38.4%). That the conversion and oil yield for reduced coal in divided cell are much higher than in undivided cell also proves the extensive hydrogenation during electroreduction in divided cell. The conversion and oil yield in HyPy of the solvated coal are less than raw coal because some soluble substances which will be converted to oil are already removed during solution.

The elemental analysis of reduced coal is also shown in Table 2. Although the H/C ratio in the reduced coal is lower than that in raw coal, it is higher than in the solvated coal. The low value of H/C in

solvated coal indicates the THF solubles with high H/C value. Considered the THF solubles is much higher in electroreduced coal than in solvated coal, a considerable of hydrogen does add to electroreduced coal.

Figure 6 gives the rate of oil formation in HyPy. The reduced coal increases the rate of oil formation in HyPy. The enhances for the reduced coal in divided cell are higher than that in undivided cell. Like catalytic HyPy using impregnated sulphided Mo catalyst³, the remarkable increase in the rate of oil formation appears in the pyrolytic and hydrogenation stages (< 800 K), while the second peak (> 800 K) formed by hydrocracking reactions is dissappeared. This indicates that the added hydrogen to coal in electroreduction enhances the amount of the intrinsic hydrogen which can saturate the free radicals at low temperature. When more free radicals are stabilized by hydrogenation, less polycondensation reactions take place and thereby less hydrocracking reactions occur at high temperatures, leading to the disappearance of the second peak in the rate of oil formation. The similar behaviours between catalyst-impregnated coal and electroreduced coal in HyPy show that hydrogenation reactions can be accelerated by using either catalyst or electrolysis. However, the functions of both processes are different. Electroreduction adds hydrogen into coal, while the promotion of hydrogenation by catalyst is related to the ability of adsorption and dissociation of gaseous hydrogen.

The problems for electrochemical reduction of coal in TBABF₄-THF-H₂O are the use of Hg and divided cell and the separation of TBABF₄ from THF solubles and reduced coal. However, electrochemical reduction of coal is an attractive method for pretreatment in hydrogenation processes of coal because of high conversion and oil yield and is worth investigating further for practical use.

References

1. Cyprès, R. and Li Baoqing, Fuel Proc. Technol., 20, 337 (1988).
2. Li Baoqing, ACS Div. Fuel Cham., preprint, 34(4), 1085 (1989).
3. Li Baoqing, Braekman-Danheux, C. and Cyprès, R., submitted to present in 'Coal Structure and Reactivity Conference', Cambridge, UK, Sep. 1990.
4. Given, P. H. and Peover, M. E., Fuel, 39, 463 (1960).
5. Sternberg, P. H., Delle Donne, C. L., Markby, R. E. and Wender, I., Fuel, 45, 469 (1966).
6. Miyaka, M., Hamaguchi, M. and Nomura, M., Energy Fuel, 3(4), 362 (1989).
7. Coleman, J. P. and Wagenknecht, J. H., Electrochem. Sci. Technol., 128(2), 322 (1981).
8. Kariv-Miller, E., Swenson, K. E. and Zenmach, D., J. Org. Chem., 48, 4210 (1983).
9. Kariv-Miller, E., Swenson, K. E., Lehman, G. K. and Andruzzi, R., J. Org. Chem., 50, 556 (1985).
10. Kariv-Miller, E. and Pacut, R. I., Tetrahedron, 42(8), 2185 (1986).
11. Svetlicic, V. and Kariv-Miller, E., J. Electroanal. Cham., 209, 91 (1986).

TABLE 1 EFFECT OF SOME PARAMETERS IN ELECTROREDUCTION OF COAL USING TBAOH SOLUTION ON PYRIDINE EXTRACTABLES, CURRENT EFFICIENCY FOR HYDROGEN AND OIL YIELD IN HyPy UNDER 3 MPa, 5 K/min, 913 K

Type	T °C	I A/dm ²	Q F/g(daf)	TBAOH C. wt%	Ex.Yield wt%,daf	Effi.for H ₂ %	Oil Yield wt%,daf
--	--	--	--	--	11,8	--	28.4
--	60	--	--	40	11,8	--	/
Undiv.	60	2	0.022	40	13,7	52,5	/
	60	4	0.022	40	17,0	52,9	29.3
	60	6	0.022	40	14,0	31,9	/
	60	4	0.022	30	7,6	68,7	/
	60	4	0.044	40	16,4	58,4	/
	60	4	0.088	40	4,2	69,9	/
	80	8	0.022	40	18,4	41,5	/
	60	4	0.022	55	N.D.*	46,6	31.2
	60	2	0.022	55	N.D.	27,8	32.6
	60	1	0.022	55	N.D.	27,3	33.8
	80	4	0.022	55	N.D.	27,2	32.3
	Divided	60	4	0.022	40	14,9	52,9
60		4	0.022	55	N.D.	68,4	31,3
60		2	0.022	40	9,7	85,9	/

* Not Determined. After electrolysis coal seems to be a mixture with TBAOH and mercury.

TABLE 2 ELEMENTAL ANALYSIS OF ELECTROREDUCED COAL

wt%, daf	Raw Coal	TBAOH*		TBABF ₄ ^	
		Undiv. Cell	Divided Cell	Solcated Coal	ER-Coal
C	84.7	81.9	84.6	84.0	85.8
H	4.9	4.8	4.9	4.1	4.4
N	1.7	1.7	2.0	1.7	1.9
S	0.9	0.9	0.9	N.D.*	N.D.
O(by difference)	7.8	10.7	7.5	10.2*	7.9*
H/C	0.69	0.70	0.70	0.59	0.62
O/C	0.07	0.10	0.07	0.08	0.06

* Electrolysis in 40% TBAOH, 60 °C, 4 A/dm², 0.022 F/g(daf)

^ Electrolysis in TBABF₄ -THF-H₂O, 0.6 A/dm², 33°C, 0.022 F/g(daf), Divided Cell

* Not Determined

• O+S: by difference

^ Assume 0.9% of sulphur content

TABLE 3 COMPARISON OF ELECTROREDUCTION PRETREATMENT AND CATALYTIC HyPy
HyPy: 3 MPa, 5 K/min, 913 K

Type	THF Soluble (wt% daf)	Oil Yield in HyPy (wt% daf)		Total Oil Yield (wt% daf)	Conversion (wt% daf)	
		as Treated	as Raw Coal		as Treated Coal	as Raw Coal
Raw Coal		/	28.4	28.4	/	42.1
Electrolysis						
TBABF ₄ Solvated	4.5	27.9	26.6	31.1	41.6	44.2
Undiv. Cell*	12.4	30.3	26.5	38.9	44.2	51.1
Divided Cell*	21.7	36.9	28.9	50.6	50.0	60.9
TBAOH [^]						
Undiv. Cell		/	31.2	30.1	/	43.3
Divided Cell		/	31.3	31.3	/	43.8
Catalytic HyPy*		/	38.4	38.4	/	47.7

* 0.5 M TBABF₄ - THF-H₂O (5 M), 0.6 A/dm², 0.022 F/g(daf), 33 °C

[^] 55% TBAOH in H₂O, 4 A/dm², 0.022 F/g(daf), 60 °C

* Impregnated MoS₂ (0.5% Mo), 3 MPa, 5 K/min, 873 K

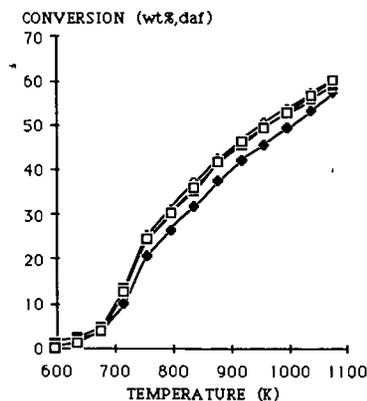


Fig. 1 Effect of Current Density in ER Pretreatment on Conversion in HyPy under 3 MPa, 5 K/min. ER: 40% TBAOH, 60 °C, 0.022 F/g(daf), Undiv. Cell.
● Raw Coal; ○ 1 A/dm²; ■ 2 A/dm²; □ 4 A/dm²

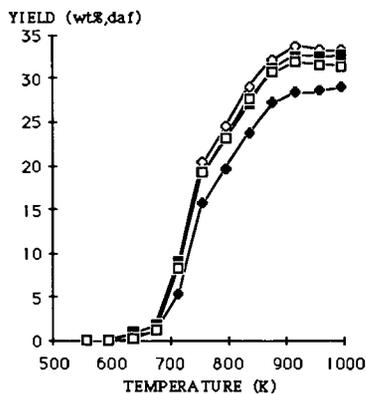


Fig. 2 Effect of Current Density in ER Pretreatment on Oil Yield in HyPy under 3 MPa, 5 K/min. ER: 40% TBAOH, 60 °C, 0.022 F/g(daf), Undiv. Cell.
● Raw Coal; ○ 1 A/dm²; ■ 2 A/dm²; □ 4 A/dm²

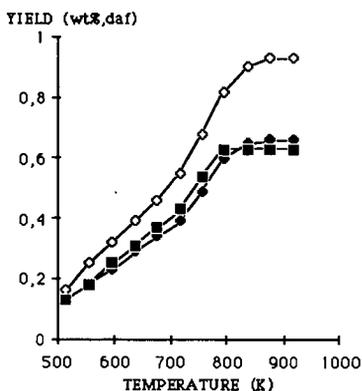


Fig. 3 Effect of Cell Type in ER Pretreatment on CO₂ yield in HyPy under 3 MPa, 5 K/min. ER: 40% TBAOH, 4 A/dm², 60 °C, 0.022 F/g(daf). ● Raw Coal; ○ ER-Undiv. Cell; ■ ER-Divided Cell.

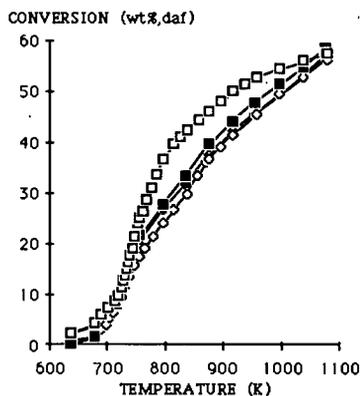


Fig. 4 Effect of ER Pretreatment on Conversion under 3 MPa, 5 K/min. ER: TBABF₄-THF-H₂O, 33 °C, 0.6 A/dm², 0.022 F/g(daf). ● Raw Coal; ○ Solvated; ■ ER-Undiv. Cell; □ ER-Divided Cell.

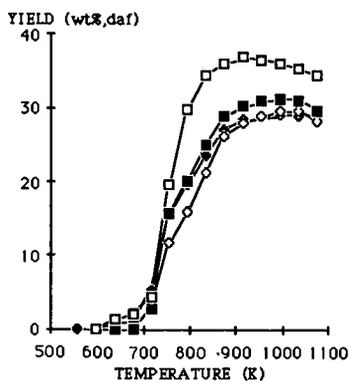


Fig. 5 Effect of ER Pretreatment on Oil Yield in HyPy under 3 MPa, 5 K/min. ER: 0.6 A/dm², TBABF₄-THF-H₂O, 33 °C, 0.022 F/g(daf). ● Raw Coal; ○ Solvated; ■ ER-Undiv. Cell; □ ER-Divided Cell.

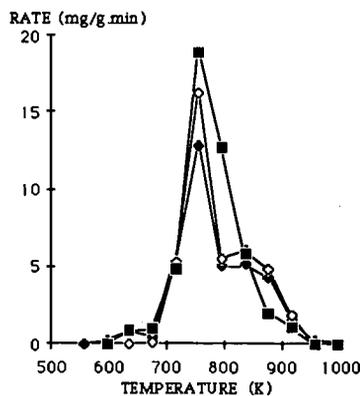


Fig. 6 Effect of ER Pretreatment on Rate of Oil Formation in HyPy under 3 MPa, 5 K/min. ER: TBABF₄-THF-H₂O, 0.6 A/dm², 33 °C, 0.022 F/g(daf). ● Raw Coal; ○ ER-Undiv. Cell; ■ ER-Divided Cell.