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Biomodification of Coal to Remove Mercury*

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Abstract

A biological process for removal of mercury from coal is under investigation. Iron and sulfur oxidizing bacteria have previously been used for desulfurization of coal and for mineral mining. We have shown that removal of mercury from coal is also possible via the same principles. Two pure cultures, *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans* and four environmental consortium samples obtained from an acid mine drainage site were studied for mercury removal from coal. Four different coal samples were included in the study and the preliminary results have shown that up to 20% of the mercury can be removed in batch cultures compared to control. Additional parameters such as media composition and inoculum size were also studied. This is the first report demonstrating successful leaching of mercury from coal using biological treatment.

Index Entries: Bioleaching; mercury; coal; ferrooxidans.

Introduction

Emissions of mercury from coal-fired burners are in the range of 0.5 to 22 lb/10^{12} Btu and there is a plausible link between emissions and mercury bioaccumulation in the food chain *(1,2)*. Current operations of coal-fired power plants do not require dedicated mercury removal equipment and emissions control in the combustion of coal has traditionally been limited to the removal of mercury from off-gases. The power-generating industry emits about 50 t of mercury each year, about a third of the total manmade emissions *(3)*. In 2003, EPA suggested two approaches to reduce mercury emissions *(4)*. In the first approach, emissions would be reduced from 48 to 34 t/yr by 2007 using existing technology and a second approach to reduce emissions by 70% by 2018. Currently, there is a debate on the amount of mercury emission

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reduction, with stress by regulators to reduce emissions beyond what has been proposed by EPA. Needless to say, new technologies capable of reducing mercury emissions significantly will be needed in near future.

Mercury is naturally present in coal from different world sources and the concentration is typically in the range of 0.02 to 0.4 mg/kg *(5)*. In the United States, coal from the Gulf Coast and Appalachian regions has the highest average concentration of mercury at 0.21–0.22 mg/kg *(6)*. In a comprehensive review by Toole-O'Neil et al. *(6)*, it was concluded that mercury in coal is most likely associated with the sulfur-containing iron compounds such as pyrite; however, a fraction of the mercury may be associated with the organic matter. It is expected that mercury and sulfur are closely associated in the coal as it is well known that mercury sulfide is a low-solubility inorganic salt *(7)*.

Analysis of trace metals in coal is sometimes based on leaching the coal with a dilute nitric acid. In unpublished studies, as much as 75% of the mercury could be removed through nitric acid leaching *(6)* and published results summarizing data from commercial cleaning facilities suggest that 12–78% removal is possible when pyrite is removed from coal via froth floatation *(8)*. Use of a two-step hydrochloric acid wash process has also been demonstrated to leach mercury from coal up to 77% *(9)*.

It is well-known that pyrite in coal can be utilized by members of the bacteria *Acidithiobacillus* (formerly *Thiobacillus*) *ferrooxidans* (*A. ferrooxidans*) *(10–12)*, and others which use both the reduced iron and sulfur in pyrite with the overall reaction

$$4\ FeS_2(s) + 15\ O_2(g) + 2\ H_2O(l) \Rightarrow 2\ Fe_2(SO_4)_3(aq) + 2\ H_2SO_4(aq).$$

This reaction is stepwise beginning with the interaction between the pyrite surface and soluble Fe(III) to liberate elemental sulfur, S(0), and Fe(II). Fe(II) and S(0) are oxidized by the bacteria, yielding the overall reaction above. The reactions are carried out by the bacterium *A. ferrooxidans* or by two bacteria (*A. ferrooxidans* and *A. thiooxidans*) working together. The generation of sulfuric acid in the process lowers the pH and helps with further dissolution of pyrite, and will aid in the dissolution of mercury-sulfur compounds. The optimal pH for iron removal from coal pyrite was determined by Torma and Olsen *(13)* to be pH 2.0 in experiments with *A. ferrooxidans*. This pH is naturally obtained through the release of sulfuric acid by the bacteria.

Another organism with good metal bioleaching capability is *Leptospirillium ferrooxidans (L. ferrooxidans)*. This organism was found to comprise more than 50% population in microbial species habitating biotopes such as mines and surrounding dump sites *(14)* at temperatures above 20°C. Other reports also suggest the dominance of *Leptospirillum* genus in acid mine drainage environments *(15)*. This is a strict chemolithoautrotroph, metabolizing ferrous iron, and pyrite.

Microbial leaching for copper and uranium recovery has been used commercially for low-grade ore *(16,17)*. Other metals including Ni, Cu, and

Table 1
ATCC 2039 Nutrient Medium and Fe(III) Medium for Experiments

Solution A	
$(NH_4)_2SO_4$	0.8 g
$MgSO_4 \cdot 7H_2O$	2 g
K_2HPO_4	0.4 g
Wolfe's mineral solution	5 mL
deionized water	795 mL
Adjust with 20% H_2SO_4 to pH 2.3.	
Solution B (prepared fresh)	
$FeSO_4 \cdot 7H_2O$	20 g
deionized water, pH 2.3 (acidified with H_2SO_4)	200 mL
Solution C (prepared fresh)	
$Fe_2(SO_4)_3 \cdot 1.5H_2O$	0.13 g
deionized water, pH 2.3 (acidified with H_2SO_4)	50 mL
ATCC 2039 [Fe (II) medium]	
Filter sterilize solutions A and B separately and combine 800 mL solution A with 200 mL Solution B.	
Fe (III) medium	
Combine 100 mL of filter-sterilized Solution A with 2 mL of filter sterilized Solution C.	

Pb have also been studied for bioleaching potential using the same organisms. The objective of this study was to investigate the feasibility of developing a biological coal modification technique based on bioleaching that will aid in the removal of mercury from coal before thermal processing.

Materials and Methods

The coals used in this study were obtained from Penn State Coal Bank (University Park, PA) and analyzed for mercury content. The coals obtained were PSOC-275 (Ohio No. 6A, Lower Freeport Seam) (0.31 mg/kg of Hg, slightly lower than published values *[18,19]*), PSOC-1286 (Ohio No. 5, Lower Kittanning Seam) (0.4 mg/kg of Hg), PSOC-1296 (Pennsylvania B, Lower Kittanning Seam) (0.26 mg/kg of Hg), PSOC-1368P (Weir-Pittsburg/Cherokee Seam, Missouri) (0.26 mg/kg of Hg), PSOC-1470 (Pratt Seam, Alabama) (0.4 mg/kg of Hg). These samples represented coals with a high-pyritic sulfur content and possibly mercury concentration based on coal seam data *(6)*. The coal samples were obtained, packaged under an inert atmosphere and were maintained under those conditions until used.

A. ferrooxidans American type culture collection (ATCC) 19859 and *L. ferrooxidans* Markosian ATCC 53993 were obtained from ATCC and grown in 5 mL of ATCC medium 2039 (Table 1) in screw-cap test tubes placed in slanted configuration in a 30°C incubator. In addition to the pure cultures mentioned earlier, a consortia of bacteria was also used for mercury leaching. Acid mine drainage from an abandoned lead and zinc mine (the Valzinco mine in Spotsylvania county, Virginia) was sampled and transferred to the

laboratory. The acid mine drainage from this abandoned mine coats the creek-banks orange (suggestive of iron-oxidizing bacteria) and downstream the acid mine drainage flows past an abandoned gold mine (the Mitchell Mine) in which mercury was used in the recovery of gold (20). Thus, there was a possibility of collecting microorganisms which have been exposed to (or not exposed to) mercury within one watershed. The microbial consortia were labeled VA No. 1, 2, 3, and 4. The VA No. 1 and VA No. 2 samples were collected from an area just downstream from the lead/zinc mine and VA No. 3 and VA No. 4 were collected downstream from the lead/zinc mine and the gold mine. The VA No. 1 and VA No. 2 were similar, except that they were collected from two different points in the same area. The same was the case with Samples VA No. 3 and VA No. 4. These natural environmental cultures were enriched by growing them in the laboratory using ATCC 2039 medium and then transferring them several times to obtain enrichment cultures. Coal biotransformation experiments were conducted using these four environmental enrichments as well.

The cultures were transferred to fresh medium every 4 wk with a 25% inoculum rate and, before an experiment, a larger (125 mL) screw-cap bottle with 20-mL ATCC medium 2039 was inoculated with 1 mL from a 4-wk-old culture.

Cell suspensions used for the studies were prepared by growing the culture in 1 L of ATCC medium 2039 in a stirred (150 rpm) batch tank reactor with pH and temperature (30°C) control. Aeration was provided via sparging with air containing 1–3% CO_2. Growth was usually noted after 24–48 h, as indicated by heavy iron(III) oxide/hydroxide precipitation. After 1 wk of incubation, the broth was centrifuged at 4000*g* for 30 min at 4°C and the supernatant removed via suction. The precipitate/cells were combined with approx 150 mL of sterile 4°C deionized water at pH 2.6 (acidified with 20% H_2SO_4) in a 250-mL media bottle. The contents were shaken vigorously for 2 min and stored at 4°C overnight. On the second day, the supernatant (containing cells) was transferred to a sterile 500-mL media bottle and store at 4°C. The precipitate (in the 250-mL bottle) was again shaken with approx 75 mL of sterile 4°C deionized water at pH 2.6 and stored at 4°C overnight. This precipitate washing protocol was repeated two more times. The supernatants collected were combined and centrifuged at 10,240*g* for 20 min at 4°C and the resulting cell pellet was resuspended with approx 15 mL of sterile 4°C deinonized water at pH 3.5 (acidified with 20% H_2SO_4). The cell suspension was centrifuged at 12,180*g* for 20 min at 4°C and, finally, the cell pellet was resuspended with approx 15 mL of sterile 4°C deinonized water at pH 3.5 (acidified with 20% H_2SO_4) and stored at 4°C until used. The OD_{600} of this culture suspension was approx 1. A similar procedure of cell harvesting has previously been reported (21). It should be noted that whereas most of the Fe(III) precipitate is removed as a result of the above procedure, it is impossible to remove all the iron particulates, as small iron precipitate particles are strongly attached to the bacteria.

Biotreatment experiments

The feasibility for biotreatment of coal was done in batch experiments by contacting 0.1 g of coal with 4.5 mL of (pH-adjusted) medium and 0.5 mL of concentrated cell culture in 15-mL (screw cap) test tubes. In controls, 0.5 mL of pH 3.5 sulfuric acid was added in place of the cultures. Two different media formulations were used, one containing Fe(II) and the other containing Fe(III) (*see* Table 1). The effect of mixing by intermittent shaking was also studied for certain experimental conditions. The tubes were shaken gently to mix the coal and the microbial culture and then incubated for 1–8 wk at 30°C.

Sampling and Analysis

The test tubes were sampled and analyzed for mercury by collecting 4 mL samples after the culture test tubes had been centrifuged. The samples were oxidized using bromine monochloride, followed by neutralization of excess oxidant with hydroxylamine hydrochloride and reduction of mercury using stannous chloride to produce elemental mercury, which was analyzed using single gold trap amalgamation and cold vapor atomic fluorescence spectroscopy (Hg analyzer, BrooksRand, Seattle, WA). The method closely followed US EPA Method 1631E and is a standard methodology which works for mercury at levels ranging from 10 ng/L to 2 μg/L. The instrument can measure sub- to low-picogram levels of mercury which allows very low levels of detection and analysis of small volumes of samples.

Results and Discussion

The goal of this work was to determine the feasibility for mercury removal under different conditions by different organisms. There was significant mercury removal in most of the experiments compared with control experiments. In the first experiment, two pure cultures *A. ferrooxidans* and *L. ferrooxidans* were compared when they were acting on the different coals. A typical result from one of those experiments are shown in Fig. 1. Duplicate analysis of a few samples was conducted and found to be within 10% of each other. As seen in the Fig. 1, *L. ferrooxidans* was found to remove more mercury from coal PSOC-1470 than *A. ferrooxidans* in a 4-wk period. Another experiment investigating an 8-wk-time period indicated that no additional mercury was removed under these conditions (data not shown). Tests were also performed with the other coal types using the two pure cultures and these tests indicated a similar or lower amount of mercury removal as compared with coal PSOC-1470. In most cases, *L. ferrooxidans* was found to remove more mercury from a particular coal than *A. ferrooxidans* after 4 wk. It is also important to note that the pH changes during the incubation in inoculated samples, dropping from an initial pH

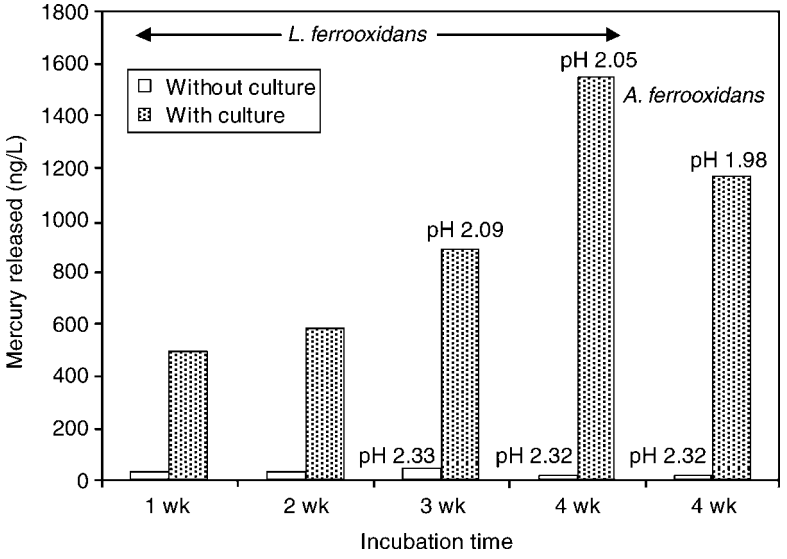

Fig. 1. Removal of mercury from coal PSOC-1470 using pure cultures at 30°C. The pH values given are at the end of the experiment. The starting pH was 2.3.

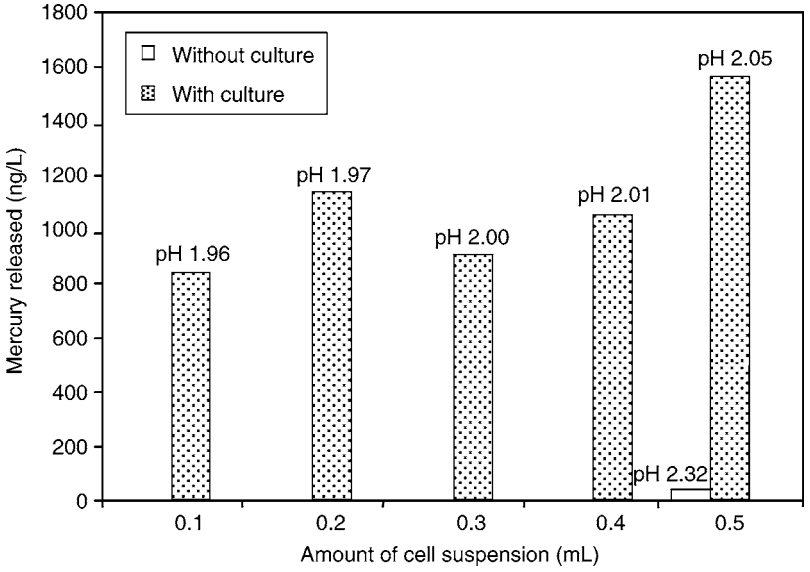

Fig. 2. Effect of culture density on removal of mercury by *L. ferrooxidans* from PSOC-1470 coal. The initial pH was 2.3.

of 2.3 to pH 2.0 or slightly below; the pH in control samples remained approximately constant during the incubation (Fig. 1).

The effect of culture density on mercury removal was studied by varying it between approx 2 and 10% by volume (0.1–0.5 mL/5 mL medium). The results are shown in Fig. 2—in general, there was little effect of culture density over the range studied. Thus, it was decided to use 0.5 mL of cell

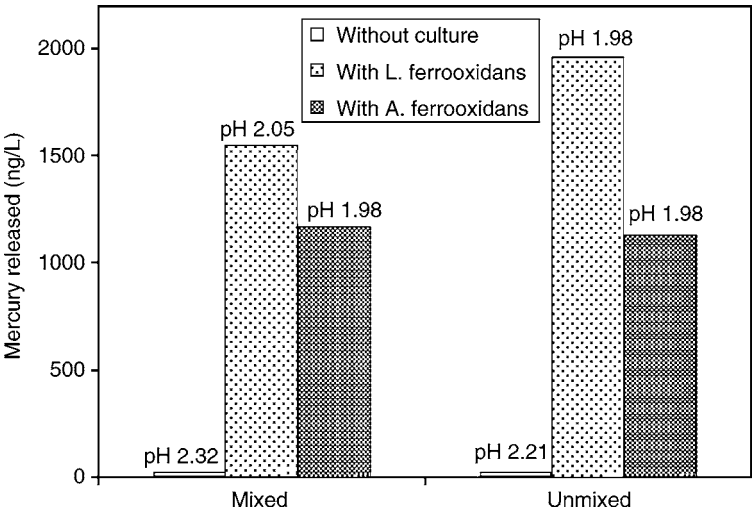

Fig. 3. Effect of mixing on the removal of mercury by *L. ferrooxidans* from PSOC-1470 coal after 4 wk at 30°C.

suspension per 5 mL coal sample slurry for all experiments. An experiment was conducted to study the effect of intermittent mixing (Fig. 3). The experiment was conducted over a 4-wk period and mixing was done on alternate days for a 5-min period via vortex mixing. The results show that there was less mercury removed under mixing conditions. The iron oxidizing bacteria are known to work by attaching themselves to ore surface and this may explain why static conditions may be better *(22)*. However, the experimental data set is insufficient to make a strong case for this observation. As the mixing appeared to have a negative effect on the release of mercury, further experiments were done without mixing.

Natural environmental cultures also proved to liberate more mercury from the coal compared with controls. As part of these studies, augmentation of different species of iron was studied. The significance of adding Fe(III) vs Fe(II) is as follows: the reduced form of iron (Fe[II]) is required for growth of microorganisms, whereas the oxidized form is the one which reacts with pyrite/FeS_2 in coal, thereby oxidizing the sulfide species known to bind mercury. This chemical reduction process also produces Fe(II) which is then available for organisms to grow on. It has been shown that Fe(III) can be helpful when it is present as the only iron species in the beginning of the chemical leaching process *(23,12)*. However, in a long-term process, it may not make a significant difference to add either.

Results from experiments with culture enrichments from the acid mine drainage sites are shown in Figs. 4–7. Removal of mercury was observed by all cultures/enrichments. In general, VA No. 1 and VA No. 2 performed better than the other two enrichment cultures. Up to 1300 ng/L of mercury was leached from the coal samples by the former enrichments. Among these two, VA No. 2 performed better under the Fe(III) media conditions.

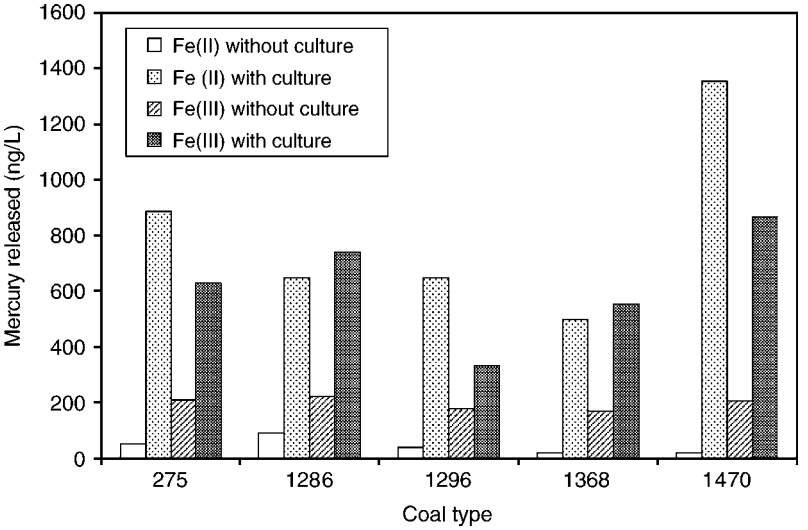

Fig. 4. Removal of mercury from various coals by environmental culture enrichment VA No. 1 with different species of iron augmentation.

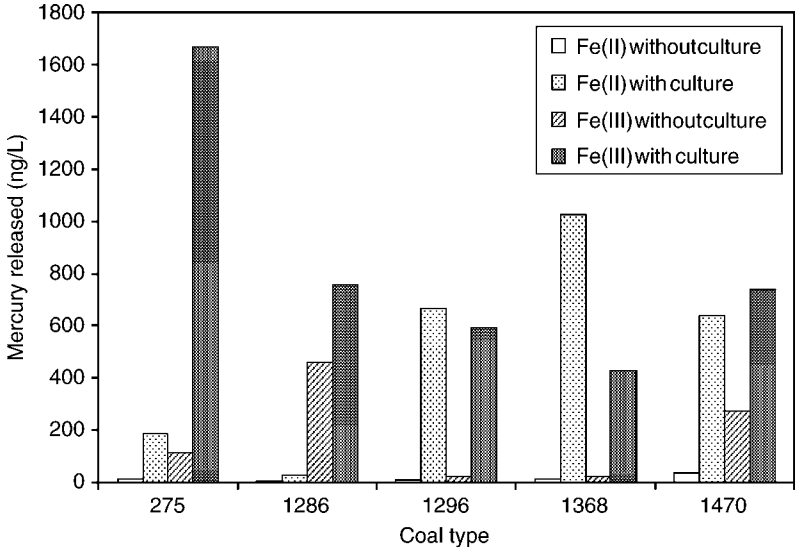

Fig. 5. Removal of mercury from various coals by environmental culture enrichment VA No. 2 with different species of iron augmentation.

A comparison of mercury removal by pure cultures vs environmental cultures can be done for coal PSOC-1470. For example, about 1548 ng/L Hg was released by *L. ferrooxidans* in 4-wk period (Fig. 1), whereas 1353, 637, 520, and 21 ng/L Hg was released by VA No. 1, 2, 3, and 4 cultures, respectively, under Fe(II) augmentation condition (Figs. 4–7). Although it may seem that the pure culture is able to remove higher level of mercury from coal PSOC-1470 than environmental cultures, this statement cannot

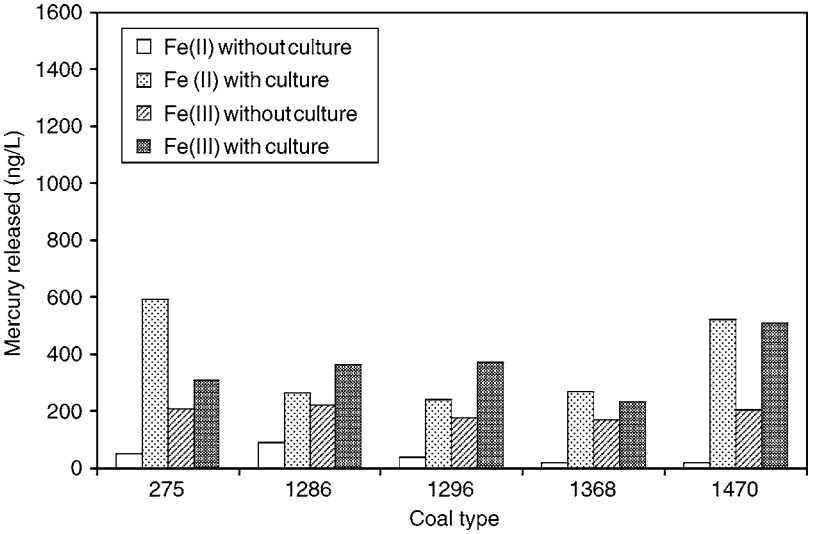

Fig. 6. Removal of mercury from various coals by environmental culture enrichment VA No. 3 with different species of iron augmentation.

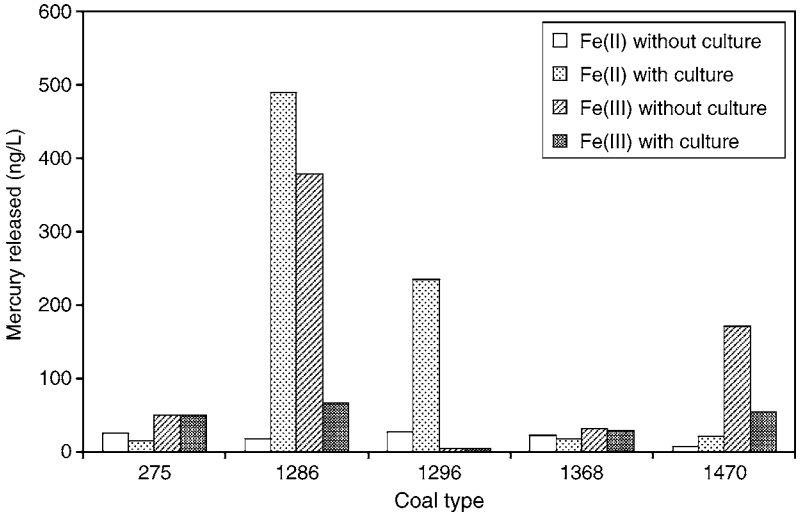

Fig. 7. Removal of mercury from various coals by environmental culture enrichment VA No. 4 with different species of iron augmentation.

be generalized. However, literature reports indicate that *L. ferrooxidans* is one of the most common organisms in acid mine *(24,25)* and metal bioleaching studies *(14)* and that it is a very effective organism for oxidation of metal sulfides such as pyrite.

Previous research has shown that mercury leaches out of coal under acidic conditions. Dronnen et al. *(9)* found that neither *A. ferrooxidans* nor *A. thiobacillus* improved the leachability of mercury from North Dakota lignite—the authors concluded that their growth medium with a pH of 2.0

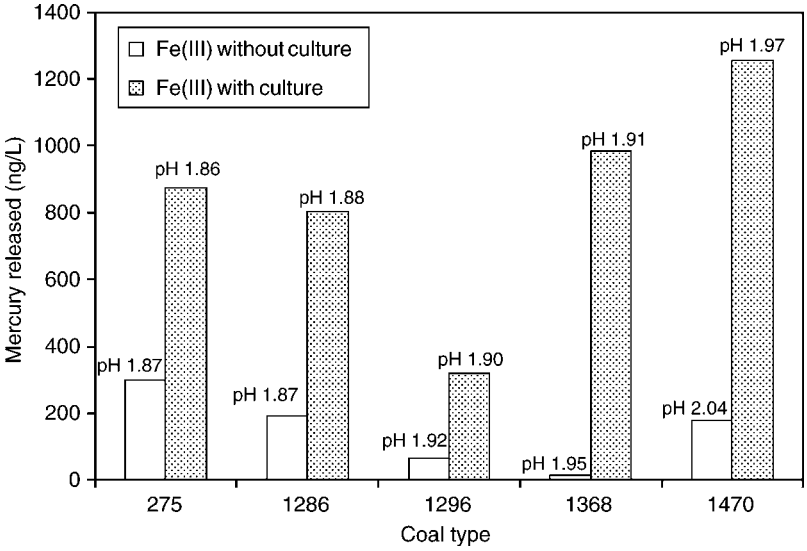

Fig. 8. Removal of mercury from various coal by environmental culture enrichment VA No. 2 with ferric iron, Fe(III), augmentation.

or 5.0 removed as much mercury as either of the organisms did when grown together with the lignite at pH 2.0 for 116 d. In our studies, the pH dropped during cultivation; consequently we performed additional studies in which the pH in the control experiments were initially reduced to a level we anticipated the inoculated experiments would reach after 4 wk of incubation. The result from such an experiment may be seen in Fig. 8. As is noted, the pH in some of the control test tube experiments ended up lower than in the inoculated test tubes and the opposite was found in other experiments. Regardless, mercury release from the coal was clearly better in the inoculated cases.

In our studies, the mercury release from the coal was detected by monitoring the concentration of mercury in the leaching liquid. In other studies, investigators have reported volatilization of mercury from soil slurries contaminated with mercury in the presence of a strain of *A. ferrooxidans* (26). In a limited number of experiments, vials with coal, medium, and pure strain organisms were incubated in a larger sealed glass vial with a Teflon-lined septum. After 4 wk of incubation, the gas phase was sampled for mercury but none detected. This suggests that the strains (both, *A. ferrooxidans* and *L. ferrooxidans*) used in these experiments do not volatilize mercury under the conditions studied, but solubilized the mercury and released it into the liquid medium.

The reproducibility of mercury removal by the bacterial strains is an important question. Although the biotreatment experiments were not run in duplicate, repeat experiments were conducted to assess mercury release from coal PSOC-1470 by *L. ferrooxidans* and have shown qualitatively similar results (data not shown). The differences observed in release of mercury

may be owing to the differences in density of the cell culture used in the three experiments with *L. ferrooxidans* (Figs. 1–3). A quantitative analysis of the amount of mercury released (g inoculated cells) per day is being conducted and will be reported in a subsequent publication targeted at kinetics and mass transfer issues. Other issues such as effect of higher concentrations of mercury on bacteria, effect of iron species on growth, and microbial growth during leaching process are also under investigation. It was also difficult to assess the exact release of mercury in noninoculated controls. For example, mercury release in the control experiments for coal PSOC-1368P with Fe(III)-augmentation ranged between 21 and 170 ng/L (Figs. 4–7). It is not entirely clear what caused this variation. It should be noted that samples for analysis were taken after centrifugation but were unfiltered and any small quantity of carbon accidentally transferred from the culture test tube to the sample container would increase the apparent mercury concentration in the sample. Further, the control experiments were done under nonsterile conditions. Some bacteria are expected to be present associated with the coal. It is possible that these indigenous bacteria result in release of mercury in coal. Regardless of the cause, the variations of mercury release in control experiments are not significant enough to change our conclusions.

The amount of mercury released from the different coal types has been observed to vary significantly. The reason for this may be the difference in porosity, pyrite distribution, and sample heterogeneity of the coal samples. Overall, the greatest mercury removal was observed from coal PSOC-1470, which contained about 0.4 mg/kg of mercury. About 1600 ng/L of mercury was observed in the leachates from this coal, amounting to about 20% removal of mercury. A process capable of continuous mercury removal using mercury-specific sorbents can potentially improve the extent of mercury removed from coal.

Conclusions

Preliminary experiments have shown the capability of pure strains, as well as environmental enrichments, to assist in the leaching of mercury from coal. Mercury was released to a different extent from each coal type. Environmental enrichment samples did as well as pure cultures. The augmentation of ferric iron at low concentration help leaching under some conditions. Under the best conditions, 20% of the mercury initially present in the coal was leached into the liquid and it was mediated by the presence of bacteria.

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