Kinetics of Biodesulfurization of a High-Sulfur Coal

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ABSTRACT

The apparent activation and deactivation energies and the corresponding frequency factors of coal desulfurization by *Thiobacillus ferrooxidans* have been determined to be $\Delta E_a = 60.9$ kJ, $A_a = 1.45$ s^{-1} and $\Delta E_d = 178.3$ kJ, $A_d = 5.65 \times 10^{27}$ s^{-1}, respectively. The thermodynamic values ($\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$) of the activated complex were calculated. Kinetic parameters of the Monod equation were determined to be $V_m = 55.9$ mg dm^{-3} h^{-1} and $K = 24.1\%$ pulp density. The maximum rate of desulfurization of coal was found to be $V_m = 55.7$ mg dm^{-3} h^{-1} for the particle size. The generalized second order regression equation relating the yield of desulfurization to the leaching parameters was shown to adequately predict coal extraction data and optimum values of process variables. Tank leaching studies using optimum conditions resulted coal desulfurization about 90%. The iron hydrolysis reactions involving the formation of mono- and polynuclear, hydroxo- and sulfato complexes of amorphous and crystalline precipitates were discussed.

Index Entries: Coal desulfurization; *Thiobacillus ferrooxidans*; hydrolysis.

INTRODUCTION

Concerns about the environmental effects of emissions (sulfur dioxide, carbon dioxide, nitrogen oxides, and solid submicron size inorganic matter) for the combustion of coal continued to increase in the last dec-
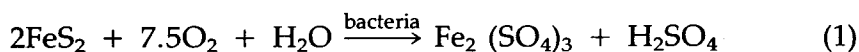
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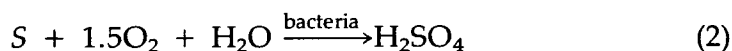†Current Address: St. George Mining Company, St. George, UT 84770

ade (1). There is agreement with regards to the emission of pollutants to the atmosphere that should be limited in accordance with the existing air quality standards. The most effective method to succeed will be the one that is technically sound and economically viable. A variety of chemical and physical coal desulfurization processes have been suggested (2,3). Within these processes a new approach is emerging that deals with the desulfurization of coal by biomediated reactions.

Coal is not a homogenous material. It is of fossil origin and formed during definite geological periods (4) from arrested decay of the remains of trees and other form of plant life (5). The mineral inclusions (predominantly silicates and pyrite) are responsible of the ash content of coal. Sulfur in coal is present in two major forms: inorganic sulfur (pyrite, elemental sulfur, metal sulfides, and sulfates), and organic sulfur (benzothiophenes, disulfide joining cyclic structures, and alkyl sulfide attached to either an aromatic or aliphatic group).

Microbial removal of inorganic sulfur from coal can be represented by the following reactions (6–8).

$$2FeS_2 + 7.5O_2 + H_2O \xrightarrow{bacteria} Fe_2 (SO_4)_3 + H_2SO_4 \qquad (1)$$

and

$$S + 1.5O_2 + H_2O \xrightarrow{bacteria} H_2SO_4 \qquad (2)$$

There is evidence that mixed cultures (9), mesophilic (10–12), and thermophilic (13–15) bacteria may be involved in the solubilization of inorganic sulfur content of coal. However, the microbial extraction of organic sulfur from coals is a difficult task and opinions are far from being uniform (16). Very often it is not known exactly how the sulfur is incorporated in the structure of coal (17). Certain soil microorganisms (18) and thermophilic bacteria such as *Sulfolobus acidocaldarius* (19,20) were found to be effective in removing organic sulfur from coal. Microbial degradation of sulfur-containing heterocyclic compounds of coal samples by bacteria was reported to have promising potential for desulfurization (21). Although the studies on inorganic sulfur removal are well advanced and extractions as high as 90–95% have been achieved, the information regarding the removal of organic sulfur content of coal by microorganisms is preliminary in nature and further studies are needed for a better understanding of these phenomena.

Bacterial leaching coupled with flotation technique has also been proposed for the removal of pyrite fractions from coal. This process consists of subjecting pulverized coal to bacterial attachment in acid media for a short period of time, then raising the pH and floating the coal by addition of oil-agglomeration flotation agents. It was suggested that during the treatment bacteria adsorb on pyrite surfaces and the surface property of coal altered favorably for flotation. The mechanism of the process

is not completely understood. However, pyrite removal from coal as high as 60–90% have been reported (24,25).

The present investigation provides information on the kinetics of biodesulfurization of a high-sulfur coal and on the formation of different iron precipitates associated with the process.

MATERIALS AND METHODS

Organisms

A culture of *Thiobacillus ferrooxidans* (26) originally isolated from mine waters was routinely maintained in our laboratory on an iron-free medium (27) in which the energy source, ferrous ion, was replaced by the pulverized coal sample. When growth reached the late logarithmic phase, an aliquot of the leach suspension was transferred into a new medium to maintain the stock culture of bacteria or this was used as an experimental inoculum.

Coal Sample

A hand-picked high-sulfur coal sample was obtained from Central Ohio Coal Company, was ground to particle size less than 37 μm and homogenized by coning and quartering four times. It contained 3.7% total iron, 5.01% S_{total}, which was composed of 4.11% S^{2-}, 0.12% SO_4^{2-}, and 0.78% organic sulfur. The mineralogical X-ray diffraction analysis indicated the sulfide content was pyrite and sulfate gypsum.

Leaching Experiments and Kinetic Determinations

The 250 cm^3 Erlenmeyer flasks were charged with varying amounts of coal samples, 70 cm^3 nutrient (iron-free) medium (27) and 5 cm^3 bacterial inoculum. In sterile controls, the bacterial inoculum was replaced by 5 cm^3 of a 2% thymol in alcohol solution. To monitor the iron extraction from coal, one cm^3 samples were removed from the swirling flasks periodically. The sample vols were replaced with one cm^3 iron-free nutrient medium. The leaching of coal-pyrite gives rise to insoluble reaction products, therefore a one cm^3 of $5N$ HCl was added to the analytical samples. These were kept for 30 min at room temperature, to dissolve iron precipitates, and after dilution and filtration, the samples were analyzed for total iron content using an atomic absorption spectrophotometer (28,29).

Tank leaching experiments were carried out in 15 dm^3 reactor equipped with control of Eh, pH, temperature, agitation (rpm), and dissolved oxygen concentration (30). The reactor was charged with 1.75 kg of pulverized coal (particle size less than 37 μm), 9.5 dm^3 nutrient medium (27), and 0.5 dm^3 bacterial inoculum. Periodically samples were re-

moved from the leach suspension and analyzed for dissolved total iron content.

The rate of iron (sulfur) removal from coal samples was determined as the slope of the straight line portion of the leaching curves representing the dissolved iron concentration as a function of time using a linear least squares regression analysis technique (31). The direct linear plot technique (32) was used to estimate the kinetic constant of Monod equation. The particle size effect was described using an empirical exponential equation.

$$V = V_m \exp(-Bd) \tag{3}$$

where V_m is the theoretically attainable maximum rate of coal biodesulfurization, B is a proportionality constant, and d is the particle size of coal size fractions. Optimization of the effect of process variables was done for the final desulfurization of coal (percent of pyrite-iron removal) using a statistical design-kinetic evaluation approach (33) and a second-order equation.

$$FY = b_o + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i<j} b_{ij} X_i X_j + e \tag{4}$$

where the b-values are the regression coefficients, X-values are the leach variables, n is the number of variables, and e is the error term. The apparent activation (ΔE_a) and deactivation (ΔE_d) energies and the corresponding frequency factors (A_a and A_d) have been derived from the effect of temperature using the linearized Arrhenius equation (31).

$$\ln k = \ln A - \Delta E_{a,d}/RT \tag{5}$$

where k is the overall reaction rate constant, R is the universal gas constant, and T is the absolute temperature.

RESULTS AND METHODS

Throughout this study the total dissolved iron concentration has been used as a measure for the removal of inorganic sulfur content from coal. This assumption is reasonable since the iron concentration corresponds to the pyrite composition that makes up almost the total inorganic sulfur content of coal. Furthermore, all shake flask experiments have been carried out in double runs for the inoculated experiments and the average value of these measurements has been used for kinetic determinations. The sterile controls were done in single runs, which resulted in about 5 to 14 times less iron extractions than the inoculated ones. Therefore, the sterile control data will not be detailed.

SHAKE FLASK EXPERIMENTS

Effect of pH

The influence of pH on pyrite removal from coal was studied in the range of pH values varying from 1–4 with leach suspensions containing 10% solids and the data are summarized in Table 1. The optimum pH appears to be at 2.0. This value is in good agreement with the findings of other investigations (29,34) for sulfur removal from coal by microbial leaching. This value (pH = 2.0) has been used in the further experiments as the initial pH.

It was found that when coal first contacted with nutrient medium and the pH of the suspension stabilized at pH = 2.0 with dilute sulfuric acid, then the inoculation with *T. ferrooxidans* resulted in immediate growth. This way of proceeding was beneficial and eliminated the formation of lag time. These findings are in good agreement with the studies of other investigators on the pH effect (37).

Effect of Temperature

The influence of temperature was studied with 20% pulp density leach suspensions in the range of temperatures 20–45°C. The data were found to follow a shrinking core model (35).

$$1 - (1 - R)^{1/3} = (k_1 C / r_0 \rho) \, t = kt \qquad (6)$$

where R is the fractions of pyrite (in coal) reacted, k_1 is the reaction rate constant, C is the acid concentration, r_0 is the initial particle size, ρ is the density of coal, k is the overall reaction rate constant, and t is the time of
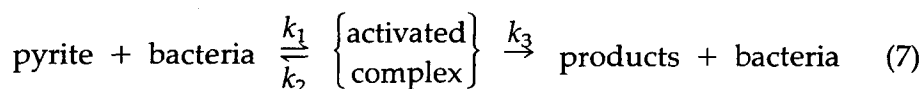
Table 1
Influence of pH on Iron Extraction from Coal

		Iron removal	
pH_i	pH_f	Rate, mg dm^{-3} h^{-1}	Final yield,[a] %
1.0	1.8	5.04	40.25
1.5	1.9	9.62	64.43
2.0	1.9	11.31	72.83
2.5	1.9	9.99	69.19
3.0	2.0	9.41	62.46
3.5	2.1	8.80	58.75
4.0	2.1	8.18	47.90

[a]The final yield was calculated after 8 d of leaching.

leaching. By plotting $1 - (1 - R)^{1/3}$ vs t for each temperature, straight lines have been obtained with slopes equal to the k-values. Using these k-values in the Arrhenius plot (36) as shown in Fig. 1, the apparent activation energy $\Delta E_a = 60.9$ kJ and $A_a = 1.45$ s^{-1} were derived for the temperature range of 20–35°C and $\Delta E_d = 178.3$ kJ and $A_d = 5.65 \times 10^{27}$ s^{-1} for 35–45°C, respectively. The activation energy of 60.9 kJ suggests that the removal of pyrite from coal was not diffusion controlled. The relatively high value of 178.3 kJ for the deactivation energy is in the order of magnitude of ΔE_d-values for protein denaturation reactions. It represents the thermal death of *T. ferrooxidans* in the range of 35–45°C.

The bacterial oxidation of pyrite in coal can be considered to proceed through the formation of an activated complex (36).

$$\text{pyrite} + \text{bacteria} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \begin{bmatrix} \text{activated} \\ \text{complex} \end{bmatrix} \overset{k_3}{\rightarrow} \text{products} + \text{bacteria} \qquad (7)$$

where k_1, k_2, and k_3 are reaction rate constants for the formation and decomposition of the activated (coal–bacteria) complex, and for the formation of products, respectively. Application of the transition state theory on the experimental data of biodesulfurization of coal allows the determination of thermodynamic values ($\Delta G^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta H^{\ddagger}$) for the formation of the activated complex. It is assumed that $k_1 \gg k_2$ and $k_3 \approx k$ (experimental reaction rate constant). Therefore, k can be related to the free energy change ($\Delta G^{\ddagger}$) of the activated complex by

$$k = (k^* T/h) \exp(-\Delta G^{\ddagger}/R) \qquad (8)$$

where $k^* = 1.381 \times 10^{-23}$ JK (Boltzmann constant), $h = 6.626 \times 10^{-34}$ Js (Planck's constant), and $R = 8.314$ JK^{-1} mol^{-1}. The entropy change of activation ($\Delta S^{\ddagger}$) is related to the frequency factor (A_d) by the following expression (29).

$$A = 2.72 \, (k^* T/h) \exp(\Delta S^{\ddagger}) \qquad (9)$$

The enthalpy change of activated complex ($\Delta H^{\ddagger}$) differs slightly from the experimental energy of activation (ΔE_a) according to

$$\Delta H^{\ddagger} = \Delta E_a - nRT \qquad (10)$$

However, the application of Eq. (10) requires that the exact value of n must be known. Since n is an unknown quantity for the bioleaching of coal-pyrite, an alternative approach can be used for the determination of $\Delta H^{\ddagger}$, given by

$$\Delta H^{\ddagger} = \Delta G^{\ddagger} + T\Delta S^{\ddagger} \qquad (11)$$

Using the experimentally determined apparent activation energy, frequency factor, reaction rate constant ($k = 4.4269 \times 10^{-3}$ s^{-1}) at temp 303°K, and Eq. (8–11), the thermodynamic values for the activated com-
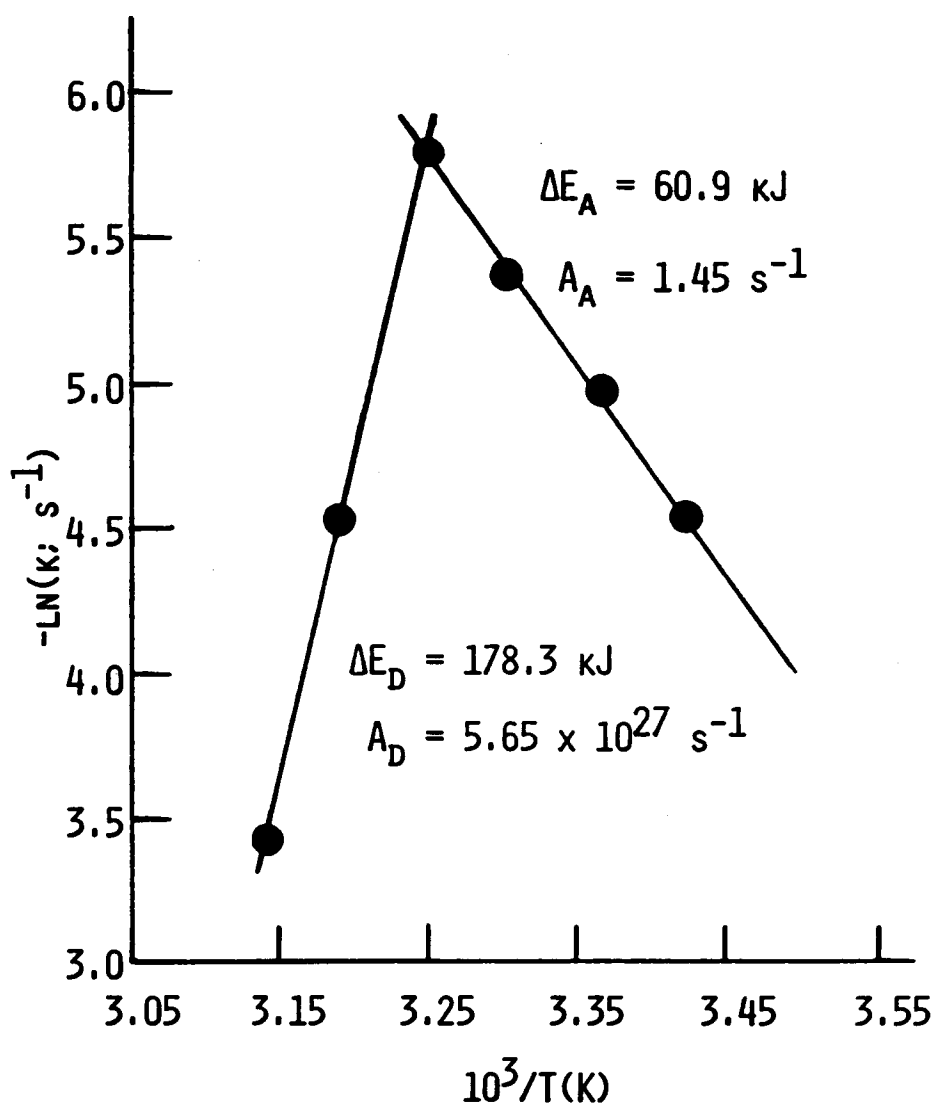
Fig. 1. Arrhenius plot.

plex have been calculated to be: $\Delta G^{\ddagger} = 86.6$ kJ, $\Delta S^{\ddagger} = -246.5$ JK^{-1}, and $\Delta H^{\ddagger} = 11.9$ kJ.

If the reaction involves a considerable loss of entropy when the activated complex is formed, then $\Delta S^{\ddagger}$ is negative, as is the case for microbial desulfurization of coal. As a consequence, the expression: $\exp(\Delta S^{\ddagger}/R)$ and the frequency factor, A, will be relatively small. During metabolism, bacterial enzymes undergo interaction with substrate molecules. The reactions involve electrically charged species that may have identical or opposite charges, with a resulting effect on the entropy of activation. When charges of interaction species are of the same electrical charge, there is a much greater loss in the entropy of the system and $\Delta S^{\ddagger}$ is negative. This

means that the activated complex has a larger charge than the reacting species alone. This reasoning is in good agreement with previous findings (29,30), however, more studies are needed to elucidate this phenomenon.

Effect of Pulp Density

The effect of substrate concentration was studied with leach suspensions containing 5–30% pulp density (mass of coal sample/total volume of leach medium). The data are presented in Fig. 2. The observations (rate of iron removal versus pulp density) are plotted as lines in parameter space (32). The kinetic parameters are estimated directly from the coordinates of Fig. 2 to be $V_m = 55.9$ mg dm^{-3} h^{-1} and $K = 24.1\%$ pulp density. The advantage of this technique (32) over the traditional evaluation methods is that this is very simple to construct, because it is composed entirely of straight lines and requires no calculation or mathematical tables (30). The rate of desulfurization of coal with respect to effect of the pulp density can be given by

$$V = 55.9[PD]/(24.1 + [PD]) \qquad (12)$$

The highest experimental rate of 33.4 mg dm^{-3} h^{-1} was obtained with a 35% pulp density suspension.
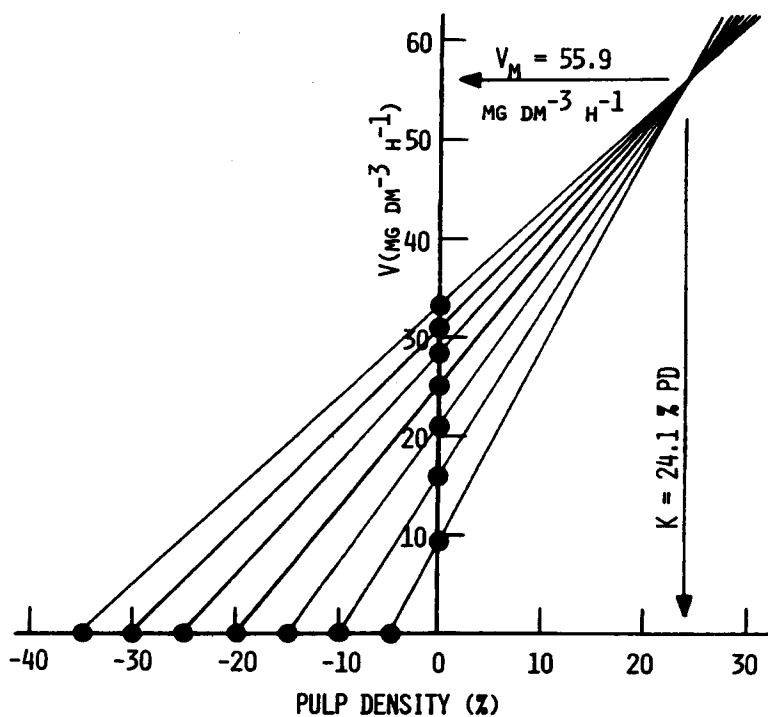

Fig. 2.   Direct linear plot for estimation of kinetic parameters of Monod Eq. (32).

Effect of Particle Size

This series of experiments was carried out with 25% pulp density suspensions at 35°C. The linearized form of Eq. (3) is presented in Fig. 3 as a semilogarithmic plot. The maximum rate (V_m) is obtained from the intercept of the straight line with the ln V-axis, while the proportionality constant (b) was determined as the slope of the straight line. Accordingly, the rate of biodesulfurization can be given by

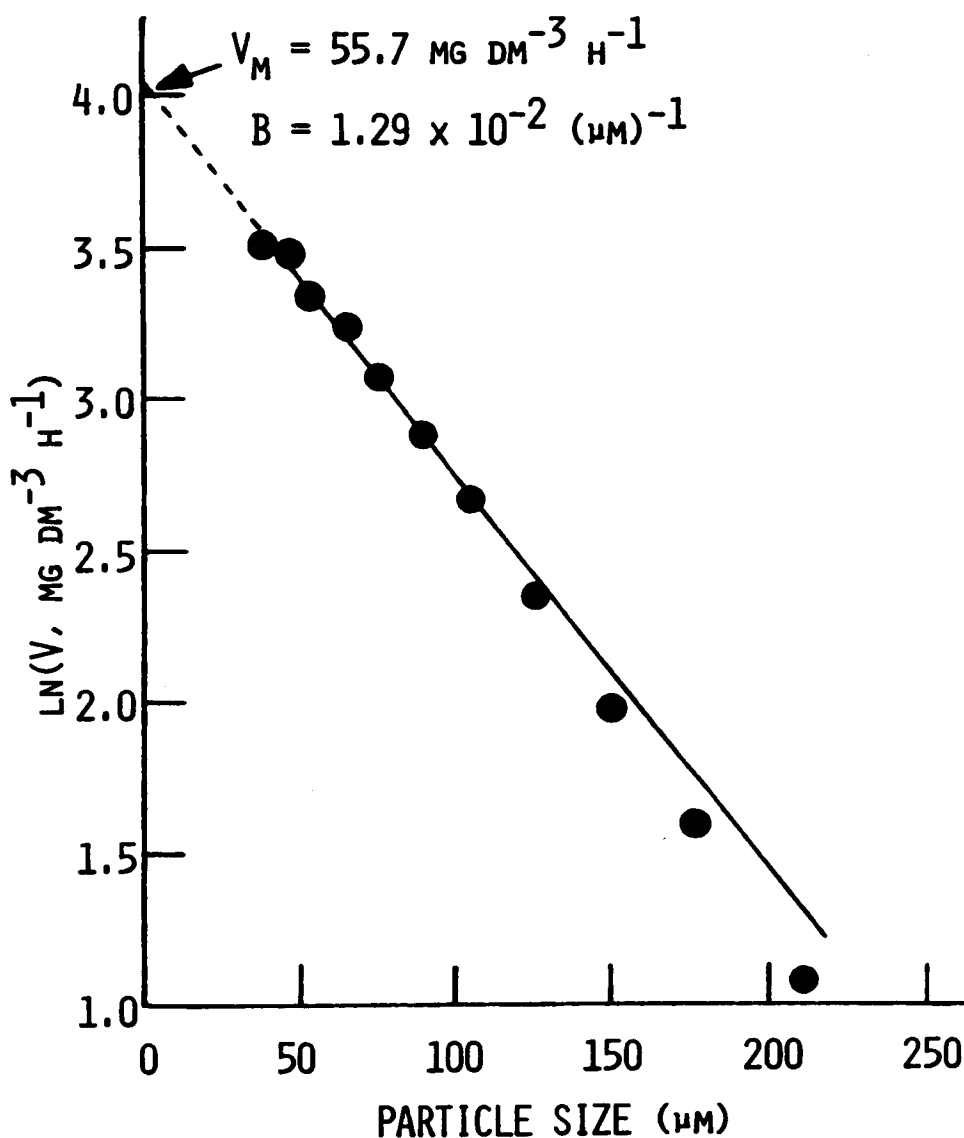$$V = 55.7 \exp (1.29 \times 10^{-2} \times d) \tag{13}$$

Fig. 3.   Effect of particle size on biodesulfurization of coal.

The slight deviation from the straight line behavior in the particle size range of 105–210 μm is expected since at larger particle size not all pyrite may have been equally exposed to bacterial action.

An excellent agreement exists regarding the maximum rate of coal desulfurization obtained in the pulp density (V_m = 55.9 mg dm^{-3} h^{-1}) and particle size (V_m = 55.7 mg dm^{-3} h^{-1}) experiments. The highest experimental rate realized in the particle size experiments was 35.5 mg dm^{-3} h^{-1} which was obtained with 37 μm particle size fraction.

Optimization of Process Variables

The final yield (Y in %) of total iron removal from the coal samples (unfractionated, particle size less than 37 μm) by bacterial action after 6 d of leaching has been optimized for the effects of X_1 = pH, X_2 = particle size, X_3 = pulp density, and X_4 = temperature. The equation derived by multiple linear regression analysis applied to the experimental data is

$$Y = 80.48 - 21.38X_1^2 = 6.08 \times 10^{-4}X_2^2 - 0.09X_3^2 - 0.11X_4^2$$
$$+ 2.98X_1X_2 - 0.05X_2X_3 + 0.12X_3X_4 \qquad (14)$$

with R^2 = 0.86 and the overall F value = 27.86, meaning the regression coefficients in the model are significantly different from zero. For this study, the particle size fractions varied from 37–600 μm. The experimental data are compared with the calculated (fitted) yield (FY in %) that was calculated by Eq. (14) in Table 2. The optimum values of the four process variables have been obtained maximizing Eq. (14) and using modification of a computer code in FORTRAN (38). The experiments were conducted in the range 1 ≤ X_1 ≤ 4, 37 ≤ X_2 ≤ 600, 5 ≤ X_3 ≤ 35, and 20 ≤ X_4 ≤ 45. The optimum values are pH = 2.2, initial particle size = 37 μm, pulp density = 16%, and temperature = 35°C. These reported optimum values are in good agreement with those for a different kind of high-sulfur coal bioleaching (29).

Large-Scale Experiments

Using optimum values of leach variables determined by the linear regression analysis method from the data of shake flask experiments, larger-scale tests were performed in sterile and inoculated runs. The results are shown in Fig. 4. After ten days of leaching the total iron removal in the inoculated run was about 91%, while in the sterile control this was only 17%. These extraction data are in good agreement with those reported for pipe reactor leaching experiments (41).
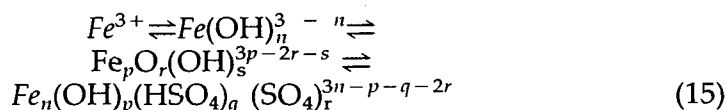
Iron Precipitates

A portion of the leached coal (leach residue) was washed with distilled water twice, dried at room temperature, then submitted for X-ray analysis. It was found that iron, which is solubilized from the coal according to Eq. (1), is partially precipitated onto the coal in well crystal-

Table 2
Comparison of Observed (Y) and Predicted (FY)
Yield (%) for the Effect of Process Variables
on Biodesulfurization of Coal

X_1, pH	X_2, μm	X_3, PD%	X_4, °C	Y, %	FY, %
1.0	37	10	35	40.25	44.57
1.5	37	10	35	68.43	70.01
2.0	37	10	35	92.83	84.76
2.5	37	10	35	89.19	88.82
3.0	37	10	35	62.46	62.17
3.5	37	10	35	58.75	64.86
4.0	37	10	35	47.90	36.86
2.0	600	25	35	10.78	18.72
2.0	425	25	35	16.41	14.91
2.0	212	25	35	20.44	23.25
2.0	150	25	35	31.43	40.94
2.0	75	25	35	44.24	68.59
2.0	54	25	35	69.04	77.56
2.0	45	25	35	84.77	81.57
2.0	37	25	35	92.83	84.76
2.0	37	5	35	94.68	91.17
2.0	37	10	35	92.83	84.76
2.0	37	15	35	92.14	85.99
2.0	37	20	35	92.09	82.89
2.0	37	25	35	74.26	75.44
2.0	37	30	35	61.34	63.65
2.0	37	35	35	47.49	47.50
2.0	37	20	20	44.27	47.34
2.0	37	20	25	59.43	64.71
2.0	37	20	30	79.41	76.56
2.0	37	20	35	92.09	86.89
2.0	37	20	40	88.75	83.71
2.0	37	20	45	70.65	78.99

lized forms including hydroxide, hydroxy-oxide, and sulfate. These iron precipitates did not impede bacterial activity, which is in good agreement with our previous results on pyrite oxidation by microorganisms (39).

The microbial oxidation of ferrous to ferric ion immediately induces hydrolysis unless selective ligands bind ferric ion by forming mononuclear [Fe(ligands)$_n^3$ $^-$ ^{n}] or polynuclear [Fe$_n$ (ligands)$_r^{3p}$ $^-$ ^{r}] complexes. In the bioleaching media, the ligands may include hydroxyl, sulfate, and hydrogen sulfate species, and there is a continuous competition between these ligands for binding the ferric ion until an equilibrium is established, as shown in the next equation.
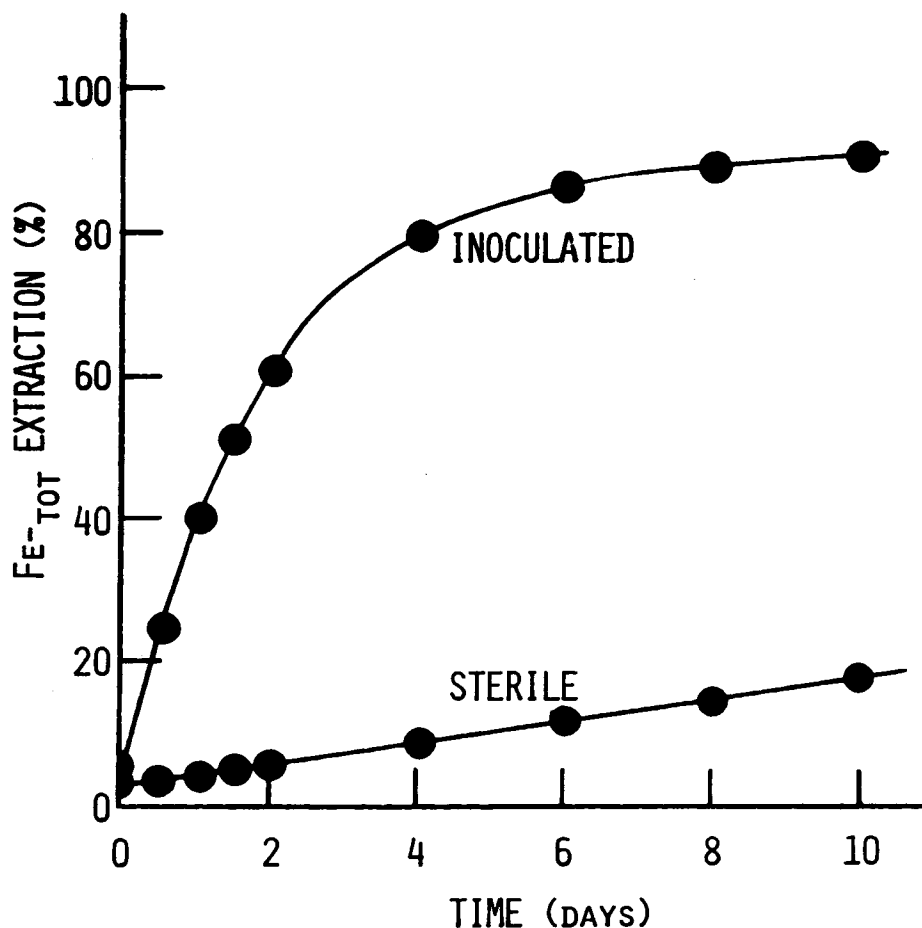
$$Fe^{3+} \rightleftharpoons Fe(OH)_n^3 \ ^- \ ^n \rightleftharpoons$$
$$Fe_pO_r(OH)_s^{3p-2r-s} \rightleftharpoons$$
$$Fe_n(OH)_p(HSO_4)_q \ (SO_4)_r^{3n-p-q-2r} \tag{15}$$

Fig. 4. Tank leaching experiments for the desulfurization of coal by *Thio-bacillus ferrooxidans*.

Eq. (15) represents three distinctly different possibilities (40).

a. No polynuclear species persist while the equilibria are shifted.

b. The acidity of the bulk solution is too low to bring about acid decomposition of the stochastically formed polynuclear aggregates.

c. Nuclei have been generated that induce the growth of crystalline polynuclears in solution.

Situation b. will lead to amorphous product formation and should be avoided. If the compact amorphous iron precipitates are deposited on the surface of coal-pyrite, then bacterial activity will be limited by the diffusion phenomena. Situation c. induces the formation of crystalline polynuclear iron precipitates, which have high porosity that will not impede the metabolic activity of microorganisms.

In batch cultures the accumulation of iron in the leach media is unavoidable and it leads to iron precipitations. The pH to a certain extent can control the iron precipitation. By reducing the pH, for example, to below 2.0 and by keeping Eh below 500 mV, more iron is kept in solution, but above a total iron content of 2 g dm^{-3} iron precipitation will begin. For coal desulfurization, it would be important to remove all sulfur content with the leach solution and to avoid partial reprecipitation of sulfur content with the ferric iron precipitates. This condition can be achieved, especially when the bioleaching is carried out in a continuous system with well-established conditions to avoid iron precipitation.

CONCLUSIONS

The efficiency of *T. ferrooxidans* in desulfurization of a high-sulfur-content coal has been demonstrated. The kinetic effects of process variables were assessed using phenomenological and statistical computational techniques. The mechanism of biodesulfurization proposed to involve the formation of an intermediate activated complex. Other investigators (42,43) have developed similar kinetic approaches, in which adsorption and desorption of bacteria onto coal-pyrite surface was considered. The larger scale experiments support previous findings (7,37,44) related to industrial applicability of the biodesulfurization process.

ACKNOWLEDGMENTS

REFERENCES

1. Markuszewski, R., and Blaustein, B. D., eds. (1986), *Fossil Fuels Utilization*, ACS Symp., Ser. 319, Am. Chem. Soc., Washington, DC, pp. 2–85.
2. Wheelock, T. D. (1977), *Coal Desulfurization, Chemical and Physical Methods*, ACS Symp. Ser. 64, Am. Chem. Soc., Washington, DC, p. 132.
3. Meyers, R. A. (1977), *Coal Desulfurization*, Dekker, NY, p. 254.
4. Misagi, F. L. (1973), *SME Mining Engineering Handbook*, 5th ed., Soc. Min. Eng., Inc., NY, pp. 50–53.
5. Perry, R. H., and Chilton, C. H. (1973), *Chemical Engineers Handbook*, 5th ed., McGraw-Hill, NY, ch. 9, pp. 3–9.

6. Hoffman, M. R., Faust, B. C., Panda, F. A., Koo, H. H., and Tsuchiya, H. M. (1981), *Appl. Envir. Microbiol.* **42**, 295.
7. Dugan, P. R. (1986), *Biotechnol. Bioeng. Symp.*, **16**, 185.
8. Kawatra, S. K., Eisle, T. C., and Bagley, S. (1987), *Coal Desulfurization by Bacteria*, Repr. No. 87–133, Soc. Mining Eng., Littleton, CO, pp. 1–7.
9. Dugan, P. R. (1984), *Microbial Chemoautotrophy*, Strohl W. R., and Tuovinen, O. H. eds.), Ohio State Univ. Press, Columbus, OH, pp. 3–9.
10. Olsen, T. M., Ashman, P. R., Torma, A. E., and Murr, L. E., (1980), *Biogeochem. of Ancient and Modern Envir.* (Trudinger, P. A., Walter, M. R., and Ralph, B. J., eds.), Springer Verlag, West Berlin, pp. 693–703.
11. Kargi, F. (1982), *Biotechnol. Bioeng.*, **24**, 749.
12. Bos, P., Huber, T. F., Kos, C. H., Ras, C., and Kuenen, J. G. (1986), *Fundamental and Applied Biohydrometallurgy* (Laurence, R. W., Branion, R. M. R., and Ebner, H. G., eds.), Elsevier, Amsterdam, pp. 129–150.
13. Kargi, F., and Robinson, J. M. (1982), *Biotechnol. Bioeng.* **24**, 2115.
14. Kargi, F., and Robinson, J. M. (1985), *Biotechnol. Bioeng.* **27**, 41.
15. Murr, L. E., and Mehta, A. P. (1982), *Biotechnol. Bioeng.* **24**, 743.
16. Finnerty, W. R., and Robinson, M. (1986), *Biotechnol. Bioeng. Symp.*, **16,** 205.
17. Kargi, F. (1982), *Enzyme Microb. Technol.* **4**, 13.
18. Mishra, A. K., Roy, P., Roy-Makapatra, S. S., and Chandra, D. (1983), *Rec. Prog. in Biohydromet.*, (Rossi, G., and Torma, A. E., eds.), Associazione Mineraria Sarda, Iglesias, Italy, pp. 491–510.
19. Kargi, F., and Robinson, J. M. (1984), *Biotechnol. Bioeng.*, **24**, 687.
20. Kargi, F., and Robinson, J. M. (1986), *Fuel*, **65**, 397.
21. Klubec, B., and Clark, D. (1986), Final Report of US Dept. of Energy. Contract No. DE-FC01-83Fe60339, S. IL. Univ., Carbondale, IL, pp. 1–19.
22. Kempton, A. G., Moneib, A., McCready, R. G. L., and Capes, C. E. (1980), *Hydromet.*, **5**, 117.
23. Doddema, H. J. (1983), *Rec. Prog. Biohydromet.* (Rossi, G., and Torma, A. E., eds.), Associazione Mineraria Sarda, Iglesias, Italy, pp. 467–478.
24. Attia, Y. A., and ElZeky, M. A. (1985), *Proc. and Util. of High Sulfur Coals* (Attia, Y. A., ed.), Elsevier, Amsterdam, pp. 673–682.
25. Dogan, Z. M., Ozbayoglu, O., Hicyilmaz, C., Saridaya, M., and Ozcengiz, G. (1986), *Fund. and Appl. Biohydromet.* (Laurence, R. W., Branion, R. M. R., and Ebner, H. G., eds.), Elsevier, Amsterdam, pp. 165–170.
26. Torma, A. E., and Gundiler, I. H. (1986), *First Int. Min. Proc. Symp.*, vol. 2, Mineral Processing, Izmir, Turkey, pp. 601–612.
27. Silverman, M. P., and Lundgren, D. G. (1959), *J. Bacteriol.* **77**, 642.
28. Atkins, A. S. (1978), *Met. Appl. Bact. Leaching and Rel. Microb. Phen.* (Murr, L. E., Torma, A. E., and Brierley, J. A., eds.), Academic, NY, pp. 403–426.
29. Torma, A. E., and Gundiler, I. H. (1987), *Bioleach. High Sulfur Coal*, repr. no. 87-2, Soc. Min. Eng., Littleton, CO, pp. 1–8.
30. do Rego Montiero Neto, O., and Torma, A. E. (1985), *Microb. Effects on Metall. Proc.* (Clum, J. A., and Haas, L. A., eds.), Metall. Soc., Warrendale, PA, pp. 15–33.
31. Singh, A. K., Torma, A. E., and Rossi, G. (1982), *Res. Assoc. Miner. Sarda*, 87(1), 1, pp. 1–8.
32. Eisenthal, R., and Cornish-Bowden, A. (1974), *Biochem. J.*, **139**, 715.
33. Singh, A. K., Singh, A., and Torma, A. E. (1984), *N. Mex. J. Sci.*, 24(2), 62.
34. Capes, E. E., McIlhinney, A. E., Sirianni, A. F., and Puddington, I. E. (1973), *CIM Bull.* **66**(739), 88.
35. Habashi, F. (1980), *Prin. Extr. Metall.*, vol. 1, Gordon and Breach, NY, pp. 129–164.