This study was part of a joint effort between the Illinois State Geological Survey (ISGS) and Northwestern University (NU), where the complementary project, Part B - Development of screening methodology and engineering, was implemented. The overall goal of the combined projects was to develop microbial methods to augment coalescent/flotation processes for physical desulfurization of Illinois coals. Historically, microbial methods for desulfurization of coal have focused on removal of sulfur by biological oxidation and subsequent leaching of sulfate. However, recent evidence suggests that bacterial cultures can wet pyrite surfaces and thus can act as suppressants in physical separation processes. The objectives for our portion of this project were: i) to determine whether cultures of various bacteria could enhance suppression of pyrite from Illinois coals and to measure the extent of suppression; ii) to determine the effects of bacterial strain, contact time, contact temperature, contact pH, solution ionic strength, and growth stage of the cultures on the extent of suppression; iii) to determine whether the effector of suppression was cell associated or an extracellular product; iv) to optimize both production of the microbial suppressant and its use for efficient pyrite rejection. This year we have made significant progress toward meeting these goals. While studies with thiobacilli are still inconclusive, we have demonstrated, using a small-volume Hallimond tube flotation assay (developed at NU), that preparations of the common coliform bacterium Escherichia coli suppress flotation of both -150+200 mesh coal-derived and mineral pyrite by 35% to 50%. Lipid or lipid-like material exuded by the cells appears to be responsible for suppression. Selective cellular attachment to pyrite was discounted as an effector of suppression because cell-free filtrates were equally effective and direct microscopic attachment studies showed cells of both E. coli and Thiobacillus ferrooxidans ATCC 23270 attach at least as well to coal as to pyrite. Using the flotation assay, very fine pyrite (i.e., -325 mesh) could not be suppressed by microbial treatments; however, failure to achieve suppression might have been the fault of the particular assay system rather than an inability to modify the pyrite surface. Preliminary attempts to use microbial treatments to separate pyrite from coal have been somewhat successful; however, further research will be necessary to understand various aspects of the phenomenon and achieve more efficient pyrite rejection.
EXECUTIVE SUMMARY

This study is part of a joint effort between the Illinois State Geological Survey (ISGS) and Northwestern University (NU). The goal is to develop microbial methods to augment coalescent/flotation processes for physical desulfurization of Illinois coals.

Historically, microbial methods for desulfurization of coal have focused on removal of sulfur by biological oxidation and subsequent leaching of sulfate. Therefore, significant pyrite removal required residence times of days. Recent evidence suggests that cultures of the iron- and sulfur-oxidizing bacterial species, *Thiobacillus ferrooxidans*, and other bacteria as well, can act as suppressants in physical separation processes, some of which can achieve efficient rejection of pyrite and ash in a matter of minutes. By including cells of *Thiobacillus ferrooxidans* in the aqueous coal slurry, it has been possible to double the percent pyrite rejected for oil agglomeration separations (Kempton et al., 1980). Whole cultures of a similar organism have produced a 40% increase in pyrite rejection for froth flotation separations (Attia and Elzekey, 1985). These results are thought to be caused by: 1) bio-oxidation of the pyrite surface; 2) bacterial cells, selectively attached to pyrite, acting as wetting agents; or 3) a pyrite wetting agent elaborated by the organisms and released into the culture medium. The precise mechanism, however, has not been elucidated. Our goal was to discover the mechanism, and help optimize the construction of a bioreactor, which would facilitate efficient pyrite rejection from Illinois coals.

In order to realize these goals, we developed the following objectives:

1. **(ISGS)** To determine whether cultures of various strains of iron- and sulfur-oxidizing bacteria and other species of bacteria can enhance suppression of pyrite from Illinois coals and to measure the extent of suppression using a small-volume assay developed at Northwestern University.

2. **(ISGS)** To determine the effects of bacterial strain, contact time, contact temperature, contact pH, solution ionic strength, and growth stage of the cultures on the extent of suppression.

3. **(ISGS)** To determine whether the effector of suppression is cell associated or an extracellular product, and to determine how the suppressive mechanism works.

4. **(ISGS)** To optimize the process further by manipulation of bacterial strains.

5. **(NU)** To provide a rapid means to screen the effectiveness of microbially derived pyrite and ash suppressants on small samples.

6. **(NU)** To provide an engineering evaluation of combined biological and physical cleaning process that maximizes pyrite removal from Illinois coal.
This year, studies were performed using small volume Hallimond tube flotation assays (Fuerstenau et al., 1957). Preliminary results using pyrite-grown cultures of Thiobacillus ferrooxidans ATCC 23270, and a mixed culture of thiobacilli obtained from Atlantic Research, Alexandria, VA, demonstrated some pyrite suppressant activity, but this activity was small and quite variable. This may have been due to the fact that cell densities were relatively dilute. Recently published work by Butler et al., (1986), suggests that cells of the common coliform Escherichia coli are as effective or superior to thiobacilli as pyrite suppressants in oil agglomeration separations. While E. coli differs in many ways from thiobacilli, the compositions of the cell and outer membranes are similar. Because E. coli cells could be grown quickly and easily to high densities, we elected to first concentrate our efforts on elucidating the mechanism of microbially enhanced suppression with E. coli K12 (wildtype) and then to return to further experimentation with thiobacilli. Compared to E. coli, thiobacilli grow more slowly, have a substantially smaller endpoint cell density, and are difficult to separate from the pyrite substrate or from the ferric hydroxide precipitates when grown on ferrous salts.

Using cultures of Escherichia coli K12 (wildtype), the following results were obtained:

1) We have determined that conditioning -150+200 mesh mineral pyrite with a preparation of E. coli cells prior to flotation can result in substantial decreases in the pyrite recovered in the float fraction. In a typical assay pyrite flotation was reduced from 78.4% to 51.2% when prefaced by conditioning pyrite for 5 minutes in washed cells (ca. 5 x 10^10 cells mL^-1) suspended in 0.1 M NaCl with pH adjusted to 2.0 with H_2SO_4.

2) In additional experiments, cells were removed from the cell suspension by microfiltration to test the effectiveness of the cell-free filtrate. The cell-free filtrate also effectively suppressed pyrite, reducing flotation from 81.5% to 50.7%. While most previous studies have implied that cellular attachment to pyrite is an important mechanism in suppression, our results with the cell-free filtrate suggest that the effector of suppression is a compound or group of compounds exuded from cells or released upon cell lysis. The data obtained from experiments in which the attachment of both E. coli and T. ferrooxidans ATCC 23270 cells to coal and pyrite was studied also discounts selective attachment to pyrite as a mechanism. Both types of cells attached equally well or better to coal.

3) Preliminary assays involving the flotation separation of pyrite from coal have shown some degree of microbially enhanced pyrite rejection; 54.4% obtained in the float in the absence of microbial conditioning as compared to 48.8% with microbial conditioning. Hopefully, a better understanding of the mechanism of the phenomenon, will allow us to increase the degree of pyrite rejection.
4) With the present experimental protocol, suppression of -325 mesh pyrite could not be demonstrated using the Hallimond tube flotation assay; approximately 70% of the pyrite was recovered in the float fraction regardless of treatment. Failure to achieve suppression may be due to the particular assay system rather than to an inability of the bacterial cultures to modify the pyrite surface.

5) In an attempt to identify the effector of pyrite suppression, an experiment was performed with a cell filtrate and a chloroform extracted cell filtrate. After being extracted with chloroform, the aqueous filtrate lost its ability to suppress pyrite, implying that lipid or lipid-like material, which may be responsible for suppression, was extracted from the aqueous phase. Chromatographic analyses of the extracted suppressant will be pursued.
OBJECTIVES

The objectives of the overall project (Parts A and B) are: (1) to determine whether cultures of thiobacilli can enhance suppression of pyrite in various separation processes involving Illinois coals and to determine the extent of suppression, (2) to determine whether suppression is caused by a chemial wetting agent released by cells during some stage of growth, or is the result of cellular adherence to pyrite particles or is effected, in part, by both mechanisms, (3) to optimize the mechanism of suppression by optimizing cellular production of the wetting agent, or optimizing cellular adherence, and (4) to determine the economy and feasibility of including the optimized suppression mechanism in the design of a separation reactor.

To achieve these objectives, the following tasks will be addressed:

Part A

Task I. Determine whether cultures of thiobacilli enhance the rate of pyrite suppression from Illinois coals.

Task II. Determine the mechanism of suppression.

Task III. Optimize the mechanism of suppression.

Part B

Task I. Develop a pyrite suppression screening test.

Task II. Define engineering scale bioreactor process parameters for microorganism growth under conditions identified in Part A.

Task III. Perform an engineering assessment of the applicability of a flotation/agglomeration system using pyrite suppression for improved Illinois coal desulfurization.

Part A tasks are principally accomplished by the ISGS research team and Part B tasks by the Northwestern University Research Team.

INTRODUCTION AND BACKGROUND

Historical Perspective. A relatively new approach to microbial desulfurization involves using the ability of thiobacilli to wet pyrite to enhance separation processes in which pyrite is removed physically, rather than biochemically. Until recently, most microbial methods for desulfurization of coal have focused primarily on removal of sulfur by biological oxidation and subsequent leaching of sulfate. This type of desulfurization is a relatively slow process (e.g., 3-10 days for 90% pyritic sulfur removal) because it proceeds at the growth rates of the microbial cells. Therefore, it is usually not cost-effective on an industrial scale (see, e.g., Bos et al., 1985, for a review). However, Capes et al., (1973) demonstrated that cultures of thiobacilli could rapidly modify the surface properties of pyrite so that pyrite particles were more easily separated from coal using an oil agglomeration
process. In 1980, Kempton et al., essentially doubled the % rejection of pyrite by including cells of Thiobacillus ferrooxidans in the aqueous coal slurry prior to oil agglomeration separation. Actia and Elzeky (1985) extended this work to froth flotation, achieving a 40% increase in pyrite suppression by including cultures of a similar organism. Both reports show that microbial enhancement of suppression occurs in a matter of minutes, and conjecture that it is caused by increased wettability of the pyrite surface. In 1985, Isbister et al., furthered this work by developing a scaled-up proprietary process, with the acronym of MAAPS (Microbially Augmented Ash and Pyrite Physical Separation); they reported that the use of thiobacilli cultures resulted in a 21% increase in ash removal with recovery of 80% combustibles.

Various mechanisms for microbial surface modification of pyrite have been suggested, including: biooxidation (Capes et al., 1973); selective adsorption of bacterial cells (McCreary and LeGallais, 1984); and the bacterial production of wetting agents such as phospholipids (Isbister et al., 1985); however, the precise mechanism has not been elucidated. One of the most recent studies (Butler et al., 1986) demonstrated that cells of the common coliform Escherichia coli work as well or better than thiobacilli. The cell membranes, composed primarily of lipids and complex lipids, of both E. coli and T. ferrooxidans are similar because they are both Gram-negative bacteria. It is possible that these compounds are the effectors and that biooxidation or selective attachment play little if any role in the surface modification process.

**Biology.** The species Thiobacillus ferrooxidans includes obligate autotrophic bacteria that oxidize reduced iron and sulfur to obtain energy. In addition to utilization of aqueous species such as ferrous or thiosulfate ions, these bacteria grow well on solid mineral substrates such as pyrite or elemental sulfur. Growth on pyrite ultimately results in the solubilization of iron as ferric ions and the solubilization of sulfur as sulfate. Historically microbial coal desulfurization processes using Thiobacillus ferrooxidans have been based upon this ability to metabolize and thus solubilize pyrite.

It is generally assumed that during biooxidation of pyrite the membrane of the bacterial cell is in direct contact with the surface of the mineral (Silverman, 1967). Because an intimate cell-mineral association is a requirement for utilization of minerals as growth substrates, it has been hypothesized that thiobacilli produce various surface-active wetting agents to facilitate contact. Characterization of wetting agents produced by thiobacilli growing on elemental sulfur or on ferrous salts has been a subject of interest for some time (Schaeffer and Umbreit, 1963; Jones and Benson, 1965; Barridge, id Shively, 1968). However, wetting agents produced during growth on pyrite remain relatively uninvestigated. It is generally agreed that phospholipids, either extracellular or cell-associated, act as surfactants allowing contact with elemental sulfur and perhaps pyrite. The rate of production and the chemical composition of these materials varies with growth substrate (Agate et al., 1969) and with the growth stage of the culture (Agate and Vishniac, 1973).
Present Work. The present study has investigated microbial enhancement of pyrite suppression from several finely ground Illinois coals. The overall goal of the project was to relate a biochemical/biophysical study of the principal mechanisms of pyrite wetting to an accompanying engineering study (see Microbial Enhanced Separation of Pyrite from Illinois Coals: Part B-Development of Screening Methodology and Engineering). The latter study determined applicability to froth flotation and to oil agglomeration separation processes. Ideally, a bioreactor can be constructed in which cultures can be grown on pyrite rejected during separation. Identification of the wetting mechanism and optimization of conditions under which it operates should allow the efficiency and economics of coal-pyrite separation processes to improve.

EXPERIMENTAL PROCEDURES

Microorganisms. Cultures of Thiobacillus ferroxidans ATCC 13598, T. ferroxidans ATCC 23270, and Leptospirillum ferroxidans were obtained from Art Harrison, University of Missouri, Columbia. A mixed culture of iron and sulfur oxidizing bacteria was obtained from Atlantic Research, Alexandria, VA. In most cultures, cases were grown and maintained on ATCC medium #64 with 0.5% mineral pyrite replacing FeSO₄·7H₂O as a growth substrate. The medium contained per litre of deionized water the following: NH₄SO₄, 0.8 g; KH₂PO₄, 0.4 g; MgSO₄·7H₂O, 0.16 g; 2 ml 1 N H₂SO₄. Cultures were incubated at 30°C in a gyratory shaker at 150 rpm. Occasionally, we attempted to prepare concentrated, iron-free suspensions of T. ferroxidans ATCC 23270 cells. In these cases, unmodified medium #64 was used. After incubation for 7 to 10 days the ferric hydroxide precipitate was allowed to settle. The supernatant was siphoned off and filtered through Whatman #1 filter paper to remove residual precipitate. Then cells were harvested at 4°C from the supernatant by centrifugation at 6000 x g, washed once in acidified saline (0.1 N NaCl with pH adjusted to 2.0 with H₂SO₄), resuspended in a small volume of acidified saline, and stored at 4°C. Concentrated washed suspensions of thiobacilli cells were used only for studies of bacterial attachment to coal and pyrite; we could not obtain large enough volumes to use in Hallimond tube flotation assays, due to the fact that thiobacilli grew more slowly, had a substantially smaller endpoint cell density, and were difficult to separate from the pyrite substrate or from the ferric hydroxide precipitates when grown on ferrous salts. As an example, 6 L of culture produced only slightly more than 10 mL of a clean concentrated cell suspension (4.8 x 10⁹ mL⁻¹, as determined with a Petroff-Hauser Counting Chamber.)

Escherichia coli K12 (wildtype) EMI was obtained from the Microbiology Department of the University of Illinois, Urbana. For studies of pyrite suppression, cells were grown in the following defined medium: per litre of deionized water, K₂HPO₄, 7.0 g; KH₂PO₄, 3.0 g; (NH₄)₂SO₄, MgSO₄·7H₂O, 0.1 g; CaCl₂·2H₂O, 0.01 g; FeSO₄·7H₂O, 0.5 mg; glucose, 2 g. Cultures were incubated at 37°C for 72 h on a gyratory shaker at 150 rpm. Cells were harvested at 4°C by centrifugation at 6,000 x g. After washing once with 0.1 N NaCl, the cells were resuspended in a volume of 0.1 N NaCl to produce a 10-fold increase over the original cell density (final density 5 to 10 x 10¹⁰ mL⁻¹.) The pH was then lowered to 2.0 with H₂SO₄. Cell preparations were stored at 4°C.
Cell-Free Filtrates. Cell-free filtrates of E. coli cell preparations were produced by passing the preparations through a 0.22 μm microfilter to remove all cells. To produce chloroform extracted cell-free filtrates, an equal volume of CHCl₃ was added to the cell-free filtrate in a separatory funnel, shaken, and the CHCl₃ layer removed. Chloroform was purged from the aqueous fraction with N₂ gas before the fraction was used in flotation assays. In order to demonstrate that removal of the suppressant and not the presence of residual CHCl₃ was responsible for the observed increase in pyrite flotation, cell-free filtrates that were saturated with CHCl₃ (80 μL/10 mL⁻¹) were also tested using the flotation assay.

Pyrite. Mineral pyrite, purchased from Sargent-Welch Scientific, was 85% pyrite. X-Ray diffraction analysis confirmed pyrite to be the principal component, with traces of quartz and galena. The mineral pyrite was ground and sieved to collect -325 and -150+200 mesh fractions, the remainder being used for growth substrate for the microorganisms. Pyrite derived from IBCSP#4 was obtained from the ISGS Applied Laboratory. The coal-derived pyrite was washed sequentially with methanol, 1 N HCl and water, then sieved to collect the same two fractions. Because this is a relatively precious commodity, the remainder was saved for future flotation experiments. In order to prevent surface oxidation, all pyrite fractions were placed in septum-stopped serum bottles, which were flushed and then pressurized to 20 lb with nitrogen. We were interested in the -325 mesh fraction because the particles were small enough to use for microbial attachment studies, which must be viewed under a microscope. The -150+200 mesh fraction was of interest because a previous study (Atkins, et al., 1986) demonstrated microbially enhanced pyrite suppression with a similar particle sized fraction; we wanted to duplicate some of this work and confirm the validity of the reported results.

Acid-cleaned Coal. Pyrite-free coal was prepared from 200 mesh IBCSP#4. Coal was refluxed for 30 min in a solution of 1 part concentrated nitric acid to 7 parts deionized water, then washed extensively with deionized water and air dried.

Coal-Pyrite Mixtures. A pyrite amended coal was prepared by mixing portions of 200 mesh IBCSP#4 with portions of the -150+200 mesh coal-derived pyrite at a ratio of 9:1. The final pyrite content of the mixture was approximately 13.5%. We decided that unamended coal did not have enough pyrite to accurately measure suppression with the small-volume Hallimond tube suppression assay.

Hallimond Tube Assay. A modified Hallimond flotation tube (Figure 1) was constructed according to the specifications of Fuerstenau et al., (1957). Because of the small volume (100–150 ml) both time and reagents were used more efficiently than would have been possible with larger separation cells.

In initial studies, optimum parameters in terms of float time, rate of N₂ flow, and frothing reagent were determined. In these cases, 15 ml of 0.01 N H₂SO₄ was placed in the base of the Hallimond tube. Stirring was initiated and the N₂ flow rate adjusted to the desired value. If a
frother was to be used, 10 μL was added at this time. Frothing agents tested were: methyl isobutyl carbinitol (MIBC), DowFroth-150, and NaCl-8834. Then, 0.5 g mineral pyrite was added. After 5 min conditioning time, flotation separation was initiated by the addition of 100 ml water. When the desired float time had expired, the float fraction was collected by removing the plug from the float collecting tube, allowing the contents to flow into a beaker. Following filtration through Whatman no. 10 filter paper, the percent pyrite floated was determined gravimetrically. All flotation assays, unless specified otherwise, were in triplicate.

Pyrite-grown cultures of thiobacilli of varying ages were tested as suppressants in the flotation assays. Cultures were filtered through Whatman no. 1 filter paper to remove pyrite, and were substituted for the 0.01 N H₂SO₄ as conditioning solutions. Uninoculated thiobacilli culture medium that had been incubated along with microbial cultures was also tested. If a conditioning solution resulted in less flotation, pyrite suppression was inferred.

Although other researchers (e.g., Atkins, et al., 1986; Attia and Elzeky, 1985) had been able to prepare large volumes of clean, iron-free, concentrated suspensions of thiobacilli to test as pyrite suppressants, we found this to be extremely difficult. In our case, over 99% of the cells were lost during attempts to separate them from the pyrite substrates or iron hydroxide precipitates present in the culture medium. Therefore, we decided to investigate the mechanism for pyrite suppression using cells of E. coli, which were easy to grow and purify in large quantities, and which, according to Butler et al. (1986) worked equally well. Cells were suspended in 0.1 N NaCl to prevent lysis, and also to provide a swamping concentration of electrolyte so that in future work the pH could be varied without major fluctuations in ionic strength. In the present work, pH was kept at 2.0 with H₂SO₄ for consistency. Conditioning solutions included the following: 0.1 N NaCl with pH adjusted to 2.0 with H₂SO₄; preparations of washed cells of E. coli K12 suspended in 0.1 N NaCl with pH adjusted to 2.0 with H₂SO₄; cell-free filtrates of the preparations of E. coli; the same cell-free filtrates after extraction with CHCl₃. In these tests, the N₂ flow rate was adjusted to 25 mL mm⁻¹ for -150+200 mesh pyrite and the coal-pyrite mixture, and 5 mL min⁻¹ for -325 mesh pyrite. After adding DowFroth-150, 0.5 g pyrite or 2.0 g of the coal-pyrite mixture was added. Floats were for 10 minutes.

Attachment Studies. These studies were performed according to methods suggested by Yeh et al., (1986). All water used was first filtered through a 0.22 μm filter and then autoclaved at 121°C for 20 minutes. Irgalan black solution was prepared by adding 2.0 g irgalan black (Ciba-Geigy) and 20 mL glacial acetic acid to 1 L water. Acidine orange solution (0.1%) was prepared by adding 0.1 g acidine orange (Sigma) to 100 ml water. This solution was stored in the dark at 4°C and filtered through a 0.22 μm before each use. Nucleopore polycarbonate filters (3.0 μm pore size) were stained for 24 h with irgalan black solution, then rinsed with water before use. A 10 mL aliquot of either the T. ferrooxidans concentrated cell preparation (4.8 x 10⁹ mL⁻¹) or an E. coli concentrated cell preparation (5.0 x 10¹⁰ mL⁻¹) was mixed with
0.5 g -325 mesh coal-derived pyrite or -325 mesh acid-cleaned coal, incubated at 28°C on a gyratory shaker at 50 rpm, and sampled at zero time, 5 min, 1 h, 1 day, and 1 wk. At the appropriate time 1.0 ml of the slurry was removed, washed gently with 9.0 ml acidified saline, and resuspended in 9.0 ml acidified saline. Then 1.0 ml of acridine orange solution was added and the mixture allowed to stain for 30 min before a 1 ml aliquot was removed and filtered. The filter was washed with 30 ml water, then placed on a drop of immersion oil on a microscope slide, covered with a drop of oil, a coverslip, and another drop of oil. Attachment was observed using a Zeiss Standard 16 Research microscope equipped with epifluorescence, a Zeiss -09 filter set, and a 100X Neofluor objective lens. For each determination, twenty 10 x 10 μm fields were counted with the aid of a net reticule.

**Chromatography.** Thin layer chromatography of concentrated CHCl₃ extracts was performed using 10 x 10 cm HLF plates (Analtech) in a solvent saturated system. The developing solvent contained CHCl₃, methanol, water, and NH₄OH (65:25:4:1, on a volume basis). Plates were sprayed with molybdate blue and observed for phospholipids before charring at 400°C for 45 sec. Molybdate blue was prepared by combining 1 part solution A, 1 part solution B, and 2 parts water before use. Solution A was prepared by boiling 40.1 g MoO₃ in 1 L 25 N H₂SO₄ until dissolved. Solution B was prepared by boiling 1.5 g powdered molybdenum in 500 mL solution A for 15 min, then decanting to remove the residue. Standards (Sigma) included neutral lipids (mono- and di-), and trioleates, and mono-, di-, and tripalmitates), phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid, and cardioplipid.

**RESULTS AND DISCUSSION**

**Determination of Optimum Parameters for Hallimond Tube Suppression Assays.** Figure 2 shows the percent mineral pyrite recovered in the float at various N₂ flow rates when no frother was used. Using these results it was decided that the optimum flow rates for suppression assays would be 25 ml/min for -325 mesh and 200 ml/min for -150+200 mesh. Figure 3 shows the effects of float time at these selected flow rates. From these data it was decided to use 10 minute floats for both mesh sizes. However, when microbial cultures were substituted for 0.01 N H₂SO₄ (Table 1.), no suppression of pyrite could be detected.

**Table 1. Per cent mineral pyrite recovered in the float. No frother.**

<table>
<thead>
<tr>
<th>Mesh Size</th>
<th>0.01 N H₂SO₄ Medium</th>
<th>ATCC 23270</th>
<th>ATCC 13598</th>
<th>Leptospiroplillum ferrooxidans</th>
</tr>
</thead>
<tbody>
<tr>
<td>-325</td>
<td>72.8±1.1</td>
<td>73.6±1.8</td>
<td>67.4±2.5</td>
<td>no data</td>
</tr>
<tr>
<td>(25 ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-150+200</td>
<td>80.3±0.7</td>
<td>80.7±7.5</td>
<td>81.5±3.6</td>
<td>81.3±5.0</td>
</tr>
<tr>
<td>(200 ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We reasoned that turbulence, rather than flotation, had been primarily responsible for carrying pyrite over to the float collecting tube. Under
these circumstances, changes in the surface characteristics of pyrite particles would have little effect on the outcome of the experiments. Therefore, three frothing agents were tested for their ability to improve pyrite flotation so that flow rates could be kept at a minimum. Results are shown in Table 2. All three frothing agents dramatically increased the per cent pyrite floated.

Table 2. Per cent mineral pyrite recovered in the float during 5 minute floats after conditioning pyrite with various frothers for 5 minutes. Minimum flow rates were used.

<table>
<thead>
<tr>
<th>mesh size</th>
<th>flow rate</th>
<th>no frother</th>
<th>NaCo-8834</th>
<th>DowFroth-150</th>
<th>MIBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>-325</td>
<td>5 ml/min</td>
<td>25.2±3.8</td>
<td>69.5±2.3</td>
<td>65.7±3.8</td>
<td>66.1±1.4</td>
</tr>
<tr>
<td>-150+200</td>
<td>25 ml/min</td>
<td>13.9±3.5</td>
<td>79.8±4.9</td>
<td>80.7±5.4</td>
<td>75.2±4.2</td>
</tr>
</tbody>
</table>

Thereafter it was decided to include 10 µL DowFroth 150 in all floatation assays so that these minimum flow rates could be used.

Pyrite Suppression Using Whole Cultures of Thiobacilli. Results obtained using pyrite-grown cultures of T. ferrooxidans ATCC 23270, and a mixed culture of thiobacilli obtained from Atlantic Research, Alexandria, VA, demonstrated some pyrite suppressant activity, but this activity was small and quite variable (Table 3).

Table 3. Effect of pyrite conditioning with cultures of iron and sulfur oxidizing bacteria on percent recovery of -150+200 mesh pyrite in Hallimond tube flotation assays.

<table>
<thead>
<tr>
<th>conditioning solution</th>
<th>pH</th>
<th>[SO₄²⁻] mg L⁻¹</th>
<th>[Fe] mg L⁻¹</th>
<th>% of original recovered in float</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 N H₂SO₄-4</td>
<td>2.00</td>
<td>480</td>
<td>0.0</td>
<td>80.6 ± 5.4</td>
</tr>
<tr>
<td>3-week-old cultures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uninoculated control</td>
<td>2.48</td>
<td>530</td>
<td>9.7</td>
<td>80.9 ± 4.6</td>
</tr>
<tr>
<td>T. ferrooxidans ATCC 23270</td>
<td>2.06</td>
<td>4693</td>
<td>1303</td>
<td>60.9 ± 1.1</td>
</tr>
<tr>
<td>Atlantic Research</td>
<td>2.12</td>
<td>3877</td>
<td>978</td>
<td>68.3 ± 5.3</td>
</tr>
</tbody>
</table>

5-week-old cultures

| uninoculated control   | 2.47 | 979            | 22.6        | 59.3 ± 3.8                     |
| T. ferrooxidans ATCC 23270 | 2.01 | 6938          | 2783        | 69.3 ± 5.1                     |
| Atlantic Research      | 2.04 | 6735          | 2779        | 66.9 ± 3.6                     |

Surprisingly, some suppression was seen with the uninoculated control incubated for 5 weeks. The pH, sulfate and iron in the supernatant were measured to see if there was any trend that correlated with suppression; however, none was obvious.

Pyrite Suppression Using E. coli. When -150+200 mesh mineral pyrite was conditioned with a preparation of E. coli cells in 0.1 N NaCl with pH adjusted to 2.0 with H₂SO₄, there was a substantial decrease in the pyrite recovered in the float fraction (Figure 4). Flotation was reduced from 78.4% to 51.2%. In this case and in the case of all the following
experiments the control flotation was conditioned with 0.1 N NaCl with pH adjusted to 2.0 with H₂SO₄. In a second experiment, the effectiveness of a cell-free filtrate was tested. The cell filtrate also effectively suppressed pyrite, reducing pyrite in the float fraction from 81.5% to 50.7% (Figure 4). While most previous studies have implied that cellular attachment to pyrite is an important mechanism in suppression, our results with the filtrate suggested that the effector of suppression is a compound or group of compounds exuded from cells or released upon cell lysis. Because E. coli is a potential pathogen, subsequent experiments were performed with cell-free filtrates, rather than with preparations containing whole cells. With the present experimental protocol, suppression of ~325 mesh pyrite could not be demonstrated using the Hallimond tube flotation assay (Figure 4); approximately 70% of the pyrite was recovered in the float fraction regardless of treatment. Failure to achieve suppression may be due to the particular assay system rather than an inability to modify the pyrite surface.

In an attempt to identify the effector of pyrite suppression, an experiment was performed with a cell filtrate and a CHCl₃-extracted cell filtrate (Figure 5). Before extraction, the filtrate reduced flotation from 82.1% to 40.0%. After extraction with CHCl₃, the aqueous filtrate produced 88.6% flotation implying that lipid or lipid-like material which might be the effector of suppression was removed from the aqueous phase. To demonstrate that removal of the suppressant was responsible for the observed increase in pyrite flotation, and not residual CHCl₃ cell-free filtrates saturated with CHCl₃ (80 μL 10 mL⁻¹) were tested using the flotation assay. While there appeared to be a slight increase in flotation when CHCl₃ was present, clearly the suppressant was still active, reducing flotation from 80.7% to 50.7%.

Beebe and Umbreit (1971) hypothesized that a major phospholipid component of bacterial membranes, phosphatidylethanolamine (PTEA), was the sulfur wetting agent produced by cultures of thiobacilli. Consequently, a crude extract of E. coli cells containing approximately 50% PTEA, was purchased from Sigma and tested in pyrite suppression assays (Figure 6). (These assays were not performed in triplicate thus the absence of a standard deviation bar.) Unlike the cell-free filtrate, no suppression was observed with three concentrations of this crude PTEA in acidified saline; in fact, it appeared that flotation was slightly enhanced. Phospholipids being amphipathic compounds, should behave as wetting agents; it has been suggested that phospholipids are active in pyrite suppression by Isbister et al., (1985). The fact that this preparation of PTEA did not cause suppression cannot be explained at this time, but it is possible that other compounds in the crude extract may have interfered with suppressant activity.

Work with mineral pyrite alone does not demonstrate the usefulness of microbially enhanced pyrite suppression in coal cleaning. Consequently, additional assays were performed with coal-derived pyrite and attempts were made to separate pyrite from coal using the microbial suppressant. The coal-derived pyrite floated more readily than mineral pyrite (Figure 7); in the absence of the microbial suppressant, 94.8% of the coal-derived pyrite was recovered in the float compared to only 80.7% of the
mineral pyrite. However, the cell-free E. coli filtrate reduced flotation to 57.8%, and 49.5% respectively. To evaluate the results of the attempts to remove pyrite from coal, both the float and tailings were digested with dilute nitric acid (1 part HNO₃ and 7 parts water), and the digest analyzed for iron colorimetrically using 1,10 o-phenanthroline (Taras et al., 1971). Because the greater part of the solubilized iron was pyritic, the iron concentration approximated the pyrite content. Results (Figure 7) were not as successful as those obtained using only pyrite. Approximately 48.8% of the pyrite was recovered in the float when conditioned by the cell-free filtrate; this result was similar to that obtained in previous assays. However, pyrite appeared to be almost equally suppressed in the absence of the microbial filtrate, with 54.4% appearing in the float. Additional work is needed to explain these results.

Bacterial Attachment Experiments. Figures 8 and 9 depict the results of the bacterial attachment experiments. The 1 wk samples could not be counted due to the presence of fluorescent debris in the preparations. Fewer thioecbacci cells were found attached to particles; however, this may have been caused by the fact that the thioecbacci cell suspension contained approximately 10-fold fewer cells than the E. coli cell suspension. Although the variance was great, overall, significantly more cells were attached to the acid-washed coal than to the pyrite. Coal was acid washed to remove pyrite particles, thus ensuring that the observed cells were attached only to coal. Unfortunately acid washing may have also increased the surface area of the coal, inadvertently promoting lodging of cells. In any case, no overt preference of cells for pyrite was observed. This contradicts certain previous reports (McCready and LeGallais, 1984; Bagdigan and Myerson, 1986) that thioecbacci selectively attach to pyrite, and supports a report by Wakao et al., (1984) that thioecbacci attach to almost any surface. We concluded from these results and from the fact that the cell-free filtrate was an effective suppressant, that bacterial attachment to pyrite particles plays no role in suppression.

Chromatography. At this time chromatographic analyses of the chloroform extracts are inconclusive. These analytical techniques are being modified.

CONCLUSIONS AND RECOMMENDATIONS

Preparations of E. coli cells enhance suppression of mineral and coal-derived -150+200 mesh pyrite by 35-50% in Hallimond tube flotation assays.

Attachment of bacterial cells to pyrite is not the principal mechanism of suppression.

The pyrite suppressant is a chloroform extractable compound released by the cells. Evidence suggests that this suppressant may not be a phospholipid.

Microbial suppression of -325 mesh pyrite cannot be demonstrated using the present assay system.
Experiments to determine whether the suppressant can be useful in separating pyrite from coal are in progress. Results to date are inconclusive.

Bio-suppression experiments should be continued with whole coals utilizing a larger volume test cell (greater than 200 ml) to assay pyrite suppression.

Chemical studies should be continued to identify the specific compound(s) in extracts from E. coli filtrates that enhance suppression.

Extend research on bio-suppressants to other bacteria particularly *Thiobacillus ferrooxidans*.

Determine optimal conditions of activity for bacterial suppressants and define the conditions and the specific compounds that inhibit suppressant activity.
LITERATURE CITED


Figure 1. Modified Hallimond tube for use in microbial suppressant assays (Fuerstenau and coworkers, 1957).
Figure 2. Effect of flow rate on flotation of (A) -325 mesh mineral pyrite and (B) -150+200 mesh mineral pyrite in a modified Hallimond tube. Float time is 5 minutes.
Figure 3. Effect of time on flotation of two mesh sizes of pyrite in a modified Hallimond tube. Flow rates are 25 ml/min for -325 mesh and 200 ml/min for -150+200 mesh. These values were found to be optimal for the two mesh sizes.
Figure 4. Effect of *Escherichia Coli* cell suspensions and cell-free filtrates of these suspensions on the flotation of mineral pyrite of two mesh sizes. Flotation assays were performed in triplicate; error bars indicate standard deviation.
Figure 5. Effect of cell-free filtrates of *Escherichia coli* cell suspensions and chloroform extracted and chloroform amended (80 μL chloroform 10 mL⁻¹ cell-free filtrate) filtrates on the flotation of -150+200 mesh mineral pyrite. Flotation assays were performed in triplicate; error bars indicate standard deviation.
PTEA = phosphatidylethanolamine (crude extract from *E. coli*)

Figure 6. Comparison of the effect of cell-free filtrates of *Escherichia coli* cell suspensions and three concentrations of a crude preparation of phosphatidylethanolamine (PTEA) on the flotation of -150+200 mesh mineral pyrite.
Figure 7. Effect of cell-free filtrates of *Escherichia coli* cell suspensions on the flotation of -150+200 mesh mineral and coal-derived pyrite and on the flotation of coal-derived pyrite mixed with 200 mesh IBCSP#4 coal. Flotation assays were performed in triplicate; error bars indicate standard deviation.
Figure 8. Surface density of cells of *Thiobacillus ferroxidans* ATCC 23270 on coal-derived pyrite and acid washed coal after incubation in a cell suspension for various periods of time. Error bars indicate the standard deviation of counts from twenty fields.
Figure 9. Surface density of cells of Escherichia coli on coal-derived pyrite and acid washed coal after incubation in a cell suspension for various periods of time. Error bars indicate the standard deviation of counts from twenty fields.