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Microbial desulfurization of coal by the thermophilic microorganism Sulfolobus Acidocaldarius

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MICROBIAL DESULFURIZATION OF COAL BY THE THERMOPHILIC MICROORGANISM *SULFOLOBUS ACIDOCALDARIUS*

by

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DATE

Professor Vikret Kargi
Advisor

Dr. John Chen
Chairman, Dept. Chem. Eng.

to my father

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I thank Dr. Fikret Kargi for the direction and motivation given during the project.

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ABSTRACT

The rate of microbial desulfurization of coal with Sulfolobus acidocaldarius was increased ten fold by adjusting the nitrogen to phosphorus and nitrogen to magnesium ratios. The effect of the inclusion of organic nutrients and chemical oxidants in the medium, as well as alternate nitrogen sources, were tested. Process variables such as pulp density, coal particle size, and initial cell number density were varied in order to find thier independent optima. A pulp density of 20%, a particle size of 49 um, and an initial cell number density of 10^{12} cells/gram pyrite in the coal were found to be optimal. Environmental conditions were optimized. Optimal values of pH and temperature were found to be 1.5 and 70 C to 75 C, respectively.

Kinetics of microbial removal of pyritic sulfur from coal and microbial oxidation of dibenzothiophene by Sulfolobus acidocaldarius were investigated.

1. INTRODUCTION

Coal is a relatively inexpensive and abundant energy resource. The worlds` increasing energy crisis, the U.S. dependency on foreign oil, and various options for converting coal into liquid fuels, has led to the consensus that coal will be one of the major energy sources in the future. However, direct combustion of coal can cause serious pollution problems due to the emission of sulfur dioxide (SO_2) into the atmosphere. Sulfur containing gases emitted into the atmosphere have adverse effects on animal and plant life [11] and also contribute to the increasing problem of acid rain.

There are several alternatives for the removal of sulfur from coal. The methods can be divided into two major categories-- precombustion desulfurization, and desulfurization after combustion (mainly stack gas desulfurization). The precombustion processes have the advantage of removing serious equipment wear and corrosion problems before it reaches the main part of the power plant.

Among the present alternatives in precombustion desulfurization are physical and chemical methods. Chemical desulfurization, however, requires high temperatures and pressures (100-500 C, 100-1000 psi) which make the process very energy intensive. The main physical method used is flotation. Flotation is more cost

effective than chemical methods but results in an energy loss by removing coal particles containing finely disseminated pyrite (FeS_2) [21]. This method is also ineffective in removing inorganic sulfur compounds.

Microbial Coal Desulfurization (MCD) has many advantages over chemical and physical methods, one being the advantage of comparatively low capital and operating costs [17, 16]. This method is a specific and sensitive means of sulfur removal and is applicable to the removal of finely disseminated sulfur compounds [13]. The process is also less energy intensive than chemical and physical methods and can easily be adapted to coal slurry pipeline systems and to the burning of coal-water slurries. Initial rates using bacteria to desulfurize coal were too low to allow the process to become economically feasible; the use of an alternative microorganism, Sulfolobus acidocaldarius, however, has provided significantly higher rates and therefore much more promise for the process.

The work described in this research report was directed to improve the rate and extent of sulfur removal from coal using the microorganism Sulfolobus acidocaldarius so the reactor size and/or residence time of the process may be reduced. Efforts have been made to elucidate the kinetics of microbial removal of pyritic sulfur from coal. The kinetics of microbial oxidation of

dibenzothiophene by Sulfolobus acidocaldarius was also investigated.

2. BACKGROUND

The sulfur content of United States' coals varies from 0.5% to over 6%. This sulfur exists as inorganic and organic sulfur compounds. The major inorganic sulfur compound is the mineral pyrite (FeS_2). Organic sulfur compounds are diverse and contain mainly thiols, sulfide, disulfide, and thiophene groups [21]. Usually, bituminous coal has a higher pyritic sulfur content than sub-bituminous coal, lignite, and anthracite. [13].

The amount of sulfate sulfur ($FeSO_4$) in freshly mined coal is less than 0.1%. The sulfate sulfur content gradually increases after mining due to oxidation (chemical and biological) of pyrite in the presence of weather [21]. Sulfate is soluble, however, and can be washed away. The presence of sulfate sulfur, therefore, causes no problem in coal desulfurization.

The microbial desulfurization of coal using the organism Thiobacillus ferrooxidans, a chemoautotrophic and autotrophic bacterium found in acid mine waters, has been studied by many researchers [5, 6, 7, 8, 9, 10, 18, 19, 20, 22]. A mixed culture of T. ferrooxidans and T. thiooxidans has also been used for the removal of sulfur compounds from coal [9]. The rates obtained in these studies, however, were too low to reduce the reactor size to a reasonable level [2]. The organisms are also ineffective in

removing organic sulfur compounds which, in some coals, is an appreciable amount of the total sulfur content.

An alternative organism which may be used for MCD is the thermophilic, acidophilic microorganism Sulfolobus acidocaldarius. This organism, a facultative autotroph, has a temperature optimum near 70 C and thrives at low pH (ph 1.5-4). It oxidizes reduced sulfur and iron compounds. The organism was originally isolated by Brierly [2] from the acidic hot springs of Yellowstone National Park. Several high temperature strains of Sulfolobus were isolated and further characterized by Brock et. al. [3, 4]. Organisms of the genus are widespread in solfatara areas and can be isolated from thermal acid hot springs. The organism Sulfolobus acidocaldarius may be an important geochemical agent in the production of sulfuric acid from sulfur in high temperature hydrothermal systems [3].

The severe environmental conditions at which Sulfolobus thrives offer many advantages to its use in MCD. Due to the high temperature and low pH, the chance of contamination is low; sterile conditions need not be maintained. Iron deposition is also greatly reduced as the pH is lowered. The rate of chemical oxidation of pyrite by the ferric ion at 70 C is more than two times greater than the reaction rate at 30 C [12]. Also at high temperatures, high cell concentrations can be used without expensive cooling systems.

As previously stated, Sulfolobus is a facultative autotroph. It can also be grown heterotrophically, and is maintained as such. This shows a potential pathway for breaking down some organic sulfur compounds present in coal. A concentrated culture of this organism has therefore been placed on dibenzothiophene, a model organic sulfur compound found in coal. Preliminary results have indicated oxidation of this sulfur compound with the release of sulfate.

3. MATERIALS AND METHODS

3.1 Coal Samples

Coal samples were obtained from the Pennsylvania Power and Light Company and were ground to desired particle sizes. Various size fractions were separated using U.S. standard sieve plates. The initial experiments were conducted with 100-150 mesh size (104 m < D_p < 147 m) coal particles. In later experiments smaller particle size ranges were used (150-200 mesh and 270-325 mesh). Two different coals were used, both from the same source-- a plant feed coal with ~4 wt% total sulfur content (2.1 wt% pyritic sulfur) and coal refuse with ~12 wt% sulfur (11.5 wt% pyritic sulfur).

3.2 Microbiological Methods

A pure culture of Sulfolobus acidocaldarius originally isolated by Brock et. al. [4] (strain 98-3) was used. The experiments and culture transfers were performed using the mineral salts medium developed by Brock et. al. [4] (see Appendix for composition). The cells were grown on several substrates: (1) heterotrophically on glucose (10 g/l) and yeast extract (1 g/l) for 3-4 days, (2) autotrophically on finely ground pyrite (20 g/l) for 10-14 days, and (3) on a 10 wt% coal slurry of plant feed coal (100-150 mesh) for 10-14 days. A concentrated culture was also kept on dibenzothiophene (DBT, 0.3 g/l) and a specially developed sulfate-free mineral salts medium for about 30 days. For all the above

mentioned cultures, 100 ml of the mineral salts medium was mixed with the desired substrate in a 500 ml baffled shake flask. The pH was adjusted to 2.5-3.0 and the flask and contents were autoclaved for 15 minutes at 121 C. The flasks were inoculated on cooling and placed at 70 C for the duration of the experiment. Stationary cultures were also maintained on yeast extract (1 g/l) in a 150 mm culture tube containing 5 ml of medium. These tubes were maintained at 70 C for 3 days, with daily shaking, and then left at room temperature for the remainder of the week.

The coal desulfurization experiments were performed in 500 ml baffled shake flasks. The flasks were charged with 100 ml of mineral salts medium and the desired amount of coal particles of known particle size. The pH adjustment and sterilization procedures are the same as previously mentioned. The flasks were inoculated with 10 ml of active cells and were placed at 70-75 C and 200 RPM in a controlled environment incubator shaker (New Brunswick Scientific Co. model G26) for the duration of the experiment. The inoculum culture was usually grown on pyrite. The samples were withdrawn daily for the analysis of soluble iron and sulfate after addition of sterile water to compensate for evaporation loss. The amount of water needed was determined by weighing the flask before sampling and subtracting the value from the weight of the flask recorded after the previous sample had been taken.

A control flask was used to determine the non-biological (acid catalyzed) sulfur and iron removal.

For experiments with recorded initial cell concentrations, heterotrophically grown cells were used. These cells were centrifuged and washed aseptically with sterile mineral salts medium and resuspended in the same for counting. The counting was done using a Petroff-Hauser counter under 40X magnification. The samples were diluted appropriately and used for inoculum for the experimental flasks.

3.3 Analytical Methods

The samples were filtered through Whatman No. 2 filter paper to remove coal particles from the liquid medium. The residual solids were washed with 0.1N hydrochloric acid (HCl) to extract adsorbed sulfate and iron from the coal surface into the filtrate [19]. The filtrate was analyzed for sulfate and total soluble iron.

Sulfate concentration was measured turbidimetrically [1, 15]. Two ml of 10% $BaCl_2$ solution was added to 2 ml of appropriately diluted sample and 0.5 ml of a conditioning solution containing alcohol and glycerine for improved suspension and NaCl and HCl for a more consistent $BaSO_4$ crystal formation. The ingredients were mixed for one minute in a Genie vortex mixer. The maximum turbidity of the final mixture over a three minute period was measured in a

spectrophotometer (Bausch and Lomb, Spec. 700) at 420 nm and was compared to a calibration line.

Total iron concentration was measured colorimetrically. One ml of 1% hydroquinone was added to 1 ml of the diluted sample to reduce the ferric iron into the ferrous form. The total iron concentration was measured by adding 2 ml of a 0.1% o-phenanthroline solution to the sample and measuring the absorbance at 500 nm in a spectrophotometer and comparing the results to those of a ferrous sulfate standard.

Total sulfur content of the coal was determined by the Eschka method [15].

Sulfate sulfur content of coal samples was determined by extracting one gram samples of coal with dilute (~0.4N) HCl by refluxing with a cold finger condenser for 30 minutes. The extracted acid was analyzed for sulfate [1].

Pyritic sulfur content was determined by refluxing 1 gram coal samples in hot 2N HNO_3 for 90 minutes. Iron concentration in the final liquid was analyzed using the o-phenanthroline method described before. The pyritic sulfur content is then determined from the difference of the total inorganic sulfur (nitric acid extraction) and the sulfate sulfur content (hydrochloric acid

extraction).

Organic sulfur content is determined indirectly from the differences between total sulfur and total inorganic sulfur content. The details of these methods are provided in the appendices.

Protein concentrations were determined using an assay developed by Bio-Rad Laboratories. The standard Lowry protein assay could not be easily used due to interference from coal. The protein concentration was correlated to cell number and dry weight. The cells were digested in 1N NaOH in a boiling water bath for 15 minutes and the pH was readjusted to 2.0 before analysis of the protein.

Total protein (attached and free cells) was analyzed using the above method and a calibration curve constructed with samples of a known cell number and the same coal pulp density. Free cells were separated from the coal-water slurry by filtration through a coarse grade filter paper (Whatman No.s 4 or 541) and washing with an equal volume of 0.1N HCl. The protein concentration was then determined using a calibration line constructed from samples with known cell number but no coal. Attached cell number was determined indirectly from the difference of the total and free cell number.

4. RESULTS AND DISCUSSION

This work was performed in order to improve the rate and extent of sulfur removal from coal using the organism Sulfolobus acidocaldarius and to elucidate the kinetics of pyritic sulfur removal. Many aspects of the process must be studied in order to determine the best set of conditions for maximum sulfur removal rate. The cell growth medium and/or desulfurization medium can be changed in many ways. The substrate which the organism is to desulfurize can be altered chemically or physically as well as changing its concentration. The organisms characteristics may also be changed by adjusting the environmental conditions. Many of these variables have been varied to find the optimal conditions. The experimental program was designed to understand the basic characteristics of the process and the general effect of each of the following variables-- the effect of simple medium components, coal concentration and particle sizes, pHs, cell concentrations, and temperatures. An attempt was made to find an optimum with respect to each of these variables independently.

4.1 Medium Improvement

The initial medium used for the coal desulfurization experiments and culture transfers was that proposed by Brock et. al. [3, 4]. The basic ingredients of the medium are 1.3 g/l $(NH_4)_2SO_4$, 0.28 g/l K_2HPO_4, 0.25 g/l $MgSO_4\ 7H_2O$, 0.07 g/l $CaCl_2\ 2H_2O$, and 0.02

g/l $FeCl_3$ $6H_2O$. Other trace minerals were also added (see appendix for complete medium composition). This is a very simple well defined medium prepared to allow good growth on glucose and yeast extract, pyrite, coal, or elemental sulfur. The fact that it provides the minerals essential for good growth, however, does not guarentee that it will allow the best desulfurization characteristics. Therefore, a few organic nutrients, chemical oxidants, and alternate minerals were tested to determine thier effects on the rate and extent of the sulfur removal from coal.

4.1.1 Organic Nutrients

The effect of the addition of yeast extract (0.02%) and peptone (0.1%) on the rate of sulfur and iron removal was tested. These organic nutrients were only supplied in small amounts to determine if there was a positive or negative effect on the rate of removal of sulfur, not to quantify this effect. A 10% coal slurry of plant feed coal (2.1% inorganic sulfur) was used. The inoculum used was a 10 ml sample of Sulfolobus acidocaldarius grown autotrophically on pyrite. The temperature was controlled at 75 C and the initial pH was adjusted to 2.5. The coal particle size was 104-147 um. The experimental results are depicted in figure 4-1. The rate and extent of sulfur removal in the presence of 0.02% yeast extract were lower than that with only mineral salts medium. A similar, more pronounced effect was seen in the flask containing both yeast extract and peptone. The experiment indicated that the organism

Figure 4-1: Effect of organic nutrients and chemical oxidants on sulfur removal

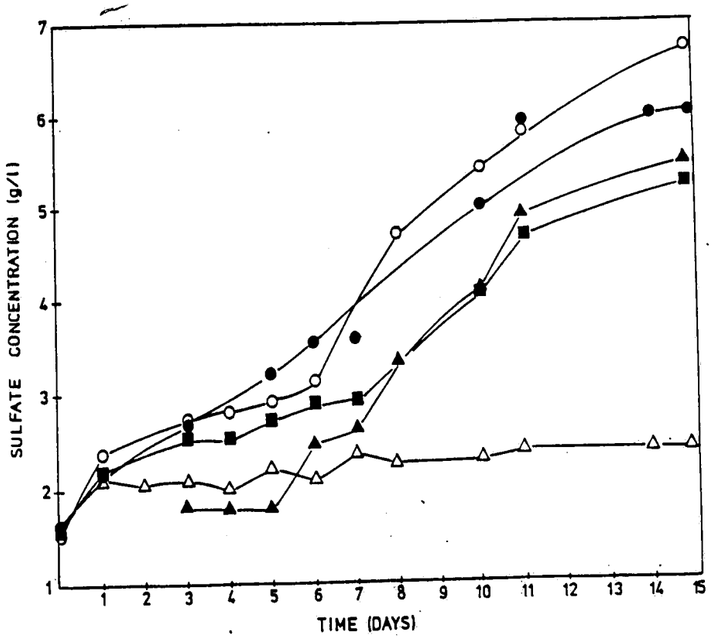
● - 0.01M $FeCl_3$

o -Mineral salts (MS) +cells

Δ -Control (MS only)

■ -MS +yeast extract

▲ -MS +yeast extract +peptone



could remove sulfur from coal in mineral salts medium alone. The inclusion of organic nutrients did not improve the rate and even had an adverse effect. The organic nutrients seem to act as an alternative substrate for growth which competes with the sulfur compounds in coal and therefore reduces the amount of sulfur and

iron utilized.

4.1.2 Chemical Oxidants

The inclusion of $FeCl_3$ into the reaction medium initiates chemical oxidation of iron, and therefore sulfur. The effect of the addition of 0.01 M $FeCl_3$ alone can be seen by comparing the sulfur removal of the flask containing the chemical oxidant to the control flask containing only mineral salts without the chemical oxidant (fig. 4-1). However, on comparing the results of the 0.01 M $FeCl_3$ flask to the flask containing only cells, one can see that after a short lag phase, the rate and extent of sulfur removal in mineral salts medium alone exceeds that of the chemical oxidant. It was also shown that on combining the two variables (cells + chemical oxidant) the high $FeCl_3$ concentration inhibits the sulfur removal when compared to the mineral salts medium and cells alone.

4.1.3 Nitrogen to Phosphorus Ratio

In order to improve the rate and the extent of pyritic sulfur removal, the N/P and N/Mg (nitrogen to phosphorus and nitrogen to magnesium ratios, respectively) were varied in the mineral salts medium. In the experiment, phosphorus was kept constant at the value proposed by Brock et. al. [3, 4]. The amount of $(NH_4)_2SO_4$ and $MgSO_4$ added was then varied according to a Box-Wilson experimental design for two independent variables. The experiment was run at 70 C and the initial pH was set to 2.5. The coal

particle size used was 147 to 104 um. The results of the experiment are presented in Table 4-1 on the following page.

Table 4-1: The influence of N/P and N/Mg ratios on pyritic sulfur removal from coal

$(NH_4)_2SO_4$ (g/l)	N/P	N/Mg	Rate (mg S/l hr)	%Sulfur Removal
3.50	54.8	22.0	17.4	88.1
0.35	5.5	22.0	----	31.7
1.93	30.1	36.4	15.6	92.1
1.93	30.1	7.3	22.1	84.1
3.04	47.5	32.0	33.1	74.6
3.04	47.5	11.5	27.4	88.1
0.81	12.7	32.5	9.9	24.6
0.81	12.7	11.5	14.3	54.8
1.93	30.1	21.9	18.2	61.9

The optimal N/P and N/Mg ratios were found to be 47.5 and 11.5, respectively, resulting in a reaction rate of nearly 28 mg S removed/1 hr and 88% pyritic sulfur removal. The maximum rate of pyritic sulfur removal obtained in the experiment was about an order of magnitude higher than our previous results obtained with a 5% coal slurry. Up to 92% of the initial pyritic sulfur was removed in a single batch.

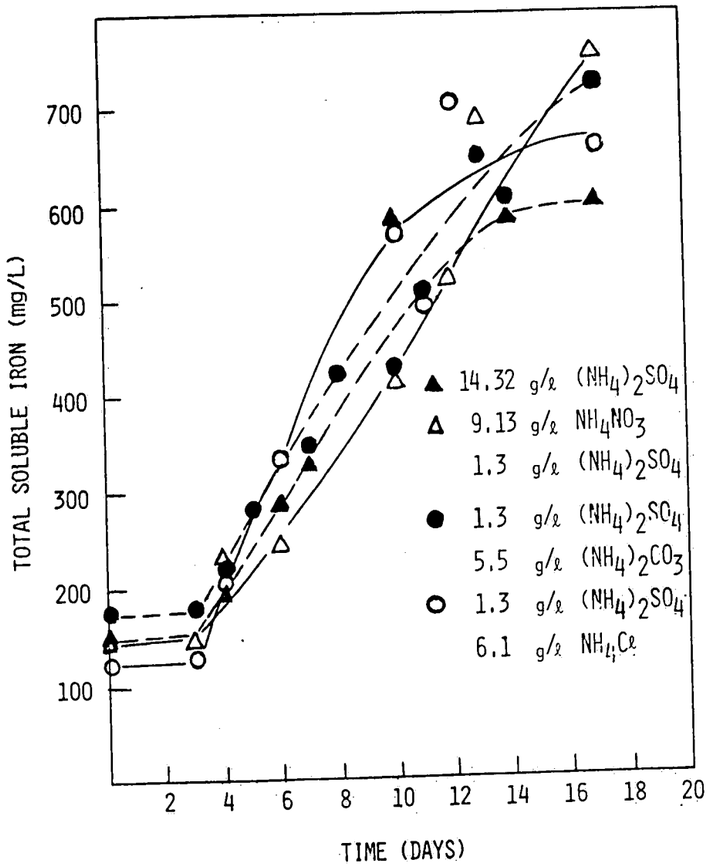
4.1.4 Alternate Nitrogen Sources

The use of $(NH_4)_2SO_4$ in large amounts (high N/P) causes some difficulty in the analysis of sulfate. The high dilution necessary to reduce the sulfate concentration to the linear portion of the calibration curve magnifies the error of the assay. Several other ammonium sources (NH_4NO_3, $(NH_4)_2CO_3$, NH_4Cl, and $(NH_2)_2CO$) were, therefore, tested to see if high initial sulfate concentrations could be avoided, without reducing the rate and extent of sulfur removal. Figure 4-2 depicts the influence of the alternate nitrogen sources on the pyritic sulfur removal from coal. The ammonium sulfate (recommended in Brock's medium) was substituted with the above nitrogen sources while keeping the N/P ratio nearly constant. The rate of pyritic sulfur removal was not significantly affected by the nitrogen source used, with the exception of urea. The $(NH_4)_2SO_4$ and NH_4Cl mixture resulted in a slightly higher rate of sulfur removal when compared to the other nitrogen sources. The use of urea resulted in a significant reduction in the rate and extent of sulfur removal as compared to $(NH_4)_2SO_4$ alone.

4.1.5 External Carbon-Dioxide Supply

An experiment was performed in which the concentration of CO_2 in the sparging air was varied. Carbon-dioxide is the carbon source used by Sulfolobus when the organism is oxidizing pyritic sulfur. If it is a rate limiting nutrient, the pyritic sulfur removal rate will be accelerated on addition of external CO_2 supply [14]. A

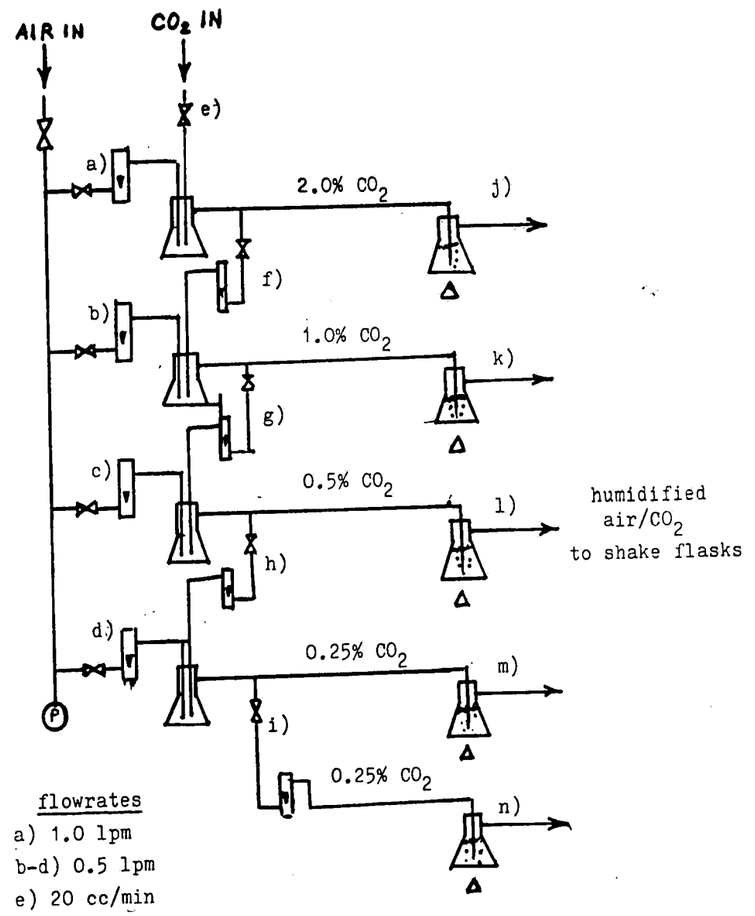
Figure 4-2: Effect of alternate nitrogen sources on sulfur removal rate



network of flasks was constructed in order to supply CO_2 in serial dilutions to a set of shake flasks. The flasks were charged with a 10% coal slurry of plant feed coal containing 2.1% pyritic sulfur(D =49 um), the initial pH was adjusted to 2.5, the flasks were inoculated with cell grown on pyrite, and they were incubated

for 2 weeks at 70 C. Daily samples were taken after compensation for evaporation loss. The gas entering the shake flask had to be pre-humidified to keep evaporation losses minimal. The flask network as designed is shown in figure 4-3. The effect of the external CO_2 supply on the pyritic sulfur removal rates is shown in figure 4-4 and figure 4-5. The rate and extent of sulfur removal was not effected by external CO_2 supply. The CO_2 concentration in the atmosphere, therefore, is not a limiting nutrient for coal samples containing 2.1% pyritic sulfur at 10% coal pulp density.

Figure 4-3: CO_2 mixing flask apparatus for varying CO_2 concentration in external CO_2 supply



flowrates

a) 1.0 lpm

b-d) 0.5 lpm

e) 20 cc/min

f-n) 0.5 lpm

Figure 4-4: Total soluble iron release profiles for various CO_2, air mixtures

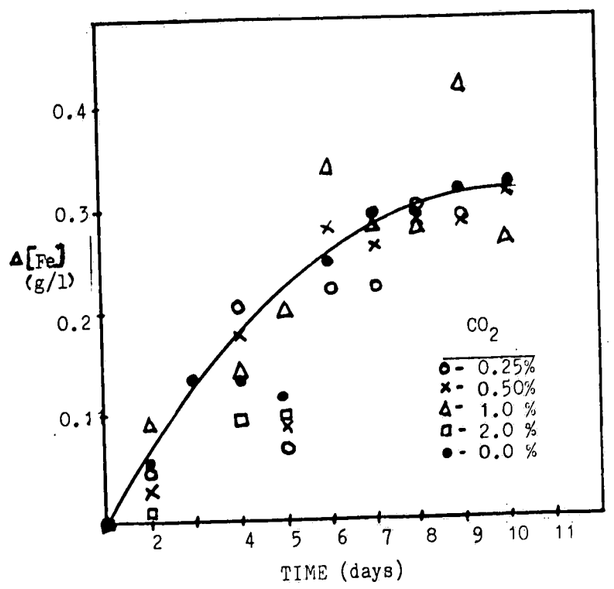
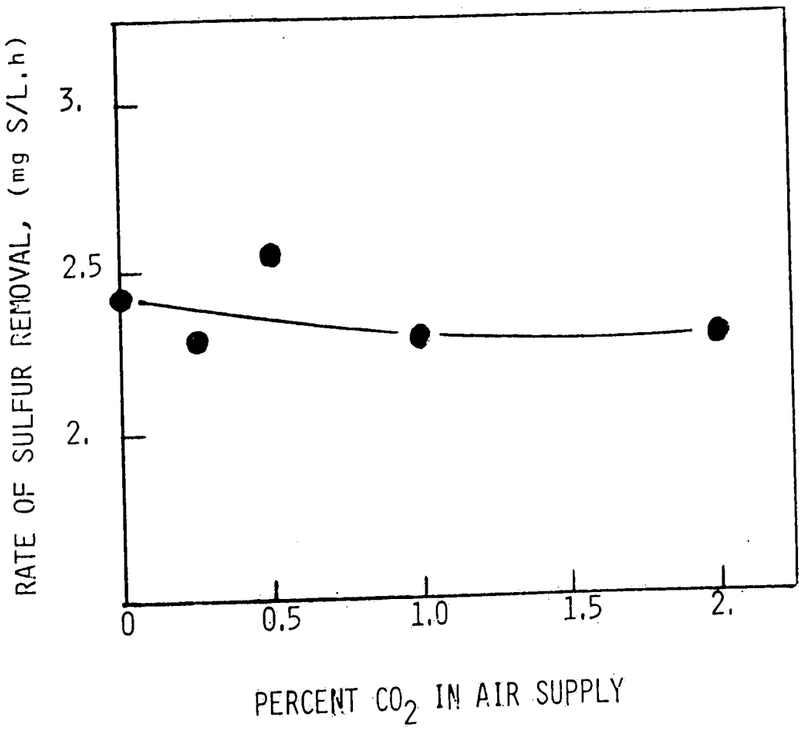


Figure 4-5: Variation of pyritic sulfur removal rate with CO_2 concentration



4.2 Process Variables

The types of coal, and thier sulfur content (both pyritic and organic) can vary widely as different coal sources are tested. The physical and chemical structures of coal can have a great effect on suliur removal characteristics.

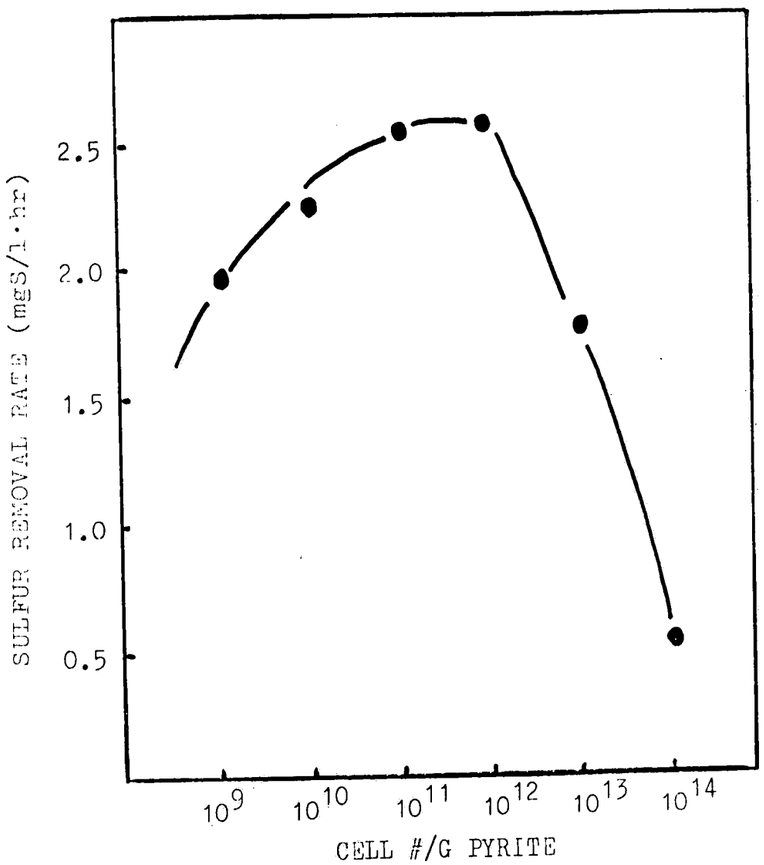
Microbial coal desulfurization using Sulfolobus acidocaldarius involves a surface reaction. Therefore variables such as pulp density and particle size become important as they effect the total amount of surface available for microbial action. This group of experiments was developed to better understand the kinetics of and the limitations on the suface reaction rate.

4.2.1 Initial Cell Concentration

In order to investigate the effect of initial cell concentration on the initial rate of pyritic sulfur removal, and to find the optimal cell number:coal surface area ratio, a 5% coal slurry of plant feed coal (2.1% pyritic sulfur) was inoculated with various cell concentrations. The average particle diameter of the coal used was 125 um. The temperature and initial pH were 70 C and 2.5, respectively. The cells used were cultivated in heterotrophic medium and were centrifuged, washed, and reconcentrated to obtain high cell densities. Serial dilutions of this stock culture were used to inoculate the experimental flasks. After appropriate dilutions, a Petroff-Houser cell counter was used to determine the

cell number density. Figure 4-6 below depicts the variation of sulfur removal rate as a function of the initial cell concentration in the reaction medium.

Figure 4-6: Variation of pyritic sulfur removal rate with initial cell number/$[FeS_2]_0$ ratio



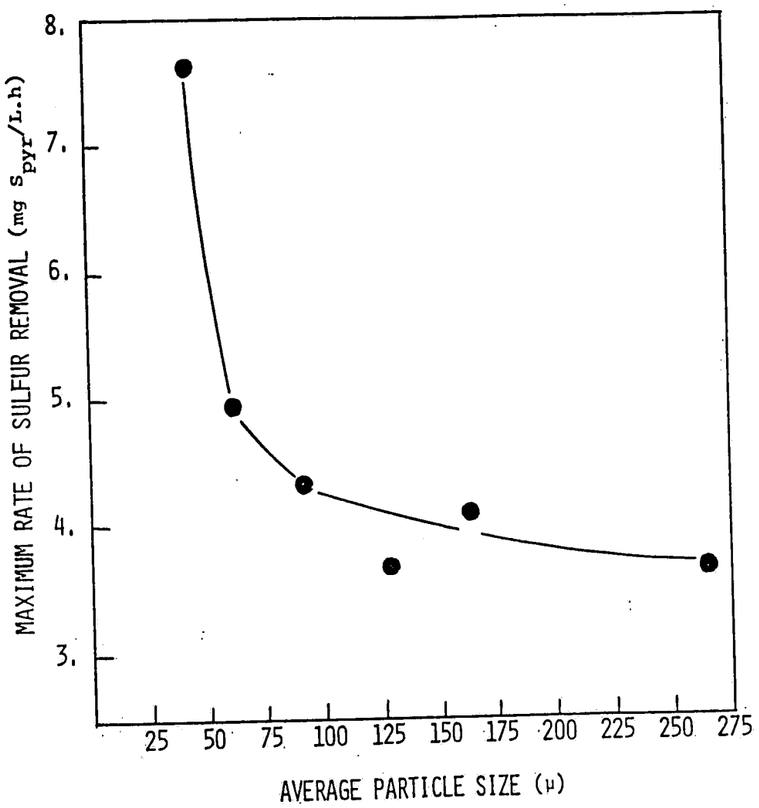
The rate increased with increasing cell concentration for cell number densities between $2x10^6$ cells/ml and $2x10^8$ cells/ml (10^9 and 10^{11} cells/g pyrite in coal). The rate was relatively constant for

cell number densities of $2x10^8$ and $2x10^9$ cells/ml (10^{11} and 10^{12} cells/g pyrite in coal, respectively). At higher cell concentrations ($2x10^{10}$ cells/ml and $2x10^{11}$ cells/ml or 10^{13} and 10^{14} cells/g pyrite) the rate of pyritic sulfur removal decreases. A reduction in the transfer of gaseous nutrients, mainly O_2 and CO_2, into the liquid medium at high cell concentrations due to heavy foaming may be the reason for this reduction in rate. The optimal cell concentration with 5% pulp density experiments was near $2x10^9$ cells/ml which is equivalent to 10^8 cells/cm surface area of coal ($4x10^{10}$ cells/g coal or 10^{12} cells/g pyrite in coal).

4.2.2 Coal Particle Size

Various size fractions of coal samples were used in order to determine the influence of particle size on the rate of removal of sulfur from coal. A 5% coal slurry of plant feed coal at 70 C and initial pH of 2.5 was used for this test. The cells used in this experiment were cultivated in heterotrophic medium, centrifuged, washed and concentrated as described before. The concentrated cells were used to inoculate the reaction medium to yield an initial cell concentration in all experimental flasks (excluding the control) of $2x10^9$ cells/ml. Figure 4-7 shows the variation of maximum rate of sulfur removal with the average coal particle diameter. The rates were calculated by determining total soluble iron concentration in the liquid medium and converting the data into pyritic sulfur

Figure 4-7: Variation of sulfur removal rate with particle diameter



removal data using the stoichiometric relationship of sulfur and iron in pyrite. The sulfur removal rate decreased with increasing particle size and seemed to reach a constant level at a particle size near 250 um. The total surface area of the coal in the flask is a function of the reciprocal of the particle radius. Reduction in the particle size increased the external surface area of the coal particles and, therefore, resulted in significant increases in the

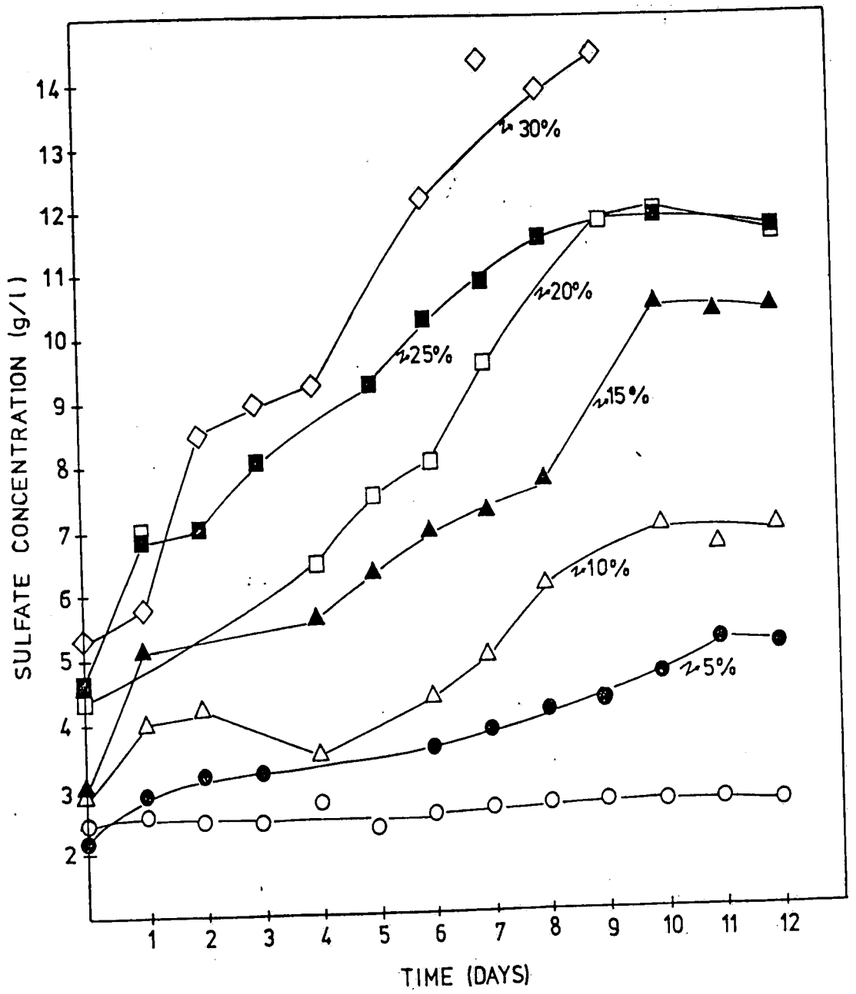
rate of sulfur leaching. Furthermore, a plot of maximum pyritic sulfur removal rate versus reciprocal diameter ($1/D_p$) produced a straight line showing a linear relationship between the two variables.

4.2.3 Coal Pulp Density

In order to test the effect of coal pulp density on sulfur removal rate, experiments were performed with different pulp densities (5%-30%) of coal with an average particle diameter equal to 125 um. Cells grown on pyrite were preconcentrated and used as inoculum. The results are shown in figure 4-8. The data in figure 4-8 was used to calculate the maximum volumetric sulfur removal rate (mg S removed/1 hr). The volumetric rate of sulfur removal was plotted against coal pulp density in figure 4-9. The volumetric reaction rate varies linearly with coal pulp density up to 15%. Above a 15% pulp density the volumetric reaction rate levels off showing a substrate limitation. This limitation may be caused by two or more things-- coal agglomeration at high pulp densities causing a reduction in effective surface area, or gas transfer limitations (mainly O_2 and CO_2) at high solids concentration. Initial experiments indicated no CO_2 limitation at 10% pulp density. The optimum pulp density with respect to volumetric reaction rate is near 20%.

The surface reaction rate can be related to the volumetric

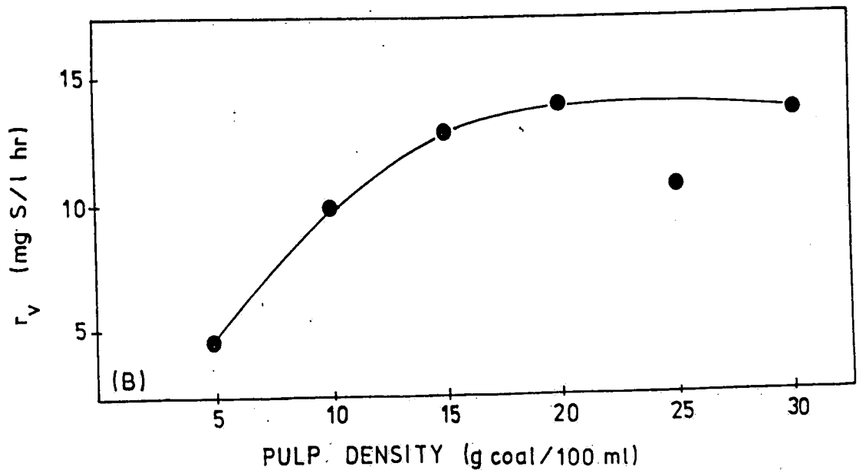
Figure 4-8: Effect of pulp density on pyritic sulfur removal



reaction rate with the following equation:

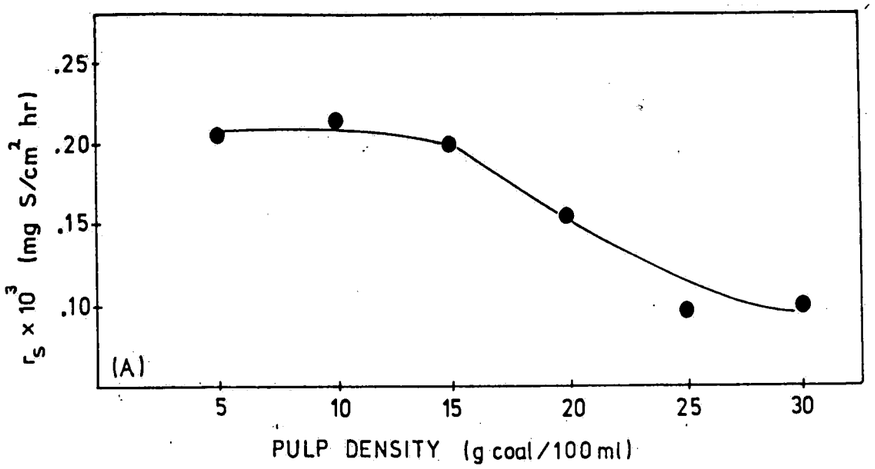
$$r_v = r_s p_d (6/ c^D p) \qquad (4\text{-}1)$$

Figure 4-9: Variation of volumetric reaction rate with pulp density



where r_v represents the volumetric reaction rate, r_s is the surface reaction rate, p_d is the pulp density, $_c$ is the density of the coal, and D_p is the average particle diameter. This equation was used to calculate the surface reaction rate for the six data points on figure 4-9. The results appear in figure 4-10 below. As previously stated, at low pulp densities (<15%) the surface reaction rate is constant. At higher pulp densities, the surface reaction rate decreases for the reasons specified earlier.

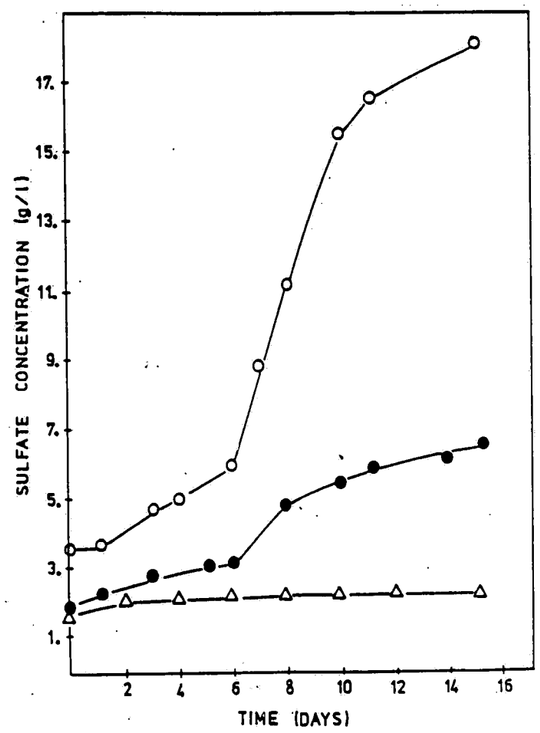
Figure 4-10: Variation of surface reaction rate with pulp density



4.2.4 Pyritic Sulfur Content

The rate and extent of sulfur removal from coal was found to depend on the pyritic sulfur content of the coal tested. Coal refuse (11.5% pyritic sulfur) and plant feed coal (2.1% pyritic sulfur) were used in the initial experiments. The high-sulfur coal refuse resulted in a rate of nearly 13 mg S/l hr while the plant feed coal resulted in a rate of 4.5 mg S/l hr for the 10% coal slurries. Autotrophic cultures were used as inoculum. This result is shown in figure 4-11. A follow-up experiment was performed to elucidate the rate as a function of sulfur content of the coal by testing coal samples of average sulfur content between 2.1% and 11.5%. The points tested were 2.1%, 4.0%, 5.0%, 6.0%, 8.0%, 10.0%.

Figure 4-11: Effect of Sulfur Content on Sulfur Removal Rate



and 11.5% sulfur. Normal mineral salts medium was used and the experiment was run at 70 C, 200 RPM, and initial pH=2.5, with cells grown on pyrite used as inoculum. The results will be discussed later in the kinetics section.

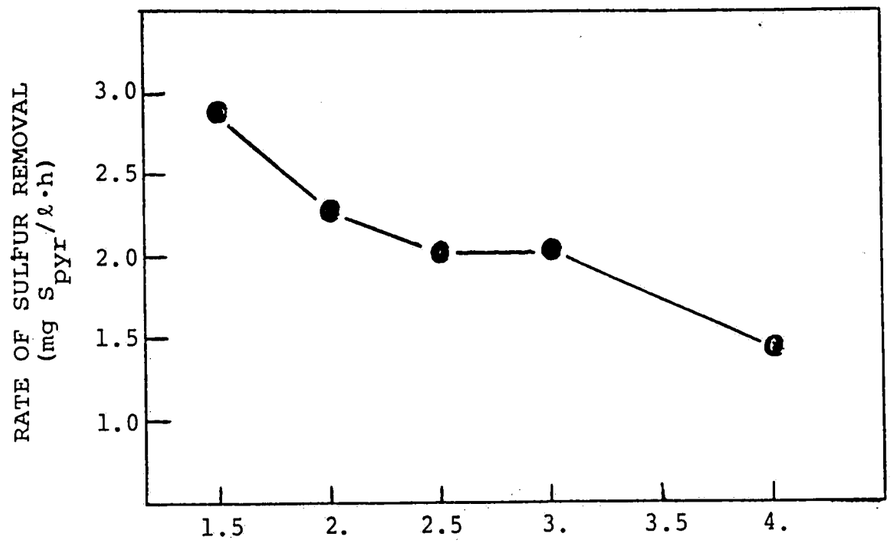
4.3 Environmental Conditions

Just as the environment has an effect on all forms of life, values of pH, temperature, and CO_2 concentration have effects on the sulfur removal rates from coal using Sulfolobus acidocaldarius. Temperature and pH had significant effects on the rate of pyritic sulfur removal. Acid-catalyzed sulfur and iron leaching is a strong function of pH. The chemical oxidation with the ferric ion is a strong function of temperature. The purpose of this section of experiments is to find the best values of these environmental conditions to improve the sulfur removal

4.3.1 Initial pH

In order to determine the influence of initial pH on the rate of pyritic sulfur removal, shake flask experiments were performed with initial pH values ranging from 1.5 to 4.0. A 5% pulp density coal slurry was used at 70 C with 100-150 mesh coal particles. Before inoculation, the cells were incubated at their respective pHs for 10 days to help prevent an adverse reaction to a large, sudden change in pH on inoculation. Daily samples were withdrawn and analyzed for sulfate and total iron. Figure 4-12 depicts the variation of the maximum rate of pyritic sulfur removal with initial pH values. The rate of pyritic sulfur removal decreased with increasing initial pH. The high leaching rate at pH=1.5 may be due to the increased acid leaching or due to higher cell activity at low pH.

Figure 4-12: Variation of Pyritic Sulfur Removal with Initial pH



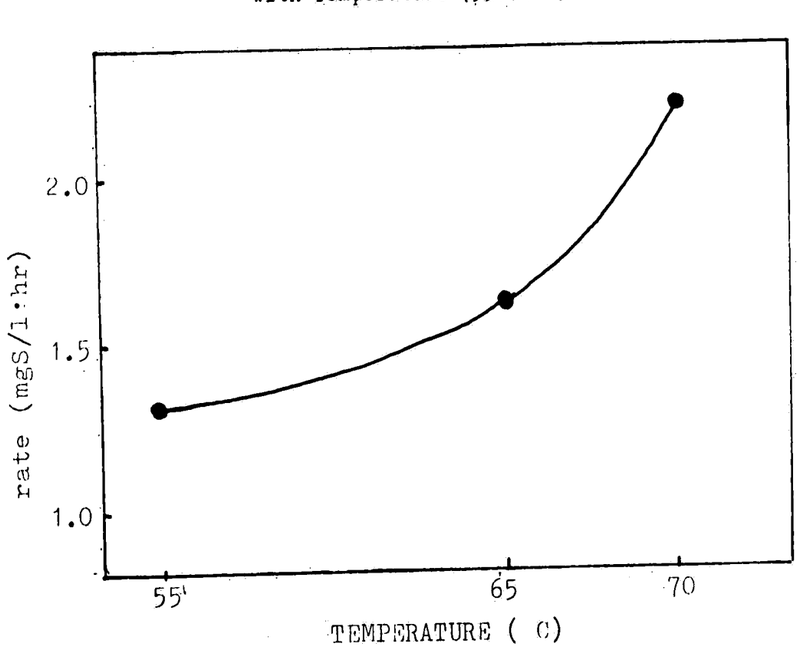
4.3.2 Temperature

Two separate shake flask experiments were performed in order to determine the best temperature for the process. First the lower temperatures (55 C, 65 C, 70 C) were tested in duplicate. In the second experiment, the rates of microbial desulfurization at 70 C and 80 C were compared. For both experiments, a 5% coal slurry of 150-200 mesh coal particles were used at the initial pH of 2.5. Two

transfers were made at each temperature before inoculation to avoid temperature shock on inoculation. At the end of the second transfer, the cells were centrifuged, washed, and counted. The same initial cell number was then added to each flask.

The first experiment resulted in rates of 1.3, 1.6, and 2.2 mg S/l hr at 55 C, 65 C, and 70 C, respectively. The second experiment resulted in pyritic sulfur removal rates of 6.2 and 4.5 mg S/l hr for 70 C and 80 C, respectively. The results of the first experiment appears in figure 4-13.

Figure 4-13: Variation of Sulfur Removal Rates with Temperature (55-70 C)



From previous experiments it has been determined that the rates at

70 C and 75 C are comparable. Therefore, the temperature optimum lies between 70 C and 75 C.

4.4 Organic Sulfur Removal

Organic sulfur removal using Sulfolobus acidocaldarius is a very slow process. However, niether T. ferrooxidans nor T. thiooxidans are capable of organic sulfur removal. Physical methods (flotation), as previously stated, also fail to remove organic sulfur. Microbial means using Sulfolobus seems to be the ideal means of removing organic sulfur compounds from coal while maintaining energy efficient conditions.

In order measure to the slow sulfate production rates more accurately, a specially formulated medium, free of sulfate, has been developed. Any sulfate detected in the liquid reaction medium, therefore, will be solely due to oxidation of dibenzothiophene. The composition of this medium is included in the appendix.

4.4.1 Oxidation of Dibenzothiophene

Dibenzothiophene (DBT) is a water insoluble powder and forms a suspension in the nutrient medium. The cells used in the experiment were grown heterotrophically, centrifuged and washed to remove residual glucose, and placed in DBT medium with a 300 mg/l initial DBT concentration for 30 days before inoculation.

A preliminary experiment was done in order to determine how well Sulfolobus acidocaldarius functioned on this organic sulfur compound. Sulfate concentration was determined over a 30 day period using the method previously discussed. The sulfate profile obtained appears in figure 4-14 below. A control flask containing the same medium and initial DBT concentration was also used to determine non-biological oxidation of DBT; no non-biological oxidation was observed. Microbial growth was not quantitatively measured but a slight increase in cell number seemed apparent under microscopic observation during the course of the experiment. The sulfate doubling time for the experiment was approximately 8 days. About 65% of the initial sulfur present in DBT was oxidized to sulfate by the organism. Sulfate release ceased about 28 days after inoculation. This may be due to limitation by some other nutrient (e.g., nitrogen or phosphorus) or as a result of complete oxidation of DBT. The total soluble sulfur had not been measured to test the presence of other soluble organic or inorganic sulfur compounds released to the medium due to oxidation of DBT.

A possible pathway theorized for the oxidation of DBT can be found in figure 4-16.

Figure 4-14: Sulfate release into medium from the oxidation of dibenzothiophene by Sulfolobus acidocaldarius

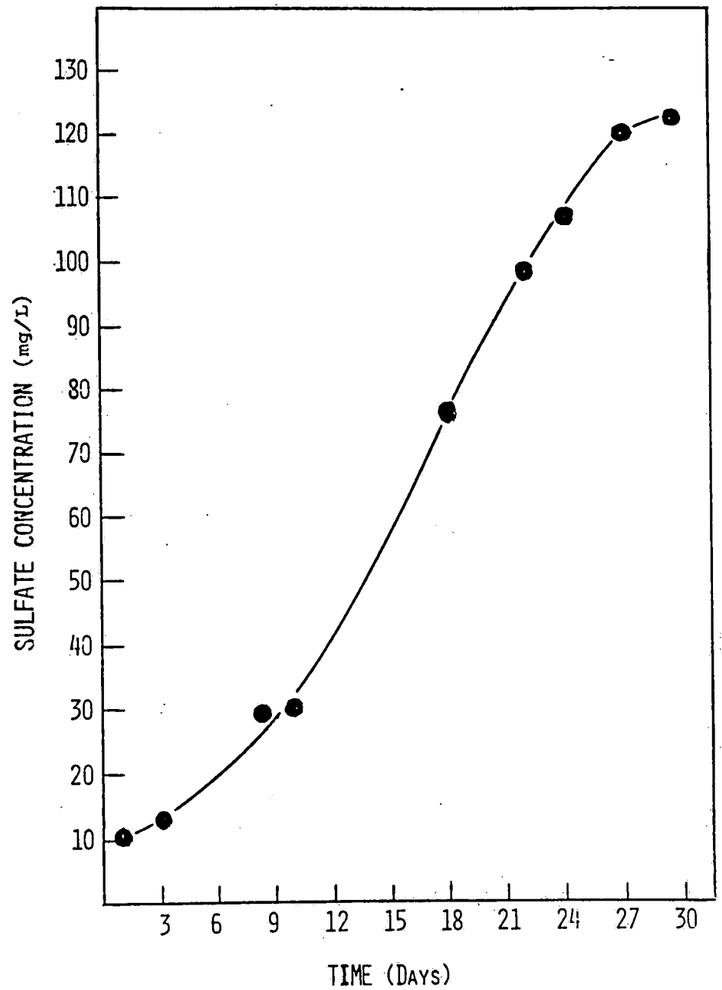
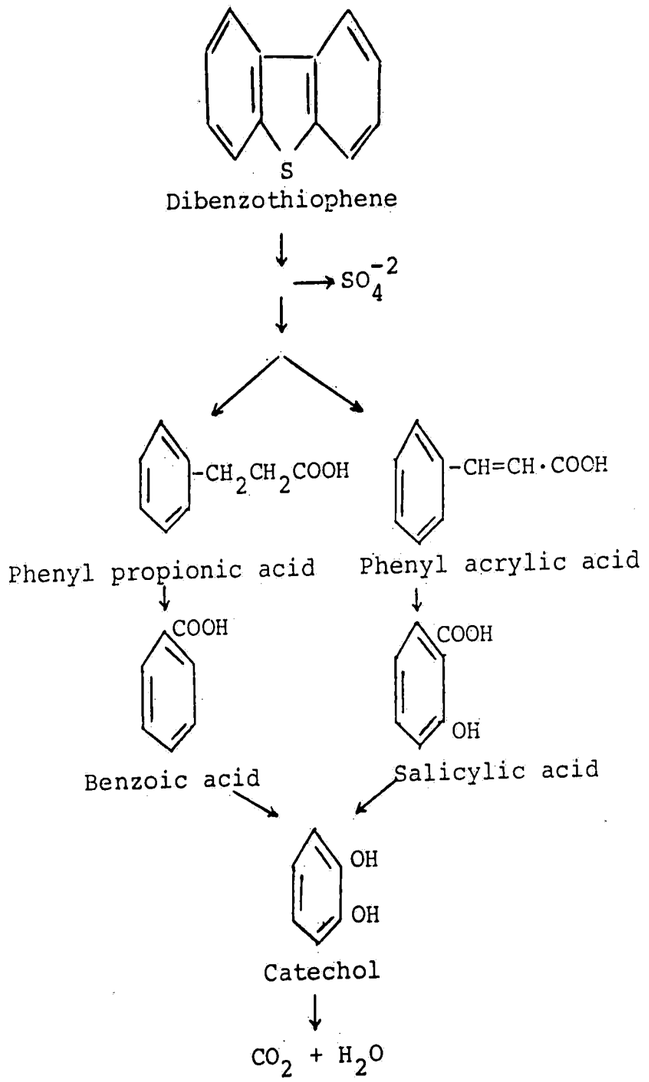


Figure 4-15: Possible pathway for microbial DBT oxidation



4.4.2 Organic Sulfur Removal from Coal

A culture which had been placed on DBT for 30 days was used to test the removal of organic sulfur compounds from coal and petroleum pitch. The sample of petroleum pitch obtained contained nearly 3.1% sulfur (all organic). The coal used had been pretreated in several different ways. One sample had been leached with hot 2 N HNO_3 for two hours. This pretreatment removed all of the pyritic sulfur and some of the organic sulfur. The residual sulfur concentration after pretreatment was 0.71% sulfur. Another sample was leached microbially and was washed and dried. The residual sulfur content after pretreatment was 2.3% sulfur (1.9% organic sulfur).

The results of the experiment are shown in table 4-2. The sulfur content of the samples were determined using the Eschka method [15]. The data also shows nearly 44% of the organic sulfur removed from acid leached coal in 28 days. It also indicates that all pyritic sulfur is removed from the microbially pretreated coal in two batches. The amount of organic sulfur removed from coal samples was between 3.1 and 3.6 mg S per gram of coal. More organic sulfur was removed from the petroleum pitch (7.66 mg S/g substrate) but the initial organic sulfur content of petroleum pitch was higher than that of the coals tested. The percent removal, therefore, was lower(24.7% organic sulfur removal).

Table 4-2: Removal of organic sulfur from inorganic-sulfur-free coal using Sulfolobus acidocaldarius preadapted to DBT

SUBSTRATE / PROPERTY	ACID TREATED COAL	MICROBIALLY TREATED COAL	PETROLEUM PITCH
PRETREATMENT	2 H TREATMENT IN BOILING HNO_3	2 WK INORGANIC SULFUR LEACHING BY SULFOLOBUS	NONE
INITIAL SULFUR CONC^N (AFTER PRE-TREATMENT)	~0.71% (ORGANIC)	2.3% (~1.9% ORGANIC)	~3.1% (ORGANIC)
FINAL SULFUR CONC^N (AFTER BIO-LEACHING 28 DAYS)	0.40%	1.54%	2.33%
% S REMOVED	43.7%	ALL PYRITIC 18.7% ORGANIC	24.7%
ACTUAL AMOUNT S REMOVED PER G SUBSTRATE	3.1 MG S/G COAL	7.6 MG S/G COAL (3.6 MG ORGANIC S)	7.66 MG S/G PET PITCH

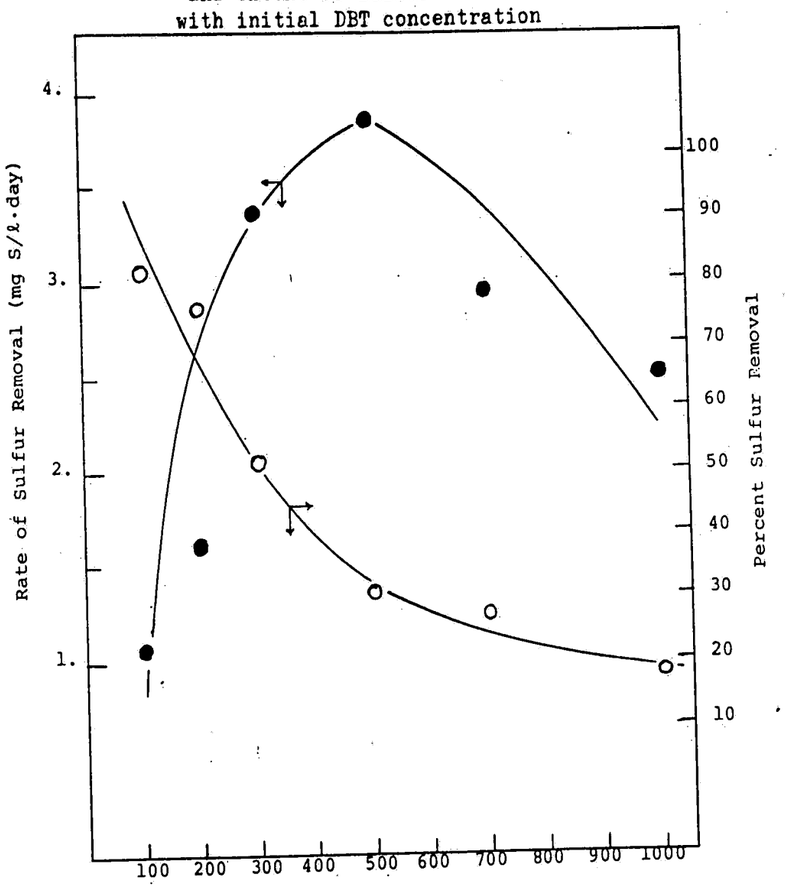
4.5 Kinetics

4.5.1 Kinetics of DBT Oxidation

An experiment was designed to determine the kinetics of microbial oxidation of dibenzothiophene by measuring the sulfate release into the liquid medium as a result of microbial oxidation of dibenzothiophene. The initial concentration of DBT was varied in order to determine its effect on the initial rate of sulfate release. A lag phase of 12 to 14 days was encountered with all experimental flasks. Initial DBT concentrations of 100, 200, 300, 500, 700, and 1000 mg DBT/l were tested. Figure 4-16 shows the variation of rate and extent of the removal of sulfur from dibenzothiophene as measured by sulfate release. The initial rate of DBT oxidation increased with increasing initial DBT concentration for concentrations between 100 and 500 mg DBT/l. The rate decreased with higher concentrations indicating the inhibitory effects of DBT for initial concentrations exceeding 500 mg DBT/l. The percent removal decreased steadily with increasing DBT concentration indicating limitation of one of the other nutrients at high DBT concentrations.

The rate of sulfate release from DBT oxidation by Sulfolobus acidocaldarius was represented by the non-competitive substrate inhibition kinetics with the following form:

Figure 4-16: Variation of sulfate release and extent of sulfur removal with initial DBT concentration



$$r_s = \frac{r_{max}}{(1+K_s/S)(1+S/K_i)} \qquad (4\text{-}3)$$

where r_s is the rate of sulfur release in the form of sulfate, S is the initial DBT concentration, and K_s and K_i are the saturation and

inhibition constants, respectively. At low initial DBT concentrations, equation 4-3 has the following form:

$$r_s = \frac{r_{max}}{(1+K_s/S)} \tag{4-4}$$

or in double reciprocal form:

$$\frac{1}{r_s} = \frac{1}{r_{max}} + \frac{K_s}{S}\cdot\frac{1}{r_{max}} \tag{4-5}$$

At high initial DBT concentrations, the rate is:

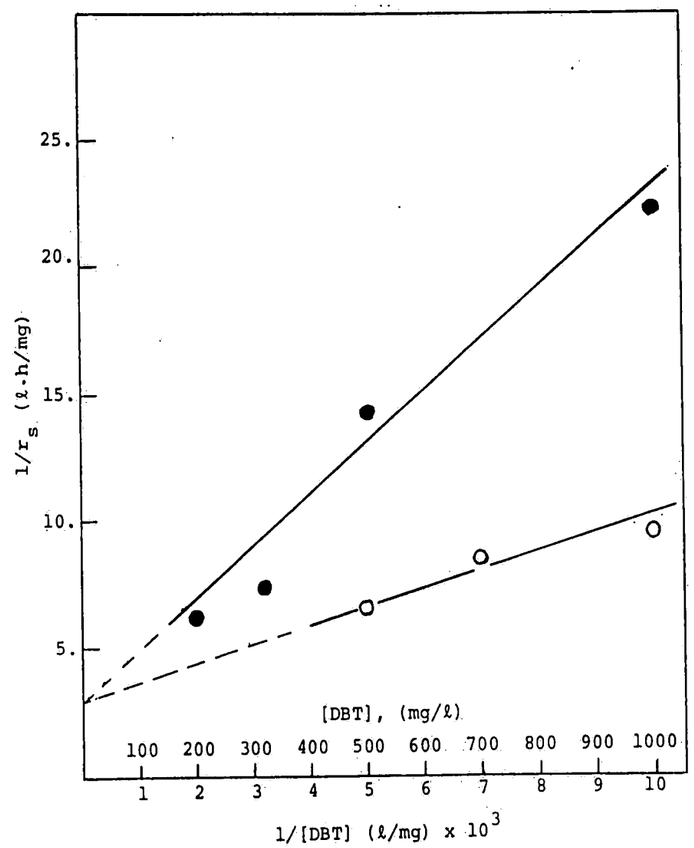
$$r_s = \frac{r_{max}}{(1+S/K_i)} \tag{4-6}$$

In double reciprocal form:

$$\frac{1}{r_s} = \frac{1}{r_{max}} + \frac{S}{K_i}\cdot\frac{1}{r_{max}} \tag{4-7}$$

Equations 4-5 and 4-7 are used to determine the kinetic constants of DBT oxidation. When $1/r_s$ is plotted against $1/S$ at low DBT concentration, $1/r_{max}$ and K_s can be found from the intercept and slope, respectively. When $1/r_s$ is plotted against S for high initial DBT concentrations, the inhibition constant, K_i, can be

Figure 4-17: Determination inhibition and saturation constants for DBT oxidation



determined from the slope. The plots are shown in figure 4-17. The following values were obtained from these plots- r_{max}= 0.333 mg S/l hr, K_s= 666 mg S/l, K_i= 480 mg S/l. The equation corresponding to these values is:

(4-8)

The rate is in the units of mg S/l hr; the sulfur concentration is in the units of mg S/l.

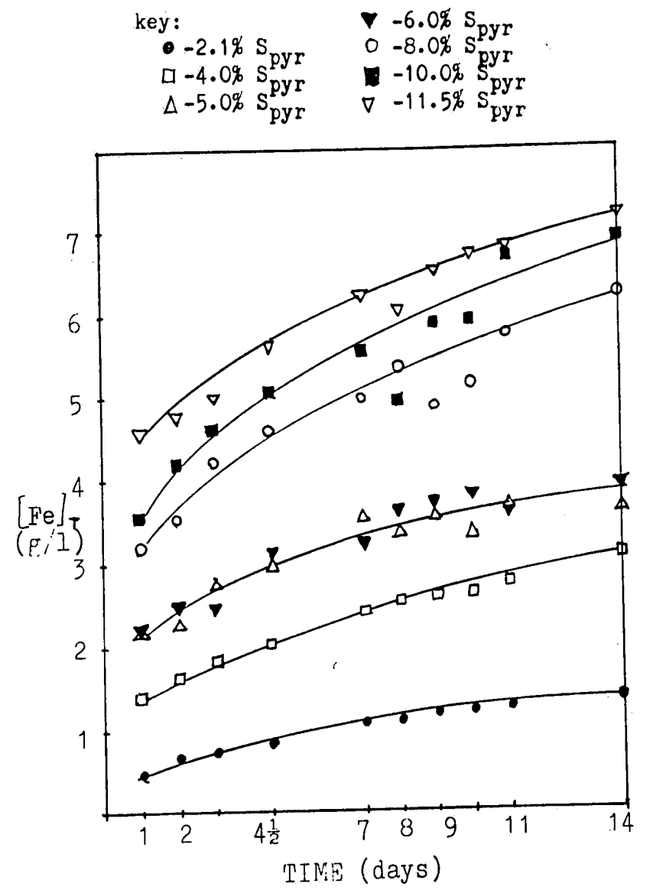
It is important to note that the only oxidation product measured in the preceeding organic sulfur removal experiments was sulfate. Other water soluble compounds containing sulfur in a partially oxidized form have not been measured due to equipment limitations. The rate of DBT oxidation, as well as the extent of DBT oxidation, may actually be greater than reported.

4.5.2 Kinetics of Pyritic Sulfur Removal

In order to test the effect of initial pyritic sulfur content of coal on pyritic sulfur removal rate, experimental flasks containing coal samples of various average pyritic sulfur contents were tested. The soluble iron and sulfate were measured daily in all flasks. In addition, free and total protein concentrations, as well as residual pyrite concentrations, were analyzed on alternate days for three of the experimental flasks.

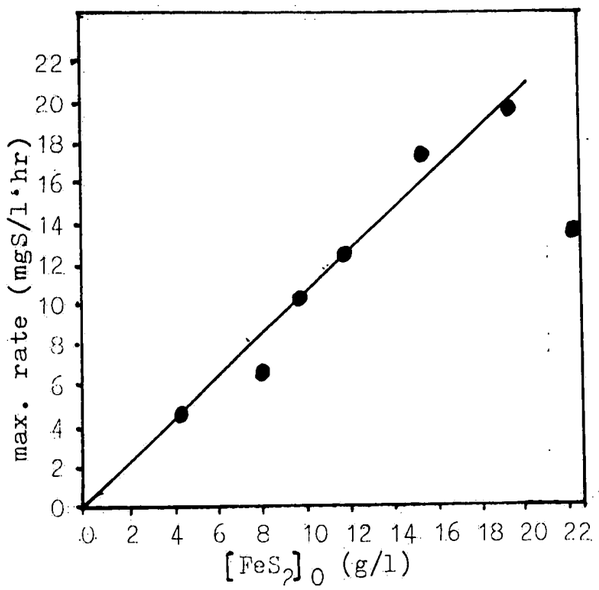
Coal samples were prepared by grinding plant feed coal and coal refuse separately and then mixing them in the desired amounts to achieve the desired pyrite concentrations in the medium. The average coal particle diameter used was 49 um (270-325 mesh). The initial pH and the temperature were set to 2.5 and 70 C, respectively. Figure 4-18 below depicts the profile of total iron

Figure 4-18: Total soluble iron profiles for coal with various initial pyrite contents



with time for the various initial pyrite contents. The rates as, determined from these curves, were plotted versus the initial pyrite concentration in figure 4-19. Note the linear functionality of rate

Figure 4-19: Variation of maximum rate with initial pyrite content

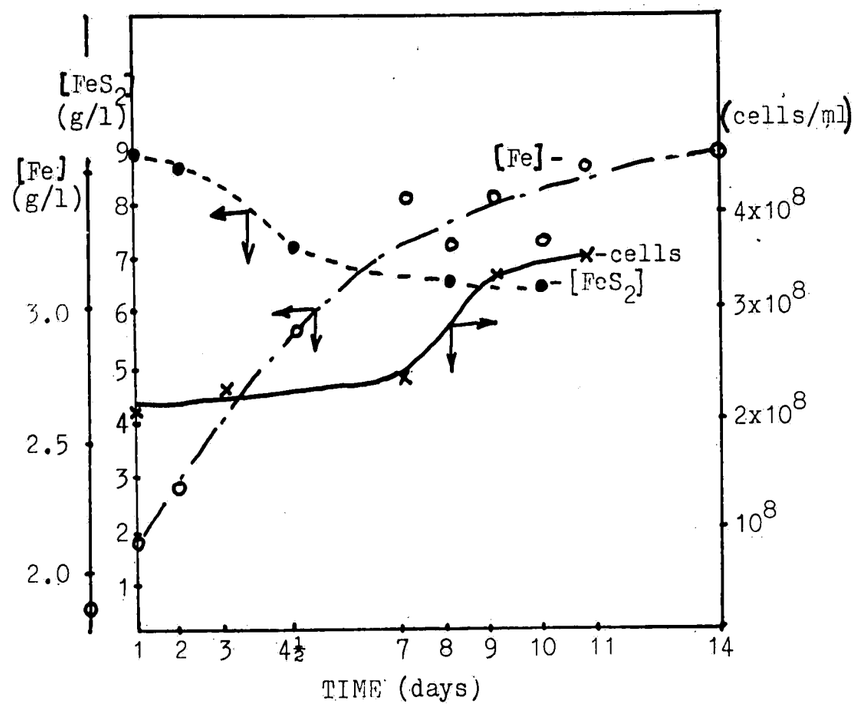


with pyritic sulfur concentration, a characteristic observed earlier in pulp density experiments where the pulp density was below 15%.

The specific sulfur removal rates (mg S/hr cell) were also calculated. The protein concentrations determined in the experimental flasks were correlated to cell number and the cell density profiles were drawn. In figure 4-20, a typical profile of the concentrations of iron released, residual pyrite concentration,

and the attached,free, and total cell number densities for the pyrite removal process are shown.

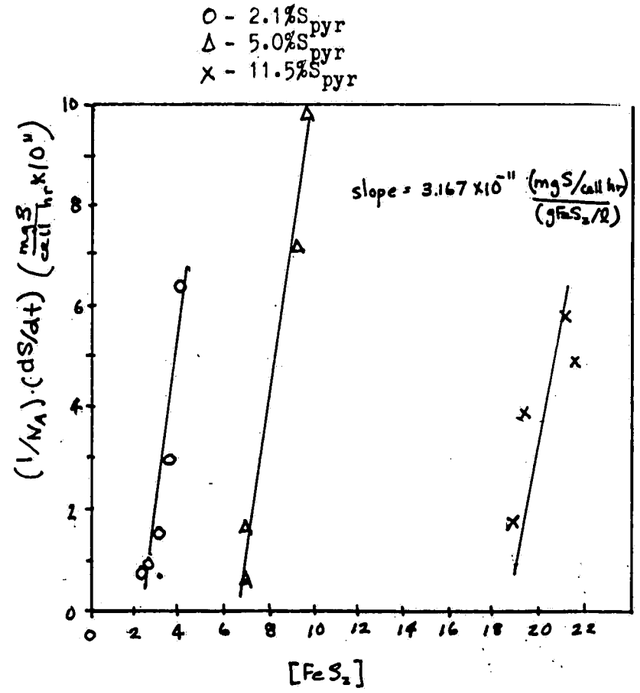
Figure 4-20: Typical pyrite, iron, and cell number profiles for coal desulfurization



The specific pyritic sulfur rates were calculated by dividing the instantaneous rates of sulfur removal at different times during the course of the experiment by the corresponding attached cell number. These specific pyritic sulfur removal rates were than plotted versus the instantaneous pyritic sulfur content of the coal to find the

variation of the rate with respect to the pyrite concentration. Figure 4-21 depicts the variation of specific sulfur removal rate with the pyrite concentration of coal for the three flasks for which the cell numbers have been determined. The three flasks produce data lying on lines of approximately the same slope (3.167×10^{-11} mg S/cell hr/(g FeS_2/l)).

Figure 4-21: Specific reaction rate as a function of pyritic sulfur content



This shows that the specific reaction rate is a linear function of

residual pyrite concentration, or:

$$r_s = k[FeS_2] + C \qquad (4\text{-}9)$$

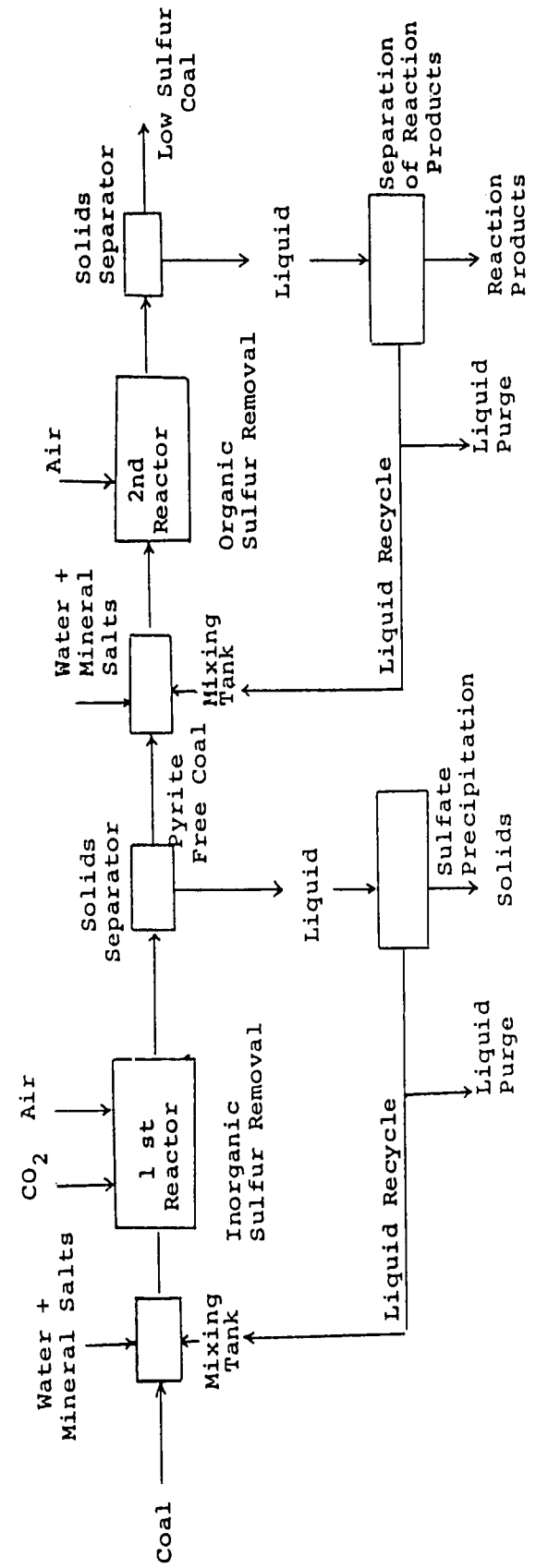
The proportionality constant is equal to the slope in figure 4-21 (3.167×10^{-11} mg S/cell hr/(g FeS_2)). The intercept (C) is a function of the initial pyritic sulfur content in the coal.

4.6 A Suggested Process Scheme

A suggested process scheme for the removal of pyritic and part of the organic sulfur from coal using Sulfolobus acidocaldarius is depicted in figure 4-22. The system can be separated into two parts. Inorganic sulfur (pyritic and sulfate sulfur) would be removed in the first section of the plant. Cell and nutrient recycle would be implimented to decreases operating costs. Sulfate would be removed after precipitation with calcium carbonate. Supplemental nutrients would be added to the recycle liquid. The residence time in the first reactor is in the order of 8 days.

The second part of the system would be arranged to remove the organic sulfur from the coal. The coal entering this section would be pyrite free. The DBT medium would be used in this part of the plant. A recycle stream would also be used in this section with supplemental nutrients added after the oxidation products are

Figure 4-22: A suggested process scheme for the use of Sulfolobus in MCD



removed. The residence time in the second reactor would be nearly 40 days. The coal effluent would be free of pyritic sulfur, but would probably contain some residual organic sulfur.

5. Conclusions

1. The inclusion of organic nutrients and chemical oxidants reduces the rate and extent of microbial coal desulfurization by Sulfolobus acidocaldarius. A simple mineral salts medium, specified in the appendix, contains all the ingredients necessary for effective sulfur removal by microbial means.

2. The optimal N/P and N/Mg ratios for MCD with Sulfolobus are 47.5 and 11.5, respectively. Ten fold rate increases were obtained with this medium over the standard mineral salts medium.

3. Alternate nitrogen sources, such as $(NH_4)_2CO_3$, NH_4Cl,and NH_4NO_3, performed as well as $(NH_4)_2SO_4$ when used while maintaining constant N/P. The initial sulfate concentration can, therefore, be lowered by using an alternate nitrogen source.

4. External carbon-dioxide supply had no marked effect on pyritic sulfur removal rates when tested with coal samples containing 2.1% pyritic sulfur at 10% pulp density. The carbon-dioxide concentration in the atmosphere was not limiting in the experiments. External CO_2 supply may be necessary at higher pulp densities and pyrite concentrations.

5. The pyritic sulfur removal rate varies directly with surface area. Therefore, the pyritic sulfur removal is maximized at minimal particle size (maximum surface area to volume ratio). The best particle size to be used will be determined by grinding costs.

6. The volumetric pyritic sulfur removal rate is maximized at 20% coal pulp density under suface aeration conditions. The surface reaction rate is constant for coal pulp densities of 15% and below.

7. The optimal initial cell concentration was found to be near $2x10^9$ cells/ml; this corresponds to about 10^8 cells/cm^2 of surface area of coal ($4x10^{10}$ cells/g coal or 10^{12} cells/g pyrite in coal)

8. The rate of pyritic sulfur removal decreases with initial pH. The optimal initial pH was found to be 1.5.

9. The optimal temperature for MCD using Sulfolobus acidocaldarius was found to be between 70 C and 75 C.

10. Organic sulfur removal from dibenzothiophene and coal sources was obtained using a culture of Sulfolobus acidocaldarius which was placed on DBT for 30 days prior to inoculation. About 65% of the initial sulfur present in DBT was oxidized to sulfate microbially. Up to 44% of the initial organic sulfur present in the coal tested was removed in a single batch run in about 28 days.

11. Saturation and inhibition consants of the oxidation of DBT with Sulfolobus acidocaldarius were found to be 666 mg S/l and 480 mg S/l, respectively. A maximum rate of 0.333 mg S/l hr was also calculated from kinetic rate data. This corresponds to a rate equation of:

$$r_s = 0.333/((1+666/S)(1+S/480))$$

12. The specific rate of pyritic sulfur removal from coal is a linear function of residual pyritic sulfur content. The proportionality constant when considering attached cells is equal to 3.167×10^{-11} mg S/cell hr/(g FeS_2/l).

13. The maximum rate of pyritic sulfur removal was found to be a linear function of the initial pyritic sulfur content of coal,or

$$r_{max} = k\,[S_{pyr}] + C$$

The first order rate constant was $k = 2 \times 10^{-3}$ hr^{-1}.

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APPENDIX

I. Media & Analytical Methods

Appendix A: Mineral Salts Medium Composition

Component	(g/l)
$(NH_4)_2SO_4$	1.3
K_2HPO_4	0.28
$MgSO_4\ 7H_2O$	0.25
$CaCl_2\ 2H_2O$	0.07
$FeCl_3\ 6H_2O$	0.02
$MnCl_2\ 4H_2O$	0.0018
$Na_2B_4O_7\ 10H_2O$	0.0045
$ZnSO_4\ 7H_2O$	0.00022
$CuCl_2\ 2H_2O$	0.00005
$NaMoO_4\ 2H_2O$	0.00003
$CoSO_4$	0.00001

appendix B: DBT Medium Composition

Component	(g/l)
NH_4NO_3	1.3
K_2HPO_4	0.28
$MgCl_2\ 7H_2O$	0.25
$CaCl_2\ 2H_2O$	0.07
$FeCl_3\ 6H_2O$	0.02

appendix C: Sulfate analysis

Conditioning solution:

mix:

50 ml glycerine
30 ml concentrated HCl
300 ml distilled H_2O
100 ml 90% EtOH (or isopropanol)
75 grams NaCl

1. Dilute samples to be tested so the concentration is between 0 and 150 mg SO_4/l.

2. Mix 2 ml of the diluted sample with 0.5 ml of the conditioning solution listed above.

3. Add 2 ml of 10% $BaCl_2$ solution and mix on a Genie vortex mixer for 1 minute.

4. Measure the maximum absorbance at 420 nm over a 3 minute period.

5. Compare the results to a calibration line prepared from standard solutions of SO_4. Multiply by the dilution factor used.

appendix D: Iron analysis

1. Dilute samples so the iron concentration is between 0 and 20 mg Fe/l.

2. Take 1 ml of te above samples and add 1 ml of 1% hydroquinone.

3. Mix the above solution with 2 ml of 0.1% o-phenanthroline and measure the absorbance at 500 nm.

4. Compare the results to a calibration line prepared from standards of known iron concentration.

appendix E: Sulfate sulfur analysis

1. Place a 1 g sample of coal with 25 ml of 4 N HCl into an erlenmeyer flask.

2. Insert a cold finger condensor and apply heat to the bottom of the flask. Allow to reflux for 30 minutes.

3. Filter the slurry on cooling through Whatman No. 2 filter paper. Wash the cold finger condensor and the residual solids with 0.5 N HCl.

4. Analyze the filtrate for sulfate using the method previously described.

appendix F: Analysis of pyritic sulfur content of coal

1. Place 1 g of coal and 25 ml of 2N HNO_3 into a wide mouth erlenmeyer flask. Insert a cold finger condensor and apply heat to the flask.

2. Boil the slurry and allow to reflux for 90 minutes.

3. Wash the condensor two times with 5 ml of 2N HNO_3 and collect the washings in the flask.

4. Filter the slurry through Whatman No. 2 filter paper and wash with 2 N HNO_3.

5. Add concentrated NH_4OH to the filtrate until all the iron has precipitated.

6. Again, filter the precipitate and the dissolve it in distilled water.

7. Analyze the iron content using the o-phenanthroline method described earlier and calculate the total inorganic sulfur content of the coal.

8. Subtract the sulfate sulfur content from this value to obtain the pyritic sulfur content.

appendix G: Analysis of the total sulfur content of coal

1. Intimately mix a pre-weighed sample of coal (0.5-1.0 g) with 1 g of Na_2CO_3 and 2 g of MgO.

2. Place the solid mixture into a crucible then place the crucible into a cold muffle furnace.

3. Heat the furnace to 800 C (25 C) in 1/2 hours.

4. Hold the temperature at 800 C for 90 minutes or until all black color is gone.

5. Wash the crucible and its contents with 25 ml of hot distilled water.

6. Place the solids and the washings in a beaker and heat.

7. Add enough concentrated HCl to dissolve all the solids.

8. Add 10 ml of 10% $BaCl_2$ and hold the temperature just below boiling for 30 minutes.

9. Two finishes can be used- centrifuge the solids from the liquid and resuspend the precipitate for turbidometric analysis, or filter and wash the solids for gravimetric determination of sulfate.