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Kinetics of the Removal of Iron Pyrite from Coal by Microbial Catalysis

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Different strains of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* were used to catalyze the oxidative dissolution of iron pyrite, FeS_2, in nine different coal samples. Kinetic variables and parametric factors that were determined to have a pronounced effect on the rate and extent of oxidative dissolution at a fixed P_{O_2} were: the bacterial strain, the nitrogen/phosphorus molar ratio, the partial pressure of CO_2, the coal source, and the total reactive surface area of FeS_2. The overall rate of leaching, which exhibited a first-order dependence on the total surface area of FeS_2, was analyzed mathematically in terms of the sum of a biochemical rate, r_1, and a chemical rate, r_2. Results of this study show that bacterial desulfurization (90 to 98%) of coal samples which are relatively high in pyritic sulfur can be achieved within a time-frame of 8 to 12 days when pulp densities are $\leq$20% and particle sizes are $\leq$74 μm. The most effective strains of *T. ferrooxidans* were those that were isolated from natural systems, and *T. ferrooxidans* ATCC 19859 was the most effective pure strain. The most effective nutrient media contained relatively low phosphate concentrations, with an optimal N/P molar ratio of 90:1. These results suggest that minimal nutrient additions may be required for a commercial desulfurization process.

To meet increasing energy requirements within the United States, utilization of domestic coal has been accelerated, even though significant environmental problems have been associated with its use. These problems include an increase in CO_2 and SO_2 emissions, an increase in particulate discharge and aerosol formation, and an overall reduction of ambient air quality. To minimize the potential deleterious impact of increased coal utilization on ambient air quality, alternative methods for coal desulfurization must be developed. Precombustion removal of sulfur from coal by microbial action (11, 16, 36) presents an attractive conceptual alternative to current chemical or physical methods (13, 23, 55). The sulfur present in a typical grade of coal, on the average, is about equally distributed between inorganic sulfur, in the forms of iron pyrite, marcasite, or sulfate, and organic sulfur. Organic sulfur is randomly distributed throughout the polymeric coal structure in the form of monosulfide, disulfide, or thiophene linkages (9), whereas inorganic sulfur is found in discrete pockets at elevated mole fractions.

In this paper, we present the results of a study of the ability of different strains of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* to catalyze the oxidative dissolution of iron pyrite, FeS_2, in the presence of a coal matrix. Determination of the kinetics and mechanisms of the oxidative dissolution of FeS_2 found in coal as catalyzed by bacterial action should establish optimal biochemical, chemical, and physical conditions for bacterial desulfurization of coal. Properly constructed coal slurry pipelines (54) could serve ideally as plug-flow, mixed-culture reactors for microbial desulfurization. Water-soluble sulfur compounds formed during microbial metabolism would be extracted during dewatering procedures before wet-cake drying and combustion.

After initial identification of *T. ferrooxidans* in acidic mine waters (10) and subsequent studies (20, 39) on its ability to promote the oxidative dissolution of FeS_2 in coal, Ashmead (4) explored the possibility of using the acidophilic iron- and sulfur-oxidizing bacteria to remove pyrite from coal. He reported that naturally occurring microorganisms accelerated the rate of oxidation of FeS_2 found in coal samples with a pyrite sulfur content of 4% by weight. Subsequent studies by Silverman and co-workers (31, 33, 35, 36) showed that *T. ferrooxidans* could be used successfully to oxidize FeS_2 found in coal. They reported that appreciable quantities of pyrite were oxidized in 3 to 4 days. The rate of oxidation or removal was

a function of particle size and the rank of the particular coal. The apparent rate of oxidation increased with a decrease in particle size. Particles with an average diameter (D_p) of <43 μm were most effectively leached, with 60% removal observed after 8 days. However, in some coal samples, FeS_2 was not readily oxidized. Addition of ferric sulfate to the batch reactor slurry increased the degree of pyrite removal. In all cases, there was a significant nonbacterial leaching as determined by control studies.

Dugan and Apel (11) studied the effectiveness of mixed enrichment cultures isolated from acid mine waters for desulfurization of pulverized coal. Using a blended coal sample, they showed that either *T. ferrooxidans* or *T. thiooxidans* alone was less effective as compared to the natural mixed-culture system. Microbial leaching was observed to be most effective when the pH of the slurry was adjusted to the range of 2.5 to 2.0 and when the nutrient medium was supplemented with NH_4^+. They reported 97% removal of pyritic sulfur, which was initially 3.1% by weight under optimal conditions, although there was a 5-day lag period.

Silverman et al. (35) reported that *T. thiooxidans* was ineffectual for the oxidative dissolution of FeS_2. However, naturally occurring mixed cultures containing both *T. ferrooxidans* and *T. thiooxidans* have been shown to be highly effective in the catalysis of FeS_2 dissolution (11). Additional evidence for symbiotic relations among acidophilic bacteria has been reported by Norris and Kelly (29). In this case, *Leptospirillum ferrooxidans*, an iron-oxidizing acidophile in mixed cultures with *T. thiooxidans*, was reported to be an effective catalytic system for the oxidative dissolution of FeS_2.

With the above research in mind, the factors affecting the rate of oxidative dissolution of iron pyrite in a variety of coal samples were systematically studied. Kinetic results of this parametric study are reported here.

MATERIALS AND METHODS

Coal sample preparation. Four discrete particle size fractions of coal—$D_p < 250$ μm (−60 mesh); $D_p < 74$ μm; 43 μm $\leq D_p \leq 74$ μm (+325 to −200 mesh); and $D_p < 43$ μm—were used in experiments with coal. FeS_2 crystals (99.9%; Cerac/Pure, Inc., Milwaukee, Wis.) were used as received ($D_p \leq 149$ μm). For bulk processing of crushed coal samples, 110 kg of coal was screened initially through a 10-mesh screen. The +10 mesh portion was crushed with a coffee mill crusher and resieved. This procedure was repeated until the majority of the initial coal sample passed through the 10-mesh screen. The sample was split subsequently into 11.0-kg portions with a basic splitter. Each batch was ground in a steel ball mill grinder for 30 min or until more than 96% of the particles were less than 74 μm. Other coal samples were crushed by hand with a mortar and pestle and shaken on a Tyler screen shaker for 15 min. In all experiments involving coal, sample preparation was performed in a way that minimized surface oxidation. In addition, to minimize soluble aqueous-phase iron at the beginning of each experiment, the coal samples were washed for 2 h in a 0.1 N HCl solution, rinsed thoroughly with distilled water (Milli-RO4, MilliQ Water Purification System; Millipore Corp., Bedford, Mass.), and subsequently dried overnight in a 60 to 70°C, N_2-purged oven.

Representative coal samples were obtained from mines in Illinois, Kentucky, and Pennsylvania. Locations of the mines, sample designations, and analyses for iron and sulfur are given in Table 1.

Bacteriological procedures. Strains of bacteria used in these experiments were obtained from multiple sources. *T. ferrooxidans* ATCC 19859, ATCC 13598, ATCC 8085, and ATCC 13728 and *T. thiooxidans* ATCC 19377 were obtained initially from the American Type Culture Collection, Rockville, Md. *T. ferrooxidans* 11Fe, *T. ferrooxidans* O.T. 6, Agnew Lake culture, Tioga River culture, and *T. thiooxidans* strains were obtained from the sources listed in Table 2.

The Agnew Lake and the Tioga River cultures were isolated in this laboratory. The Agnew Lake culture was a mixed culture containing primarily *T. ferrooxidans* and a small amount of mold. No attempt was made to isolate a single strain of *T. ferrooxidans* in this particular case. The Tioga River culture was a pure culture of *T. ferrooxidans*; it was derived from a single colony from a membrane filter on ferrous iron agar (22).

All *T. ferrooxidans* cultures were maintained selectively on one of the following media. A standard ferrous sulfate medium (51, 53) was prepared for maintenance of pure stock cultures with the following composition, per liter: 0.4 g of K_2HPO_4, 0.4 g of $(NH_4)_2SO_4$,

TABLE 1. *Coal samples and their pyrite content*

Sample no.	Name and location of mine	% FeS_2[a]
1, 2	Broken Aro Mine, Coshocton, Ohio	4.00 4.90
3, 4	Simco Peabody Strip, Coshocton, Ohio (Ohio no. 6 coal (raw); 6″ × 0 "Run of Mine")	3.16 2.90
5	Simco Peabody Deep, Coshocton, Ohio (Ohio no. 6 coal (raw); 6″ × 0 "Run of Mine")	4.19
6	Sun Spot Mine, Amax Coal Co., Vermont, Ill.	5.36
7	Ayrgem Mine, Amax Coal Co., Central City, Ky. (576 Pit Seam no. 11)	0.82
8	Renton Mine, Consolidation Coal Co., Pittsburgh, Pa.	0.62
9	Ayrgem Mine, Amax Coal Co., Central City, Ky. (576 Pit Seam no. 12)	2.13

[a] Methods used for analysis were adopted from test procedures D3177-75 and D2492-68 from the American Standards of Testing Materials, Part 26 (2).

TABLE 2. *Bacterial strains used in oxidative dissolution studies of FeS_2 entrained in coal*

Organism and strain designation	Source	References
T. ferrooxidans		
ATCC 19859	ATCC[a]	49, 50
ATCC 13598	ATCC	
ATCC 13728	ATCC	
11Fe	N. Tomizuka, Fermentation Research Institute, Chiba, Japan	40–42
O.T. 6	O. H. Tuovinen, Ohio State University, Columbus	51–53
Agnew Lake culture		
Tioga River culture	Tioga River, Blossburg, Pa.	
T. thiooxidans		
UM	Microbiology Department, University of Minnesota, Minneapolis	
ATCC 8085	ATCC	
ATCC 19377	ATCC	

[a] ATCC, American Type Culture Collection, Rockville, Md.

0.4 g of $MgSO_4 \cdot 7H_2O$, and 27.8 g of $FeSO_4 \cdot 7H_2O$. This medium was adjusted to pH 2.1 with the addition of concentrated H_2SO_4. The iron (II) sulfate was filter sterilized separately and added after the basal salts were autoclaved. The 9K medium of Silverman and Lundgren (34) was adjusted to pH 2.1 with sulfuric acid and sterilized by autoclaving. The 9K medium (34) was used with and without added $(NH_4)_2SO_4$ in initial survey experiments on FeS_2 autoxidation rates and for tests of possible bacterial mutualism between *T. ferrooxidans* and the nitrogen-fixing bacteria *Beijerinckia lactogenes*. Tsuchiya and co-workers (49, 50) have observed a positive mutualism between *T. ferrooxidans* and *B. lactogenes* and pronounced increases in the rate of oxidative dissolution of $CuFeS_2$ and $(Fe,Ni)_9S_8$ in ore concentrates. As an alternative to the conventional 9K medium (34) a basal salts medium called LOPOSO, an acronym for low phosphate (PO_4^{3-})-low sulfate (SO_4^{2-}), was developed with the following composition, per liter of distilled water: 0.10 g of KH_2PO_4, 0.85 g of $MgCl_2 \cdot 6H_2O$, 0.10 g of KCl, 0.008 g of $CaCl_2 \cdot 2H_2O$, and 0.123 g of NaCl. Variations of the LOPOSO medium, LOP10 and LOP10N, were used. LOP10 was a medium of LOPOSO basal salts diluted by a factor of 10, and LOP10N was the LOP10 medium with NH_4Cl added in a mole ratio ($[NH_4^+]_T$: $[PO_4^{-3}]_T$ of 16:1). Finally, strains of *T. thiooxidans* were maintained on a medium with the following composition, per liter: 0.2 g of $(NH_4)_2SO_4$, 0.5 g of K_2HPO_4, 1.5 g of KH_2PO_4, and 0.5 g of $MgSO_4 \cdot 7H_2O$. To the basal salts of the *T. thiooxidans* medium, 0.5 g of elemental sulfur was added per 100 ml after sterilization by ethylene oxide.

Chemical reagents used in media preparation were analytical grade. *T. ferrooxidans* cultures used as the inocula were maintained on the LOPOSO medium with FeS_2 as the energy source. Reaction flasks, which were run in duplicate, were covered with gas-permeable siliconized caps and placed on a reciprocating shaker (5-in. [ca. 12.1-cm] stroke and 76 rpm) at 25°C in a constant-temperature room. Samples were taken for chemical analysis at well-defined time intervals. Bacteria, substrate, and solid products were removed by filtration through 0.4- and 0.22-μm membrane filters. pH measurements were made with an Orion 701 Ionalyzer. Kinetic samples were acidified with 2% Ultrex HNO_3 (J. T. Baker) for preservation before chemical analysis.

Both sterile and unsterilized coal samples were used. For sterile experiments the media and coal samples were autoclaved at 120°C and 1.02 atm (103.38 kPa) for 15 min. Unsterilized coal samples were added to sterilized media to examine the effect of alteration of reactive surface sites of FeS_2 by autoclaving at elevated temperatures. The inoculum was transferred twice with a 10% inoculum (vol/vol) (i.e., 30 ml of inoculum per 300 ml of total slurry volume) at 7-day intervals before a specific experiment involving coal in the same medium. Correction for evaporation during autoclaving was made before inoculation by the addition of distilled water. The rate of evaporation was found to be fairly constant over the duration of each test, with a mean value of 0.0138 ml/h. During sampling of the coal slurries, the flasks were thoroughly mixed to ensure a relatively constant proportion of coal to medium in the sample.

Chemical and instrumental procedures. Conventional wet-chemical procedures used for sulfur analysis of coal samples were adapted from American Society of Testing Materials procedures (2). The Eschka method (D3177-75) was used for total sulfur analyses, and the D2494-79 procedure was used for sulfate and pyritic sulfur analyses.

Total iron content was determined by an alternative to the above procedure which involved leaching of 2.0 g of the coal sample with 100 ml of 13% HNO_3 after pretreatment with dilute 5% HCl. After samples were leached for 24 h, the total iron concentration in the leachate was determined by atomic absorption spectrophotometry with a Varian model 175 or 375 spectrophotometer with deuterium background correction.

Kinetic data were obtained by analysis of the filtrates of the coal-water-pyrite-bacteria slurries. The rate of appearance of soluble iron was determined by measuring iron with atomic absorption spectrophotometry. Iron precipitates formed after oxidation of FeS_2 were dissolved in dilute (10^{-1} M) HCl. Total iron release is defined as the sum of the iron in the solid and nominally soluble fractions. FeS_2 is relatively insoluble in dilute HCl. Soluble sulfate concentrations were determined by a standard turbidimetric procedure (1). Turbidity was measured at 420 nm with a 5-cm cell and a Beckman 26 UV-VIS spectrophotometer. Quantification was achieved with standard calibration curves. Sulfate $S_2O_3^{2-}$, SO_3^{2-}, and $S_4O_6^{2-}$ were measured chromatographically with a Dionex model 10 Ionchromatography (38). Readily oxidizable, intermediate sulfur oxyanions were measured polarographically in both differential and normal pulse modes with a Princeton Applied Research 174 polarographic analyzer (30).

RESULTS

Iron pyrite, FeS_2, is susceptible to natural weathering processes when exposed to oxygen-

ated water. It is readily oxidized by O_2 and Fe(III) according to the following stoichiometric equations:

$$FeS_2 + 7/2\ O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (1)$$

$$Fe^{2+} + 1/4\ O_2 + H^+ \rightarrow Fe^{3+} + 1/2\ H_2O \quad (2)$$

$$FeS_2 + 14\ Fe^{3+} + 8\ H_2O \rightarrow 15\ Fe^{2+} + 2SO_4^{2-} + 16\ H^+ \quad (3)$$

where equation 1 is linearly dependent on equations 2 and 3 (i.e., equation 1 = 14 × equation 2 + equation 3). Equation 1 can be used to represent the overall reaction stoichiometry for kinetic purposes, and equations 2 and 3 can be used to reflect overall mechanistic sequences. Singer and Stumm (37) have proposed that equation 2 is catalyzed by *T. ferrooxidans* under environmental conditions and that Fe(III) is the principal oxidant of FeS_2 according to equation 3. They came to this conclusion because the observed rates of pyrite dissolution were significantly faster than the rates of the noncatalyzed autoxidation of Fe(II).

For a heterogeneous reaction, such as the oxidative dissolution of FeS_2, the specific reaction rate, ν, is normally defined (3, 14) as:

$$\nu \equiv -\frac{1}{A_s}\frac{dN_s}{dt} \quad (4)$$

where N_s is the number of moles of solid present, and A_s is the interfacial surface area. The units for the rate of reaction, ν, are expressed as mole/centimeter-squared-second. Alternatively, when the interfacial area is unknown, the specific rate can be defined as:

$$\nu \equiv -\frac{1}{W}\frac{dN_s}{dt} \quad (5)$$

where W is the weight of the solid particles dispersed in the fluid phase. Taking the heterogeneity of the FeS_2 reaction in water and the overall stoichiometry of equation 1 into account, the specific reaction rate can be written in terms of the rate of disappearance of FeS_2 or O_2. Alternatively, the reaction rate can be expressed in terms of the rate of appearance of Fe(II) or the rate of appearance of SO_4^{2-} as follows:

$$\nu = -\frac{1}{A_s}\frac{dN_{FeS_2}}{dt} = -\frac{1}{3.5A_s}\frac{dN_{O_2}}{dt} = \frac{1}{A_s}\frac{dN_{Fe^{n+}}}{dt} = \frac{1}{2A_s}\frac{dN_{SO_4^{2-}}}{dt} \quad (6)$$

where N_{FeS_2}, N_{O_2}, $N_{Fe^{n+}}$, and $N_{SO_4^{2-}}$ are the moles of FeS_2, O_2, Fe(II), and SO_4^{2-}, respectively. When the reactor volume, V, remains constant during the course of the reaction, the rate of reaction can be expressed conveniently in terms of concentration as follows:

$$\nu = \frac{d[Fe^{n+}]}{dt} = \frac{kA_s}{V} \quad (7)$$

where k is the specific reaction rate or rate constant. When FeS_2 is present as a finely divided solid, the rate of its disappearance may be written as:

$$-\frac{V}{A_s}\frac{d[FeS_2]}{dt} = -\frac{1}{A_s}\frac{dN_{FeS_2}}{dt} \quad (8)$$

An initial set of experiments were performed with pure FeS_2 (99.9% Cerac) in the 9K medium (34) to determine suitable operating conditions for subsequent coal-FeS_2 experiments.

The 9K medium developed by Silverman and Lundgren (34) is high in total orthophosphate and sulfate. Alternative media containing high phosphate concentrations appeared to inhibit the rate of oxidative dissolution of FeS_2, and those containing high initial sulfate concentrations introduced analytical uncertainty in turbidimetric and ion chromatographic analyses. Therefore, the LOPOSO medium was tested with variable amounts of NH_4Cl. This medium proved to be preferable.

Experimental results indicated that an inoculated LOPOSO medium (*T. ferrooxidans* ATCC 19849) with low phosphate concentration (0.7 mM) and no NH_4^+ oxidized pyrite more efficiently than an inoculated 9K medium with higher phosphate (2.9 mM) and higher NH_4^+ and SO_4^{2-} [3 g of $(NH_4)_2SO_4$ per liter]. Similar results were observed when *T. ferrooxidans* strains O.T. 6 and 11Fe were examined. An inoculated 9K medium without ammonium sulfate showed a complete inhibition of pyrite oxidation. Addition of $(NH_4)_2SO_4$ at elevated concentrations may counteract apparent phosphate inhibition by formation of soluble ion-pair and coordination complexes of iron and sulfate. Insoluble iron phosphates, $(Fe)_3(PO_4)_2 \cdot 18H_2O$ and $FePO_4 \cdot 2H_2O$, may form either on the surface of FeS_2 or in the bulk aqueous phase as indicated by chemical equilibrium computations of the dominant soluble and insoluble chemical species in the nutrient media (26).

Addition of soluble Fe(III) or Fe(II) in the range of 1.0 to 10 mM at the beginning of a reaction showed little impact on reaction rates. Fe(III) addition proved to be slightly inhibitory, as shown previously by Duncan and Drummond (12), whereas Fe(II) addition resulted in a slight acceleration of the observed reaction rates, as reported previously by Kovrov et al. (19). These trends are consistent with the relative tendency of Fe(III) and Fe(II) phosphates to precipitate for the reaction conditions used in the experiments described above.

To study effectively the kinetics of oxidative dissolution of FeS_2 entrained in a coal matrix, it was necessary to find coal samples which were relatively high in pyritic sulfur. Seven different coal samples were obtained from different mines and were analyzed to determine their iron content. Results of this analysis are presented in Table 1. Parametric studies were carried out primarily with Broken Aro (samples 1 and 2) and Simco Peabody (sample 4) coal. Parameters selected for study included: particle size, pulp density or slurry concentration, initial pH, nitrogen requirement, inoculum age, bacterial strain, partial pressure of carbon dioxide, and the coal source.

According to equation 4, the rate of dissolution of a solid should exhibit a first-order dependence on available reactive surface area. To verify a dependence of the reaction rate on surface area, the effects of decreasing paricle size (i.e., increased surface area) and total surface area (i.e., pulp density or slurry concentration) on the reaction rates and extent of reaction were examined. Results of these experiments using the LOPOSO medium are summarized in Fig. 1 through 3. Figure 1 illustrates that the relative rate of dissolution increases with a decrease in particle size (i.e., and increase in specific surface area) for a fixed mass of FeS_2. This relationship can be expressed according to equation 9 (14) for spherical particles:

$$A = \frac{6}{\rho D_p} \qquad (9)$$

where ρ is the density of FeS_2, D_p is the particle diameter, and A is the specific surface area. From the data presented in Fig. 1, the initial rates of dissolution are 22.8 and 40.4 mg of SO_4^{2-} $liter^{-1}$ day^{-1}, respectively, for the particle size fractions 43 $\mu m < D_p < 74$ μm and $D_p < 43$ μm. Assuming that the average particle size can be represented by the screen opening for the U.S. Standard Sieve sizes of 200 and 325 mesh, the estimated surface areas of FeS_2 in each fraction are 162 and 280 cm^2/g, respectively. From these estimated specific surface areas, given that the total mass remains relatively constant, it can be seen that as the surface area doubles, the dissolution rate approximately doubles (i.e., $A_2/A_1 = 1.73$ and $\nu_2/\nu_0 = 1.77$). This relationship suggests a first-order dependence of the dissolution rate on surface area as predicted by equation 7. Identical results were found when the rate of reaction was measured as $d[Fe^{n+}]/dt$.

An alternative way of ascertaining the reaction rate dependence on surface area is to study the impact of total surface area when the particle size fraction is held constant (i.e., 43 $\mu m < D_p < 74$ μm). The impact of increasing slurry concentration is illustrated in Fig. 2 for a mixed-culture reaction involving *T. ferrooxidans* ATCC 19859 in a LOPOSO medium diluted by one-tenth with distilled water with NH_4Cl added in an N/P molar ratio of 16:1. A linear relationship (Fig. 3) was observed between the initial

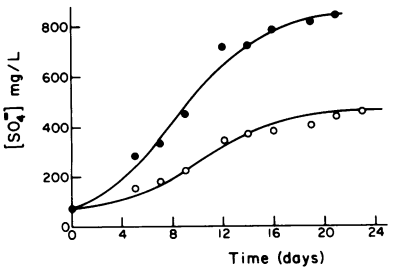

FIG. 1. *Relative rates of oxidative dissolution by T. ferrooxidans ATCC 19859 of FeS_2 entrained in Broken Aro no. 2 coal samples as a function of particle size. [SO_4^{2-}] versus time. Particle size: (○) 43 $\mu m < D_p < 74$ μm; (●) $D_p < 43$ μm. LOPOSO, 1% slurry.*

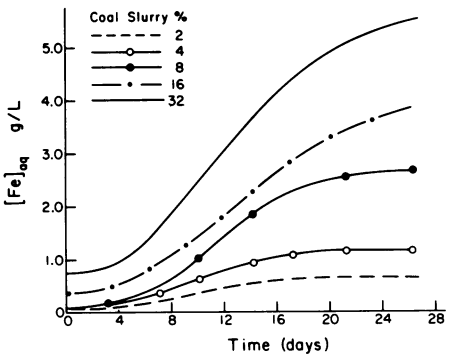

FIG. 2. *Rate of oxidative dissolution of FeS_2 as a function of total surface area for a fixed particle size fraction (43 $\mu m < D_p < 74$ μm). Slurry concentrations of 2, 4, 8, 16, and 32% by weight.*

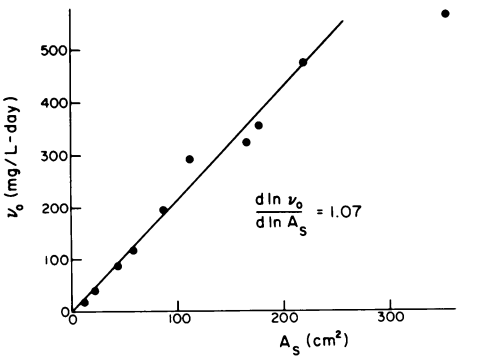

FIG. 3. *Plot of the initial leaching rate, ν_0 (mg $liter^{-1}$ day^{-1}), as a function of total surface area, A_s (cm^2), for coal slurry concentrations of 1, 2, 4, 5, 8, 10, 15, 16, 20, and 32% by weight.*

rate of leaching (determined from the slope of the concentration versus time profile at the beginning of the exponential rise) and the total estimated surface area. The slope of a double-logarithmic plot for these values (1, 2, 4, 5, 8, 10, 15, 16, 20, and 32% slurries by weight) is close to 1, as shown in Fig. 3. This result corroborates the previous observation of a first-order dependence of the reaction rate on the total reactive pyrite surface area, A_s, where A_s is given by $A \times$ (mass of FeS_2).

The impact of the initial pH of coal-water-medium slurry on the rate of leaching was investigated (Fig. 4). The rate of leaching appeared to be independent of the initial pH when $pH_0 \geq 2.0$, although the apparent lag time increased with higher pH_0 when $pH_0 > 3.5$. After 10 days the pH of all slurries except the one at $pH_0 = 1.5$ decreased to the narrow range of 1.9 to 2.3. These results are consistent with reported pH optima for the activity of *T. ferrooxidans* with various substrates (11, 41, 45). An initial pH of 1.5 is clearly inhibitory to bacterial activity. In this case, the leaching rate was identical to the sterile control.

As reported above for reactions with pure FeS_2, the LOPOSO medium without supplemental NH_4^+ was superior to the 9K medium as a nutrient medium. Subsequent experiments showed that dilution of the LOPOSO medium by one-tenth produced shorter lag times and faster initial leaching rates. This medium was labeled as LOP10. Further experiments were carried out to determine the optimal N/P ratio for maximum acceleration of the rate of leaching and minimum inhibition by phosphate complexes and precipitates. In these experiments, a 10% coal slurry with a particle size fraction of 43 $\mu m < D_p < 74$ μm in an LOP10 medium was used with a variable N/P molar ratio. Results of these experiments are summarized in Fig. 5, where the initial rate of leaching is plotted versus the molar ratio of the initial ammonium ion concentration to the initial dihydrogen phosphate concentration. As the N/P molar ratio was increased initially, the leaching rate increased until a maximum at N/P $\simeq$ 90 was obtained. After this apparent maximum was reached, the initial rate decreased to a steady value at higher molar ratios.

The nitrogen requirements of *T. ferrooxidans* ATCC 19859 and the Agnew Lake culture in the LOPOSO medium were examined using $(NH_4)_2SO_4$, NH_4Cl, $(NH_4)_2CO_3$, and $(NH_2)_2CO$ as nitrogen sources. Addition of all four sources of nitrogen gave enhanced dissolution rates as compared to the LOPOSO medium without nitrogen (Table 3). The rate of leaching was relatively independent of the nitrogen source when the source was an ionic salt. Overall, urea was less effective as a nitrogen source (Table 3).

Since *T. ferrooxidans* and *T. thiooxidans* are chemolithotrophic bacteria, the availability of CO_2 in the reaction medium may limit the level of microbial activity and consequently the rate of oxidative dissolution of FeS_2. To examine the reaction rate dependence on the partial pressure of CO_2, the conventional shake flasks were mod-

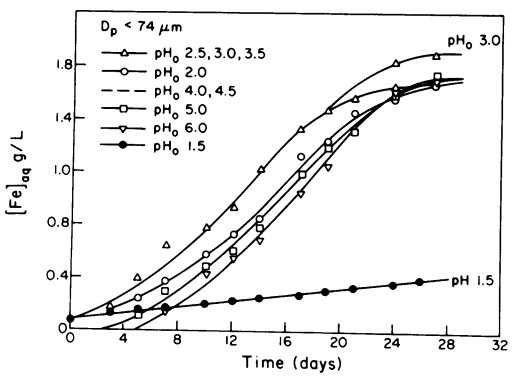

FIG. 4. *Impact of initial coal-water slurry pH on the activity of T. ferrooxidans and T. thiooxidans toward Broken Aro no. 2 coal with a D_p of ≤74 μm.*

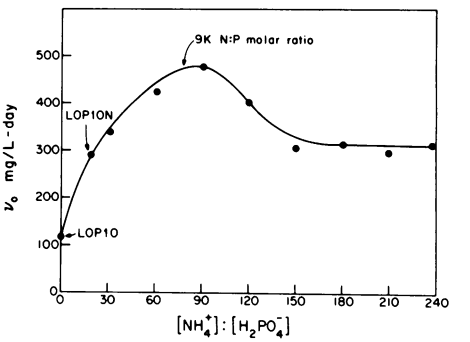

FIG. 5. *Initial rate of leaching, v_0 (mg liter^{-1} day^{-1}), plotted against the N/P molar ratio of the LOP10 medium.*

TABLE 3. *Nitrogen source and requirement for T. ferrooxidans ATCC 19859 in LOPOSO medium*[a]

N/P (molar ratio)	v_0 (mg liter^{-1} day^{-1}) with source:			
	$(NH_4)_2SO_4$	NH_4Cl	$(NH_4)_2CO_3$	$(NH_4)_2CO$
0	58.0	58.0	58.0	58.0
7.5	94.4	92.1	116.2	73.4
15.3	94.1	103.6	109.7	79.8
30.6	102.3	110.9	119.7	103.3
61.0	103.3	114.5	128.4	74.0

[a] $[HPO_4^-]_0$ = 0.75 mM; D_p (−60 mesh) <250 μm; coal sample no. 1.

ified slightly to provide a constant flow of air with variable CO_2 concentrations into the head space of the flask. The air-CO_2 mixtures were humidified in glass-bead columns and passed through two successive bacteria traps and a flow meter.

Ten percent slurries of coal with a particle size range of 43 $\mu m < D_p < 74$ μm were inoculated with *T. ferrooxidans* ATCC 19859 (Table 4). It was apparent that the rate of leaching increased slightly with an increase in P_{CO_2}. For example, the rate was enhanced by 31% when the P_{CO_2} was increased from atmospheric pressure ($10^{-3.5}$ atm) to 0.1 atm. The sterile control flasks showed that the linear chemical dissolution rate was independent of P_{CO_2}.

As pointed out by Bailey and Ollis (6), the length of the lag phase will depend on both the changes in nutrient-substrate composition and the age and size of the inoculum. Experiments were designed to study the impact of the inoculum age and the stock culture substrate. Based on initial studies, an optimal transfer time of 7 days was selected when FeS_2 was the primary substrate and two successive 7-day transfers were made before inoculation of the test medium. Later, the impact of inoculum ages of 3, 5, 7, and 10 days and substrate sources of either pure (99.9%) FeS_2 or FeS_2 in coal was systematically investigated with the inoculum size held constant at 10% (vol/vol). Experimental results for *T. ferrooxidans* ATCC 19859 showed that the leaching rate was independent of the inoculum age or substrate composition; however, the lag time was reduced somewhat by an increase in inoculum age from 7 to 10 days when pure FeS_2 was used as the stock culture substrate. A dramatic reduction of the lag time was observed when the stock culture was maintained on a coal-FeS_2 substrate. When the stock culture was adapted to the particular coal used in the actual tests, the lag time was reduced from 5 days to 1 day or less.

When the *T. ferrooxidans* 11Fe and O.T. 6 strains were tested on pure FeS_2, inoculum ages of 5, 7, and 10 days resulted in shorter lag times ($t_{lag} \simeq 5$ days) than the 3-day-old inoculum ($t_{lag} \simeq 12$ days). When stock cultures of Agnew Lake and Tioga River *T. ferrooxidans* were grown on FeS_2-coal, there was no detectable impact of inoculum age on the lag time (i.e., $t_{lag} \leq 1$ day).

The experiments described above were performed primarily with coal sample no. 2 and to some extent sample no. 4. As an extension of the parametric study, the efficacy of each strain of *T. ferrooxidans* listed in Table 2 for leaching FeS_2 from the coal samples listed in Table 1 was determined.

T. ferrooxidans ATCC 19859 and Agnew Lake and Tioga River strains were tested on seven different coal samples. An initial pH of 2.5 was established for each coal slurry. Coal samples 1, 3, and 6 required no pH adjustment, whereas coal samples 5, 7, 8, and 9 had to be adjusted with the addition of HCl. Representative results for the activity of the Agnew Lake culture are shown in Fig. 6 for −60-mesh coal samples from the various sources listed in Table 1. The rate of leaching was most rapid for samples 1 and 5, which were samples with high FeS_2 content. A relatively rapid leaching rate for these samples is consistent with the first-order dependence of the reaction rate on the total reactive surface area of FeS_2. In general, the rate of leaching for each sample appeared to be an approximate function of its FeS_2 content, with the major exception of sample no. 6. Sample 6, which had a relatively high FeS_2 content, exhibited a lower leaching rate, and the actual extent of leaching was reduced to a fraction (~24%) of the maximum extent as calculated from the experimentally determined total available iron concentrations. Analysis of the leachates by inductively coupled plasma emission spectroscopy showed that significant levels (milligrams per liter) of Pb (2.5), Cr (0.2), Ni (1.0), Cd (1.0), B (16.9), Zn (0.9), Co (1.5), and Cu (5.6) were leached during the course of the reaction with sample 6. Cadmium, chromium, and boron may inhibit the activity of *T. ferrooxidans* to some extent and thus account for the lower leaching rates and reduced extent of leaching in this particular sample. The other samples showed measurable concentrations of these components in their leachates, but the total concentrations were significantly lower, with the exception of sample 8. In all cases, the initial rates of leaching exhibited in flasks inoculated with the naturally isolated

TABLE 4. *Reaction rate dependence on the partial pressure of carbon dioxide based on air/CO_2 mixtures*[a]

	P_{CO_2} (% atm)	ν_0 (mg liter^{-1} day^{-1})
A.	0.032	124.0
	Control (0.032)	20.8
B.	0.1	142.0
	Control (0.1)	26.0
C.	1.0	144.0
	Control (1.0)	23.3
D.	10	162.0
	Control (10.0)	21.0

[a] Broken Aro coal sample no. 2; 10% slurry; 43 $\mu m < D_p < 74$ μm; *T. ferrooxidans* ATCC 19859; pH_0 = 2.5; medium, LOP10N.

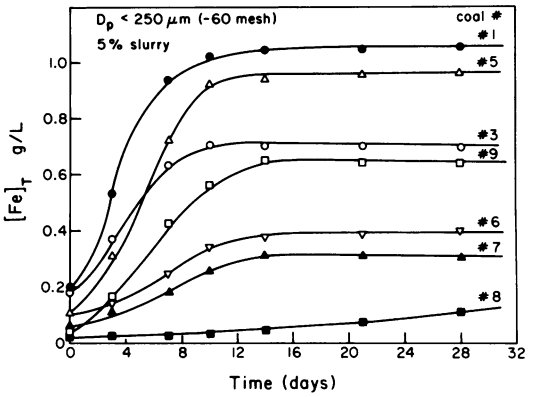

FIG. 6. *Activity of the Agnew Lake culture as a function of various coal samples listed in Table 1 as measured by $[Fe]_T$ released to solution with time.*

cultures, Tioga River and Agnew Lake, were significantly higher than those inoculated with *T. ferrooxidans* ATCC 19859.

With a constant coal sample, the efficacy of each strain was determined (Fig. 7). Clearly, the natural mixed cultures, Agnew Lake and Tioga River, were the most effective strains in terms of both the rate and extent of leaching, although strains ATCC 19859 and O.T. 6 were comparable in terms of the degree of conversion. Strains 11Fe, ATCC 13598, and ATCC 13728 showed significantly lower activities as measured by the relative rate (Table 5) and extent of leaching.

In separate experiments, several strains of *T. thiooxidans* (ATCC 8085, ATCC 19377, and UM) were combined with *T. ferrooxidans* ATCC 19859 and the Agnew Lake and Tioga River cultures. In each combination the addition of *T. thiooxidans* exhibited little impact, within reasonable limits of experimental error, on either the rate or extent of leaching (Table 6).

In the experiments involving coal reported herein, there was no evidence for the formation of jarosite as was the case in experiments involving pure FeS_2 in the LOPOSO medium. X-ray analysis of the solid residue remaining on the 0.22-μm membrane filter pads in the coal experiments showed no X-ray diffraction response, whereas part of the solids collected in experiments with pure FeS_2 were shown to be potassium jarosite as determined by X-ray diffraction (CuK_α). For reactions of pure FeS_2, precipitation of jarosite in the pH range of 2 to 4 may be expected to occur as follows:

$$3Fe^{3+} + 2SO_4^{2-} + K^+ + 6H_2O \rightarrow KFe_3(SO_4)_2(OH)_6 \downarrow + 6H^+$$

Jarosite can exist also in the H^+, NH_4^+, or Na^+ forms; however, these forms were not evident in the X-ray analysis. The quantity of jarosite precipitated with pure FeS_2 reactions was shown to be a function of the initial sulfate concentration in the medium.

Further evidence for the absence of jarosite or amorphous iron precipitates in coal reactions was obtained by measuring both the filtrate iron concentrations in the samples and the total iron obtained after digestion of the samples in 2 N

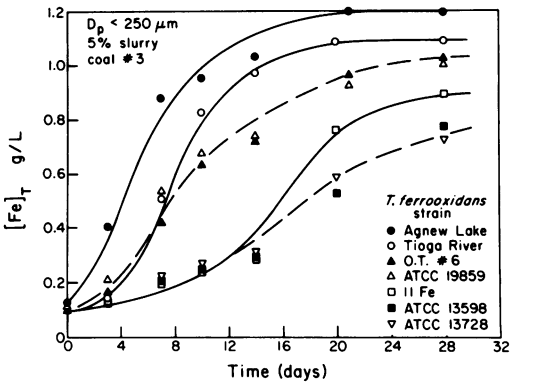

FIG. 7. *Activity of various strains of T. ferrooxidans toward coal sample no. 3 (Simco Peabody Strip) as measured by $[Fe]_T$ released as a function of time.*

TABLE 5. *Leaching as a function of bacterial strain for coal sample no. 3 (D_p < 250 μm)*

T. ferrooxidans culture	v_0 (mg liter^{-1} day^{-1})	pH at day:						
		0	3	7	10	14	21	28
ATCC 19859	47	2.5	2.5	2.3	2.2	2.1	2.0	1.9
ATCC 19859 + *T. thiooxidans* UM	46	2.5	2.5	2.5	2.3	2.2	2.0	1.9
11Fe	13	2.5	2.5	2.5	2.4	2.3	2.0	1.9
O.T. 6	54	2.5	2.5	2.4	2.3	2.2	2.0	1.9
ATCC 13598	12	2.5	2.5	2.5	2.4	2.3	2.0	1.9
ATCC 13728	14	2.5	2.5	2.5	2.4	2.3	2.0	1.9
Tioga River	64	2.5	2.5	2.4	2.2	2.0	1.9	1.9
Agnew Lake	81	2.4	2.3	2.2	2.1	2.0	1.9	1.8

TABLE 6. *Bacterial combinations on coal sample no. 3 (D_p < 250 μm) with T. ferrooxidans and T. thiooxidans*

Strain combination		$\Delta[Fe]_T$ (g/liter) at 28 days	v_0 (mg liter^{-1} day^{-1})
T. ferrooxidans	*T. thiooxidans*		
ATCC 19859	None	0.65	47
ATCC 19859	UM	0.69	45
ATCC 19859	ATCC 8085	0.70	35
ATCC 19859	ATCC 19377	0.72	49
Tioga River	None	0.77	68
Tioga River	UM	0.73	65
Tioga River	ATCC 8085	0.75	68
Tioga River	ATCC 19377	0.72	43
Agnew Lake	None	0.83	78
Agnew Lake	UM	0.76	77
Agnew Lake	ATCC 8085	0.76	67
Agnew Lake	ATCC 19377	0.80	80

HCl and removal of the remaining coal and pyrite particles. Within experimental error, the total iron released and the filtered iron concentrations were identical, whereas, in experiments with pure FeS_2, depending on the pH and time of reaction, the soluble iron released varied from 0 to 65% of the total iron released. As the reactions proceeded and the pH decreased, the fraction of total iron in the solid phase also decreased.

DISCUSSION

Results of the parametric study of factors affecting the dynamics of microbial catalysis of the autoxidation of FeS_2 at a fixed P_{O_2} show that the rate and extent of leaching are a function of the total reactive surface area, the bacterial strain, the N/P molar ratio, the partial pressure of carbon dioxide, and the coal source.

The reaction rate was found to be virtually independent of the initial pH, the inoculum age, the substrate of the stock culture, and the source of inorganic nitrogen.

The lag time in batch reactors was found to be a function of the initial pH, the inoculum age, the stock culture substrate, the bacterial strain, the initial phosphate concentration, and the coal source.

The rate of oxidative dissolution of FeS_2 exhibited an apparent first-order dependence on total reactive surface of FeS_2:

$$\frac{-\mathrm{d}[FeS_2]}{\mathrm{d}t} \propto kA_s \tag{10}$$

Other investigators (32, 43–49) have shown that the rate and extent of microbial leaching are proportional to the initial surface area and pulp density of oxidizable metal sulfides. Usually an optimal pulp density in the range of 15 to 20% is observed for batch reactor systems. Similar results were observed in this study.

Silverman et al. (36), Nelson et al. (28), and Dugan and Apel (11) have reported that the oxidation rate of FeS_2 entrained in coal samples increased with decreasing particle size (i.e., increasing surface area for a fixed mass). Torma and co-workers (43, 46, 47) have shown that initial rate of microbial autoxidation of NiS, CoS, ZnS, CdS, and CuS by *T. ferrooxidans* can be expressed in terms of a modified Monod (23, 24) expression of the following form:

$$\mu = \frac{\mu_m A_s}{K + A_s} \tag{11}$$

where μ, μ_m, and K are the conventional terms for specific growth rate constant, maximum growth rate constant, and the half-saturation constant, respectively, and A_s represents the total initial surface area.

Based on experimental observations made at constant P_{O_2}, it is reasonable to express the rate of microbial autoxidation of FeS_2 entrained in coal in terms of the following differential equations:

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \frac{\mu_m A_s}{K + A_s} B \tag{12}$$

$$\frac{\mathrm{d}[Fe]_T}{\mathrm{d}t} = -\frac{\mathrm{d}[FeS_2]}{\mathrm{d}t} = \frac{\mu_m A_s}{K + A_s} \frac{B}{Y} \tag{13}$$

where A_s is the reactive surface area of FeS_2 or, for finely divided particles, the effective concentration of FeS_2; $[Fe]_T$ is the total concentration of iron released from FeS_2 at time t; μ_m is the maximum specific growth rate constant (t^{-1}); K is the concentration of FeS_2 supporting half-maximum growth; B is the concentration of active microorganisms; and Y is the cell yield coefficient defined by:

$$\frac{\mathrm{d}B}{\mathrm{d}t} = -Y \frac{\mathrm{d}[FeS_2]}{\mathrm{d}t} \tag{14}$$

This treatment assumes that the rate of oxidative dissolution of FeS_2 is catalyzed by the release of extracellular enzymes produced by *T. ferrooxidans*, the active catalytic agent in the reaction system. In addition, this development assumes that the concentration of enzymes is directly proportional to the bacterial biomass concentration. The bacterial growth rate according to Monod (24) for the ideal case of a pure bacterial culture and a single growth-limiting substrate can be expressed as:

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \mu B \tag{15}$$

where μ, in this particular case, would have the following form:

$$\mu = \frac{\mu_m [FeS_2]}{K + [FeS_2]} \tag{16}$$

Assuming that, under the experimental conditions of this study, the substrate concentration is nonlimiting (i.e., $[FeS_2]_0 >> K$ and $\mu = \mu_m$) such that B is not constant with time, equation 15 can be integrated from B_0, the microorganism concentration at time 0, to B, the concentration at time t. This integrated expression is substituted into equation 13 to give:

$$\frac{-\mathrm{d}[FeS_2]}{\mathrm{d}t} = \frac{\mathrm{d}[Fe]_T}{\mathrm{d}t} = \frac{\mu_m B_0 e^{\mu_m t}}{Y} \tag{17}$$

which can be integrated, in turn, from $[FeS_2]_0$ to $[FeS_2]_t$ and $t = 0$ to t to yield:

$$[FeS_2]_0 - [FeS_2]_t = \frac{B_0}{Y} (e^{\mu_m t} - 1) \tag{18}$$

Taking the logarithm of both sides of equation

18 and rearranging gives:

$$\ln\{([FeS_2]_0 - [FeS_2]_t) + B_0/Y\} = \ln(B_0/Y) + \mu_m t \quad (19)$$

or, when $e^{\mu_m t} > 1$ in equation 18:

$$\ln\{([FeS_2]_0 - [FeS_2]_t)\} \simeq \ln(B_0/Y) + \mu_m t \quad (20)$$

Graphical analysis of data obtained for a 10% slurry of coal no. 2, which was inoculated with *T. ferrooxidans* ATCC 19859, according to equation 20 is shown in Fig. 8. By least-squares analysis the equation for the apparent linear relationship was found to be $\ln\{\Delta[FeS_2]\} = 0.22\ t - 1.56$. Provided that the assumed conditions, $e^{\mu_m t} > 1$ and $K << [FeS_2]_0$, were satisfied, the slope of Fig. 8, 0.22, should be a reasonable estimate for μ_m. Alternatively, values for μ_m and K were obtained from experimental information shown in Fig. 9, where μ represents the slope of a $\ln[Fe]_T$ versus time relationship for the initial exponential rise in iron concentration with time for reactions in which $[FeS_2]_0$ was varied. The average value for μ in the asymptotic region was $0.22 \pm 0.2\ day^{-1}$. This value can be taken as μ_m. The half-saturation constant was estimated graphically to be in the range of 200 to 400 mg of FeS_2 per liter.

Using these values for μ_m and K, and making the following assumptions—(i) the initial total substrate concentration is equivalent to the total aqueous-phase iron concentration at infinite time according to mass balance considerations; (ii) the overall process of oxidative dissolution is autocatalytic either through microbial or chemical action; (iii) the cell mass concentration can be approximated by a simpler linear function of the concentration of iron released from FeS_2 at time t (i.e., $B \propto [Fe]_T$); (iv) the autoxidation of FeS_2 produces predominately Fe(II) and Fe(III) in soluble forms; and (v) when the solid substrate is finely divided, the reactive surface area can be expressed in terms of the concentration of FeS_2—equations 12 and 13 can be rewritten as:

$$\frac{dB}{dt} = \frac{d[Fe]_T}{dt} = \frac{\mu_m[FeS_2][Fe]_T}{K + [FeS_2]} \quad (21)$$

$$\nu_1 = \frac{-d[FeS_2]}{dt} = \frac{\mu_m[FeS_2][Fe]_T}{K + [FeS_2]Y} \quad (22)$$

Equations 21 and 22 were solved simultaneously by using a computer program based on the fourth-order Runge-Kutta method (17). A typical solution for a 10% slurry is shown in Fig. 10. Optimal values for K equal to 290 mg/liter and for Y equal to 0.9 were determined by sensitivity analysis. The calculated concentration-versus-time profile for the 10% slurry concentration agrees reasonably well with the experimental data. Variation of the K value has the net effect of shifting lag time horizontally along the abcissa of Fig. 10. Variation in the cell yield coefficient, Y, determines the value of the maximum extent of leaching given by $[Fe]_{T,\infty}$. Inhibition or stimulation of growth and substrate utilization can be modeled empirically by modifying K with appropriate terms that take into account the concentrations of influential metabolites, toxins, or deleterious surfaces.

Equation 13 would be consistent with a mechanism in which the rate-limiting step in the sequence is the oxidation of Fe(II) by O_2. Singer and Stumm (37) have postulated that the rate of autoxidation of Fe(II) at low pH limits the rate of autoxidation of FeS_2 in coal. In the ab-

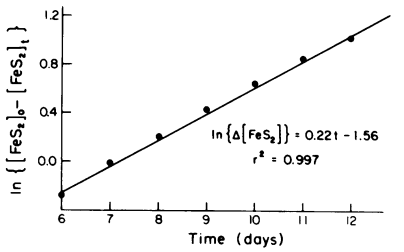

FIG. 8. *Graphical analysis of the integrated Monod relationship of equation 20 of the text for a 10% slurry of coal no. 2 (D_p < 74 μm) inoculated with T. ferrooxidans ATCC 19859.*

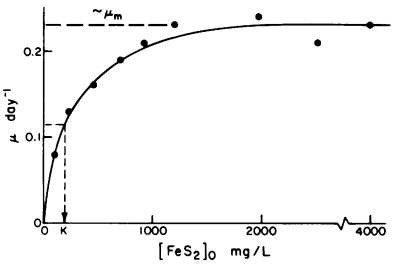

FIG. 9. *Estimation of the maximum specific growth rate constant and half-saturation constant for the autoxidation of FeS_2 in coal.*

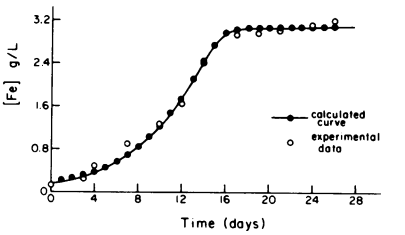

FIG. 10. *Mathematical model results for the oxidative dissolution of FeS_2 from a 10% slurry of Broken Aro no. 2 coal assuming that the rate is a direct function of bacterial growth. $\mu_m = 0.22\ day^{-1}$; $K = 290$ mg/liter.*

sence of catalytic influences, the rate law and rate constants determined by Huffman and Davidson (18) and George (15) at low pH, atmospheric pressure, and 30.5°C give a pseudo-first-order rate constant of approximately 10^{-5} day^{-1}. When this value is compared with the first-order constant ($\mu_m = 0.22$ day^{-1}) reported herein, it is clear that catalysis of Fe(II) autoxidation is needed to produce reaction rates observed in microbial systems.

In the absence of catalytic activity, as represented by the sterile control reactions shown in Fig. 11, a slow linear leaching of FeS_2 was observed. This slower oxidative dissolution reaction may represent the reaction of O_2 directly with the FeS_2 since the noncatalytic regeneration of Fe(III) under these conditions was prohibitively slow.

A mechanism consistent with linear release rates observed in control experiments can be proposed as follows:

$$FeS_2 + O_2 \underset{\leftarrow}{\overset{K_a}{\rightarrow}} FeS_2 - O_2 \quad (23)$$

$$FeS_2 - O_2 \underset{\text{slow}}{\overset{k}{\rightarrow}} FeS_2 - O_2^* \quad (24)$$

$$FeS_2 - O_2^* + O_2 \underset{\leftarrow}{\overset{K_a'}{\rightarrow}} FeS_2 \cdot 2O_2^\dagger \quad (25)$$

$$FeS_2 \cdot 2O_2^\dagger \overset{k'}{\leftarrow} Fe(II) + SO_4^{2-} + S^0 \quad (26)$$

where K_a is an equilibrium absorption constant for the rapid chemisorption of O_2 on reactive surface sites of FeS_2; k is the reaction rate constant for a rate-determining electron transfer and formation of a reactive surface complex; K_a' is the rapid attachment of an additional O_2 molecule to the reactive surface site; and k' is the rate constant for the irreversible loss of Fe(II) and the oxidized sulfur species, SO_4^{2-} and S^0, from a surface site. This proposed scheme is consistent with the experimental results of McKay and Halpern (22) for the autoxidation of FeS_2 at elevated temperatures and partial pressures of O_2. They determined a reaction rate law of the following form:

$$r_2 = -\frac{d[FeS_2]}{dt} = kA_{FeS_2}P_{O_2} \quad (27)$$

where A_{FeS_2} is the macroscopic surface area of FeS_2 and P_{O_2} is the partial pressure of O_2. The experimentally determined value for k as a function of temperature was reported as:

$$k = 0.125 \exp\{-13{,}300/RT\} \quad (28)$$

with units of moles per liter per centimeter squared per atmosphere per minute. At 25°C, the rate constant k is 2.1×10^{-11} mol cm^{-5} atm^{-1} min^{-1}. With A_{FeS_2} and P_{O_2} constant, k can be reduced to a pseudo-zero-order constant of $k^0 = 2.5 \times 10^{-4}$ mol liter^{-1} day^{-1} for $A = 540$ cm^2/g and 0.66 mol of FeS_2 per liter (8% slurry) or, equivalently, $k^0 = 14$ mg liter^{-1} day^{-1} released to solution as iron. This value can be compared with linear release rates observed in this study for sterilized coal samples ranging from 15 to 18 mg liter^{-1} day^{-1} at a 10% slurry concentration.

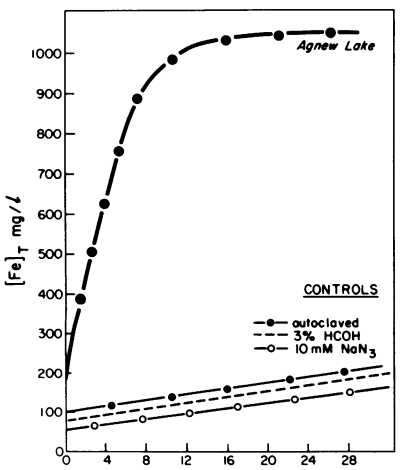

FIG. 11. *Effect of sterilization method on the rate of oxidative dissolution of FeS_2 in inoculum-free samples of Broken Aro no. 2 coal.*

Equation 27 above predicts a first-order dependence of the control flask leaching rate on active surface area which initially seems to be contrary to the observed linear kinetics. Inherently, it should be predicted that as leaching takes place there should be a corresponding reduction in the mass of the FeS_2 present per liter and a progressive reduction in the observed reaction rate. However, if there is a simultaneous reduction in particle size or an increase in surface roughness as observed by some investigators (8, 27), there will be a compensating increase in specific surface area, as predicted by equation 9, and therefore a net linear rate should be observed.

Results of this study show that bacterial desulfurization of various coal samples, which are relatively high in pyritic sulfur, is a potentially viable means for reduction of the total sulfur content before combustion. Removal of 90 to 98% of FeS_2 from some eastern coals can be achieved within a reasonably short time-frame of 8 to 12 days when the slurry concentration or pulp densities are ≤20% and the particle size fractions have a D_p of ≤74 μm. Increased pulp densities and larger particle size fractions result in slower leaching rates and reduced extents of leaching. This problem can be overcome, as shown by Atkins (5), by suspending the reactive

solids in a fresh medium with a new inoculum of *T. ferrooxidans*.

The most effective strains of *T. ferrooxidans* were those that were isolated from natural systems. *T. ferrooxidans* ATCC 19859 was clearly the most effective of five pure strains tested. Addition of equal volumes of *T. thiooxidans* to *T. ferrooxidans* cultures had no effect on either the leaching rate or extent of leaching, although some enhancement in the rate of appearance of sulfate was detected for mixed cultures containing *T. thiooxidans*.

Over the course of a 2-year experimental program it was observed that the lag time in successive experiments was reduced gradually from 6 days to less than 1 day. This effect may be due in part to bacterial strains that were adapted to a coal-pyrite medium. During this extended period of successive 7-day transfers the strains may have evolved genetically such that longer adaption periods or lag times were no longer necessary.

From a practical standpoint, it was discovered that high phosphate concentrations were inhibitory. This effect had been previously reported by Beck (7). The most effective nutrient media contained relatively low phosphate concentrations with an N/P molar ratio of 90:1. Phosphate inhibition at low pH may be due to the formation of iron phosphate precipitates on the reactive surface of FeS_2. The saturation index for both $FePO_4 \cdot 2H_2O$ and $Fe_3(PO_4)_2 \cdot 18H_2O$ was exceeded for reactions in which there were elevated concentrations of orthophosphate as determined by equilibrium computations using the program REDEQL2 (26). These results suggest that minimal nutrient additions would be required for a commercial desulfurization process provided that the waters used for slurry preparation had moderate background concentrations of the essential nutrients.

Reaction rate laws for the autoxidation of FeS_2 in coal as determined in this study may be used to predict relative FeS_2 conversion rates or O_2 depletion rates in either a plug-flow or continuous-flow stirred-tank reactor. Proposed coal slurry pipelines, in which coal and FeS_2 particles may have a median D_p of approximately 180 μm (21), may be used as biological reactors for which the volume required to achieve a certain degree of treatment will be given by the following steady-state expression (2):

$$V_{PFR} = Q \int \frac{d[Fe]_T}{\nu_T} \qquad (29)$$

where V_{PFR} is the differential volume element in the plug-flow reactor, Q is the volume rate of flow, and $\nu_T = \nu_1 + \nu_2$. Similarly, the volume required for the continuous-flow, stirred-tank reactor under steady-state conditions will be given by:

$$V_{CFR} = \frac{Q([Fe]_{T,e} - [Fe]_{T,0})}{\nu_T} \qquad (30)$$

where $([Fe]_{T,e} - [Fe]_{T,0})$ is the concentration difference between the influent and effluent streams.

In general, for reaction rate laws greater than zero-order the volume of water required to achieve the same extent of reaction in a plug-flow reactor will be significantly less than that in a single continuous-flow reactor (3). However, a major drawback to a pressure conduit slurry pipeline reactor will be the need for numerous reaeration stations as predicted from the overall stoichiometry of equation 1. These problems and comparative models for different reactor configurations will be addressed in a sequel to this paper, which will focus on a reaction network model incorporating stepwise reaction mechanisms.

ACKNOWLEDGMENTS

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