

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.