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KINETIC STUDY OF MICROBIAL COAL DESULFURIZATION USING THERMOPHILIC MICROORGANISMS

The Ohio State University

Ph.D. 1986

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KINETIC STUDY

OF

MICROBIAL COAL DESULFURIZATION USING THERMOPHILIC MICROORGANISMS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

by

Chi-Yu Chen, B.S., M.S.

The Ohio State University
1986

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Chi-Yu Chen was born in Tainan, Taiwan August 18, 1956. He was graduated from Tunghai University in June, 1978 with a B.S. in Chemical Engineering. Then he served two years in the Army of the Republic of China and worked as an assistant at Tunghai University for one year before he came to the United States in 1981. Since then he has been with Professor Duane R. Skidmore working in the area of coal research. He received his M.S. degree in 1983; he is now a PH.D. candidate in Chemical Engineering at the Ohio State University.
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ABSTRACT

Microbial coal desulfurization was investigated using a thermophilic microorganism Sulfolobus acidocaldarius. The rate of ferrous iron oxidation by the microbes was studied. Microbial leaching of iron pyrite from coal was investigated and a kinetic model was proposed. The effects of basal salts, pyrite grain size, prior washing of coal and initial cell density on sulfur removal was experimentally determined. Experimental results showed that both chemical and microbial oxidation of pyritic sulfur were significant. A single dose of basal salts, high cell density ($10^8$ cells/ml) and fine pyrite grain size (100% < 10 µm) gave 2 to 3 times higher sulfur leaching rates. Cell attachment to coal and other particles was investigated to define the phenomenon of cell-particle interaction. Microbial cells attached to the coal surface at high rates where equilibrium was reached in 5 min and the adsorption isotherms fitted the Langmuir Model in some cases. A kinetic model which assumed a first order rate equation for chemical oxidation of pyrite and the Monod Model for biological oxidation of pyrite along with cell adsorption was proposed to fit the experimental data.
CHAPTER 1
INTRODUCTION

Coal provides cheap and abundant energy for the United State. In this country, 56% of the coal was consumed generating electricity in 1984 (Galuszka, 1986). Much of the coal produced and consumed east of the Mississippi River had high sulfur content. Combustion released sulfur as SO$_2$ some of which was converted ultimately into mineral sulfates and sulfuric acid. The acid precipitated as acid rain and caused serious problems in the northeast and in Eastern Canada (Krug et al., 1983). To reduce acid precipitation, the Clean Air Act with Amendments required SO$_2$ emissions to be less than 1.2 lb/million Btu, which is equivalent to a sulfur content of less than 0.72% for coal with a heating value of 12,000 Btu/lb.

Many solutions have been proposed for the problem of high sulfur coal. In general, desulfurization methods can be categorized according to the timing of sulfur removal related to the combustion step. Sulfur may be captured before, during or after combustion. Precombustion desulfurization methods remove sulfur before coal is burned and the desulfurization methods during combustion capture the emitted sulfur.
inside the combustion chamber while the coal is being burned. The post-combustion cleaning methods scrub the flue gas and absorb the $SO_2$ before it is emitted into the atmosphere.

Precombustion desulfurization methods include physical cleaning, chemical leaching, and microbial leaching. Physical cleaning methods make use of the differences between the physical properties of sulfur-containing materials (usually pyrite) and the coal particles to separate one from the other. For instance, float-sink methods make use of differences in specific gravity to separate heavier pyrite particles from coal-pyrite mixtures (Liller, 1985). Magnetic fields can be applied to separate magnetic sulfur-bearing materials from coal (Oder, 1985). Some investigators have modified the surface properties of coal and pyrite to separate one from the other by froth flotation or selective flocculation (Attia, 1985). Chemical leaching methods use chemical reagents to leach out sulfur-containing compounds. These reagents could be acids, bases, oxidizing agents or others. Numerous reports have been published about chemical leaching. However, high costs of chemical reagents and of reaction vessels are common to them all.

The during-combustion desulfurization methods use $SO_2$-absorbing agents to capture the pollutants before they leave the combustion chamber. Limestone and dolomite are the reagents most commonly used. These methods are commonly associated with the technology of fluidized bed combustion where good contact between absorbent and pollutants can be obtained. Post-combustion cleaning methods scrub $SO_2$ to clean the flue gas before release to the atmosphere. The scrubbing system is dry.
or wet and the scrubbing reagents vary widely but include lime water and caustic (Raymond et al., 1978, Martin, et al., 1981, Noll et al. 1975).

As mentioned previously, a problem for chemical leaching methods and for some of the other methods is the high cost of difficult-to-recover chemical reagents. To overcome the problem, a renewable leaching agent, such as a microorganism, offers some advantages. As long as appropriate substrates (primarily sulfur) are provided, the microbes grow and replenish themselves.

Detz and Barvinchak (1979) estimated the costs of some chemical and microbial desulfurization processes as follows:

<table>
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<td>Microbial desulfurization process</td>
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</tr>
<tr>
<td>TRW ferric leaching (Meyers) process</td>
<td>$20/ton</td>
</tr>
<tr>
<td>Battelle hydrothermal process</td>
<td>$20/ton</td>
</tr>
<tr>
<td>Kennecott O₂ leaching process</td>
<td>$22/ton</td>
</tr>
<tr>
<td>Solvent refined coal</td>
<td>$30/ton</td>
</tr>
<tr>
<td>Flue gas scrubbing</td>
<td>$16/ton</td>
</tr>
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The estimated costs shown above indicate that microbial leaching has the lowest cost compared with other methods. Improvements in the microbial desulfurization process can further reduce the cost and make it more competitive.

This research investigates some technical aspects of microbial coal desulfurization using a thermophilic microorganism. The work is part of an effort toward understanding and development of the biological coal desulfurization process. Chapter 2 is a literature review which gives an
overview of microbial leaching. Since the amount of literature related to the subject is very large, only the references which are closely related to the experimental study are reviewed. Chapter 3 describes experimental methods and materials and Chapter 4 gives experimental results and interpretation. Chapter 5 summarizes conclusions and Chapter 6 makes recommendations for future research.
CHAPTER 2

BACKGROUND INFORMATION AND LITERATURE REVIEW

2.0 Introduction

Topics of interest to microbial coal desulfurization include coal structure, mechanisms of sulfur removal, characteristics of the microbes, sulfur forms, phenomenon of cell attachment, and design of slurry reactors. Information in these areas provides a sound basis for the development of a commercial, continuous leaching process. Provided here is a step by step approach which starts from an understanding of cell influence upon sulfur oxidation and ends with information about the design of an air-lift fermenter. Since the topics cover microbiology, coal and mineral surface properties and transport phenomena, numbers of papers are available in the literature. Only a few pertinent references are reviewed here.

2.1 Characteristics of some sulfur-oxidizing microbes

Many microorganisms have been identified as able to utilize sulfur oxidation as an energy source. Very few of these microbes have been used to remove sulfur from coal. Some microbes able to reduce the sulfur content in coal have not been characterized well (Chandra et al., 1979).
Species for microbial coal desulfurization come from the genera: 
*Thiobacillus*, *Pseudomonas* and *Sulfolobus*.

The Gram-negative, acidophilic chemolithotrophic species *Thiobacillus ferrooxidans* has been studied since 1947 (Colmer and Hinkle, 1947) for its effects on pyrite in coal. The bacteria are rod shaped, and able to oxidize ferrous iron into ferric iron and to oxidize mineral sulfides to sulfate. The organisms were first discovered in a study to control acid mine drainage produced when the microbes oxidized mineral sulfides to iron sulfate. Later, biological oxidation of pyrite was purposefully used to reduce sulfur content in coal (Silverman, 1967; Dugan, 1978; Detz and Barvinchak, 1979; Andrews et al., 1982). The microbes also found application in the leaching of metals from waste ores (Ebner, 1978; Atkins, 1978; Murr, 1980). Typical sulfate release curves are shown in Figure 1. Often a mixed culture of *T. ferrooxidans* and *T. thiooxidans* remove sulfur faster than does a pure culture of *T. ferrooxidans*. Most of the *Thiobacillus* species are mesophilic (25-35 °C) with a few exceptions of a thermophilic sort (Murr and Berry, 1979). The thermophilic species can grow at temperatures up to 55 °C.

Applications of *Pseudomonas* bacteria to coal cleaning are rarely mentioned. Some of the species tried on coal for sulfur removal, *P. aeruginosa* and *P. putida*, were shown to remove sulfur from coal and lignite without a pH change in the medium (Rai and Reyniers, 1985). The *Pseudomonas* is a rod-shaped, Gram-negative mesophile with optimum pH at 7.0. The microbes differ from *Thiobacillus* species in their metabolism which does not produce sulfuric acid. The organisms utilize some organic
Figure 1. Sulfate Release Curve in Microbial Leaching of Coal Using Thiobacillus ferrooxidans.
sulfur compounds in crude oil (Finnerty, 1985). However, removal of organic sulfur from coal by this species has not been reported.

The Sulfolobus species provides more promising organisms. The Sulfolobus acidocaldarius microbe was employed throughout this study and will be described in more detail. The species was first isolated from Yellowstone National Park (Brock, 1972; Brierley et al., 1973). It was also found in thermal hot springs in Iceland, Dominica, El Salvador, New Zealand and Italy (Brierley, 1980). The organism is described as:

1) spherical with frequent lobes
2) a facultative autotroph, when grows on elemental sulfur and some simple organic compounds,
3) acidophilic, with pH range of 0.9-5.5, optimum of 2-3,
4) thermophilic, with temperature range of 55-85 °C with optimum of 70-75 °C,
5) without peptidoglycan-containing cell wall, and
6) characterized by DNA base composition of 60-68% guanine plus cytosine (Brock, 1972).

Since the discovery of the first strain of the genus Sulfolobus, four species, S. acidocaldarius, S. brierleyi, S. solfataricus and S. ambivalens, have been isolated and characterized. The genus is currently classified as a member of the archaebacteria, which consist of Sulfolobus, extreme halophiles, methanogens and Thermoplasma (Woese, 1981). Some interesting features about this genus have been reported in the literature. The Sulfolobus are highly resistant to some antibiotics.
such as vancomycin, while it is sensitive to novobiocin (Brook et al., 1972). A cell structure with an S-layer was reported by some investigators (Taylor et al., 1982). One of the strains of *S. acidocaldarius* harbors a UV-inducible virus called SAV 1. However the process of infection has not been identified (Martin et al., 1984). *S. ambivalens* can either oxidize elemental sulfur into sulfate or reduce elemental sulfur into hydrogen sulfide depending on the supply of oxygen and of hydrogen (Segerer et al., 1985, Zillig et al., 1985). Another species, *S. solfataricus*, can grow at temperatures up to 93 °C and can break down glucose into pyruvate without phosphorylation steps (De Rosa et al., 1984). The microorganism *S. brierleyi*, the least thermophilic among the species of the family, is able to leach metals from ore (C. L. Brierley, 1980).

*S. acidocaldarius* is able to oxidize ferrous iron, pyrite, chalcopyrite and an organic sulfur compound dibenzothiophene (DBT) (Brook et al., 1972; Brierley, 1980; Brook et al., 1975; Brierley and Brierley, 1980; Kargi et al., 1984). With these characteristics this species is suitable for leaching of sulfur from coal. Compared with *Thiobacillus* and *Pseudomonas* species, it has several advantages:

1. Its extremely thermophilic, acidophilic, aerobic and autotrophic character make it resistant to contamination as indicated in a review article of extreme thermophiles (Brock, 1985).

2. The rates of chemical reaction between ferric iron ions and pyrite are higher at high temperature, which benefits the process of sulfur removal.
3. The solubility of some mineral sulfates is higher at low pH and high temperature, which reduces reprecipitation in a process for sulfur reduction.

2.2 Characteristics of sulfur compounds in coal

The sulfur forms in coal are defined as sulfate, pyritic and organic sulfur based on the ASTM standard leaching procedures (ASTM, 1985). According to the procedures, sulfate sulfur is the HCl leachable portion, pyritic sulfur is determined from nitric acid leachable iron and the organic sulfur is the difference between the total sulfur content and the sum of the two leachable parts.

Sulfate sulfur occurs in small amounts in most fresh coals. The pyritic sulfur (FeS₂) is in the form of iron pyrite or marcasite, which is the dominating mineral sulfide in coals. However, other inorganic sulfides are also present in coal as galena (PbS), chalcopyrite (CuFeS₂), arsenopyrite (FeAsS), and spalerite (ZnS) (Kneller et al., 1985). Physically, pyrite appears in coal in particles from submicron size to large masses several feet in diameter. Pyrite size distributions in some Ohio coals show a near normal distribution with mean size between 8 and 16 microns (Kneller et al., 1985). The chemical structure of organic sulfur is still not clear. Organic sulfur compounds such as sulfide, disulfide, thiophenes, and thiols were obtained from the products of coal liquefaction (Eliot, 1978). Recently some researchers reported that a few organic sulfur compounds can be identified with X-ray absorption spectroscopy (Spiro et al., 1984).
2.3 Mechanism of sulfur removal

The mechanisms are different for the removal of different sulfur forms from coal. The mechanisms for pyritic sulfur removal have been studied since the discovery of the functioning of Thiobacillus species in 1947 and will be given here. On the other hand, the mechanism of organic sulfur removal is not clear since the structure of organic sulfur is ill defined. However, the proposed mechanism for the oxidation of a model compound such as DBT, will be reviewed briefly.

2.3.1 Mechanism for the oxidation of pyritic sulfur

The mechanism for pyritic sulfur oxidation was first proposed by Silverman (Silverman, 1967) for Thiobacillus species as comprised of direct and indirect oxidative components. The direct mechanism requires direct contact between bacteria and pyrite since no extracellular enzymes are involved. In the indirect mechanism, the ferric iron chemically reacts with pyrite to give ferrous iron and elemental sulfur. The bacteria then oxidize ferrous iron to ferric iron and oxidize elemental sulfur to sulfate. The chemical and biological reactions are summarized as follows (Silverman, 1967):

\[ 2 \text{FeS}_2 + 7 \text{O}_2 + 2 \text{H}_2\text{O} \xrightarrow{\text{Bacteria}} 2 \text{FeSO}_4 + 2 \text{H}_2\text{SO}_4 \quad \text{(1)} \]

\[ 2 \text{FeSO}_4 + 1/2 \text{O}_2 + \text{H}_2\text{SO}_4 \xrightarrow{\text{Bacteria}} \text{Fe}_2(\text{SO}_4)_3 + \text{H}_2\text{O} \quad \text{(2)} \]

Overall reaction:

\[ 2 \text{FeS}_2 + \text{H}_2\text{O} + 15/2 \text{O}_2 \xrightarrow{\text{Bacteria}} \text{Fe}_2(\text{SO}_4)_3 + \text{H}_2\text{SO}_4 \quad \text{(3)} \]
Indirect mechanism:
\[
\text{FeS}_2 + \text{Fe}_2(\text{SO}_4)_3 \rightarrow 3 \text{FeSO}_4 + 2 \text{S} \quad \quad \quad \quad \quad \quad (4)
\]
\[
2 \text{S} + 3 \text{O}_2 + 2 \text{H}_2\text{O} \xrightarrow{\text{Bacteria}} 2 \text{H}_2\text{SO}_4 \quad \quad \quad \quad \quad \quad (5)
\]
\[
2 \text{FeSO}_4 + \frac{1}{2} \text{O}_2 + \text{H}_2\text{SO}_4 \xrightarrow{\text{Bacteria}} \text{Fe}_2(\text{SO}_4)_3 + \text{H}_2\text{O} \quad \quad \quad \quad \quad \quad (2)
\]

In the absence of bacteria, the regeneration of ferric iron is the rate limiting step for pyrite oxidation. The bacteria increase the pyrite oxidation rate by oxidizing ferrous iron to ferric iron.

The bacterial mechanisms of iron-sulfur oxidation were investigated by Silver (1978). For ferrous iron oxidation, electrons are transferred from \text{Fe}^{2+} to molecular oxygen catalyzed by cytochrome c and cytochrome a. For the oxidation of elemental sulfur, a more complicated pathway was proposed (Silver, 1978). The oxidation of \text{Fe}^{2+} and \text{S}^{2-} occur in the cell envelope of Gram-negative bacteria catalyzed by iron oxidase and sulfur oxidase (Lundgren and Tano, 1978). These bacteria have a typical three-layered cell-envelope which consists of lipopolysaccharide, peptidoglycan and a cytoplasmic membranes. Oxidation of \text{Fe}^{2+} to \text{Fe}^{3+} results in the removal of one electron by the cell. A proton must be drawn from the environment to maintain a balanced electrical charge (Dugan and Apel, 1978). This reaction may explain the obligate acidophilic nature of the microorganism.

The application of the bacterial mechanisms derived for \textit{Thiobacillus ferrooxidans} to \textit{Sulfolobus acidocaldarius} is still dubious.

One of the reasons is that the cell wall structure of \textit{Sulfolobus} is
different from that of *Thiobacillus* by the absence of a peptidoglycan layer. However, direct or indirect mechanisms, which are the result of bacterial activities, can be assumed to be valid for *Sulfolobus* species because:

1) *Sulfolobus* oxidizes $Fe^{2+}$, $S^0$ and pyrite at acidophilic conditions with production of $Fe^{3+}$ and $SO_4^{2-}$.

2) Selective cell attachment of *S. brierleyi* to pyrite has been observed (Murr and Berry, 1976).

### 2.3.2 Mechanism for the oxidation of organic sulfur

Dibenzothiophene (DBT) has been used as a model organic compound in the study of the mechanism of organic sulfur removal from coal. *Pseudomonas, Acinetobacter, Beijerinckia, Rhizobium* and *Sulfolobus* genera have been reported able to oxidize DBT. The degradation of DBT by members of the genus: *Pseudomonas*, is mediated by a plasmid in the cell. The introduction of the plasmid into a suitable cell enables the cell to degrade DBT (Finnerty, 1985). The proposed mechanism for the degradation of DBT into sulfate, $CO_2$ and $H_2O$, is shown in Figure 2. Different products from oxidation of DBT by other microorganisms have been reported in the literature (Kargi, 1984). As shown by the work of Rai et al. (1985), the oxidation of sulfur in coals by members of *Pseudomonas* species did not lower the pH of the medium. This suggests that the mechanism of sulfur removal by *Pseudomonas* microbes is different from that of *Sulfolobus* and *Thiobacillus* genera.

### 2.4 Adsorption of cells on solid surfaces
Figure 2. Possible pathway for the microbial oxidation of dibenzothiophene.
Fundamental understanding of microbial coal desulfurization requires information about the cell-coal surface interaction. The direct mechanism for pyrite oxidation suggests that a direct bacterial contact with the pyrite surface is necessary (Silverman, 1967; Brierley, Brierley, Norris and Kelly, 1980). Consequently, the rate of oxidation can be correlated with the number of cells attached to the pyrite surface. Information about cell attachment is important when a continuous process is designed with recycled medium. The cell balance around the continuous reactor requires definition of the cell density in the recycled medium and the number of adsorbed cells per unit weight of coal. Because the cells which attach to coal particles leave the process and are considered a loss, the cell growth rate must exceed the loss rate in order to attain and maintain steady state. Therefore, mathematical modeling to predict the performance of continuous processes requires the information about the rate and extent of cell adsorption.

2.4.1 Surface structure and properties of the microbial cell wall

The three species of microorganisms under development for microbial coal desulfurization can be categorized into two types, according to their cell wall structures. Both _Thiobacillus_ and the _Pseudomonas_ types are Gram-negative bacteria with a 3-layered cell wall. The _Sulfolobus_ species is an Archaebacterium with a special cell containment construction. Figure 3 portrays schematically the cell wall structure of Gram-negative bacteria. The outer cell membrane contains lipopolysaccharide, phospholipids and proteins (Stanier et al., 1976). Gram-negative bacteria usually produce a wide variety of glycocalyces.
Figure 3. Cell Wall Structure of *T. ferrooxidans*.
that enable the cell to attach to solid surfaces (Murr, 1980). Some surface appendages have been observed on cell surfaces. The most common ones are flagella, pili, fimbriae and prostheca. The archaeabacteria usually contain no peptidoglycan but possess an S-layer structure (Taylor et al., 1982). Some appendages were also observed on the cell walls of these bacteria (Weiss, 1973).

Generally, the bacterial cell surfaces have a net negative charge at acidic pH (Rutter et al., 1980, Ward, 1980). For Gram-negative bacteria, the lipopolysaccharides and proteins are the sources of acidity and negative charge on the outer surface of the cell wall. The bacteria show a large variety in hydrophobicity and hydrophilicity. The Thiobacillus types show a strong hydrophilicity as indicated in the literature (Kempton et al., 1980).

2.4.2 Attachment of microbial cells on solid-water interfaces

The mechanism of cell attachment is determined by the surface properties of both cell wall and solid surface. Most solid surfaces found in natural habitats have a net negative surface charge (Marshall, 1985). How a negatively-charged cell can attach to a negatively-charged surface depends on the existence of other forces to overcome the repulsive force. These forces could be long-range or short-range.

For reversible adsorption, the force could be a resultant of long-range forces such as van der Waal forces or London forces. Once the cell is adsorbed, the Brownian motion, the shear of water flow or the rotational movement of motile bacteria could remove the cell from the surface. On the other hand, in the case of irreversible adsorption,
short-range forces may dominate. The phenomenon of "irreversible adhesion" was defined by Marshall et al. (Marshall et al., 1985) as a time-dependent, firm adhesion in which bacteria can not be washed away from the surface. The bridging between the cell wall and the surface may come from an extracellular polymer, pili, fimbriae or flagella (Marshall, 1985).

Irreversible adhesion of Thiobacillus ferrooxidans on substrate surfaces has been studied by many investigators (Jones and Starkey, 1961; Takakuwa et al., 1979). It is generally agreed that a wetting agent is responsible for cell adhesion. Murr and Berry studied cell attachment on mineral ores by scanning electron microscopy and concluded that adhesion is selective on sulfide surfaces (Murr and Berry, 1976). Adsorption of T. ferrooxidans on coal and other particles was studied by some investigators (Myerson and Kline, 1983; DiSpirito et al., 1983). Figure 4 shows results from Myerson et al. Some researchers even found that surfactants enhanced contact between cells and the sulfide surface and therefore increased sulfur removal rates (Wakao et al., 1983). The pyrite-selective adsorption characteristics were applied to improve physical separation of coal and pyrite as described in the literature (Kempton et al., 1980; Attia et al., 1985). In those studies, the properties of pyrite particle surfaces was modified by cell adsorption. Then the coal was separated by oil agglomeration, selective flocculation or froth flotation.

The attachment of Sulfolobus to solid particles has been reported by some investigators (Brook et al., 1972; Weiss, 1973; Murr and Berry, 1976). In their study of sulfur oxidation by S. acidocaldarius, Shivvers
Figure 4. Adsorption of Thiobacillus ferrooxidans on Coal.
(Reference: Myerson, A. S. and P. Kline., "The Adsorption of
Thiobacillus ferrooxidans on Solid Particles.", Biotech. Bioeng., 25,
(1983), 1669-1676.)
and Brock observed that the cells were attached to sulfur crystals until the late exponential stage and stationary stage were achieved (Shivvers and Brock, 1973). Another researcher studied the phenomenon of cell attachment for *S. acidocaldarius* and found that the cells attached to sulfur crystals by means of pili (Weiss, 1973). He indicated that cell attachment was not necessary for sulfur oxidation. However, attachment provided a means for colonization in natural habitats (Weiss, 1973). In his study, he observed that cells obtained from bubbling pools did not attach to sulfur crystal while those from a flowing stream did. Usually the attached cell was separated from the solid surface by a short distance (Weiss, 1973).

Murr and Berry applied scanning electron microscopy to the study of cell attachment to a mineral surface. They reported that a *Sulfolobus*-like bacterium (later named *S. brierleyi*) preferentially adsorbed on the pyrite surface. No pili were observed to effect cell attachment (Murr and Berry, 1975, 1976, 1976). Similar conclusions were drawn for chalcopyrite, molybdenite and low grade sulfide ores in their study. Based on an attachment study, Murr and Berry proposed that attached cells promoted direct oxidation of pyrite, while the freely suspended cells oxidized the pyrite by an indirect mechanism (Murr and Berry, 1976).

2.5 Mathematical models for microbial coal desulfurization

Development of mathematical models is necessary for many purposes. First, a model can be applied in computer simulation of continuous processes so that a computer control algorithm can be designed. The
model can also be employed in the design and scale-up of bioreactors. The models can be classified into kinetic models, diffusion models and continuous-leaching models.

2.5.1 Kinetic model

Since the system involves solid substrates, a Monod type model which is valid only for soluble substrates, is not applicable. Consequently, kinetic models must be developed based on the direct and indirect mechanisms. For the direct mechanism, a first order rate equation related to pyrite concentration is considered. For the indirect mechanism, rate equations are considered separately for chemical reactions and microbial activity.

In the direct mechanism, the microbial cells adsorb onto the substrate surface and oxidize the substrate (usually pyrite) into sulfate and ferrous iron. Through many decades of study on the oxidation of iron pyrite by the Thiobacillus species, this mechanism is well documented and modeling has been performed in detail. Detz and Barvinchak (Detz and Barvinchak, 1979) assumed first order kinetics in pyrite concentration to describe pyrite oxidation. Other researchers (Gormely et al. 1975, Chang and Myerson, 1982 and Hoffmann et al. 1981) proposed a rate equation which is first order in pyrite surface area. According to the model proposed by Detz and Barvinchak, the rate equation is written as:

\[
\frac{dS}{dt} = - k S...........................................\text{(6)}
\]
where $S$ is the leachable pyrite concentration and $k$ is the first order specific rate constant. Integration of this equation yields an exponential equation as follows:

$$S = S_0 e^{-kt} \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots (7)$$

where $S_0$ is the initial concentration of leachable sulfur. If sulfate is the only product of pyrite oxidation, the sulfate concentration can be described as:

$$\left[SO_4^2-\right] = \left[SO_4^2-\right]_0 (1 - e^{-kt}) \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots (8)$$

where $\left[SO_4^2-\right]_0$ is the initial sulfate concentration in the leaching medium. This equation fits most of the kinetic data in the literature where the initial cell density (inoculum size) was sufficiently high. However, for lower initial cell densities, this model fails to fit the data.

Huber et al. (Huber et al., 1984) modified the model by assuming two regimes according to the number of cells which existed in the system. In the biomass-limited regime, the cell density was so low that the pyrite surface was not fully covered with cells. In that case the rate of sulfur removal was first order in the number of adsorbed cells. The microorganism was in its exponential growth phase since the substrate was sufficiently supplied. On the other hand, in the substrate limited regime, the pyrite surface was completely covered by cells and the pyrite was not sufficient to support microbial growth. Consequently
the rate was first order in available pyrite. The rate equation can be expressed as follows:

**Biomass limited regime**

\[
\frac{dS}{dt} = -k_1 X \hspace{1cm} (9)
\]

\[
\frac{dX}{dt} = \mu X \hspace{1cm} (10)
\]

where \(X\) is the cell density, \(k_1\) is the rate constant and \(\mu\) is the specific growth rate of the microbes. Integration of these two equations gives the following equation:

\[
S = S_0 - \frac{k_1 X_0}{\mu} (e^{\mu t} - 1) \hspace{1cm} (11)
\]

**Substrate limited regime**

\[
\frac{dS}{dt} = -k \ S \hspace{1cm} (12)
\]

Integration of the equation gives an equation similar to equation (7) derived previously.

\[
S = S^* e^{-k(t-t^*)} \hspace{1cm} (13)
\]
Where $S^*$ is the unreacted pyrite at time $t^*$, the time when the regime is changed from biomass-limited to substrate-limited.

In the indirect model, the $\text{Fe}^{3+}$ ions attack the pyrite to produce $\text{Fe}^{2+}$ and elemental sulfur. Then the microbes oxidize $\text{Fe}^{2+}$ into $\text{Fe}^{3+}$, and oxidize the $S^0$ into sulfate. Some investigators studied the mechanism of reaction for the change from ferric to ferrous iron reaction and proposed the following mechanism (Smith and Shumate, 1970):

(Adsorption of $\text{Fe}^{3+}$ on active sites of pyrite)  
$$ \text{Fe}^{3+} + S \rightarrow \text{Fe}^{3+}.S^* \quad \text{(14)} $$

(Adsorption of $\text{Fe}^{2+}$ on active sites of pyrite)  
$$ \text{Fe}^{2+} + S \rightarrow \text{Fe}^{2+}.S^* \quad \text{(15)} $$

Decomposition of activated complex)  
$$ \text{Fe}^{3+}.S^* + e^- \rightarrow \text{Fe}^{2+}.S \quad \text{(16)} $$

Desorption of adsorbed ferrous iron)  
$$ \text{Fe}^{2+}.S \rightarrow \text{Fe}^{2+} + S \quad \text{(17)} $$

Application of Hougen-Watson concepts gives the following rate expression (Smith and Shumate, 1970):

$$ \text{Rate} = \frac{k_{3F} K_1 - K_2 \frac{\text{Fe}^{2+}}{\text{Fe}^{3+}}}{\frac{1}{\text{Fe}^{3+}} + K_1 + K_2 \frac{\text{Fe}^{2+}}{\text{Fe}^{3+}}} \quad \text{(18)} $$
where

\[ K_{3f} = \text{rate constant for reaction (16)} \]
\[ K_1 = \text{adsorption equilibrium constant for ferric ions} \]
\[ K_2 = \text{adsorption equilibrium constant for ferrous ions} \]
\[ K = \text{equilibrium constant for equation (17)}. \]

At low Fe\(^{3+}\) concentration, the rate is first order in Fe\(^{3+}\) concentration.

2.5.2 Models for the leaching system with diffusion control

Since coal is a porous solid, reaction could occur inside the pores beneath the external surface. To describe these phenomena, many diffusion models are available in the literature (Joshi et al., 1981). The models proposed by Joshi et al. (Joshi et al., 1981) for oxydesulfurization of coal can be applied here with the oxygen replaced by Fe\(^{3+}\). Depending on the controlling step, which could be the chemical reaction, diffusion in the leached layer or diffusion in the liquid film, the model is presented in three cases:

**Case I: Surface reaction control**

In this case, the rate of chemical reaction is so low that the concentration of Fe\(^{3+}\) is the same everywhere inside the particle. The rate expression is similar to that for a homogenous reaction,

\[ \frac{d[Fe^{3+}]}{dt} = -k' [Fe^{3+}] \] \hspace{1cm} (19)
Case II: Control by diffusion through leached layer

In this case, the surface reaction rate is much greater than the diffusion rate through the leached layer. The concentration of Fe\(^{3+}\) on the surface of the unreacted core is zero due to the high chemical reaction rate. The diffusion equation can be expressed as:

\[
\frac{d[Fe^{3+}]}{dt} = D_{\text{eff}} \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial [Fe^{3+}]}{\partial r} \right) \quad \text{.........(20)}
\]

Case III: Control by diffusion in the liquid film

In this case, diffusion is so slow in the liquid film around the particle that the overall rate depends on the diffusion rate of reactants through this film. This is the case when mixing in the liquid phase is poor and a thick film develops between the bulk liquid phase and the solid surface. The diffusion equation is given by equation (20) except that the boundary conditions are different.

In a reactor with good mixing, and with the assumption that neither the surface reaction nor the diffusion is controlling, a combination of case I and case II yields the following equation:

\[
\frac{d[Fe^{3+}]}{dt} = D_{\text{eff}} \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial [Fe^{3+}]}{\partial r} \right) - k'[Fe^{3+}] \quad \text{....(21)}
\]

where \(D_{\text{eff}}\) is the effective diffusivity and \(r\) is the radius of the particle.

2.5.3 Models for continuous leaching
Erickson et al., (1970) proposed a model for microbial systems with two liquid phases. The concept of the model is that the cells adsorbed on the substrate surface undergo different growth kinetics from the cells in suspension. Another researcher modified the model to predict the continuous leaching of zinc sulfide by *Thiobacillus ferrooxidans* (Gormely, 1975). In this model, cell attachment to the mineral surface was assumed to be necessary for leaching. Consequently, only the attached cells could grow and the growth rate was at its maximum (Gormely, 1975). The rate of leaching, \( r_x \), could then be defined as:

\[
    r_x = \mu_m \sigma \tag{22}
\]

where \( \sigma \) is the concentration of attached cells, which is determined by the adsorption-desorption equilibrium:

\[
    k_1 (s - \sigma a)(X - \sigma) = k_{-1} \sigma \tag{23}
\]

where \( k_1 \) and \( k_{-1} \) are the adsorption and desorption rate constants, respectively. The \( s \) is the surface concentration of cells and \( a \) is the surface area occupied per unit of attached bacteria. In a continuous culture at steady state, \( r_x/X = \mu = D \), where \( D \) is the dilution rate. The parameters in the equations can be evaluated from steady-state data obtained with a continuous culture (Gormely et al. 1975).

To model the leaching of pyrite, the growth of the bacteria both in the solution and on the surface of pyrite are considered. In the
solution, microbes oxidize ferrous iron into ferric iron. On the pyrite surface, bacterize oxidize pyrite into ferrous iron and sulfate. The Monod model can be applied to describe the exponential growth of the bacteria on ferrous iron (Chang and Myers, 1982; Myers and Kline, 1984):

\[
\mu = \frac{\mu_m [Fe^{2+}]}{K_m + [Fe^{2+}]} \tag{24}
\]

where \( \mu \) is the specific growth rate defined as:

\[
\frac{dX}{dt} = \mu X
\]

and \( K_m \) is a constant.

The surface growth rates were defined by the cell balance in a continuous culture:

\[
FX_b + AX_s = U_s X_s A_t + U_b X_b V \tag{25}
\]

where \( F \) is the volumetric flow rate (ml/h), \( X_b \) is the bacterial concentration in solution (ug protein/ml), \( A \) is the surface area flow rate, \( X_s \) is the concentration of bacteria on the solid surface (ug protein/m²), \( A_t \) is the total amount of solid surface in the reactor (m²), \( U_b \) is the specific growth rate in the liquid (h⁻¹), and \( V \) is the liquid volume (ml). The relation between \( X_s \) and \( X_b \) is defined by an equation derived by modifying equation (25):
\[ k_1 (1 - \theta) X_b = k_{-1} \theta \] .................................(26)

Combining these two equations, with the dilution rate \( D \) defined as \( F/V \) and the dilution rate of solid \( D_s \) defined as \( A/A_t \):

\[
D = \frac{U_s X_s S}{X_b} + \frac{U_m [Fe^{2+}]}{K_m + [Fe^{2+}]} - \frac{D X_s S}{X_b} \] ..............................(27)

A different approach for the modeling of microbial leaching of pyritic sulfur from coal was proposed by Kargi and Weissman (Kargi and Weissman, 1984). In this model, material balances of free cells and attached cells are considered. With some assumptions, the following equations were derived (Kargi and Weissman, 1984) for a single coal particle:

**Cell balance for attached cells**

\[
\frac{dN_A}{dt} = \frac{\mu_m S}{K_S + S} N_A + \frac{K_A N_F (N_S - N_A)}{K_D N_A} - K_D N_A \] ..............................(28)

where \( N_A \) is the number of absorbed cells on a single coal particle; \( S \) is concentration of pyritic sulfur in a single coal particle; \( N_S \) is the number of cells required to completely cover the surface of the coal particle; \( N_F \) is the number concentration of free cells in liquid phase; \( K_A \) and \( K_D \) are the adsorption and desorption coefficients, \( \mu_m \) and \( K_S \) are respectively the maximum specific growth rate of cells and saturation constant defined in a similar way to that in equation (24).
Cell balance for free cells

\[
(1 - F) \frac{dN_F}{dt} = K_D N_A n - K_A F (N_S - N_A) n \quad \text{(29)}
\]

where \( F \) is the volume fraction of coal-water mixture that is occupied by coal particles and \( n \) is the number of coal particles per unit volume of the slurry.

All the models mentioned above contain adsorption-desorption terms in the equations and all of them assume the "direct oxidation mechanism" to be the dominating mechanism. These assumptions are not necessarily true in a real microbial leaching system. Chang and Myerson (1982) reported that the adsorption of \( T. \) ferrooxidans on coal and pyrite was irreversible. If that is the case, then all the desorption terms are meaningless. On the other hand, much of the literature supports the idea that the direct and indirect mechanisms operate simultaneously. Without considering the chemical reactions, such as reaction (28), the model is meaningless. Especially for the case of pyrite leaching by thermophiles, the rate of chemical reaction is high due to high temperature. The model proposed by Kargi and Weissman (1982) did not consider growth in the solution, which deviates considerably from reality since both \textit{Thiobacillus} and \textit{Sulfolobus} bacteria oxidize soluble ferrous iron. Finally, the model of Kargi and Weissman assumed a Monod type of model for pyrite oxidation. In a real case, pyrite is a nonsoluble solid and the rate of oxidation depends on the available surface area, not the concentration.
CHAPTER 3
EXPERIMENTAL EQUIPMENT AND PROCEDURES

The experiments were directed toward two distinct problems. One set of experiments investigated the chemical characteristics of the microorganism and the chemical mechanisms of sulfur leaching. The second portion of the experiments studied cell attachment to coal and other solid particles.

3.1 Culture method and pure culture isolation

The medium for microbial growth was made of a basal salts solution, and an energy source with or without supplements. The basal salt solution had the following composition (g/l): \((\text{NH}_4\text{)}_2\text{SO}_4, 1.3; \text{KH}_2\text{PO}_4, 0.28; \text{MgSO}_4 \cdot 7\text{H}_2\text{O}, 0.25; \text{CaCl}_2 \cdot 2\text{H}_2\text{O}, 0.07\); in 1 liter of tap water. In some cases, yeast extract was added as a supplement, and sublimed sulfur was added at 2.0 g/l as the energy source. Without the yeast extract supplement, growth was slow and most of the cells attached to the sulfur particles (Shivvers and Brock). The pH of the medium was adjusted to 2.0 with 3.0 N \(\text{H}_2\text{SO}_4\).

Eight hundred ml. of the medium were placed in a three necked, round bottomed flask equipped with a condenser. The system was autoclaved at 121 °C for 15 min and then inoculated with a culture of \(\text{S. acidocaldarius}\). The system was kept in a water bath at 76 °C. The flask
was continuously aerated with air to provide oxygen and carbon dioxide for microbial activities. The condenser served the purpose of condensing the moisture evaporated with the air leaving the flask so that the medium would not be dried out. Figure 5 demonstrates the configuration of the fermenter. The air was saturated in a saturator before entering the fermenter. The cooling water leaving the condenser flowed through the water jacket of the saturator to keep the temperature of the saturator at the temperature of the exit air. With this design, the liquid level inside the flask remained constant for a week, neither dropping nor rising. With temperature of the water bath controlled at 76 °C, the temperature of the medium in the flask was 72 °C. A cooling effect occurred from water evaporation. The culture was transferred every week. Mechanical agitation was provided by stirrers to enhance particle mixing and mass transfer of gases.

The original culture of *S. acidocaldarius* from Dr. Brierley at the New Mexico Institute of Mining and Technology and Dr. Kargi at Lehigh University was used throughout the investigation. The culture had been transferred in coal slurry since 1983. Sulfur leaching of coal by *S. brierleyi* had also been studied in the same laboratory by other researchers. The two species of *Sulfolobus* showed similar sulfur removal rates at 70 °C. It is possible that a mixed culture could exist in the coal slurry. Therefore, the first step in the experiment was to isolate a pure culture of *S. acidocaldarius*. The optimum growth temperature of *S. brierleyi* (60-65 °C), was lower than that of *S. acidocaldarius* (70-75 °C), as reported in the literature (Brierley, 1977; and Beck, 1981;
Figure 5. Fermenter for Batch Experiments.
Rawlings, 1981). A pure culture was isolated from the coal slurry by transferring in a sulfur medium at 72 °C several times.

To isolate a pure culture of \textit{S. acidocaldarius}, a coal slurry containing the microorganism was inoculated to a sulfur medium supplemented with 0.02% (w/v) yeast extract. After 10 days of incubation, the culture was transferred to another flask with the same sulfur medium. Cell density and sulfate concentration in this new flask were followed to ensure the presence of the thermophile. Sulfate concentration was determined by the standard turbidometric method specified by the ASTM. The procedures of sulfate analysis are given in Appendix C. Cell growth was detected by the measurement of optical density for the supernatant. The sample taken from the flask was first centrifuged at 1000x g for 5 min. and then the optical density was measured as absorbance at 550 nm in a Bausch and Lomb Spectronic 2000 spectrophotometer. The culture obtained was maintained in sulfur medium supplemented with yeast extract and transferred every 5 days. This culture served as the stock culture which provided cells for inoculum and cell attachment experiments in a later study.

The harvest of cells occurred 3-5 days after inoculation. As indicated in the literature, the cells attached to the sulfur surface in the late exponential phase and stationary phase (Shivvers and Brock, 1973). The maximum number of cells was recovered from the solution in 3-5 days. The culture was first centrifuged at 1000x g for 5 min to remove the sulfur particles. Then, the cell solution was centrifuged at 4000x g for 20 min to separate the cells. The cells were resuspended in basal salt solution without an energy source. These cells were used as
inoculum for sulfur leaching from coal or for the cell attachment study, as will be described later.

More accurate cell counts were achieved by protein analysis calibrated with visual cell count under a phase contrast microscope equipped with a Petroff-Hausser counting chamber. The total protein was assayed by the modified Bradford Method suggested by Peterson (Peterson, 1983). This method, commercialized and known as the Bio-Rad method, used coomassie brilliant blue G to stain the protein for quantitative analysis. Detailed procedure for hydrolyzing the cells and for protein assay are given in appendix A.

3.2 Ferrous iron oxidation

These experiments studied the oxidation of ferrous iron by the microbes. Oxidation of ferrous iron into ferric iron was one of the steps in both the "direct" and "indirect" mechanisms for pyrite leaching. The rate of ferrous iron oxidation and the effect of yeast extract supplement were examined.

Medium with ferrous sulfate as the energy source was prepared by adding 10 g/l and 3.18 g/l of FeSO$_4$. $7\ H_2O$ to the basal salt medium to yield an initial iron concentration of 2000 and 700 ppm. Experiments were conducted with and without yeast extract supplement. An uninoculated control flask was run in parallel to show the rate of chemical oxidation of ferrous iron by oxygen in the air. Samples were taken every 12-24 hours for the assay of ferrous and ferric iron concentrations. The analysis followed a procedure suggested by Christian (1977), who used 1,10 phenanthroline hydrochloride as the indicator for iron.
concentration. Combination of Fe$^{2+}$ with the indicator in an aqueous solution gave a strong absorption at 510 nm., which was used to indicate iron quantitatively. Appendix B gives details of the analytical procedures.

3.3 Effect of basal salts

Kentucky #9 coal was treated with the microbes in media with different amounts of added basal salts. The medium with the basal salt composition given in 3.1 was designated as "single strength". The medium with a double amount of basal salts was called "double strength". In the double strength basal salts medium, the amount of all the 4 salts, (NH$_4$)$_2$SO$_4$, KH$_2$PO$_4$, MgSO$_4$.7H$_2$O and CaCl$_2$.2H$_2$O, were doubled. Again, samples of the slurry were taken from each flask every 12-24 hours for sulfate analysis. Experiments were conducted with both 5% and 10% coal slurries.

3.4 Removal of organic sulfur

Sulfur leaching experiments studied the possibility of removal of organic sulfur by the microorganism. A very fine Kentucky #9 coal, in which most of the pyritic sulfur had been removed by other methods, and a petroleum coke from Amoco Co., were used in this experiment. Basal salts solution with the composition given in 3.1 and a solution with double amounts of basal salts were used. Sulfate in the solution and total sulfur content in the solid particles (the fine coal or the coke) were measured periodically throughout the experiment.
In the other experiments with Kentucky #9 coal, levels of different sulfur forms (sulfate, pyritic and organic) were determined before and after microbial leaching. The sulfur analyses followed the standard testing methods specified by the ASTM with minor modification. Appendix F gives the procedures for sulfur form analysis.

3.5 Effects of prior washing of the coal

In this experiment, the Kentucky #9 coal of 270-325 mesh was washed with the basal salt solution at 70 °C before leaching. This test showed whether water soluble materials from the coal affected the sulfur removal rate of pyrite from coal with a narrow size distribution. This set of experiments was part of the effort toward the modeling the kinetics of microbial pyrite leaching from coal.

Leaching experiments were conducted in a three-necked flask as described in 3.1. In each flask, 40 g of Kentucky #9 coal were mixed with 760 ml of basal salts solution. The slurry was inoculated with the cells harvested from the stock culture. Every 12-24 hours, 3 ml of slurry were taken from each flask and filtered through Whatman #1 filter paper. Concentrations of sulfate, ferrous, and ferric iron in the solution and total sulfur content in the coal were measured. Concentration of sulfate was determined by the methods specified in Appendix C. The sulfur content in coal was measured by combustion and titration using a LECO sulfur analyzer. Detailed procedures for sulfur analysis are given in Appendix E.

In some runs, cell densities in the coal slurry, both in the solution and those attached to the coal particles, were measured. To
measure the number of attached cells, 3 ml of the slurry were centrifuged at 4000x g for 20 min. to separate all the cells (free and attached) and coal particles from the solution. Then protein assay was conducted according to the procedures in Appendix A. A separate coal slurry sample was centrifuged at 300 rpm to separate the coal particles from the solution. Cell density in the solution was measured by protein assay. The number of attached cells was calculated as the difference between the total number of cells and the number of free cells.

3.6 Effects of pyrite grain size

The coals were Kentucky #9 coal from the Babcock and Wilcox Co. in Alliance, Ohio, and an Ohio coal mixture sampled from a pneumatic transport line in a power plant in Pickaway County, Ohio. The analyses of the coals are shown in Appendix D. The original Kentucky #9 coal and the part with sizes ranging from 270 to 325 mesh (45-53 micron) were used in this experiment.

3.7 Effects of initial cell density

This set of experiments was performed with Ohio Clarion #4A coal. The coal was sampled from a surface mine in McArthur, Ohio. The coal was first crushed in a roll mill and then ground in a ball mill with steel balls. Only the very fine part of the pulverized coal, that smaller than 200 mesh (74 micron), was used for microbial leaching.

For the batch leaching experiments, different amounts of inoculum were introduced to produce different initial cell densities. The initial
cell densities ranged from $10^7$ to $10^8$ cells/ml. Concentrations of sulfate, iron and sulfur contents in coal were measured.

3.8 Cell attachment to coal and other particles

Attachment of the cells of *S. acidocaldarius* to coal and other particles was investigated. The objective of this set of experiments was to study 1) the rate of cell attachment, 2) the extent of cell attachment, 3) the effect of process parameters such as agitation, temperature and pH, on cell attachment, 4) the effect of prior acid leaching of coal on cell adhesion 5) adsorption isotherm for cell attachment, 6) the theory of preferential adsorption of cells on pyrite.

Cell adsorption on different coals and other particles was investigated. Kentucky #9 coal, an Ohio coal mixture, Ohio #6, and a European coal from Sulzer Brothers Co. were used. Besides coals, activated carbon from Darco and a pure pyrite from Dr. Attia in the Mining Division of The Ohio State University, were also employed. The first two coals mentioned above were high sulfur coals with more than 2% pyritic sulfur and about 1.4% organic sulfur. Ohio #6 was a coal with organic sulfur as the major sulfur form. The Sulzer coal was a European low sulfur coal also with organic sulfur as the major sulfur form. Particles for adsorption experiments ranged narrowly in size: 270 to 325 mesh (45 to 53 μm). All the solid particles were washed with sterilized basal salt solution at 72 °C for 30 min. prior to adsorption tests in order to reduce possible analytical interferences by colored extract from the coal.
Adsorption tests were carried out in a series of test tubes maintained in a water bath controlled at 72°C. In each test tube, 5 ml of the harvested cells were mixed with 1 g of coal particles or 3.43 g of pyrite, and the cell density in the solution was determined at 1, 5, 15, and 30 min. The test tubes were periodically agitated to keep the particles in suspension. A parametric study was conducted on Kentucky #9 coal with an initial cell density of $8.20 \times 10^8$ cells/ml. The pH of the solution was varied from 1.5 to 5 and the temperature of the water bath was varied from 60°C to 80°C. The effect of agitation speed was studied in a 500 ml 3-necked, round bottom flask equipped with a propeller. The stirring rate of the propeller was varied from 0 to 600 rpm and the cell density was measured after 30 min of cell-coal contact.

To study the effect of acid leaching of the coal on adsorption, coal was leached with nitric acid or with hydrochloric acid. The concentrations of the acids were those specified by the ASTM standard procedures for sulfur form analysis of coal (ASTM, 1985). The nitric acid not only oxidized the pyritic sulfur but also oxidized the coal surface and made it more hydrophilic. The hydrochloric acid dissolved sulfate and cleaned up the pyrite surface. Only Kentucky #9 was used in the study of acid leaching effects. To obtain the adsorption isotherm, different initial cell densities were employed and the cell density in the solution was determined over the 30 min. of cell-solid contact time. Amounts of adsorbed cells were calculated by the difference between the initial and final cell concentrations. The specific adsorption was calculated as number of adsorbed cells per unit volume of solid particles. The volume of each sample was computated from known weight
and density. If spherical particle shape were assumed, unit volumes of different solid particles should have given the same external surface area.

Desorption of cells from solid particles after attachment was investigated. One gram of coal or pyrite particles was mixed with a cell solution of $3.6 \times 10^9$ cells/ml for cell attachment. Cell density in the solution was determined and the number of adsorbed cells defined by difference between the initial and final cell densities. Then the particles were separated and resuspended in 5 ml of basal salt solution. The mixture was thoroughly agitated and set aside for 15 min. Cell density in the solution was then measured.

Adsorption of a pyrite-selective adsorbate called polyacrylic acid/xanthate (PAAX) obtained from Dr. Attia (Attia et al., 1982) was used to define the available pyrite surface on the coal. The adsorbate is a low molecular weight polymer (average molecular weight about 1800) with xanthate functional groups which selectively adsorb on pyrite surfaces. Detailed descriptions of xanthate adsorbates and the preparation of PAAX are available in the literature (Attia, et al., 1982; Miller, 1982). A polymeric xanthate permitted, by its large size, adsorption on external pyrite surfaces to be distinguished from entrapment inside the pores of the coal. The PAAX molecules were too large to diffuse into the pores in coal which ranged from 3 to 300 Å with significant differences among the coals (Walker 1972). The microbial cells were about 1 µm in size and considered to adsorb on external surfaces only. An aqueous PAAX solution at pH 11 was used with concentrations varied from 100 to 800 ppm. Ten ml of the solution were
mixed with 0.2 g of coal (or 0.686 g of pyrite) in a test tube and the concentration of PAAAX in solution was measured after 1.5 hr. The test tube was continuously agitated to ensure good mixing. The amount of adsorbed PAAAX was calculated from the difference between initial and final concentrations in the solution. The PAAAX concentrations were derived from calibrated absorbance measurements at 308 nm in a Bausch and Lomb Spectronic 2000 spectrophotometer.
CHAPTER 4
RESULTS AND DISCUSSION

The results from different sets of leaching experiments are presented and interpreted in this chapter. The first set of experiments was designed to study the oxidation of ferrous iron by the microorganism. Oxidation of ferrous iron into ferric iron was observed in several batches with ferrous sulfate as the energy source. The second set of experiments was designed to observed the release of iron and sulfate from coal during microbial leaching process. Runs with a coal, Kentucky #9, and with different amounts of added basal salts were conducted. Another set of data were obtained from the leaching of a very fine Kentucky #9 coal and a petroleum coke, in which organic sulfur was the major sulfur form. The forth set of data demonstrates the effects of prior washing on sulfate release from coal. The next set of data gave the results of microbial leaching from different coals.

Results of the study of cell attachment on coal and other particles were obtained. An adsorption model was proposed to describe the isotherms. The theory of preferential adsorption of cells on pyrite particles was also tested based on the experimental data. Finally, with all the information gathered in this study, a mathematical model was
proposed to describe the microbial leaching system. The meaning and the function of the model are discussed.

4.1 Isolation of the microbes from coal slurry

The medium with elemental sulfur as the energy source and supplemented with yeast extract was inoculated with 10 ml of coal slurry derived from previous microbial leaching experiments. After 5 days the turbidity of the medium increased rapidly and spherical cells were observed under microscope. The culture was then transferred several times in the same elemental sulfur medium. It was reported that at 72 °C the growth rate of *S. acidocaldarius* was much higher than that of *S. brierleyi* (Beck, 1981). Figure 6 shows a typical growth curve for the species, which was believed to be *S. acidocaldarius*, in the elemental sulfur medium supplemented with 0.04% w/v yeast extract. The cell density in the solution dropped when the cells began to attach to the sulfur particles in the stationary phase, as reported in the literature (Shivvers and Brock, 1976). The doubling time was about 5 hours according to the data. This was the fastest way to obtain a large number of free suspended cells for leaching or adsorption experiments. Since this microorganism showed little or no time lag when transferred from one type of substrate to another (Brierley, 1973), in later experiments all the batches were inoculated with the cell concentrate obtained from an elemental sulfur medium.
Figure 6. Growth curve of *S. acidocaldarius* on elemental sulfur supplemented with yeast extract.
4.2 Microbial oxidation of ferrous iron

The concentration profiles of ferrous iron in the runs with 3.1 g ferrous sulfate per liter medium are shown in Figure 7. Results show that the rate of oxidation of ferrous iron is many times faster in the presence of bacteria. The medium was the basal salt solution plus yeast extract. The initial ferrous iron concentration was about 700 ppm. The curve with the lowest iron oxidation rate is the one for the uninoculated control. The control was autoclaved and 5% sodium chloride was added to prevent contamination. The pH in the medium was adjusted to 2.4 with dilute HCl. The yeast extract was reported to have a buffering effect (Brock, 1972), which required much more acid to further lower the pH of the medium. Run #2 was the batch inoculated with 5 ml of coal slurry. Run #3 was inoculated with 5 ml of culture obtained from run #2 and run #4 was inoculated with 5 ml of culture from run #3. Increasing rate of iron oxidation was apparent from run #2 to run #4.

Total iron and sulfate ion concentrations dropped with time. The molal ratio of loss of iron to loss of sulfate was between 0.9 and 1.1 calculated by measured concentrations in solution. However, analysis of the yellowish precipitate yielded a ratio of 1.6. Consequently reaction (38) is not proper to describe the phenomenon of precipitation. More accurate measurement is necessary for better understanding of iron-sulfate precipitation.

Cell density in the coal slurry was not available due to several difficulties in measurement. First, the cell density was very low (about $10^5$ cells/ml), which was beyond the accuracy of the protein assay.
Figure 7. Microbial oxidation of ferrous iron in a ferrous sulfate medium.
method. About $10^8$ cells give one microgram of protein. The other difficulty was the limitation in microscopic counting. Some of the cells attached to the fine particles formed by precipitation of sulfate and ferric iron. However, the cell density in the medium near the end of the batch run was near $10^6$ cells/ml, which made visual cell counts possible. In run #3 the final cell density was near $5 \times 10^5$ and in run #4 the final cell density was close to $7 \times 10^5$. The final cell density in run #2 was too low for accurate microscopic counting.

Another set of experiments was conducted at higher levels of ferrous iron concentration. In the runs, 10 g/l of FeSO$_4$ was added to the medium as an energy source. In two runs, yeast extract was added as the carbon source while in another two runs no organic carbon source was added. According to the literature, yeast extract only slightly enhanced the microbial growth (Brock, Cook and others, 1976). To prevent precipitation of ferric iron, the pH value of the two runs without yeast extract was adjusted to 1.7. The concentration profiles of ferrous, ferric and total iron in the yeast extract supplemented medium were obtained and shown in Figure 8. Since the pH was 2.4, precipitation of ferric iron kept the ferric concentration low at a constant value of 200 ppm. Figure 9 shows the concentration profiles of iron in the batches without organic supplement. In the two runs, the inoculum was a cell concentrate harvested from an elemental sulfur medium. The initial cell density was measured to be $2.35 \times 10^7$ cells/ml. It had been tried many times to harvest cells from ferrous sulfate medium for inoculation but the attempts failed because of low cell densities in the medium. In Figure 9, one curve obtained from the medium with yeast extract and
Figure 8. Microbial oxidation of ferrous iron in a medium of high ferrous iron concentration.
Figure 9. Iron concentration in solution versus time in two batch runs with ferrous sulfate as energy source.
similar initial ferrous iron concentration is shown for comparison. It was indicated that with yeast extract, cell growth was much better. However, this may not be true when cell density is as low as the one shown in run #3 in Figure 7. One possible explanation is the low pH. The species was reported to have maximum growth at pH 2 to 3. With the yeast extract, the pH remained constant at 2.4, which is preferred for microbial growth. The other explanation may be the limitation of carbon availability. At low cell densities, the carbon dioxide in the air was enough to provide adequate carbon. However, when the cell density was as high as $10^7$ cells/ml, the carbon present in the air may not have been enough for microbial growth.

To model ferrous iron oxidation, several factors should be considered. The first one is the chemical oxidation of ferrous iron to ferric iron by oxygen in the air. The second is the microbial oxidation of ferrous iron. Precipitation of ferric iron should also be considered. The simplified reactions are listed as follow:

$$2 \text{Fe}^{2+} + 2 \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow 2 \text{Fe}^{3+} + \text{H}_2\text{O} \quad \text{(30)}$$

$$\text{Fe}^{2+} \xrightarrow{\text{Bacteria}} \text{Fe}^{3+} \quad \text{(31)}$$

$$2 \text{Fe}_2(\text{SO}_4)_3 + \text{K}_2\text{SO}_4 + 12 \text{H}_2\text{O} \rightarrow 2 \text{KFe}_3(\text{SO}_4)_2(\text{OH})_6 + 6 \text{H}_2\text{SO}_4 \quad \text{(32)}$$

Rate equations can be written based on a few assumptions. It was assumed that reaction (30) was a reversible first order reaction which reached equilibrium at a certain ratio of ferrous to ferric iron concentration. The ratio depended on the pH of the solution and the
partial pressure of oxygen. The microbial oxidation can be assumed to follow the monod type model, as proposed by some researchers (Chang and Myerson, 1982). Precipitation of ferric iron was assumed to be first order on ferric iron concentration. The rate equations were derived as follow:

Rate of chemical oxidation of Fe\(^{2+}\) = \(k_1 \ [Fe^{2+}] - k_{-1} [Fe^{3+}] \) ..........(33)

Rate of microbial oxidation of Fe\(^{2+}\) = \(\frac{\mu_{M, Fe} \ [Fe^{2+}] \ * \ X}{K_{Fe} \ + \ [Fe^{2+}] \ * \ \frac{X}{Y_{Fe}} \) ....(34)

Rate of Fe\(^{3+}\) precipitation = \(k_p \ [Fe^{3+}] \) .........................(35)

where the first two terms to the right of the equal sign in equation (33) are the forward and reverse rates of reaction (30). In equation (34), \(\mu_{M, Fe}\) is the maximum specific growth rate and \(K_{Fe}\) is the saturation constant in the Monod model; \(X\) is the cell density and \(Y_{Fe}\) is the yield factor defined as the number of cells obtained per mM of iron consumed. In equation (35), the rate of iron precipitation was denoted by a simple first order rate expression with the rate constant \(k_p\).

4.2.1 Oxidation of ferrous iron in an uninoculated control

In the absence of microorganisms, the chemical reaction will reach an equilibrium. As shown in the uninoculated controls in Figure 7 and 8. With the help of information about equilibrium, equation (33) and (34) can be simplified as follow:
At equilibrium, \( \frac{d[Fe^{2+}]}{dt} = 0 \) and
\[
k_1[Fe^{2+}]_{eq} = k_{-1}[Fe^{3+}]_{eq}
\] .............................................................(36)

\[
k_{-1} = k_1 \times \frac{[Fe^{2+}]_{eq}}{[Fe^{3+}]_{eq}} = k_1 \times K
\] .............................................................(37)

Rate of iron oxidation = \( k_1 ( [Fe^{2+}] - \frac{[Fe^{2+}]_{eq}}{[Fe^{3+}]_{eq}} \) \times [Fe^{3+}] \) ............(38)

The equilibrium ratio of ferrous and ferric iron, \( K \), can be evaluated from the two control runs. From the control run shown in Figure 7, by assuming that the ratio was close to the equilibrium near the end of the experiment, the ratio was calculated as 5.37. With the same assumption, from the run conducted at 2000 ppm initial iron concentration shown in Figure 8, the ratio was calculated as 4.36. These two values give an average of 4.865. Introduction of \( K \) from equation (37) into equation (33) and (34), without microbial activity,

\[
\frac{d[Fe^{2+}]}{dt} = k_1 ( [Fe^{2+}] - K \times [Fe^{3+}] )
\] .............................................................(39)

\[
\frac{d[Fe^{3+}]}{dt} = k_1 ( [Fe^{2+}] - K \times [Fe^{3+}] ) - k_p [Fe^{3+}]
\] .............................................................(40)

Equations (38) and (39) can be solved simultaneously to give the concentration profiles of ferrous and ferric iron. The Runge-Kutta method was chosen as the numerical method for solving the equations.
The two adjustable constants, \( k_1 \) and \( k_p \), are varied to fit the calculated values to the experimental data. Figure 10 and 11 shows the curve-fitting of the kinetic model to the data. Table 1 gives the values of the constants of the two control runs.

<table>
<thead>
<tr>
<th>initial ([\text{Fe}^{2+}]) (ppm)</th>
<th>( k_1 ) (1/day)</th>
<th>( k_p ) (1/day)</th>
<th>( K )</th>
</tr>
</thead>
<tbody>
<tr>
<td>control #1</td>
<td>664</td>
<td>0.91</td>
<td>0.325</td>
</tr>
<tr>
<td>control #2</td>
<td>1716</td>
<td>1.12</td>
<td>0.305</td>
</tr>
</tbody>
</table>

4.2.2 Bacterial oxidation of ferrous iron

In the presence of microorganisms, the Monod expression in equation (34) can be added to equations (39) and (40) to yield equation (41) and (42).

\[
\frac{d[\text{Fe}^{2+}]}{dt} = -k_1([\text{Fe}^{2+}] - K*[\text{Fe}^{3+}]) - \frac{v_{M,Fe}*[\text{Fe}^{2+}]}{K_{Fe} + [\text{Fe}^{2+}]} \quad \text{.........(41)}
\]

\[
\frac{d[\text{Fe}^{3+}]}{dt} = k_1([\text{Fe}^{2+}] - K*[\text{Fe}^{3+}]) + \frac{v_{M,Fe}*[\text{Fe}^{2+}]}{K_{Fe} + [\text{Fe}^{2+}]} - k_p[\text{Fe}^{3+}] \quad \text{.........(42)}
\]

\[
\frac{dx}{dt} = \frac{v_{M,Fe}*[\text{Fe}^{2+}]}{K_{Fe} + [\text{Fe}^{2+}]} \quad \text{.........(43)}
\]
Figure 10. Curve-fitting of experimental data on chemical oxidation of ferrous iron at low iron concentrations.
Figure 11. Curve-fitting of experimental data on chemical oxidation of ferrous iron at low iron concentrations.
Where equation (43) is the rate equation for microbial growth on ferrous iron. It is assumed here that the oxidation of iron is "growth associated". Again, the Runge-Kutta method was used to solve the three equations simultaneously with the constants varied to fit the data. When solving equation (43), an initial cell density was required. As mentioned earlier, the cell density was so low, only near the end of the experiment were the cells barely countable with the microscope. Since run #3 was inoculated with culture from run #2, the initial cell density could be calculated from the final cell density of run #2. Similar procedures for run #4 were followed since run #4 was inoculated with inoculum from run #3. Even so, the cell density was not accurately defined since the cell density was below $10^6$ cells/ml. The model calculated concentration profiles to compared with the experimental data as shown in Figures 12 and 13. Table 2 gives the values of the parameters for the curve-fitting represented in Figures 18 and 19.

<table>
<thead>
<tr>
<th>run no.</th>
<th>$\mu_{M,Fe}$ (1/day)</th>
<th>$K_{Fe}$ (ppm)</th>
<th>$k_1$ (1/day)</th>
<th>$Y_{Fe}(10^5$ cells/mg Fe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2.11</td>
<td>48</td>
<td>0.91</td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>2.05</td>
<td>45</td>
<td>0.91</td>
<td>3.1</td>
</tr>
</tbody>
</table>

The calculated curves fit the data to an acceptable degree. The rapid drop of iron was associated with rapid growth of the
Figure 12. Curve-fitting of experimental data on microbial oxidation of ferrous ion, run #3
Figure 13. Curve-fitting of experimental data on microbial oxidation of ferrous iron, run #4
microorganism. However, there are some differences between run #3 and run #4. As indicated by the concentration profile of ferric iron, there was more iron precipitated in run #3 than in run #4. As mentioned in Chapter 3, precipitation of ferric iron with sulfate and other species (such as potassium and $\text{H}^+$) depends on many factors. This study did not investigate the mechanism of iron precipitation. However, complex iron sulfate precipitation is very common in microbial leaching experiments and, later, will be discussed briefly.

4.3. The effect of basal salts on microbial coal desulfurization

Basal salts provide the basic nutrients for microbial activities. However, some side effects of the salts were not well understood. The data obtained in batch leaching of Kentucky #9 coal in media with different doses of basal salts and at different slurry density may be useful in defining the basic mechanisms and in designing the process.

4.3.1 The effect of basal salts on microbial leaching of 5% slurry

The performance of the bacteria in sulfur leaching was studied by observing sulfate release and iron release from coal. Figure 14 shows the iron release curves in a 5% slurry of Kentucky #9 coal without basal salts. Figure 15 shows the sulfate release curves for the same runs. The initial pH of the medium was 2.2. The ferrous iron concentration first increased and reached a maximum. Then, near the end of the leaching cycle, ferrous iron concentration dropped rapidly to a very low level. As indicated by the curves of ferric iron, significant amounts of iron precipitated back to the coal. The sulfate release curve, on the other
Figure 14. Iron release in batch leaching of Kentucky #9 coal in 5% slurry with no added basal salts.
Figure 15. Sulfate release in batch leaching of Kentucky #9 coal in 5% slurry with no added basal salts.
hand, did not drop significantly. This could mean more sulfate could have been released from coal if precipitation had not occurred. Murphy (1982) studied the phenomenon of sulfate reprecipitation and identified the precipitate as jarosite. The most common jarosite is potassium jarosite, which contains potassium, ferric iron, hydroxyl ions and sulfate. Solubility of this precipitate is very low at high pH. Figure 16 shows the concentration profiles of iron in the uninoculated control. The scattering of the data make it inappropriate for kinetic evaluation.

The iron and sulfate release curves obtained from batch leaching in the medium with single strength basal salts are shown in Figures 17 and 18. The precipitation of sulfate and iron was even more significant in these runs. A drop in sulfate concentration was observed in run #2. However, the maximum sulfate release rates in these runs are higher than those obtained in the medium without basal salts. The extent of sulfur removal was also higher. A time lag of 1 day was observed in those runs. Similar to the runs without basal salts, the sulfate release rate in run #1 decreased as the leaching went on. On the other hand, the sulfate release rate in run #2 increased rapidly in 6-9 days after inoculation. The increase in sulfate release rate was associated with the drop of ferrous iron concentration and increase of ferric iron concentration. This behavior can be explained in two ways. A higher microbial growth rate which resulted in a higher pyrite oxidation rate and released more iron. In the meantime, higher ferric iron concentration gave a higher chemical reaction rate between the ferric iron and pyrite. As shown in Figure 17, the ferric iron concentration in run #2 was twice as high as that of run #1 at day 9. Scattering of sulfur content values determined
Figure 16. Iron concentrations versus time in an uninoculated 5% slurry of Kentucky #9 coal with no added basal salts.
Figure 17. Iron release in batch leaching of Kentucky #9 coal in 5% slurry with a single dose of basal salts.
Figure 18. Sulfate release in batch leaching of Kentucky #9 coal in 5% slurry with a single dose of basal salts.
for coal was no surprise since the coal had a wide distribution of particle sizes and the finer coal particles were desulfurized faster. If the sample contained more finer coal particles, then the sulfur content would be lower.

When the amount of added basal salts increased further, there was not much gain in sulfate and iron release. The experimental data are shown in Figures 19 and 20. Precipitation of iron and sulfate was even more pronounced. A longer lag time was observed in these runs. Some chemicals in the basal salts could be inhibitory to the microbial activities. In these experiments, HCl was used to acidify the medium, and the Cl\(^-\) could contribute to the longer lag time, since Cl\(^-\) inhibits the growth of *S. acidocaldarius* (Brook et al., 1976).

For a better comparison, the maximum sulfur removal rate, extent of sulfur removal, maximum iron release rate, extent of iron released and \% of iron precipitated were evaluated and compared. Averages of two duplicate runs were used. Scattering data make it difficult to evaluate the rates. To evaluate the maximum rates, a smooth curve was drawn through the points, and then a tangent line was drawn through the curve. The extent of sulfur removal was calculated from the difference between the initial and final sulfate concentrations. The maximum iron release rate was calculated by the same method. The \% of iron precipitated was calculated from the difference between the maximum iron concentration and the iron concentration in the last day of the batch leaching, that is, 14 days after inoculation. Table 3 summarizes the calculated values of the rates and extent of sulfur and iron release.
Figure 19. Iron release in batch leaching of Kentucky #9 coal in 5% slurry with a double dose of basal salts.
Figure 20. Sulfate release in batch leaching of Kentucky #9 coal in 5% slurry with a double dose of basal salts.
The calculated values in Table 3 indicate that the medium with a single dose of basal salts gave the best performance. The maximum sulfur removal rate was highest. The single dose of basal salts yielded a higher maximum release rate, higher extent of iron release and smaller percentage of iron precipitation. The smaller rate and extents obtained from the runs with no added basal salts may be due to the shortage of necessary nutrients. The sources of mineral salts were the coal and tap water when no basal salts were added.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Max. (-\frac{dS}{dt})</th>
<th>Extent of S removal(g S/l hr)</th>
<th>Max. (-\frac{dFe}{dt})</th>
<th>Extent of Fe ppt'ed removal(mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.86</td>
<td>0.713</td>
<td>4.86</td>
<td>538</td>
</tr>
<tr>
<td>1</td>
<td>14.60</td>
<td>1.123</td>
<td>6.38</td>
<td>756</td>
</tr>
<tr>
<td>2</td>
<td>6.57</td>
<td>0.870</td>
<td>3.04</td>
<td>441</td>
</tr>
</tbody>
</table>

On the other hand, with too much basal salts added, the high concentration of potassium enhanced the precipitation of iron and sulfate. This possibility was supported by the lower extent of iron release and higher extent of iron precipitation.

4.3.2 The effect of basal salts on sulfate and iron release in 10% slurry

At higher slurry pulp density, more sulfur was available for microbial activities and the microbes could require more nutrient. The
data collected in batch leaching of Kentucky #9 coal in 10% slurry with different doses of added basal salts were analyzed to clarify mechanisms.

In Figures 21 and 22, the data show a maximum rate at the beginning of the experiment without lag time, as observed in 5% slurry (Figures 14 and 15). However, the ferrous iron concentration did not drop during the experimental period (10 days). Pyrite was still available from the coal and the microbes did not switch their substrate from pyrite to ferrous iron. Figure 22 also shows the sulfate release curve obtained from an uninoculated control. Some of sulfate was released from coal even without the microorganism. This means at higher pulp density (or higher sulfur concentration), mineral sulfate was leached or chemical oxidation was not negligible. In most of the studies reported in the literature, chemical oxidation was neglected (Chang and Myerson 1982; Kargi and Weissman, 1984) since the leaching experiment was done at ambient temperature with *Thiobacillus* species. In our case, the reaction temperature was at 72°C and chemical oxidation of pyrite was probably significant.

The sulfate and iron release curves obtained from 10% slurry with a single dose of basal salts are shown in Figures 23 and 24. Similar to the data from 5% slurry, both the iron and sulfate release showed a lag time. Then the rate increased steadily, reached a maximum and dropped when sulfur in coal was depleted. In Figure 24, sulfate release curves from the inoculated run are identical with the uninoculated runs in the first three days after inoculation. Then the rate started to increase and exceeded the rate of the uninoculated run.
Figure 21. Iron release in batch leaching of Kentucky #9 coal in 10% slurry with no added basal salts.
Figure 22. Sulfate release in batch leaching of Kentucky #9 coal in 10% slurry with no added basal salts.
Figure 23. Iron release in batch leaching of Kentucky #9 coal in 10% slurry with a single dose of basal salts.
Figure 24. Sulfate release in batch leaching of Kentucky #9 coal in 10% slurry with a single dose of basal salts.
Figure 25 shows iron release curves in the uninoculated control. The rate of iron release slowed down after 10 days although the sulfur content in the coal was still high. To model the kinetics of chemical oxidation of pyrite, the rate expression in equation (18) is too complicated to be practical. However, the concept in that model can be used and a simpler model can be derived. The basic concept of the model for chemical oxidation of pyrite is the adsorption-desorption of ferrous and ferric iron on the active sites of pyrite. When the ferrous iron concentration reaches a certain point, more of the active sites were occupied and prevent the ferric iron from attacking the pyrite. Consequently, it may be assumed that the rate depends on the ferric iron concentration and the ratio of ferrous iron and available pyrite. Oxidation of ferrous iron to ferric iron also occurred simultaneously. The simplified reactions are shown as follows:

$$2 \text{Fe}^{3+} + \text{FeS}_2 + 4 \text{O}_2 \rightarrow 3 \text{Fe}^{2+} + 2 \text{SO}_4^2^- \quad \text{(44)}$$

$$\text{Fe}^{2+} + \frac{1}{2} \text{O}_2 + 2 \text{H}^+ \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O} \quad \text{(45)}$$

Equation (45) is an oversimplified form for pyrite oxidation. The real mechanism of pyrite oxidation has been studied since 1901 (Stokes, 1901) and it is beyond the scope and interest of this study. The effort here is to fit the data with some simple kinetic equations and very few adjustable parameters for reactor and process design purposes.

The rate equations are then written as:
Figure 25. Iron release in batch leaching of Kentucky #9 coal in 10% slurry with a double dose of basal salts.
Equation (38) is also applied here for ferrous iron oxidation. Since the data in Figure 25 show little iron precipitation, the precipitation terms in equations (38) and (39) are dropped. By varying the values of \( k_s \) and \( k_1 \) in equations (38), (39) and (46), the concentration profiles can be calculated. Figure 26 shows the results of curve-fitting. The values of \( k_s \) and \( k_1 \) are 14.9 (1/day) and 1.10 (1/day), respectively. The value of \( k_1 \) here is comparable to the values obtained from ferrous sulfate medium results. (see Table 3).

The experimental data from batch leaching of Kentucky #9 coal in 10% slurry with a double dose of basal salts are shown in Figures 27 and 28. The iron concentration increased almost exponentially and then reached a plateau and longer time lag was observed. The iron or sulfate release rate in the first few days was less than that of an uninoculated control. This suggests that the basal salts may inhibit the chemical oxidation of pyrite. Reprecipitation of iron and sulfate was not observed since the ferric iron concentration remained low during the experiment period.

The maximum sulfate release rate, extent of sulfur removal, maximum rate of iron release and extent of iron release at the end of the experimental period were determined and listed in Table 4. The rates and extents of sulfur and iron release shown in Table 4 could be subject to a large error. As indicated in the data, the day to day fluctuation of the concentration profiles were quite large compared to the data from
Figure 26. Curve-fitting of experimental data on an uninoculated control of 10% slurry of Kentucky #9 coal with a single dose of basal salts.
Figure S7. Iron release in batch leaching of Kentucky #9 coal in 10% slurry with a double dose of basal salts.
Figure 28. Sulfate release in batch leaching of Kentucky #9 coal in 10% slurry with a double dose of basal salts.
the 5% slurry. Some of the variation is associated with the measurement method itself. The sample was diluted 100 times before analysis, and human error could also affect the final results. The other possibility is the precipitation of iron. It was noticed that a drop in sulfate concentration usually occurred with a drop in iron concentration.

Another possible reason is poor mixing in the 10% slurry. Some fine coal tended to agglomerate while the larger or heavier coal particles tended to settle. A sudden breakdown of the agglomerated or settled coal particles is equivalent to a sudden addition of pyrite to the bulk slurry and results in a high rate of sulfate or iron release. On the other hand, the extent of sulfur and iron removal are less sensitive to fluctuation and can be used to judge performance.

---

Table 4. Rates and extents of sulfate and iron release in 10% slurry.

<table>
<thead>
<tr>
<th>Salts doses</th>
<th>$\frac{dS}{dt}$ Max. (mg S/l hr)</th>
<th>Extent of S removal (g S/l)</th>
<th>$\frac{d[Fe^{2+}]}{dt}$ Max. (mg/1 hr)</th>
<th>Extent of Fe removal (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16.8</td>
<td>1.21</td>
<td>12.4</td>
<td>1017</td>
</tr>
<tr>
<td>1</td>
<td>16.7</td>
<td>1.42</td>
<td>7.7</td>
<td>1278</td>
</tr>
<tr>
<td>2</td>
<td>11.0</td>
<td>1.17</td>
<td>9.4</td>
<td>982</td>
</tr>
</tbody>
</table>

As shown in Table 4, the runs with a single dose of basal salts again gave the highest extents of sulfur and iron removal. If the values in Table 4 are normalized to the same basis, that is, per unit weight of coal, both the rate and extent of sulfate and iron removal are slightly
smaller. Kargi and Robinson (1985) reported that at high slurry pulp density, CO$_2$ enrichment was necessary to reach the maximum sulfur removal rate. That means that with a sufficient supply of energy (sulfur in coal), the carbon source becomes the limiting growth factor.

4.4 Removal of organic sulfur

The data collected in batch leaching of a fine Kentucky #9 coal and a petroleum coke showed little evidence of significant sulfur removal. Figure 29 shows the iron release from the fine Kentucky #9 coal. This coal had been pretreated to removal almost all the pyritic sulfur. Results from sulfur form analysis of the coal are given in Appendix D. Sulfur balance before and after microbial treatment showed that small amounts of organic were removed. In Figure 30, neither the sulfate release to solution nor the drop in sulfur content of the coal showed significant reduction of sulfur in the coal.

The data collected from batch leaching of petroleum coke are given in Figure 31. No appreciable amount of sulfur was removed from the coke. One of the difficulties in the leaching experiments was the hydrophobicity of the coke. Since the surface of the coke surface was so hydrophobic, it did not wet well with the aqueous solution. The small bulk density of the coke made the coke particles float on the surface of the slurry. A surfactant such as a trace amount of ethanol was added to assist the formation of stable slurry. However, significant sulfur reduction was not observed.
Figure 29. Iron release in batch leaching of -325 mesh precleaned Kentuck #9 coal in 5% slurry with no added basal salts.
Figure 30. Sulfate release in batch leaching of -325 mesh precleaned Kentucky #9 coal in 5% slurry with no added basal salts.
Figure 31. Sulfate concentration in solution and solid sulfur content in batch leaching of an Amoco petroleum coke.
4.5 Effects of prior washing

Figures 32 and 33 show the concentration profiles of ferrous and ferric iron. Since the particles had very small variations in size, the possibility of sampling error was reduced. Prior wash reduced the amount of soluble iron and soluble organic matter. In these, the inoculum was a cell concentrate harvested from an elemental sulfur medium. The initial cell density of the run with unwashed coal was $7.94 \times 10^7$ and that in the prewashed coal was $6.81 \times 10^7$ cells/ml.

The maximum iron release rate calculated from Figure 32 was 10.3 mg Fe/l hr, which is much higher than the values shown in Table 4. In previous leaching experiments, the pulverized coal derived from grinding was used. The coal samples contained a very wide range of size distribution, as shown in Appendix D. The -325 mesh part of the coal samples contributed to the high rate of iron release while the portion of +200 mesh coal particles yielded lower rates. A direct comparison was not appropriate.

The maximum iron release rate evaluated from Figure 33 was 7.17, which is lower than the value from the unwashed sample. The high ferrous iron concentration present in the unwashed coal may stimulate the microbial growth on ferrous iron, which in turn provide large amounts of ferric iron to oxidize the pyrite in coal. As indicated in Figure 32, the ferrous iron concentration started dropping at day 1.5, while the same phenomena occurred at day 4.5 in Figure 33. The drop of ferrous iron indicated that the rate of ferrous oxidation exceeded the rate of ferrous iron provided by pyrite oxidation. In Figure 32 and at the point where ferrous iron concentration started dropping, only 60.4 % of the
Figure 32. Iron release in batch leaching of an unwashed -270 +325 mesh Kentucky #9 coal, run #1.
Figure 33. Iron release in batch leaching of a prewashed -270 +325 mesh Kentucky #9 coal.
leachable iron had been leached. The high ferric iron concentration hastened the oxidation of the remaining 40% of pyrite. However, in Figure 33, 75.9% of the leachable iron had been leached. With lower ferric iron concentrations and smaller amounts of pyrite, the leaching rate is therefore lower. Comparison of the sulfate release curves given in Figure 34 further supports this argument.

4.6 The effect of pyrite grain size on microbial leaching

Two different coals with different pyrite grain sizes were studied. Figure 35 gives the iron release curves in a batch run of Ohio coal mixture and Figure 36 shows the concentration profiles in the batch leaching of Kentucky #9 coal. The Kentucky #9 coal had a fine pyrite grain size. According to the information obtained from the Babcock and Wilcox Co., Alliance, Ohio, the pyrite grain size was 100% -10 micron. The Ohio coal mixture, however, was assumed to have a pyrite grain size of 60% -10 micron according to the statistics of pyrite grain size in Ohio coals (Kneller et al., 1985). The initial cell densities of these two runs were about the same. The initial cell density in the batch leaching of Ohio coal mixture was 7.71 x 10^7 cells/ml, while that of Kentucky #9 coal was 7.27 x 10^7 cells/ml.

The ferrous iron concentration started dropping at 2.5 days after inoculation for the run of Kentucky #9 coal, while that in the run of Ohio coal mixture did not drop until the 12th day. The maximum iron release rate from the Ohio mixture was 8.25 while that from Kentucky #9 coal was 21.8 mg/l hr. The difference is even more evident in Figure 37. The rates of sulfate release were identical in the first 2.5 days.
Figure 34. Sulfate release in batch leaching of an unwashed and a prewashed -270 +325 mesh Kentucky #9 coal.
Figure 35. Iron release in batch leaching of a -270 +325 mesh Ohio coal mixture.
Figure 36. Iron release in batch leaching of an unwashed 
-270 +325 mesh Kentucky #9 coal, run #2.
Figure 37. Sulfate release in batch leaching of unwashed -270 +325 mesh Kentucky #9 coal and Ohio coal mixture.
However, the rate in the run with Kentucky #9 coal (finer pyrite grain size) exceeded that in the run of Ohio coal mixture. The sulfur levels in the coals were similar.

The major effect of pyrite grain size on sulfur removal was the pyrite surface area for oxidation. Finer pyrite grain size gave a higher oxidation rate as long as the coal was ground fine enough to expose the pyrite. The model proposed by Huber et al. (1985) explains the effect of pyrite surface area available. In that model, the cells attached to the surface underwent exponential growth until the surface was saturated with cells; the so called "cell limiting regime". In this regime, the rate was determined by microbial growth, not the amount of available pyrite. Once the surface was saturated with microorganisms, no more cell increase will be expected and the rate of sulfate release depended on the surface area of pyrite. The cells per unit area of pyrite remained constant in this regime and the rate of pyrite oxidation per unit surface area of pyrite remained constant. Consequently the coal with finer pyrite grain size gave a higher sulfate release rate.

4.7 The effect of initial cell density

Data on sulfate and iron release were collected in batch leaching of Clarion #4 A coal. In these experiments, -200 mesh coal was used. In Run #1, the coal was inoculated with cell concentrate to give an initial cell density of $5.13 \times 10^6$ cells/ml while in run #2 the coal slurry was inoculated to give an initial cell density of $8.20 \times 10^7$ cells/ml. The basal salt medium was modified according to previous findings. The amount of potassium phosphate was reduced to 1/2 of that the original to
prevent precipitation of jarosite. The solution pH was adjusted to 1.7 instead of 2.0 to reduce further sulfate reprecipitation.

The data are shown in Figures 38 and 39. The difference in the concentration profiles is pronounced. In Figure 38, the batch with a high cell density showed a higher ferrous iron oxidation rate, which was indicated by the drop in ferrous iron concentration and the rapid increase in ferric iron concentration. The total iron release curves for the two runs were indistinguishable in the first 2 days. There followed a rapid rise in ferric iron concentration accompanied by a high rate of sulfate release, as indicated in Figure 39. The indistinguishable rates indicate that the iron release rate (or sulfate) in the first 2 days of leaching was limited by something other than the cell density. The major contribution to pyrite oxidation could come from chemical oxidation instead of microbial.

The scattering of sulfur analyses came from sampling error since the slurry contained wide distributions of particle sizes. The data suggest that the cells induced pyrite oxidation in two ways. The cells attached to the pyrite surface and oxidized the pyrite to give ferrous iron, while the cells which did not attach to pyrite oxidized ferrous iron to yield ferric iron. Kargi and Robinson (1985) reported that the number of attached cells increased while the freely suspended cells did not increase during batch leaching experiments. However, as indicated in Figure 38, the rate of iron oxidation increased and that result suggests a growth of microorganisms on ferrous iron. Therefore, it may be assumed that some of cells attached to coal grew on ferrous iron, not on pyrite.
Figure 38. Iron release in batch leaching of an -200 mesh Ohio Clarion #4 A coal at two different initial cell densities.
Figure 39. Sulfate release in batch leaching of an ~200 mesh Ohio Clarion #4 A coal at two different initial cell densities.
4.8 Sulfur forms in desulfurized coals

Sulfur form analyses were conducted on the coals involved in the experiments of 3.5 and 3.7. The coal studied in 3.5 was a weathered Kentucky #9 coal with a size range of -270 +325 mesh. In the experiments of 3.5, one coal sample was washed with basal salt solution at 72 °C to remove soluble sulfate and iron. Sulfur form analysis was conducted on the washed coal sample before and after microbial leaching. The coal involved in 3.7 was Ohio Clarion #4A with a size of 100% -200 mesh. Sulfur form analysis was performed on the coal sample from the leaching experiment conducted at higher cell density. Results are shown in the following table.

Table 5. Sulfur forms in treated and untreated coals.

<table>
<thead>
<tr>
<th>Coal type</th>
<th>Sulfur forms (% as S) Before leaching</th>
<th>After leaching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kentucky #9</td>
<td>Total Sulfur</td>
<td>3.87</td>
</tr>
<tr>
<td></td>
<td>Sulfate</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Pyritic</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>1.75</td>
</tr>
<tr>
<td>Ohio Clarion #4A</td>
<td>Total Sulfur</td>
<td>8.31</td>
</tr>
<tr>
<td></td>
<td>Sulfate</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Pyritic</td>
<td>6.80</td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>1.33</td>
</tr>
</tbody>
</table>
Significant amounts of "organic sulfur" were removed from the Kentucky #9 coal, while only a small amount was removed from the Clarion #4A coal. Exact percentage of organic sulfur removal should be calculated on "ash free basis". Extent of organic sulfur removal was calculated to be 33% for the Kentucky #9 coal and near 5% for Clarion #4A coal.

Results on organic sulfur removal obtained in this study are comparable with those reported in the literature (Chandra et al., 1985; Kargi and Robinson, 1982; and Isbister and Kobylinski, 1985). The mesophilic microorganism employed by Chandra et al. was not characterized, but it was able to remove up to 20% of organic sulfur. Using *S. acidocaldarius*, Kargi and Robinson (1982) reported the removal of organic sulfur, which was determined by the difference between total sulfate production and sulfate produced from pyritic sulfur calculated from the amount of leached iron. However, sulfur forms analysis was not available in their study (Kargi and Robinson, 1982). A microbe designated as CB1, selected from mutant colonies of naturally occurring soil microorganisms, was claimed to be able to remove up to 47% of organic sulfur from coal depending on the coal types (Isbister and Kobylinski, 1985).

4.9 Adsorption of cells on coal and other particle surfaces

The adsorption experiments in this study provided some information about the "short term" cell-particle interaction. Figure 40 shows the solution cell densities versus contact time of cells with different solid particles. Adsorption was a very fast process in that equilibria were reached in less than 5 min. for most of the solid particles
Figure 40. Cell adsorption on different particles. (Δ) Kentucky #9; (○) Sulzer coal; (●) Ohio #6; (□) Ohio coal mixture; and (x) Activated carbon.
studied. Activated carbon reduced cell density in solution to an undetectable level in less than 1 min. The Sulzer coal which contained only 0.12% pyritic sulfur adsorbed more cells than the Kentucky #9 (containing 2.68% pyritic sulfur), as indicated in the figure. The Ohio #6 coal and the Ohio coal mixture adsorbed comparable amounts of cells despite the difference in pyritic sulfur contents.

A comparison with prior work is difficult since published literature is not available for S. acidocaldarius adsorption on coal. Furthermore, for T. ferrooxidans, none of the reports (Myerson et al., 1983, Dispirito et al., 1983) mentioned the mixing condition (whether the mixture of coal and cell solution was agitated to provide good contact) so that direct comparisons of the adsorption rates with T. ferrooxidans could not be made. Differences in coal types, in microbial species, and in cell concentrations also prevented significant comparisons. Nonetheless, the results showed a very high rate of cell attachment and a profound difference in extent of adsorption for different particles.

Figure 41 shows the effects of pH, temperature and agitation speed on the extent of cell adsorption. The rates of S. acidocaldarius adsorption between the runs at different pH and temperature were indistinguishable, so the rate data are not shown here. The pH and temperature did not significantly affect the extent of cell adsorption. At pH higher than 8.0, cell dissolution was observed under the microscope and the adsorption tests were disregarded. Increasing
Figure 41. Effects of pH, temperature, and agitation speed on extent of cell adsorption.
agitation speed only slightly decreased the amount of cell adsorption. This result indicates that the bonding force is strong between the cell and the coal surface.

Figure 42 shows the effects of prior acid leaching on cell adsorption. Nitric acid treatment reduced both the rate and extent of adsorption. However, hydrochloric acid leaching did not affect cell adsorption. Whether the reduction of cell adsorption by nitric acid treatment is due to the elimination of pyrite surface or to the oxidation of the coal surface is yet to be determined.

The effects of initial cell density on both adsorption rate and extent of attachment were investigated with Kentucky #9 coal. Results are shown in Figure 43 where the equilibrium cell density in the solution increased with increasing initial cell density. Figure 44 shows a plot of the number of adsorbed cells versus equilibrium cell density in the solution for different coals, carbon and pyrite particles.

In Figure 44, among the solid particles tested, only Kentucky #9 and Ohio coal mixture showed reversible cell attachment. However, the levels of specific adsorption obtained from desorption experiments were higher than those derived from adsorption. This result indicated that some adsorbed cells developed an irreversible attachment through contact with the particles. Similar phenomena have been reported in the literature, where some researchers showed that more Sulfolobus cells attached to elemental sulfur or mineral sulfides after longer incubation
Figure 42. Cell adsorption on pyrite, Kentucky #9 coal and acid-leached Kentucky #9 coal (o) pyrite, (A) Kentucky #9 coal, (o) nitric acid leached coal, (x) HCl leached coal.
Figure 43. Cell adsorption on Kentucky #9 coal with different initial cell densities. Initial cell density (10^6 cells/ml): (○) 16.5 (□) 8.20, (△) 5.27, (x) 2.75
Figure 44. Adsorption isotherms for cell adsorption on coals and pyrite.

(Φ) Sulzer coal, (○) Kentucky #9, (●) pyrite, (Δ) Ohio coal mixture, (▽) Ohio #6.
(+: desorption data from Kentucky #9
(x: desorption data from Ohio coal mixture)}
Interpretation of such experimental results is difficult without precise information on surface properties of the microbial cell wall and the solid surfaces. These properties are total external surface area of the solid particles, surface charge, hydrophobicity, glycocalyx or surface appendages on the cell wall and others (Savage et al., 1985).

**Adsorption modeling**

Simple adsorption-desorption kinetics can be applied to describe reversible attachment. A Langmuir model analysis is proposed as follows:

\[
- \frac{dX}{dt} = k_1 X (1-\theta) - k_{-1} \theta
\]

where \( X \) is the cell density in the solution, \( k_1 \) and \( k_{-1} \) are the rate constants for the adsorption and desorption steps, respectively, \( \theta \) is the fraction of coverage defined as \( v/v_m \), \( v \) is the number of cells adsorbed and \( v_m \) is the maximum number of cells that the surface can adsorb. The number of cells adsorbed was calculated from the difference between the initial cell density and the measured cell density in the solution at time \( t \). At \( t=0 \), \( \theta \) is 0, and the \( k_1 \) can be evaluated from the initial adsorption rate. However, the adsorption rate was so high in this work that the evaluation of the initial rate was beyond the accuracy of measurement. At equilibrium, where the net rate of adsorption was 0, equation (47) can be rearranged. With the definition
of $\theta$ as $v/v_m$, the Langmuir isotherm in linearized form is obtained as follows

$$\frac{1}{v} = \frac{1}{v_m} + \frac{1}{Kv_m} \frac{1}{X} \tag{48}$$

where $v$ is the specific adsorption (cells/cm$^3$ solid), $K$ is the equilibrium constant defined as $k_1/k_{-1}$, and $X$ is the equilibrium cell density in solution. For a suitably modelled system, a plot of $1/v$ versus $1/X$ gives a straight line with the parameters evaluated from the intercept and slope of the line. Figure 45 shows such a plot. The data fit the model to a reasonable level. The solid lines in Figure 45 were calculated from equation (48) with the parameters evaluated from plots similar to Figure 45 by the least squares curve-fitting technique. The calculated values of $K$ and $v_m$ for Kentucky #9 coal and Ohio coal mixture are shown in Table 6.

Adsorption isotherms of PAAx on coals and pyrite are shown in Figure 46. Amounts of PAAx adsorbed were related to the pyrite surface area. The coals with less pyritic sulfur adsorbed lesser amounts of PAAx, as demonstrated by the isotherms. The Kentucky #9 coal contained 2.68% pyritic sulfur, which is slightly higher than that of the Ohio coal mixture at 2.12% of pyritic sulfur. The relationship is not quite linear because pyrite area is also a function of pyrite particle size. For example, the pyrite grain size in the Kentucky #9 coal was smaller than 5 $\mu$m. The adsorption isotherms indicated that the Kentucky #9 coal
Figure 45. Data fitting with Langmuir model for the cell adsorption on Kentucky #9 coal.
Figure 46. Adsorption isotherms of PAAx on coals and pyrite.

(△) Kentucky #9 coal, (○) Ohio coal mixture (x) pyrite, (○) Ohio #6, (○) Sulzer coal.
had a higher pyrite surface area. That was a finding supported by the fact that the finer the pyrite particle, the larger the surface area for a constant mass. The maximum adsorption of PAAX, designated as $v_{mp}$, was evaluated directly from the isotherms in Figure 46.

Table 6 compares the data in Figures 44 and Figure 46. In this table, the amounts of pyritic sulfur and organic sulfur of each coal are shown together with the calculated values of $K$, $v_m$ and $v_{mp}$. The data show that Sulzer coal strongly adsorbed the *S. acidocaldarius* cells even though the pyrite surface was relatively low. The theory of preferential cell adsorption on pyrite did not apply in this case. The results suggest that the adsorption of *S. acidocaldarius* on solid particles is directly related to the broad surface properties, not necessary to the amount of pyrite surface. Further adsorption experiments with surfactants are planned to permit better definition of coal surface in order to clarify the mechanism of cell adsorption.

4.10 Modeling the kinetics of microbial leaching

Microbial oxidation terms can be added to the model of chemical leaching alone. Equation (46) is then modified as:

$$- \frac{d[FeS_2]}{dt} = k_{s}[Fe^{3+}](-\frac{[Fe^{2+}]_{eq}}{[FeS_2]_{eq}} - \frac{[Fe^{2+}]}{[FeS_2]}) + \frac{\mu_{M,S} \cdot X_A \cdot [FeS_2]}{Y_S} \quad (49)$$

The attached cells are assumed to have a maximum specific growth rate of $\mu_{M,S}$, which is independent of the amount of pyrite. As suggested by
Table 6. Sulfur contents and calculated values of $K$, $v_m$ and $v_{mp}$.

<table>
<thead>
<tr>
<th>Sulfur contents</th>
<th>$K$</th>
<th>$v_m$</th>
<th>$v_{mp}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% pyritic</td>
<td>% organic</td>
<td>$10^{-8}/$cell</td>
</tr>
<tr>
<td>Kentucky #9</td>
<td>2.68</td>
<td>1.49</td>
<td>1.063</td>
</tr>
<tr>
<td>Ohio coal mix.</td>
<td>2.12</td>
<td>1.29</td>
<td>0.933</td>
</tr>
<tr>
<td>Ohio #6</td>
<td>0.68</td>
<td>1.37</td>
<td>-</td>
</tr>
<tr>
<td>Sulzer</td>
<td>0.12</td>
<td>0.54</td>
<td>-</td>
</tr>
<tr>
<td>Pyrite</td>
<td>53.40</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Huber et al. (1984), the Monod type of model was not appropriate here since pyrite is not a soluble substrate. The value, $X_A$, is the density of attached cells, defined as cells/mM pyrite, and $Y_S$ is the yield factor for microbial oxidation of pyrite defined as cells/mM FeS$_2$ oxidized. The microbial growth is considered as occurring in two ways. One is the growth on pyrite surface, as described by the last term in equation (49). The other term is the growth on ferrous iron in solution. For the growth on pyrite surface, the number of attached cells is limited by a saturation cell density designated as $X_A^*$. For the growth on ferrous iron, the Monod expression in equation (49) can be applied. The density of the cells which did not attach to the pyrite surface (could be free suspended or attached to surfaces other than pyrite) is designated as $X_F$.

\[
\frac{d[Fe^{2+}]}{dt} = -k_1([Fe^{2+}] - K[Fe^{3+}]) + \frac{\mu_{M,Fe}^* [Fe^{2+}]}{K_F + [Fe^{2+}]} + \frac{3 \mu_{M,Fe}^* X_A^*[FeS_2]}{[FeS_2]_{eq} Y_S} \quad \cdots \cdots \cdots \cdots \cdots \cdots (50)
\]

The factor 3 came from the assumption that 3 moles of ferrous iron were released when one mole of FeS$_2$ was oxidized according to reaction (44).

\[
\frac{d[Fe^{3+}]}{dt} = k_1([Fe^{2+}] - K[Fe^{3+}]) + \frac{\mu_{M,Fe}^*[Fe^{2+}]}{K_F + [Fe^{2+}]} - k_p[Fe^{3+}] \quad \cdots \cdots \cdots \cdots \cdots \cdots (51)
\]
The second term in equation (53) is the rate at which cells switch from pyrite to ferrous iron as the energy source. Equations (49) through (53) can be solved simultaneously, with assumed values of the parameters.

The batch leaching of -270+325 mesh Kentucky #9 coal (Figure 42 and 43) was simulated with this model. The initial cell density was $7.27 \times 10^7$ cells/ml. The cell density in solution was measured as $2.15 \times 10^7$ cells/ml. The difference between the two values is assumed to be the number of cells attached to the coal. To simulate the model, the initial cell densities, both on the pyrite surface and not on the pyrite surface are necessary. Two cases were assumed. Case I describes cells preferentially adsorbed on the pyrite surface. In other words, all the attached cells were on pyrite sites in the coal. Case II has only part of the attached cells on the pyrite surface while others were on coal surface.

Figure 47 shows the simulation for case I. The predicted ferrous iron concentration was higher than the experimental data. Figure 48 shows the simulation results with the assumption of case II. The predicted ferrous iron concentration in the first two days of leaching was lower than the experimental data, which means that fewer cells were utilizing ferrous iron as energy. In Figure 48 the number of cells which
Figure 17. Curve-fitting of experimental data on batch leaching of -270 +325 mesh Kentucky #9 coal, case I.

Sulfate concentration (g/l)

Iron concentration (ppm)
Figure 46. Curve-fitting of experimental data on batch leaching of -270 +325 mesh Kentucky #9 coal, case II.
were attached to pyrite surface was assumed to be 10% of the cells attached to coal particles. Figure 49 shows the simulated cell densities. More details of the modeling work are given in Appendix G. The model predicted the general trend, or the dynamics of the leaching system, but more information is required to develop further the model for a better fit to the experimental data.
Figure 49. Calculated cell densities versus time in batch leaching of -270 +325 Kentucky #9 coal.
CHAPTER 5
CONCLUSIONS

1. In the absence of microorganisms, oxidation of ferrous iron to ferric iron by oxygen is significant. Ferric iron ions provided in this way oxidized pyrite to yield ferrous iron and sulfate. At elevated temperature near 72°C, chemical oxidation significantly contributed to the removal of pyritic sulfur from coal. A simple kinetic model proposed to simulate the reaction system gave satisfactory results.

2. The species *S. acidocaldarius* oxidized ferrous iron into ferric iron for growth. The growth could be described with a Monod model. Although information on cell counts was not precise, the model showed concentration profiles near those obtained experimentally to an acceptable degree.

3. The basal salts, which were considered as nutrients for microbial growth, increased both iron and sulfate release rates from the Kentucky #9 coal. However, adverse effects were observed at high doses of added basal salts. Evidence indicated that the basal salts promoted reprecipitation of ferric iron and sulfate. The larger amounts of HCl required to acidify stronger basal salts solutions inhibited microbial activities. The medium with a single dose of basal salts gave the best performance.
4. Leaching experiments conducted in 10% coal slurry yielded lower rates and extents of iron and sulfate release based on unit mass of the coal. The higher possibility of iron and sulfate reprecipitation presented in the 10% slurry during batch leaching experiments reduced the apparent leaching rates. The medium with a single dose of basal salts also showed the best performance.

5. This study did not provide evidence of significant removal of organic sulfur from a fine, pre-cleaned Kentucky #9 coal (100% -325 mesh with organic sulfur as the major sulfur type) or from an Amoco petroleum coke. However, sulfur forms analysis conducted on another -270 +325 mesh Kentucky #9 coal before and after microbial leaching showed 33% organic sulfur removal. Similar analysis conducted on -200 mesh Ohio Clarion #4 A coal showed 5% organic sulfur removal.

6. Batch leaching conducted on unwashed and prewashed -270 +325 mesh coal indicated that unwashed coal yielded a higher rate. The performance was interpreted to show microbial growth enhanced by soluble iron and water leachable nutrients present in the coal.

7. Batch leaching of -270 +325 mesh Kentucky #9 and Ohio coal mixtures showed that the Kentucky #9 coal, with a finer pyrite grain size, gave higher iron and sulfate production rates. The results were interpreted in terms of higher pyrite surface area allowing for increased microbial oxidation rates.

8. Ohio Clarion #4 A coal was treated with different initial microbial cell densities. The higher cell densities increased the pyrite oxidation in two ways: some cells oxidized pyrite to yield ferrous iron
and sulfate, while some cells oxidized ferrous iron in solution to give ferric iron, which in turn oxidized the pyrite in coal and yielded a higher leaching rate.

9. Cell attachment to coal and other particles was evaluated. The rate of cell attachment was so high that cell adsorption was completed in less than 5 min of cell-solid contact. More cells attached to activated carbon and to a low sulfur coal. Prior leaching of the coal with nitric acid reduced both the rate and extent of cell attachment. The relation between the solution cell density and specific adsorption was described with a Langmuir model. Comparing cell adsorption to the adsorption of pyrite-selective adsorbate indicated that the number of cells attached depends on broader aspects of surface properties, not on the number of available pyrite sites alone.

10. A kinetic model was proposed to describe the bacterial leaching system. The cells attached to pyrite sites grew on pyrite and yielded ferrous iron and sulfate as the products. The cells which were not on the pyrite sites, either attached to the coal surface or freely suspended utilized ferrous iron as an energy source. Chemical oxidation of pyrite by ferric iron and chemical oxidation of ferrous iron were also considered. The model gave a good description of the dynamics of the leaching system.
CHAPTER 6

RECOMMENDATIONS

1. The model for microbial oxidation of ferrous iron should be refined. The precipitation of ferric iron with sulfate was not properly described by the model. Two important factors of the precipitation, pH and potassium ion concentration, should be considered.

2. The study of basal salts should be repeated with greater precision.

3. The measurement of microbial activity was based on iron and sulfate release. Measuring the CO₂ or O₂ uptake with high precision may be possible to give a better measure of cell activity.

4. Leaching experiments should be conducted at different ratios of nitrogen sources to potassium source to define the optimum composition of the medium.

5. The removal of organic sulfur from petroleum coke was not shown. Poor cell-coke contact could be the reason for poor sulfur removal. The surface of the coke should be modified by adding surfactants to improve performance.

6. To test the effect of iron concentration on pyrite oxidation, ferrous or ferric iron can be added to the medium before inoculation. After the coal is washed with the basal salts solution, the solution may be added to pure pyrite which could then be subjected to microbial
leaching in order to test the possibility of sulfur removal by leachable matter in coal.

7. The pyrite grain size was not measured in this experiment. The results obtained are qualitative rather than quantitative. An accurate measurement of pyrite grain size or pyrite surface area would improved the correlation with the sulfur removal rates.

8. In the adsorption experiment, the surface area of the coals and other particles was not measured. With information about the surface area and surface properties, the theory of preferential attachment of microbial cells on pyrite surfaces can be further tested.

9. The mathematical model can be improved with new data and modified to fit the data better. The effects of pH, pyrite grain size and mass transfer rates can be included more explicitly.
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by the thermophilic Organism Sulfolobus acidocaldarius.," Biotech.
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APPENDIX A

PROTEIN ASSAY FOR THE DETERMINATION OF BACTERIAL CELL DENSITY.
APPENDIX A

PROTEIN ASSAY FOR THE DETERMINATION OF BACTERIAL CELL DENSITY

Total protein assay was adopted as the indirect measure of bacterial cell density in slurry of coal or other particles. Many researchers used the same approach in their studies of the attachment of Thiobacillus cells to coal or other particles. Dugan et al. (1983), Chang and Myerson (1983) employed the Folin Phenol Method (or Lowry Method) to determine the total bacterial protein and use that as a measure of bacterial cell density. Kargi and Robinson (1985) applied the Modified Bradford Method (commercialized as the Bio-Rad method) to determine the cell density during batch leaching experiments. Both methods have been tested in this study in order to identify the better one for our case.

A.1 Modified Lowry method

This method is based on the reduction of the phosphomolybdic-tungstic mixed acid chromagen in the reagent of Folin and Ciocalteu, the reduced form of which has an absorption maximum at 750 nm. Details about of reactions involved in the analysis are available in the literature (Lowry et al., 1951). In this study, a protein assay kit was obtained from Sigma Chemical Co. and the analytical procedures
accompanied with the kit were followed. The kit contained several solutions for protein precipitation and analysis. Solution of Bovine Serum Albumin (BSA) was provided with the kit as a standard for calibration. The procedures are summarized as follow:

1. To one ml of protein solution, 0.1 ml of sodium deoxycholate (DOC) solution was added. The mixture was allowed to stand for 10 min.

2. Add 0.1 ml of Trichloroacetic Acid (TCA) solution and centrifuge the sample at 27,000x g for 10 min.

3. Discard the supernatant and redissolve the precipitate with 1 ml of Reagent A (as designated in the instruction accompanied with the kit, the reagent was a mixture of CuSO₄, Na₂CO₃, potassium tartarate, NaOH and sodium dodecyl sulfate), allow the solution to stand for 10 min.

4. Add one ml of Reagent B (as designated in the instruction with the assay kit, the reagent was a diluted Folin-Ciocalteu phenol reagent). Allow the solution to stand for 30 min and read the absorbance at 750 nm.

5. For calibration, prepare a series of standard protein solutions by diluting the BSA solution provided with the assay kit. Follow step 1 through 4 and prepare a calibration curve.

This protein assay method was very sensitive to interferences from hundreds of organic and inorganic chemicals (Perterson, 1983). Step 1 and 2 of the procedures described above were designed to precipitate the protein from the solution such that the interferences can be eliminated.
However, the procedures were time consuming (it took more than one hour to finish one sample) and had higher risk of human error.

A.2 Modified Bradford Method

The blue dye Coomassie Brilliant Blue G shows an absorption maximum at 465 nm which shifts to 595 upon binding to protein. This method is more specific and less sensitive to many interfering substances. Preparation of the dye solution followed the procedures proposed by Peterson (1983). Step by step procedures for preparation and analysis of protein are summarized as follow:

1. 1.3 g of Coomassie Brilliant Blue G obtained from Sigma Chemical Co. was dissolved in 100 ml of pure ethanol and 200 ml of 88% phosphoric acid.

2. Filter the above dye solution through a piece of Whatman #1 filter paper and store it in an amber bottle.

3. To prepare the working solution, take 30 ml of the stock solution, mix it with 40 ml of pure ethanol and 80 ml of phosphoric acid (88%). Dilute the solution to 600 ml with double distilled water. The amount of soluble dye in this solution will decline slowly. The solution may not be usable after a time period of 4 or more weeks. At that situation, prepare a new working solution from the stock solution.

4. The pH of the protein solution was adjusted to 2.0 before analyses. To assay the total protein, add 1.5 ml of the dye solution prepared in step 3 to 1 ml of protein solution and mix them quickly.

5. Read absorbance at 595 nm between 2 and 10 min.
6. Calibration was accomplished with BSA standard solutions following the same procedures described in step 4 and 5.

At high protein concentration and low pH, the protein tends to precipitate. Nonlinear reading is possible in that situation. However, this method is superior to the Lowry methods in many ways. It is simpler and requires less than 5 min to finish one sample. Sensitivity is twice higher than that of Lowry Method, as shown in the calibration curves in Figure 50. This method is also less sensitive to most of the chemicals which interfere the Lowry Method. The disadvantage of this method is that it has not been well studied as far as interference is concerned.

A.3 Curve-fitting of the calibration data

The nonlinear calibration curves shown in Figure 50 can be fitted with a hyperbolic function. A double reciprocal plot was made from the calibration curves and shown in Figure 51. The calibration equations are listed as follows:

**Lowry Method:**

\[
\frac{1}{A} = 0.61 + 83.47 \times \frac{1}{P} \tag{54}
\]

where \(A\) is the absorbance at 750 nm and \(P\) is the protein concentration in (\(\mu g/ml\)).

**Modified Bradford Method:**
Figure 50. Calibration curve for total protein analysis.

- Modified Bradford method, at 595 nm
- Lowry method, at 750 nm
Figure 51. Hyperbolic curve-fitting for total protein analysis.
$$\frac{1}{A} = 0.58 + 41.38 \times \frac{1}{P} \quad \cdots \cdots \cdots \cdots \cdots \cdots (55)$$

where $A$ is the absorbance at 595 nm.

A.4 Bacterial cell counts

Microscopic cell counting was conducted on a microscope equipped with a phase contrast condenser and several lenses. A 10x eye piece combined with 100x object piece to give 1000x of magnification. Oil immersion was necessary to yield a good resolution. One drop of cell solution was placed on a Petroff-Hauser counting chamber and covered with a piece of cover glass for visual cell counts. The counting chamber had squares on the slide and the volume of each square was $5 \times 10^{-8}$ ml. The numbers of cells in more than 10 squares were obtained and the average number of cells per square was used to calculate the cell density in cells/ml.

For the calibration of cell density with amount of total protein, a series of cell solutions with different densities were prepared. Cell densities in the solution were determined by microscopic counting and the concentration of total protein in each solution was assayed by the methods described above. The cells were first hydrolyzed with NaOH solution to release the bacterial protein. The procedures are listed as follow:

1. Transfer 3 ml of cell solution into a 10 ml test tube.
2. Add 0.1 ml of 3 N NaOH solution to the cell solution.
3. Allow the test tube to stand in a 95 °C water bath for 20 min.
4. Adjust the pH of the solution to 2.0 with 3 N H_2SO_4.

5. Follow the procedures for protein analysis.

Figure 52 gives the calibration curves. The two methods responded differently toward the bacterial protein. Since the Modified Bradford Method exhibited higher sensitivity, it was applied as the protein assay method throughout this study. The straight line in Figure 52 for the Modified Bradford method gives a slope of $9.26 \times 10^{-8}$ μg protein/cell.
Figure 52. Calibration curve for cell counts.
APPENDIX B

ANALYSIS OF IRON CONCENTRATION IN WATER.
APPENDIX B
ANALYSIS OF IRON CONCENTRATION IN WATER

Concentration of ferrous and ferric iron in water were determined by the 1,10 phenanthroline method. Ferrous iron (Fe\(^{2+}\)) combines with 1,10 phenanthroline to form a complex, which exhibits a peak absorbance at 510 nm. This method for iron analysis is sensitive and specific (Christian, 1977). A simplified version of the method was applied in this study. The procedures are summarized as follow:

Reagents

1. 1,10 phenanthroline solution: Dissolve 0.4 g of 1,10 phenanthroline into 100 ml of distilled solution.
2. Sodium citrate solution: Dissolve 20 g of sodium citrate into 100 ml of distilled water.
3. Hydroxylamine hydrochloride solution; dissolve 10 g of hydroxylamine hydrochloride into 100 ml of distilled water.

Procedures

1. Mix 4 ml of sodium citrate solution with 1 ml of 1,10 phenanthroline solution in a 25 ml volumetric flask.
2. Add 1 ml of iron solution to the flask, dilute to the mark with distilled water. Let the solution to stand for 15 min and read the absorbance at 510 nm. The reading gives the concentration of ferrous iron in the solution.

3. Add 1 ml of hydroxylamine hydrochloride solution to the above solution. Let the solution to stand for 20 min and read the absorbance at 510 nm. The reading gives the concentration of total iron in the solution.

Step 3 in the above procedures was to reduce the Fe$^{3+}$ into Fe$^{2+}$ with hydroxylamine hydrochloride. The difference between the ferrous iron concentrations obtained from step 2 and and total iron concentration from step 3 was the concentration of ferric iron. The calibration was done with a standard ferrous sulfate solution. Hydroxylamine hydrochloride solution was added to the standard to make sure all the iron was in the form of ferrous. Figure 53 shows the calibration curve. The curve can be fitted into a hyperbolic function as described in Appendix A. The function derived in this way was:

$$\frac{1}{A} = 0.07 + 4.33 \times \frac{1}{[\text{Fe}^{2+}]} \hspace{1cm} (56)$$

where $A$ is the absorbance at 510 nm and $[\text{Fe}^{2+}]$ was in ppm.
Figure 53. Calibration curve for iron concentration.
APPENDIX C

ANALYSIS OF SULFATE ION IN WATER
APPENDIX C

ANALYSIS OF SULFATE ION IN WATER

This was the standard method for the determination of sulfate ion concentrations in water (ASTM, 1986). Barium chloride precipitates with sulfate in acidic medium to form uniformly sized barium sulfate crystals:

\[
\text{SO}_4^{2-} + \text{BaCl}_2 \rightarrow \text{BaSO}_4 + 2 \text{Cl}^-(57)
\]

The transmittance of the barium sulfate suspension was measured on a spectrophotometer and was compared with a calibration curve to determine the sulfate concentration.

Reagents

1. Conditioning reagent was prepared by mixing 50 ml. of glycerol with a solution containing 30 ml. concentrated HCl, 300 ml. water, 100 ml. 95% ethanol and 75 gm. NaCl.

2. Barium chloride crystals.

3. Standard sulfate solution was prepared by dissolving 0.7394 g anhydrous \( \text{Na}_2\text{SO}_4 \) in distilled water and diluting to one liter. The concentration of sulfate in this solution was 0.5 g/l.
Procedures

1. One ml. of sample was diluted to 100 ml. in a 100 ml. volumetric flask and then transferred to a 250 ml. Erlenmeyer flask.

2. Five ml. of conditioning reagent was added and mixed on a magnetic stirrer. Upon addition of one scoop (2 ml.) of BaCl₂ crystals, the mixing was timed with a stop watch.

3. After 1 min, 4 ml of the solution was poured into a cuvette and placed in a spectrophotometer. After a total of 5 min the transmittance at 420 nm was recorded.

4. The sulfate concentration was read from the calibration curve.

Following the above procedures, calibration was conducted with the standard sulfate solution. The calibration curve is shown in Figure 54. The calibration curve was nonlinear and a polynomial function was fitted to the data.

\[
\left[\text{SO}_4^{2-}\right] = A (x-100)^5 + B(x-100)^4 + C(x-100)^3 + D(x-100)^2 + E(x-100) \ldots (58)
\]

where \([\text{SO}_4^{2-}]\) : sulfate ion concentration, g/l

\[x\ : \text{transmittance at 420 nm}\]

\[A \approx -2.592 \times 10^{-7}\]

\[B \approx -4.789 \times 10^{-5}\]

\[C \approx -3.255 \times 10^{-3}\]

\[D \approx -9.678 \times 10^{-2}\]

\[E \approx -2.017\]
Figure 54. Calibration curve for sulfate analysis.
The solid line in Figure 54 shows the values calculated by the above equation.

This sulfate analysis was very sensitive to timing of mixing and reading. Stirring speed during conditioning was also crucial to the final results. To get a consistent result, the procedures of analysis should be exactly the same that of calibration.
APPENDIX D

ANALYSES OF COALS
APPENDIX D
ANALYSES OF COALS

Many coals were involved in this study but the analyses are available for only a few of them. Full analyses were available for Kentucky #9 coal, Ohio Coal mixture and Ohio #6. Sulfur analyses were available for the -325, precleaned Kentucky #9 coal and Ohio Clarion #4 A coals.

D.1 Analyses of Kentucky #9 coal
(Source: Advanced Products)

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<tr>
<td><strong>Proximate Analysis(%)</strong></td>
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<td>Moisture</td>
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<td>Ash</td>
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**Surfurs forms, % as S**

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**Sulfur forms, as % S**

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Size analysis of Kentucky #9 coal

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D.2 Analysis of Ohio Coal Mixture

(Source: Murphy, J., M.S. Thesis, The Ohio State University)
### Size Analysis

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D.3 Analyses of Ohio #6 coal  
(Source: Advanced Products)

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### D.4 Sulfur analysis of -325, precleaned Kentucky #9 Coal

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### D.5 Sulfur analysis for Ohio Clarion #4 A coal

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<tr>
<td>Organic (Difference)</td>
<td>1.33</td>
</tr>
<tr>
<td>Total</td>
<td>8.31</td>
</tr>
</tbody>
</table>
APPENDIX E

ANALYSIS OF TOTAL SULFUR CONTENT IN COAL
This procedures was a standard ASTM method for sulfur determination, adapted for specialized equipment. Coal samples were combusted under controlled conditions and then the effluent gases were titrated for SO$_2$ content according to the following reactions:

\[
\begin{align*}
\text{KIO}_3 + \text{KI} + \text{HCl} & \rightarrow 6 \text{KCl} + 3 \text{I}_2 + 3 \text{H}_2\text{O} \\
\text{SO}_2 + \text{I}_2 + 2 \text{H}_2\text{O} & \rightarrow \text{H}_2\text{SO}_4 + 2 \text{HI}
\end{align*}
\]

Starch solution was added to reaction solution to serve as the indicator for iodine. The blue color created by starch-I$_2$ complex faded when SO$_2$ gases passed through the titration vessel and lower the free iodine concentration. The amount of KIO$_3$ which must be added to the titration vessel to maintain the blue color is used to define the amount of SO$_2$ which had passed through the vessel.

**Equipments**

a. LECO Model 777-300 induction furnace

b. LECO Model 532-500 sulfur titrator
Preparation of titration solution and coal samples

1. Starch solution: Two grams of iodometric quality starch was dissolved in 50 ml. of water. The starch solution was poured slowly into 150 ml. of boiling water with continuous stirring. Later 0.05 grams of KOH was added to the solution. After cooling 6 grams of KI was added.

2. Hydrochloric acid solution was prepared by diluting 15 ml. to a total volume of 1 liter.

3. Potassium iodate solution was prepared to give a concentration of 0.444 g/l.

4. Coal samples:
   a. A large scoop of $V_2O_5$ was added to a crucible.
   b. Onto the crucible, 50 mg of coal was weighed on an analytical balance.
   c. Then, one small scoop of iron powder and one small scoop of Lecocel accelerator. Finally one large scoop of $V_2O_5$ was added and the crucible was covered with a piece of porous lip.

Procedures

1. Oxygen supply was turned on and maintained at 1.0 to 1.5 l/min. The oxygen passed through a purifying train of sulfuric acid, followed by ascarite and anhydrone before it got into the induction furnace.

2. The titration vessel was filled to approximately one-half inch above the constriction point with hydrochloric acid solution. Five ml. of starch solution were added to the solution.
3. The titrator was turned on and switched to "endpoint". The titrator automatically added KIO$_3$ until the solution reached a blue color.

4. The heating jacket between the induction furnace and the titrator was turned on.

5. The crucible was placed in the induction furnace and then the furnace was turned on.

6. Reading on the titrator was taken at the end of titration and the sulfur content was calculated based on calibration factor obtained from standard sulfur compound.
APPENDIX F

SULFUR FORMS ANALYSIS IN COAL
SULFUR FORMS ANALYSIS IN COAL

Sulfur forms analysis in coal was based on ASTM standard procedures (ASTM, 1985). Amount of sulfate sulfur and pyritic sulfur were determined by consecutive leaching experiments. The reagents and procedures were summarized in the following.

Reagents

1. Amonium hydroxide: concentrated NH₄OH.
2. Barium Chloride solution: One hundred grams of BaCl₂ were dissolved in 1 liter of water.
3. Saturated bromine water: Excess amount of bromine to 1 liter of water.
4. Hydrochloric acid solution: Two volume of concentrated HCl were mixed with 3 volume of water.
5. Hydrogen peroxide solution: The concentration was 30% by volume.
6. Nitric acid: One volume of concentrated HNO₃ was added to 7 volume of water.

Procedures
1. Sulfate sulfur
   a. Two to 5 grams of coal were weighed and transferred to a 250 ml. erlenmeyer flask.
   b. Fifty ml. of hydrochloric acid were added to the flask, then the flask was boiled gently for 30 min.
   c. The contents of the flask were filtered through double-acid washed filter paper.
   d. The HCl extract was boiled and 5 ml. of bromine water were added.
   e. Iron was precipitated by adding the ammonium hydroxide slowly until the solution became basic.
   f. The solution was neutralized with concentrated HCl.
   g. Sulfate was precipitated by adding 10 ml. of BaCl₂ solution. The solution was filtered with ashless filter paper.
   h. The filter paper was burned in a tared crucible and the amount of sulfate was determined gravimetrically.

2. Pyritic sulfur
   a. The extracted residue from step a in sulfate sulfur analysis was transferred into a 50 ml. Erlenmeyer flask and 50 ml. of nitric acid solution were added.
   b. The mixture was boiled gently for 30 min and the filtered through a double-acid washed filter paper.
   c. Two ml of 30% H₂O₂ solution were added to the extract and boiled for 5 min.
d. The iron in the nitric acid extract was precipitated with ammonium hydroxide and the concentration was determined by atomic absorption.
APPENDIX G

ADJUSTABLE CONSTANTS EVALUATED FROM MODELING
### APPENDIX G

**ADJUSTABLE CONSTANTS EVALUATED FROM MODELING**

The model was simulated with two different cases. Case I assumed that all the attached cells measured by protein analysis were attached to pyrite sites. Case II assumed that 10% of the attached cells attached to the pyrite sites. The values of the adjustable constants are listed as follow:

<table>
<thead>
<tr>
<th>Case I</th>
<th>Case II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate constant for chemical oxidation of pyrite, $k_s$ (mM/day ppm$^2$)</td>
<td>14.9</td>
</tr>
<tr>
<td>Rate constant for chemical oxidation of ferrous iron $k_1$ (1/day)</td>
<td>0.91</td>
</tr>
<tr>
<td>Maximum specific growth rate of cells on ferrous iron, $\mu_{MF}$ (1/day)</td>
<td>0.00092</td>
</tr>
<tr>
<td>Saturation constant in the Monod model, $K_{Fe}$ (mg Fe/l)</td>
<td>41</td>
</tr>
<tr>
<td>Yield factor for cell growth on ferrous iron, $Y_{Fe}$ (cells/nM Fe)</td>
<td>$1.8 \times 10^7$</td>
</tr>
<tr>
<td>Specific growth rate on pyrite</td>
<td></td>
</tr>
<tr>
<td>$\mu_{M,S}$ (1/day)</td>
<td>1.44</td>
</tr>
<tr>
<td>---------------------</td>
<td>------</td>
</tr>
<tr>
<td>Yield factor for growth on pyrite (cells/mM FeS$_2$)</td>
<td>$4.02 \times 10^9$</td>
</tr>
</tbody>
</table>