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# Microbial Factor in Acid Mine Drainage Formation



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# ***Microbial Factor in Acid Mine Drainage Formation***

MICROBIOLOGICAL FACTOR IN ACID MINE DRAINAGE FORMATION

Microbiological Factor in Acid Mine Drainage Formation: II. Further  
Observations from a Pilot Plant Study

by

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## ABSTRACT

### MICROBIOLOGICAL FACTOR IN ACID MINE DRAINAGE FORMATION

by Robert A. Baker and Albert G. Wilshire

The role of chemoautotrophic organisms (*Ferrobacillus ferrooxidans*, *Ferrobacillus sulfooxidans* and *Thiobacillus thiooxidans*) in the formation of acid mine drainage from pyritic materials associated with coal mining has been investigated by pilot plant techniques. Dynamic flow, controlled environment units which served as models of mines were used.

It was demonstrated that the concentration of acidity, ferrous and total iron and sulfate in effluent from aerobic, biologically-seeded or unseeded pyritic beds is zero order with respect to flow, except at low flow rates where mass transport is diffusion limited. Algal growth occurred in the acidic, aerobic environment but did not affect acid production.

Nonaerobic systems produce acidity consisting only of ferrous iron. Total acidity is lower from biologically-seeded than -nonseeded, aerobic systems at retention times exceeding three hours because microorganisms consume acid. At higher hydraulic rates, lower retention time, total acidity is greater in the aerobic-nonseeded effluents. Under aerobic-nonseeded conditions acid mine drainage release reaches a maximum rate at a specific flow. Further increase in flow only dilutes the concentration with total mass discharge remaining constant. Under aerobic-seeded conditions, a dynamic equilibrium exists between the rates at which pyrite dissolves, organisms reproduce and are flushed from the system. Maximum release occurs at a specific hydraulic rate. Higher or lower flows result in decreased discharges.

Acid mine drainage release (a) was not significantly affected by seeding with the individual or a mixture of the three organisms; (b) increased directly with recycle ratios up to 4:1 of forward flow rate; (c) increased appreciably under forced aeration; and, (d) is directly related to available pyritic surface area.

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**Key Words:** Mine Drainage/Chemoautotrophic Microorganisms/*Ferrobacillus*/*Thiobacillus*/Pyrite/Pilot Plant/Dynamic Equilibria

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## Section 1

### CONCLUSIONS

The role of chemoautotrophic microorganisms (*Ferrobacillus ferrooxidans*, *Ferrobacillus sulfooxidans*, and *Thiobacillus thiooxidans*) in the formation of acid mine drainage from pyritic materials associated with coal mining has been studied using pilot plant reactors. The units were charged with crushed pyrite from actual mining sites. Most of the tests were conducted with horizontal, flooded-bed reactors through which water of fixed mineralization was passed at a controlled rate of flow.

Dissolution of pyrite,  $\text{FeS}_2$  or iron sulfide, leads to ferrous (Fe II) ion and the sulfur species,  $\text{S}_2^{-\text{II}}$ . These are subsequently oxidized to ferric and sulfate reaction products. When the microorganisms were added to the system the unit is described as being seeded. Nonseeded is used to describe the condition when the organisms were not added.

The conclusions are:

1. The concentration of acidity, ferrous ion and total iron and sulfate in effluents from pyritic beds is zero order with respect to flow if the flow is expressed in reciprocal time units,  $\text{Day}^{-1}$ , (total volume of water per day ÷ volume of pyrite bed) over the range of 4.6 to 13.8,  $\text{Days}^{-1}$  or 1.5 to 4.3 hours retention time. At low flow rates of water flow the reaction products are mass transport limited and increase in concentration as flow decreases.

2. There is continuous release of Fe (II) and  $\text{S}_2^{-\text{II}}$  from non-aerobic pyritic systems. The mass per unit time discharged is constant if the flow of water is not so low as to be diffusion rate limited. Pyrite dissolution takes place in the absence of air (oxygen) although at a lower rate than under aerobic conditions. Since the Fe (II)- and  $\text{S}_2^{-\text{II}}$ -utilizing chemoautotrophic microorganisms are aerobes they are not active and do not affect release of these ions under nonaerobic conditions.

3. Under aerobic conditions a mycelial growth may develop in the pyritic beds despite acidic pH 3 to 4.5. There was no evidence that the presence of the heterotrophic organisms affected chemoautotrophic organism activity or altered the mass of acid mine drainage released per unit time. Acid mine drainage discharge was similar in periods of high or low algal growth.

4. The effect of flow rate on effluent characteristics was examined for nonaerobic, aerobic-nonseeded and aerobic-seeded operating conditions. Flow was varied to provide retention times through the pyritic beds of 1.5 to 10 hours.

- a. The effluent pH under nonaerobic conditions increased slightly over feedwater pH of 4.5 because of dissolution of alkaline materials in the pyritic mineral. The pH of the seeded and nonseeded aerobic effluents were 3.8 to 4.1 over the entire hydraulic flow range.

b. Effluent acidity under nonaerobic conditions was solely that contributed by ferrous ion. For aerobic effluents the total acidity is lower for seeded than nonseeded systems at flows providing retention times greater than approximately three hours. This is attributed to consumption of acid by the chemoautotrophic microorganisms. At higher flow rates or lower retention times the total acidity is greater in the aerobic-nonseeded effluents. Acidity, corrected for ferrous ion and expressed as  $H_2SO_4$ , decreases as flow increases and retention time decreases from 7 to 1.5 hours for the aerobic-seeded system. The corrected acidity is much greater in aerobic-nonseeded systems at 7 hours retention but decreases to concentrations equivalent to aerobic-seeded systems at retention time of 1.5 hours.

c. Ferrous ion and total iron release is greater from the aerobic-seeded than from the aerobic-nonseeded systems at all retention times greater than 1.5 hours. Most of the total iron content of the effluent is Fe (II). Ferric compounds precipitate in the bed. Mass release of iron reaches a maximum at approximately 2 hours retention time in the aerobic-nonseeded system then remains constant as flow rate increases. For the aerobic-seeded system the iron release reaches a higher maximum value than for the aerobic-nonseeded system at approximately the same hydraulic rate. With increasing flow, lower retention times, there is a drop in mass release of iron until the mass release from the aerobic-seeded and aerobic-nonseeded systems is comparable at <1.5 hours retention time.

d. There is no sulfate in nonaerobic system effluents. For aerobic systems, the sulfate mass release is greater for the seeded than for the nonseeded condition over most of the hydraulic range studied. Maximum release is at approximately 2 hours retention time in each case. For the nonseeded system, increasing flow does not alter the mass release of sulfate. For the seeded system, the sulfate release drops as retention time decreases to <2 hours and approaches the nonseeded system mass release at retention times <1.5 hours. Sulfate required to balance nonferrous acidity, expressed as sulfuric acid, is in slight excess and increases with flow rate for aerobic-nonseeded systems but decreases from a great excess at low flow rates to values comparable to the aerated-nonseeded system at retention times of 1.5 hours.

e. In aerobic systems the mass release of sulfur as sulfate and of total iron approximates the stoichiometric ratio expected from pyritic dissolution; however, because of reaction product accumulation within the pyritic beds, the effluent concentrations are not indicative of the pyrite dissolution rate.

f. The effect of chemoautotrophic organisms on formation of acid mine drainage is the result of a dynamic equilibrium between the rate at which their cells reproduce, their flushout rate within the effluent and the dissolution of pyrite. It is not possible from these results to establish whether these microorganisms directly affect pyrite surface reactions. They enhance oxidation of Fe(II) but the increased Fe (II) in the effluent of seeded systems may be an indirect result of higher ferric:ferrous ion ratios at the mineral surface rather than a direct microbial action.

5. The standard chemoautotrophic microorganism mixture used to seed the pilot plant systems contained equal numbers of *Thiobacillus thiooxidans*, *Ferrobacillus ferrooxidans* and *Ferrobacillus sulfooxidans*. In tests made at flow rates which provided 4.3 hours retention it was found that the effluent showed little difference in composition whether the seed was the individual organism or the ternary mixture.

6. When effluent is recycled at 1:1 and 4:1 times the forward flow rate, the mass release of acidity, ferrous ion and total iron and sulfate increases directly with the total flow through the pyritic bed.

Supplemental studies made with flooded, vertical, packed-bed columns of pyrites operated as aerobic-seeded systems demonstrated that:

7. Forced aeration of the pyritic bed raised acidity 35-fold. The corresponding pH changed from 5.2 to 2.8 in comparison to a nonaerated system. Ferrous ion, total iron and sulfate discharge increased by approximately 30-, 70-, and 120-fold respectively with aeration. Passing gaseous carbon dioxide through the pyritic bed raised the acidity 12-fold. Although pH remained comparable, iron concentration increased 3- to 4-fold and sulfate only 50%.

8. If reactant (chemical or biological) depletion or reaction product concentration is not rate limiting, the release of acid mine drainage is proportional to the available pyritic surface area for a given hydraulic flow rate. This relationship does not apply if flow is so low that transport is diffusion rate limited.

## Section 2

### RECOMMENDATIONS

Despite recent significant contributions from investigators engaged in biological and chemical research on the subject of pyritic oxidation there is still a lack of knowledge of the nature of the reaction mechanism at the mineral surface. The need for defining this oxidation mechanism is of practical, as well as academic consequence. For example, application of bacteriocidal agents to mineral surfaces is predicated on the proposition that the chemoautotrophic organisms will be destroyed, thus retarding or preventing ferrous iron and sulfide release. The validity of this premise is in doubt. Research should be undertaken to firmly establish the nature of the pyritic oxidation at the mineral surface in the presence and/or absence of chemoautotrophic microorganisms.

There is a serious problem in interpreting the results obtained in fundamental chemical and biological investigations for actual field use. Basic studies are usually made in small-volume, batch systems. In coal mining operations which release acid mine drainage, a dynamic equilibrium exists between the dissolution of pyrite and the transport of the released mineralization as a function of water flow. Thus, small pilot plants, such as those employed in this study, which serve as models of mines should be put to greater use for testing the postulates derived from fundamental studies. Typical is the need to clarify the interrelationship between the limiting water:pyrite ratio, ferric:ferrous ion concentration, and microbial density.

The major emphasis was placed on establishing the effect of environmental conditions and hydraulic rate on acid formation and release in this study. The effect of surface area; differences in pyrite source; supplemental CO<sub>2</sub> (by a partial pressure increase of the gaseous environment rather than by forced gasification); biocides to arrest chemoautotrophic activity; and dark reactions (eliminate sunlight or artificial light which promotes algal activity); and the effect of varying feedwater composition merit further pilot plant investigation.

This study demonstrated a need for development of accurate and reliable miniature probes which could be operated remotely in closed systems to measure oxidation-reduction potential and dissolved oxygen. At the present time it is not possible to make an accurate oxygen balance on the reactor or to determine the Eh at which pyrite dissolution takes place, particularly under nonaerobic conditions.

The nature of the sulfur species which is released on dissolution of pyrite and its fate in subsequent oxidation reactions is unknown and should be elucidated if the pyritic oxidation processes are to be understood.

## Section 3

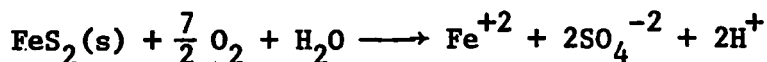
### INTRODUCTION

The objective of this research was to advance the understanding of the effect of microbiological factors in the formation of acid mine drainage from pyritic materials associated with coal mining. The study was made in pilot plant reactors amenable to environmental and hydraulic control which were charged with pyritic material taken from actual mining locations. Thus, the pilot plants served as models of mines. Results obtained under dynamic equilibrium conditions are directly relevant to the particular mining locations from which the pyritic samples were obtained. Specific factors which were investigated included the effect of: aerobic versus anaerobic environments; liquid flow rate; biological seeding; nature of chemoautotrophic microorganism seed; supplemental gasification by carbon dioxide or air; surface area; and recycle of effluent.

Although significant advances in the basic knowledge of chemical and biological factors have been made in recent years, these results have not been readily applicable to acid mine drainage problems in the field. The pilot plant concept was advocated to bridge the gap between fundamental studies and field needs. It is suggested that this concept, once demonstrated, could be applied to other laboratory and field locations as needed in the study of regional problems. The technique could be used by operators to determine acid-producing potential in advance of mining. This should enhance pollution abatement activities by indicating the most readily controlled factor(s) for a specific site. Since major operators presently take core samples ahead of operation, this screening step could be a complementary activity.

Table-top and batch systems used in basic studies are often rate limiting because of consumption of reactants, depletion of vital nutrient or accumulation of reaction products. A continuous pilot plant permits attainment of dynamic equilibrium and better represents field conditions. However, there are disadvantages. In this study, the effect of the variables tested was determined by monitoring the composition of the effluent from the reactors. It was not possible to make material balances on specific ions or compounds. Simultaneous dissolution, oxidation and precipitation processes occur. Thus, information obtained from batch and continuous pilot plant experimentation is necessary.

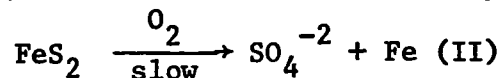
The oxidation of coal mine pyrite,  $\text{FeS}_2$ , in the presence of moisture leads to acidity. Half of the acidity is attributable to  $\text{S}_2^{\text{II}}$  oxidation to sulfate and half to Fe (II) oxidation to ferric iron and its hydrolysis. The overall reaction suggested by Leathen, et.al. (1953a) was



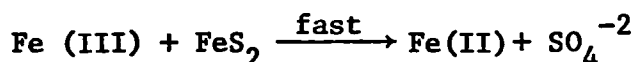
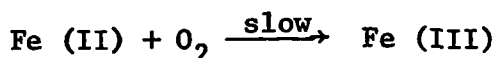
These authors further postulated that the reaction may be biologically promoted to increase the rate of oxidation and subsequent hydrolysis of  $\text{FeSO}_4$  in the presence of sulfuric acid to yield ferric sulfate, ferric hydroxy-sulfate and ferric hydroxide. In the colloquial language of the coal industry, these resulting yellow-brown reaction products are called "yellowboy" (Girard, 1965). Elucidation of the interrelationship between the chemically- and biologically-promoted oxidation reactions has been the basis of considerable effort in recent years. Stumm and Lee (1961) found the ferrous



oxidation rate to be proportional to  $[\text{OH}^-]^2$  above pH 4.5 for bicarbonate solutions comparable to natural waters. A transition occurs at pH 3. At lower pH the oxidation rate is independent of pH. Hem and Cropper (1959) and Hem (1960) defined the solubility field diagrams for ferrous ion release from pyrite. At pH  $\leq 6$ , Fe (II) solubility is high and its concentration may change by several orders of magnitude over a narrow Eh range. Singer and Stumm (1968) found that sulfate retards Fe (II) oxidation. Though the oxidation rate continues to be first order with respect to Fe (II) it is slower than in the absence of sulfate. The rate of ferric hydrolysis is linear second-order with respect to Fe (III) and faster in the presence of sulfate. They proposed that in the absence of bacteria the rate determining step is the Fe (II) oxidation. Schematically:



and



The Fe (II) oxidation rate is dependent upon the anionic species under acidic conditions (Huffman and Davidson, 1956). The rate increases in the same order as the complexing affinity for Fe (III): perchlorate, sulfate, chloride, phosphate and pyrophosphate. Since the hydroxyl ligand has a strong Fe (III) affinity it was postulated to be a factor in Fe (II) oxidation. The hydrolysis of free Fe (III) is therefore involved in Fe (II) dissolution from the pyrite. Garrels and Thompson (1960) studied pyrite oxidation by iron sulfate and showed that the rate is independent of total iron content and is controlled by differential adsorption of Fe (III) and Fe (II) ions on the pyritic surface. Oxidation of pyrites to release ferrous and sulfate ions was observed only at sites occupied by ferric ions. This oxidation rate is slow relative to the adsorption process hence the latter controls. They postulated that pyrite oxidation may involve release of molecular sulfur.

The specific role of acidophilic chemoautotrophic bacteria in pyritic conversion to acid mine drainage is undefined. These Fe (II)- and  $\text{S}_2^{-\text{II}}$ -utilizing organisms are active at pH 2 to 4.5 and use carbon dioxide as their carbon source. Since oxidation of  $\text{FeS}_2$  may proceed solely by chemical routes the microorganisms are not essential to acid formation. Their role may be (1) as a direct catalyst to alter the overall chemical reaction rates or (2) as specific catalytic agents which alter the rate of intermediate reactions and the nature of the resulting byproducts but not the overall rate. The microorganisms may remove electrons from surface pyritic iron to start a reaction chain and/or catalyze sulfur oxidation or they may simply increase Fe (III) concentration and hence the Fe (III) to Fe (II) ionic ratio. The Fe (III) is reduced by the pyrite and releases Fe (II).

Colmer, Temple and Hinkle (1950) reported an organism which oxidized Fe (II) to Fe (III) and thiosulfate to sulfuric acid. Later Temple and Colmer (1951) named this organism *Thiobacillus ferrooxidans*. Leathen, et.al. (1953a) could not confirm that a single organism was capable of oxidizing both the Fe (II) and thiosulfate energy sources. Leathen, et.al. (1953b) demonstrated that *Thiobacillus thiooxidans* increased acidity and

sulfate formation when elemental sulfur was present in the medium. Leathen and Braley (1954) subsequently described an organism capable of oxidizing ferrous to ferric sulfate and named it *Ferrobacillus ferrooxidans*. Leathen, et.al. (1956) further characterized this organism showing that neither sulfur nor thiosulfate are oxidized by it, thus distinguishing it from the genus *Thiobacillus*.

Unz and Lundgren (1961) examined the nutritional requirements of *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans*, and *Ferrobacillus ferrooxidans* and found them to be similar. Morphologically, these organisms are indistinguishable and these authors suggest that they cannot be classified by their nutritional differences. Kinsel (1960) reported a sulfur-oxidizing, iron chemoautotroph, *Ferrobacillus sulfooxidans*. The dispute about the classification of these organisms remains unresolved. Lundgren and Schnaitman (1965) examined cultural characteristics and kinetics of biological iron oxidation. They showed that Fe (II) oxidation was directly proportional to cell count of *Ferrobacillus ferrooxidans*.

The role of bacteria in the oxidation of pyritic materials has been the focus of much debate. Silverman and Ehrlich (1964) outlined two alternate mechanisms for bacterial conversion of  $S_2^{-II}$ . One involves Fe (III) oxidation of pyrites to obtain Fe (II) which the organism oxidizes back to Fe (III). The alternate mechanism is independent of Fe (III) and requires only contact between the bacteria and  $S_2^{-II}$ . It is likely that both mechanisms could take place in a given system depending on the nature of the pyritic material. Silverman (1967) further elaborated on the mechanism of the bacterial action and proposed that two mechanisms of bacterial pyrite oxidation operate concurrently. These were termed the direct contact and indirect contact mechanisms. The direct contact mechanism requires physical contact between the bacteria and the pyrite particles. The indirect contact mechanism requires that the bacteria oxidize Fe (II) to the Fe (III) state thereby regenerating the Fe (III) ions required for chemical oxidation of pyrite. During this investigation it was reported that Fe (III) oxidizes pyrite in the absence of bacteria and oxygen. Smith and Shumate (1970) claim that direct oxygen oxidation and Fe (III) oxidation of pyrite are independent processes and that the latter is a chemical analogy of the microbially-enhanced pyrite oxidation process.

The role of  $S_2^{-II}$  and the ferrous ion is two fold in the metabolism of these bacteria. They supply both the energy and reducing power for carbon dioxide fixation. Dugan and Lungren (1965) have proposed, in the case of the Fe (II), a model to explain the coupling of the energy released from Fe (II) in the form of an electron to the carbon reduction mechanism within the cell. In this model, an initial iron and oxygen complex is oxygenated but not oxidized in the absence of electron transport. Subsequently this complex reacts with iron oxidase or oxygenase to affect the electron transport. It is not evident from this study whether the complex is formed in solution or on the pyrite surface.

These often unreconcilable reports prompted the initial pilot plant study. In the first phase of this research (Baker and Wilshire, 1968) it was demonstrated that:

1. Chemoautotrophic microorganisms significantly accelerate the oxidation of Fe (II) and  $S_2^{-II}$  released from the pyrites but apparently do not directly alter the rate of pyrite dissolution in an aerobic environment.

2. Excluding oxygen does not prevent dissolution of pyrites. Under dynamic conditions a steady-state release of Fe (II) and  $S_2^{-II}$  takes place; however, the rate is very slow. The practice of sealing mines to exclude air may reduce but will not eliminate acid formation. Since the autotrophic organisms are aerobes they are not active and have no effect in an anaerobic environment of Fe (II) or  $S_2^{-II}$ . Fe (II) emitting from sealed mines must ultimately be oxidized in the receiving body of water with the production of acidity and "yellowboy" precipitate.

3. In the absence of biological organisms, effluent Fe (II) content is greater under an aerobic than anaerobic environment. Effluent Fe (III) content is negligible under highly acidic, aerobic conditions since it is precipitated.

4. Organic carbon if available from coal or its associated hydrocarbons will supply energy for heterotrophic microorganisms. Certain heterotrophs can exist in the highly acidic environment of an aerobic system. A mycelial growth of the species *Penicillium* developed in the aerated reactors of the pilot plant. These heterotrophs may affect the autotrophic oxidation processes through a succession reaction mechanism. They release carbon dioxide and enzymes. Since autotrophs utilize inorganic carbon this supplemental carbon source could be available to increase autotrophic metabolism in cases where  $CO_2$  availability is rate limiting.

5. Total acidity released by aerobic-nonseeded systems exceeds that from aerobic-seeded systems since Fe (II) content is greater. However, the acidity corrected for Fe (II) equivalent is greater from the seeded systems. The acidity released from nonaerobic systems consists almost entirely of Fe (II).

6. Sulfate release in the nonaerobic reactor effluents was essentially nonexistent. Under aerobic environments the biologically seeded systems release lower sulfate effluent concentrations than nonseeded systems.

7. Viable autotrophic organisms capable of utilizing Fe (II) were native to each of the pyritic materials tested. These have been found to survive extended periods of relative inactivity pending establishment of favorable environmental and other conditions.

These conclusions were reached from pilot plant studies at a specific hydraulic rate. It was recognized that more extensive testing was required to determine the validity of these findings over a range of hydraulic rates. The heterogeneous reaction of pyrite oxidation could be limited by mass transport or reaction kinetics depending upon the particular limitations imposed on the experimental system.

## Section 4

### EXPERIMENTAL UNITS

The microbiological factor in acid mine drainage formation was studied with continuously operating pilot plants. These units served as models of mining sites. They provided for controlled environment, hydraulic flow of preestablished composition and rate over pyritic minerals of known composition. Microbiological seeding provisions were made. Three versions of packed bed reactor units were employed: horizontal, flooded-bed; horizontal, flooded-bed with recycle chamber; and, vertical, flooded-bed.

#### A. Horizontal, Flooded-Bed Reactors

The unit used to make most of the tests provided four identical horizontal, flooded, packed bed reactors in controlled environmental chambers. The reactors and associated control systems are depicted schematically in Fig. 1. This drawing shows two reactors each under aerobic and nonaerobic conditions.

All four may be operated under identical environments by making the appropriate line adjustments. Feedwater flow to the aerated and non-aerated reactors is from 45-liter pyrex carboys (C) each under 3 psig Cono-flow regulated (R) air or nitrogen pressure (P). Liquid flow control is adjusted by Manostat teflon needle valves. Liquid-(aerated or nonaerated, (L<sub>a</sub>) or (L<sub>n</sub>)) containing lines are 7 mm glass tubing connected by Tygon tubing sections. Filtered air (A) or nitrogen (N) is delivered via a Gelman model 1235 filter holder containing 47 mm, 0.1 $\mu$  millipore filters which are changed each time feedwater is prepared. Filtered, house compressed air is used. Nitrogen is obtained from tank supplies. The carboy tops are securely wired during operation but are removed during feedwater preparation. A fritted-glass sparger permits air or nitrogen as desired to be bubbled through the vented carboys during the liquid transfer and chemical addition steps. Once the carboys are filled and the flow to the spargers terminated, the caps are repositioned and the uniform pressure head maintained. The aerobic and nonaerobic reactors are kept under a slight positive (approximately 2 to 4 cm water) pressure of air or nitrogen respectively measured by U-tube manometers (U) filled with water. Effluent liquid (E) passes through a loop which serves as a seal to maintain environmental control. Biological organisms are added by syringe through septa mounted in holders on the inlet end of small glass tubes leading into the reactors at inoculation points (I). Inoculations are made without disrupting the atmosphere. An outer shell serves as an environmental chamber and an internal reactor contains the mineral and feedwater. This chamber consists of a 4-inch I.D., 4.25-inch O.D. plexiglass section 36 inches long. It is fitted with 0.5 inch thick plexiglass end caps machined to accept standard pipe thread fittings. The liquid and environmental gas service lines, manometer and inoculation septa are mounted on these end caps. It is supported on a spring-loaded mount for leveling adjustment. The reactor is a 30-inch long plexiglass tray cut from a 2.25-inch I.D., 2.5-inch O.D. tube. A longitudinal section has been cut away to create a tray 1 13/16-inches high. Overflow and underflow weirs are used to reduce short circuiting of liquid through reactor. These are cut from 1/32-inch cellulose acetate and are cemented into the reactor. Overflow weirs at the inlet, center and exit and underflow weirs at the 25 and 75% points between the inlet and exit weirs are provided. The beds are filled with 625 ml or 1350 g of mineral

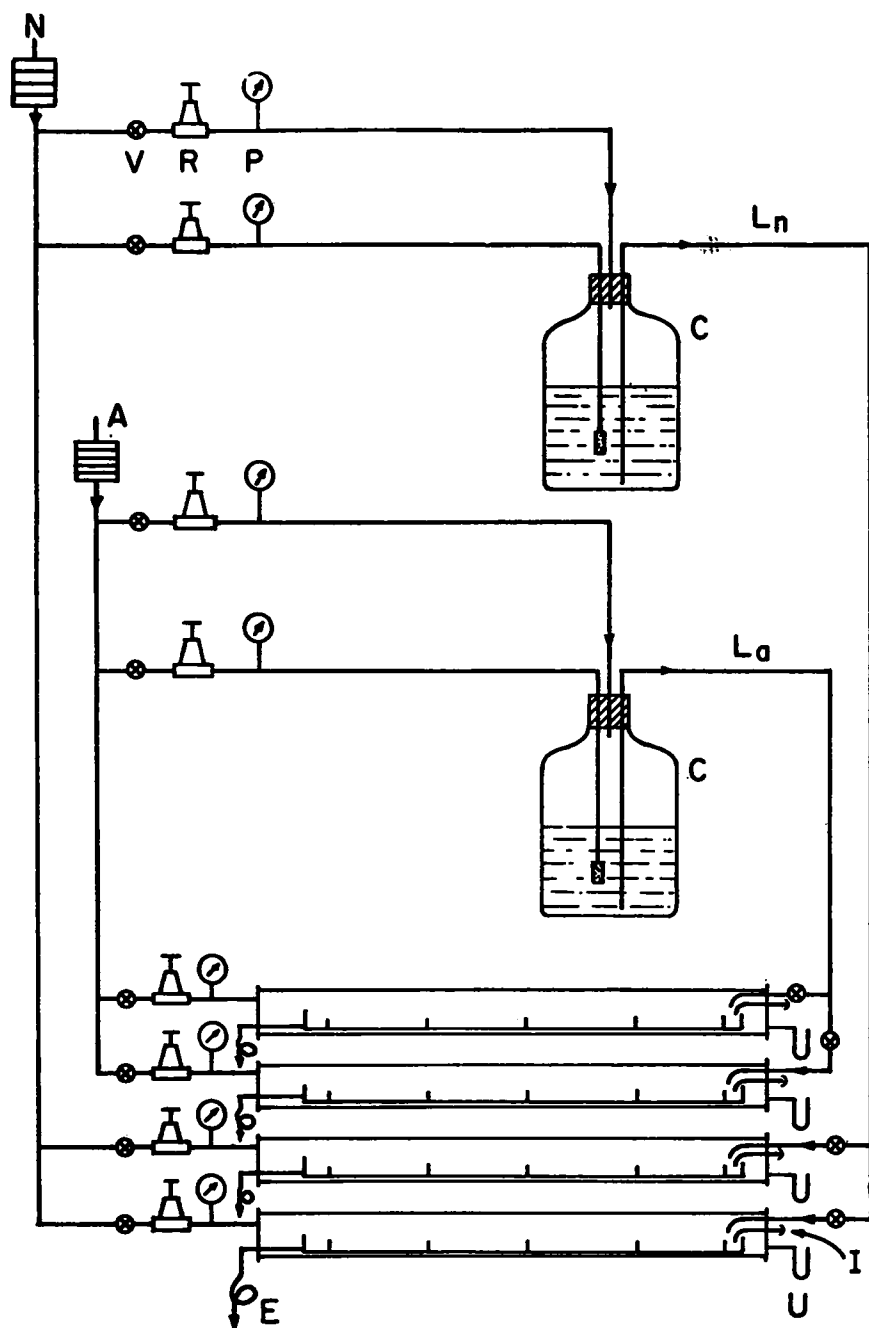


Figure 1. Schematic of Horizontal Reactor Pilot Plant

A	filtered air	$L_n$	nonaerated feedwater
C	carboy	N	filtered nitrogen
E	effluent	P	pressure guage
I	inoculation septum	R	regulator
$L_a$	aerated feedwater	V	valve

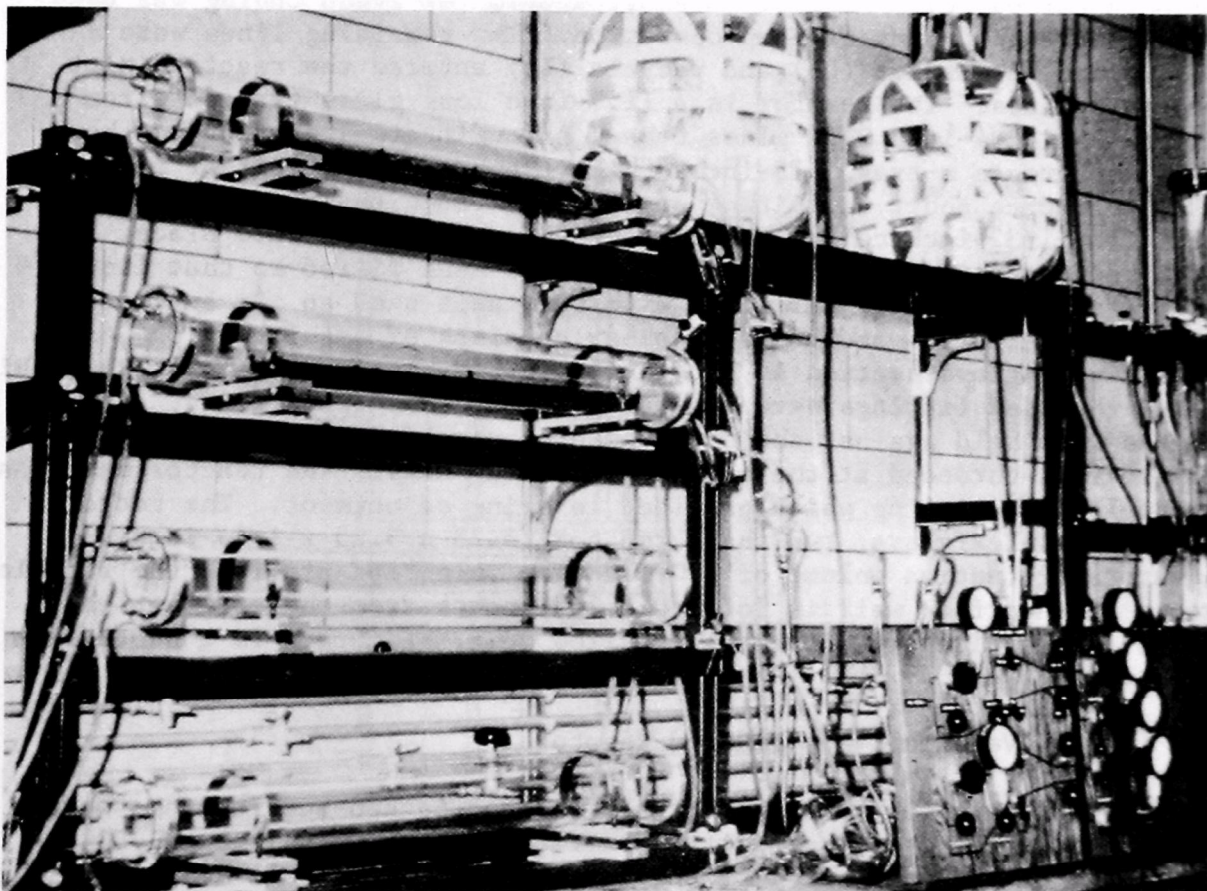


Figure 2. Horizontal Reactor Pilot Plant



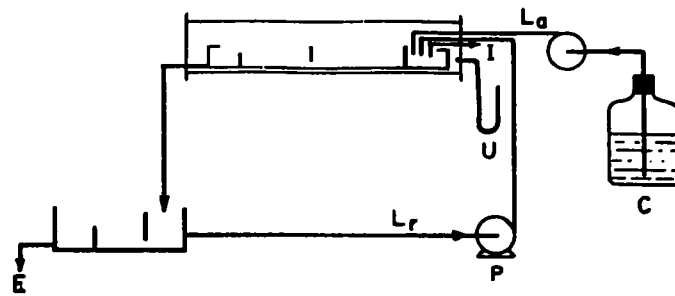
and have a liquid retention volume of 520 ml. When the reactors are operating the liquid just covers the surface. The bed is flooded. Fig. 2 is a photograph of this unit.

#### B. Horizontal, Flooded-Bed Reactor with Recycle Chamber

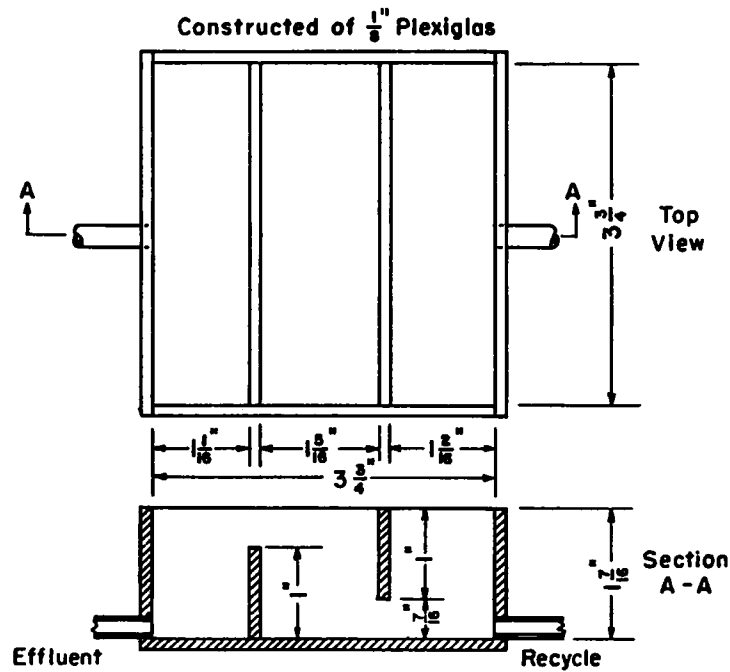
The effect of recycling effluent was determined with a horizontal, flooded, packed bed reactor unit similar, except for size, to the aforementioned units. Feedwater was added to the reactor by a peristaltic pump (P) from a carboy (C). Fig. 3. A short section of Tygon tubing was used in the line at the point of pump operation. The remaining lines were glass tubing. Feedwater ( $L_a$ ) and recycle ( $L_e$ ) entered the reactor inlet by separate lines. The reactor is a 11.7-inch long glass tray cut from a 2.2-inch I.D., 2.4-inch O.D. glass tube. A longitudinal section has been cut away to create a tray 1.75-inches high. An underflow weir at midpoint is used to reduce short circuiting of liquid through the reactor. These are cut from 1/32-inch cellulose acetate and are cemented into place. The flooded beds contain 230 ml of pyritic mineral when filled so that liquid just covers the surface. All tests with this unit used an air environment. The exterior environmental chamber consists of a 4-inch I.D., 4.25-inch O.D. plexiglass section 14 inches long. End plates containing the tubing and pipe threaded fittings were made of 0.5 inch thick plexiglass. The endplates were held against the environmental chamber by four stainless steel tie rods threaded at the ends to take wing nuts. The reactor base was a spring-loaded mounting which provided leveling adjustment. The recycle chamber was a plexiglass, two-chambered box (3.25 x 3.25 x 1.44 inches) with a total retention volume of 370 ml. The inlet end provides the recycle source. An underflow weir is located 1.125 inches from the effluent end. Effluent (E) is discharged over an overflow weir. Figure 4 is a photograph of this unit.

#### C. Vertical, Flooded-Bed Reactors

Three to four small vertical, flooded packed-bed reactors were used to examine the effect of pyritic surface area and supplemental  $CO_2$  (Fig. 5). The units were made of acrylic tubing with overall height of 51 cm and internal diameter of 6.3 cm. The columns were mounted on a plexiglass base. They were first packed with 4 cm polystyrene and glass beads. This layer was arranged around a polyethylene gas dispersion tube and a glass feedwater inlet tube coming down the center of the column. The inert beads serve to promote uniform cross sectional flow distribution. Pyrites were then added to a depth of approximately 44 cm (925 ml of pyrite per unit). Feedwater is added by a peristaltic pump from a carboy. Effluent is discharged via a sidearm. Microrotameters were calibrated and used to measure tank supplied gases, either carbon dioxide or air.



a. Schematic



b. Recycle Chamber Size

Figure 3. Schematic of Horizontal Reactor Pilot Plant with Recycle

C carboy  
E effluent  
I inoculation septum

$L_a$  aerated feedwater  
P pump  
U manometer

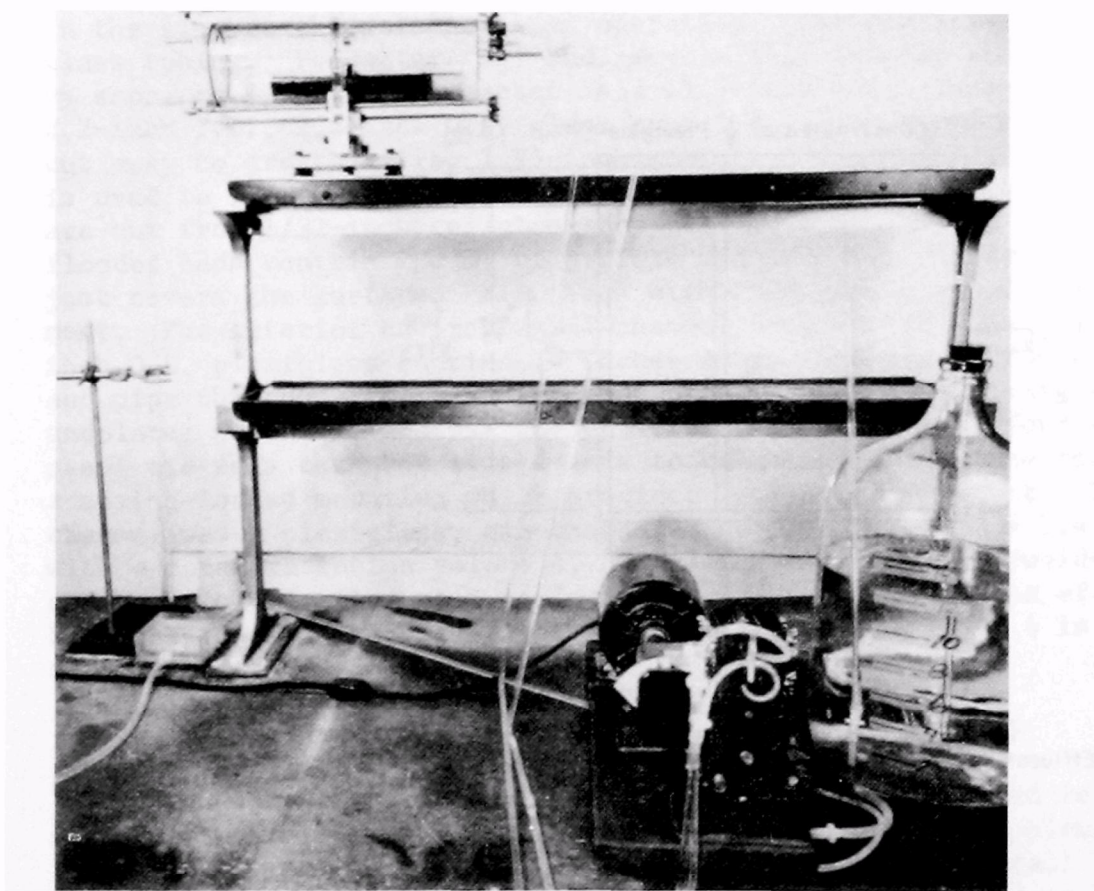


Figure 4. Horizontal Reactor Pilot Plant with Recycle Chamber

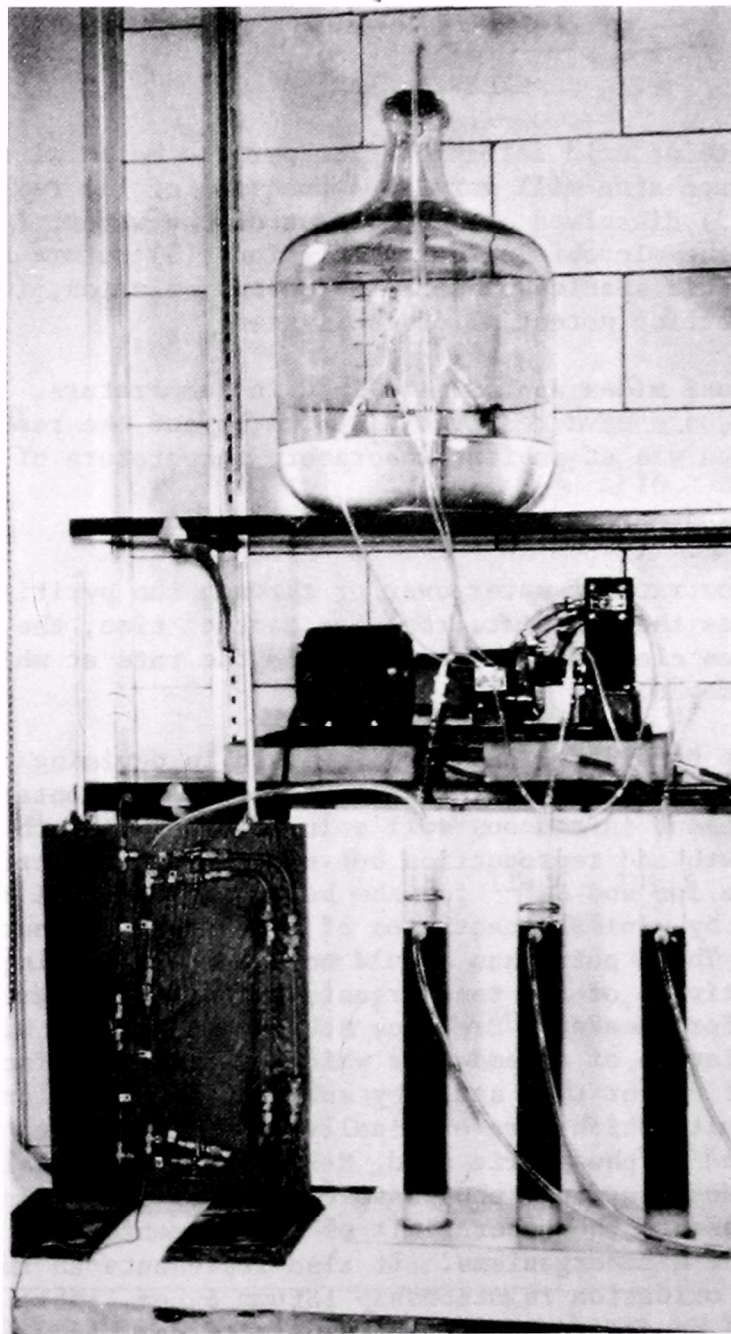


Figure 5. Vertical Reactor Pilot Unit

## Section 5

### EXPERIMENTAL

The rate of acid formation from pyritic material of given composition and surface area will vary as a function of (1) temperature, (2) hydraulic rate, (3) dissolved oxygen content of the water, (4) nature and concentration of the microbiological population, (5) nature and composition of the inorganic species present in aqueous solution, (6) pH, and (7) the oxidation-reduction potential of the system.

Deep coal mines approximate 15°C in temperature. Simulation of this temperature would have required a system beyond the resources of this project. Operation was at ambient laboratory temperature of 23° to 26°C.

#### A. Feedwater

The flow rate of water over or through the pyritic mineral material determines the immediate reaction contact time, the rate at which nutrients and trace elements are supplied and the rate at which reaction byproducts are removed.

Various considerations were involved in devising a feedwater formulation. It should be of constant composition and contain typical mineralization present in aqueous soil solutions, provide the ions necessary for bacterial growth and reproduction but not contain the primary energy sources of ferrous ion and  $S_2^{-II}$  for the bacteria. Typical mine waters are characterized by minimal quantities of the biological nutrients nitrogen and phosphorous. These nutrients should not be rate limiting factors for the biological activity of the test organisms in this research. Higher levels were therefore needed. Previous studies, Baker and Wilshire (1968), resulted in formulation of a feedwater which contained sulfate. This formulation was modified for this study by substitution of chloride as the anion for those salts which were originally added as sulfate (Table 1). The pH was adjusted by phosphoric acid,  $H_3PO_4$ , to a final value of 4.5 to 4.7. This produced a final phosphate content of approximately 30 mg/l. The final pH represents the upper limit of environmental suitability for reproduction of the microorganisms. It also represents an important point in the pH-ferrous oxidation relationship (Stumm & Lee, 1961). It was demonstrated that: (a) no deleterious osmotic effects were induced by organism (*Thiobacillus thiooxidans*, *Ferrobacillus sulfooxidans*, and *Ferrobacillus ferrooxidans*) transfer from culture media to feedwater or vice versa; (b) biological growth was maintained in the feedwater; and (c) the feedwater need not be sterilized since no iron- or sulfur-utilizing organisms are present. Despite the last result, every care was exercised to avoid contamination of the feedwater supplies.

The deionized water used to prepare the feedwater was either aerated by air or deaerated by tank nitrogen. Final dissolved oxygen content was approximately 7 and 0 mg/l respectively. This was done in separate 13-gallon Nalgene polyethylene tanks. The deionized conditioned water was transferred to the feedwater carboys under their respective gas heads (air or nitrogen). The chemicals were added to the carboys immediately and the carboys were sealed. Feedwater characteristics were routinely monitored to assure constancy of composition and oxygen level.

TABLE 1  
Feed Water Composition

<u>Component</u>	<u>Composition, mg/l</u>
Ca	76
Mg	17
K	13
Mn	6.5
NH <sub>4</sub>	6.8
NO <sub>3</sub>	10
Cl	199.5
PO <sub>4</sub> <sub>1</sub>	16
PO <sub>4</sub> <sup>*</sup> <sub>2</sub>	30
pH <sub>1</sub>	~6.7
pH <sub>2</sub> <sup>*</sup>	~4.5
Specific Conductance (μmhos/cm)	690

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\* After adjustment with H<sub>3</sub>PO<sub>4</sub>; with a resulting acidity of approximately 20 to 25 mg/l as H<sub>2</sub>SO<sub>4</sub>.



## B. Pyrite

The iron sulfide associated with coal seams is chiefly pyritic. Another mineralogical form, marcasite, is usually less abundant at locations where acid mine drainage is a problem. The oxidation rate of pyrite is affected by such factors as the crystal size, nature and concentration of impurities. The pyrite used in these studies was obtained from Shawville, Pennsylvania. This mineral was also used in preliminary investigations, Baker and Wilshire (1968). It contains 45% iron, 0.05% magnesium, 0.26% manganese and 0.08% calcium. Loss on ignition is 27.8%. This loss includes entrained coal and conversion of carbonates or other anionic forms to oxides. X-ray analysis indicates pyrite content of 90% or more with minor quantities of siderite, marcasite and quartz. If all the iron were assumed to be present as  $\text{FeS}_2$  then the material would be 98% pyrite.

Surface area and pore size characteristics were measured by a BET apparatus using nitrogen as sorbate. The sample was first reduced to 60/80 mesh screen size and degassed for four hours at  $200^\circ\text{C}$ . Sorption capacity is low. Sorption and desorption loops do not close and the sorption isotherm is classified as Type I in the Brunauer-Deming-Deming-Teller classification. Type I characterizes nonporous and microporous solids with capillaries which have a width not exceeding a few molecular diameters. Data between 0.02 and 0.20 relative pressure where linearity exists were used to calculate a specific surface of  $1.09 \text{ m}^2/\text{gm}$ . For a cubic particle with edge length of the average size screened the specific surface is  $0.006 \text{ m}^2/\text{gm}$ . The BET value is 184 times as great which appears excessive for the triple product of shape factor, size distribution factor and surface roughness factor. The density was measured by helium displacement and by mercury displacement. Helium which penetrates all voids with access from the surface gave a density of  $4.12 \text{ gm/ml}$ . Mercury at a pressure equivalent to penetration into pores 9 microns in radius gave a density of  $3.588 \text{ gm/ml}$ . From these densities the calculated pore volume is  $0.0358 \text{ ml/gm}$  or on a volume basis  $0.128 \text{ ml/ml}$ . In summary, the pyritic material is a microporous solid with at least  $0.036 \text{ ml/gm}$  internal voids. These voids have an internal specific surface of approximately  $1 \text{ m}^2/\text{gm}$ .

The sulfur ball and similar pyritic material as received was not washed with acid, solvents or water. The outer surfaces were rejected during crushing operations and the freshly-exposed, ground mineral was screened to the desired size range. Most studies were made with 3.5 to 7 mesh particle size. The freshly-ground pyrite was sterilized to eliminate native organisms by exposing the particles to an atmosphere of carboxide (10% ethylene oxide and 90% carbon dioxide) for at least 24 hours prior to charging reactors (Allison, 1951). Studies were made which demonstrated that the gaseous sterilization did not affect activity of the pyrite in acid formation reactions. Sterilization effectiveness was monitored by placing representative mineral in sulfur and iron culture media and checking for autotrophic activity over a period of at least ten days.

## C. Chemoautotrophic Organisms

Certain autotrophic organisms have long been known to occur in mine drainage. The specific role of these bacteria in the acid formation reactions has not been adequately defined. Three specific organisms were

used: *Ferrobacillus ferrooxidans*, FF, *Ferrobacillus sulfooxidans*, FS, and *Thiobacillus thiooxidans*, TT. They do not use the same energy sources. FF oxidizes only ferrous ion. FS oxidizes ferrous ion and  $S_2^{-II}$ . TT oxidizes sulfur and thiosulfate but not ferrous ion. All three species are chemo-autotrophic. They obtain carbon from carbon dioxide and carry on metabolic processes at pH 2 to 4.5.

The original FF and FS organisms were obtained from cultures maintained at the Mellon Institute by Dr. W. Leathen. TT was culture number 8085 obtained from American Type Culture Collection. Subcultures were prepared every seven days and maintained at 26°C. Waksman's media (Waksman, 1922) was used for the TT. Leathen's media (Leathen, 1951) was used for the FF and FS. An inoculum of 1 ml was transferred aseptically to 99 ml of appropriate medium in a 250 ml Erlenmeyer flask. It was determined by dilution and culturing tests that one week old cultures each contained at least  $10^7$  with maxima of  $10^{11}$  organisms per ml. These counts were essentially constant for the next few weeks so cultures 7 to 14 days old were used to seed the biological reactors.

#### D. Operating Conditions

The effect of biological seeding on acid formation was the primary factor to be examined in this research. Preliminary studies (Baker and Wilshire, 1970) had established that in the absence of oxygen, biological seeding was without effect since the autotrophic organisms are aerobic. Consequently, major emphasis was placed on assessing acid production under the following operating modes: aerobic biologically-seeded; aerobic-nonseeded; and, nonaerobic-nonseeded. These operating modes were tested in the horizontal flooded-bed reactors. The secondary variables of recycle, surface area,  $CO_2$  supplement and nature of seed were tested in the horizontal-recycle and vertical units. In each case, pyrite was charged to the units, the atmosphere adjusted and feedwater added. Seeding, except for the specific study of the nature of biological seed, was by a ternary mixture of *Thiobacillus thiooxidans*, *Ferrobacillus ferrooxidans* and *Ferrobacillus sulfooxidans*. Equal volumes of stock culture containing  $10^7$  to  $10^{11}$  organisms/ml were first settled to remove excessive sediment and energy sources. The clarified supernatant was withdrawn by syringe for inoculation into the units through septa-covered ports. One ml of seed was added then a 5 ml rinse of deionized water to flush the organisms into the reactors. Seeding was practiced for 14 days then the organisms were allowed to adapt to the particular experimental conditions. Equilibrium, judged by steady-state effluent characteristics, generally required 10 to 90 days. The primary operating modes were evaluated in the four-reactor, horizontal, flooded-bed unit at flows from 1.44 to 8.64 liters per day or 8.7 to 1.5 hours retention time. The effect of recycle at 0:1, 1:1 and 4:1 ratio of recycle to forward flow rates, was determined in the smaller, flooded-bed reactor with recycle chamber. The effect of varying pyrite surface area and adding supplemental  $CO_2$  or air was tested in the vertical packed-bed columns.

## E. Analytical

### 1. Chemical Analyses

The chemical analytical procedures used to monitor the reactions were chiefly those described by ASTM (1968). Table 2. Acidity was measured to an end point of 7.3 pH after oxidizing Fe (II) with hydrogen peroxide by the procedure described by Salotto, Barth, Ettinger and Tolliver (1967). Their studies demonstrated that hydrolysis of Fe (III) and Al (III) was complete at pH 7.3 before formation of aluminate ion. Carbon dioxide acidity is removed by aeration and sodium hydroxide is the titrant. The alkalinity was measured to an end point of 4.5 pH. Dissolved oxygen calibration of the Yellow Springs instrument was by the Alsterberg-Azide modification of the Winkler test. Sulfate in preliminary studies was measured indirectly by measurement of excess barium after addition of barium chloride, formation of barium sulfate, 24-hour growth of precipitate, filtration, and atomic absorption analyses of barium to measure the extent of barium consumed. However, the feedwater formulation was adjusted to eliminate the sulfate anion for this study and the effluents often contained <20 mg/l sulfate. It was necessary to increase sample size prolonging sampling time at the low flow rates which was considered undesirable. Therefore, an alternate turbidimetric analytical procedure was used for sulfate at <20 mg/l as described in Standard Methods (1965), pp. 291-293. Ferric ion was occasionally measured by D-1068-62T as an analytical check but generally by atomic absorption analyses from an acidified (HCl) solution. Additional cations present in the pyritic samples were measured by atomic absorption analyses after solution of the material. A pyritic sample of known weight was digested with phosphoric and nitric acids. The residue was removed by filtration, ashed, and treated with concentrated hydrofluoric acid to remove silica. The filtrates were combined and diluted with de-ionized water. An equivalent acid blank was also prepared to serve as a reference in atomic absorption analyses. Carbon dioxide content of the water could not be routinely monitored. Phosphates were measured as an indicator of biological activity but significant depletion only occurred at the beginning of biologically seeded, aerobic test series (Baker and Wilshire, 1968).

### 2. Biological Analyses

The presence of viable chemoautotrophic organisms in the effluent from the reactors was determined by inoculating tubes containing 10 ml of either Waksman's or Leathen's media with 1 ml of effluent. The tubes were incubated in the dark at 26°C. They were examined after 7 and 10 days incubation. A drop of more than one pH unit from 4.5 for the Waksman medium was considered positive evidence of the presence of *Thiobacillus thiooxidans* or *Ferrobacillus sulfooxidans*. *Thiobacillus thiooxidans* do not oxidize Fe (II) and *Ferrobacillus ferrooxidans* do not use elemental sulfur as an energy source. A positive potassium thiocyanate reaction indicating oxidation of ferrous to ferric ion in the Leathen medium was considered evidence of *Ferrobacillus ferrooxidans*, *Ferrobacillus sulfooxidans* or both.

TABLE 2

## Chemical Analytical Procedures

<u>Component</u>	<u>ASTM<sup>*</sup> Designation</u>	<u>Instrument</u>
pH	D-1293-62T	Beckman Zeromatic
Fe II	D-1068-62T	
Specific Conductance	D-1125-64	
NH <sub>4</sub> <sup>+</sup> Nitrogen	D-1426-58	
NO <sub>3</sub> <sup>-</sup> Nitrogen	D-992-52	Yellow Springs
PO <sub>4</sub>	D-515-62T	
Dissolved Oxygen	D-1589-60	
SO <sub>4</sub>	Turbidimetric <sup>**</sup>	
Fe, Total	Atomic Absorption	Perkin Elmer 303

<sup>\*</sup>ASTM Book of Standards, Part 23, 1968.

<sup>\*\*</sup>Standard Methods, 12th Edition, APHA, 1965.

Supplemental analyses were made of the biological growth present in the water and on the pyritic surface at the end of some of the test series. A microscopic examination and a plate culturing procedure were used. Plates of Leathen's and Waksman's media solidified with #2 ion agar were streaked with sample to isolate the chemoautotrophic organisms. Heterotrophic organisms were isolated by streaking plates of Sabouraud dextrose agar and subsequently making transfers to specific media including: lead acetate agar; phenol red broths containing sucrose or dextrose or lactose; gelatin; and EMB agar.

## Section 6

### RESULTS

#### A. Horizontal, Flooded-Bed Reactors

##### 1. Effect of Environment and Seeding

The reactors were operated over intervals varying from 60 to 140 days at fixed forward flow rates during which time the effluent characteristics were monitored. Once equilibrium was achieved, as indicated by constancy of all analytical measurements, the tests were terminated. Typical effluent measurements are shown for an aerobic-seeded horizontal reactor test: pH, Fig. 6; acidity, Fig. 7; total and ferrous ion, Fig. 8; and sulfate, Fig. 9. The units generally required approximately 30 days to approach equilibrium. Initially high values of effluent components are the result of flushout of pyritic fines of greater surface area and hence greater immediate oxidation potential. The acidity values presented in Fig. 7 are uncorrected for feedwater acidity which varied slightly from batch to batch, Table 1. These data were obtained at feedwater flow rates corresponding to  $2.3 D^{-1}$  volume ratio or 8.7 hours retention. In the presentation of results which follows, only the equilibrium characteristics are given although each point is the result of a comparable time-analyses series.

Effluent characteristics (pH, acidity, sulfate, total and ferrous ion) were measured as a function of water flow rate and environmental operating modes. The latter include: nonaerobic-seeded, Fig. 10; aerobic-nonseeded, Fig. 11; and, aerobic-seeded, Fig. 12. Preliminary tests, Table 3, demonstrated that since the chemoautotrophic organisms are aerobic, there is no difference in acid mine drainage emitted from pyrite under nonaerobic-seeded and nonaerobic-nonseeded modes. Hence, only the combination of nonaerobic-seeded was examined in this study. The pyrite used in the preliminary test differs from the mineral used in the rest of the experiments. Therefore, effluent characteristics differ from those obtained at comparable conditions. Furthermore, the preliminary test was made at a flow rate which was diffusion limited with respect to transport as determined in subsequent studies.

The effluent characteristics are essentially linear functions of flow rate (expressed as the ratio,  $Q \div V = D^{-1}$ , where  $Q$  = volume of water per day and  $V$  = volume of pyrite) except for the very lowest flows. Since only two values are available at low flow (high retention time) operation, the regression in this zone is depicted by a dashed line. Figs. 10-12. The entire regressions of sulfate and acidity concentration vs. flow under non-aerobic conditions are dashed to indicate limits of analytical reliability as values approach zero. A summary of the average effluent characteristics for each flow rate tested under each operating mode is given in Table 4. The concentration,  $C$ , of components of the effluent fit the general equation

$$C = a - b (D^{-1})$$



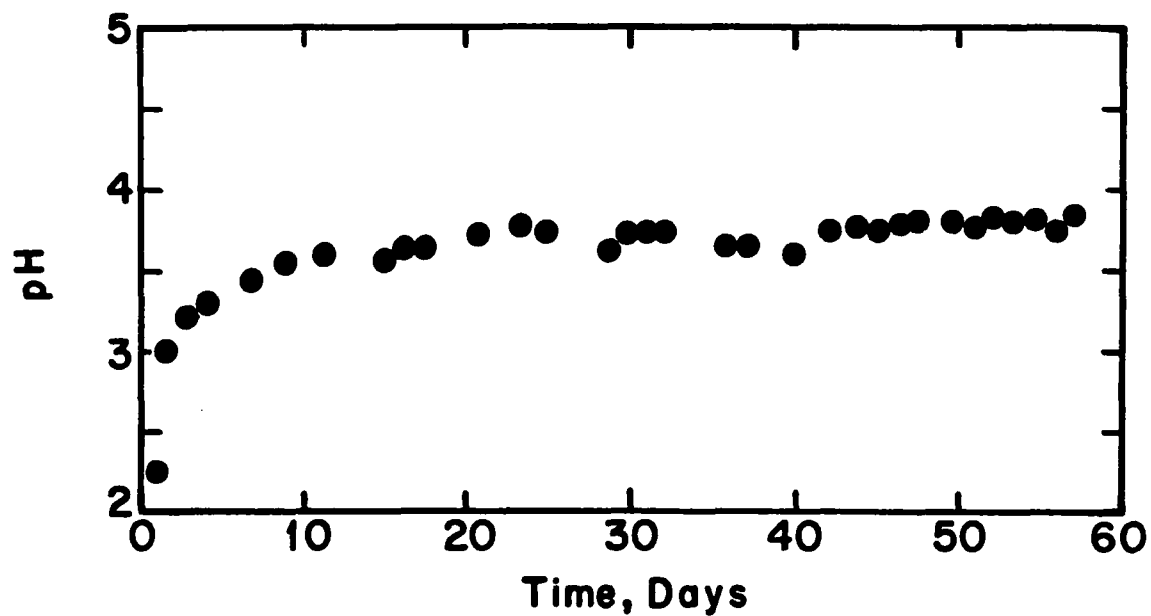


Figure 6. Effluent pH from Aerobic-Seeded Horizontal Reactor

Flow,  $2.3 \text{ D}^{-1}$ , volume water per day + pyrite volume. Shawville pyrite, 625 ml, 3.5 to 7 mesh size. Retention time, 8.7 hours. Influent pH, 4.5.

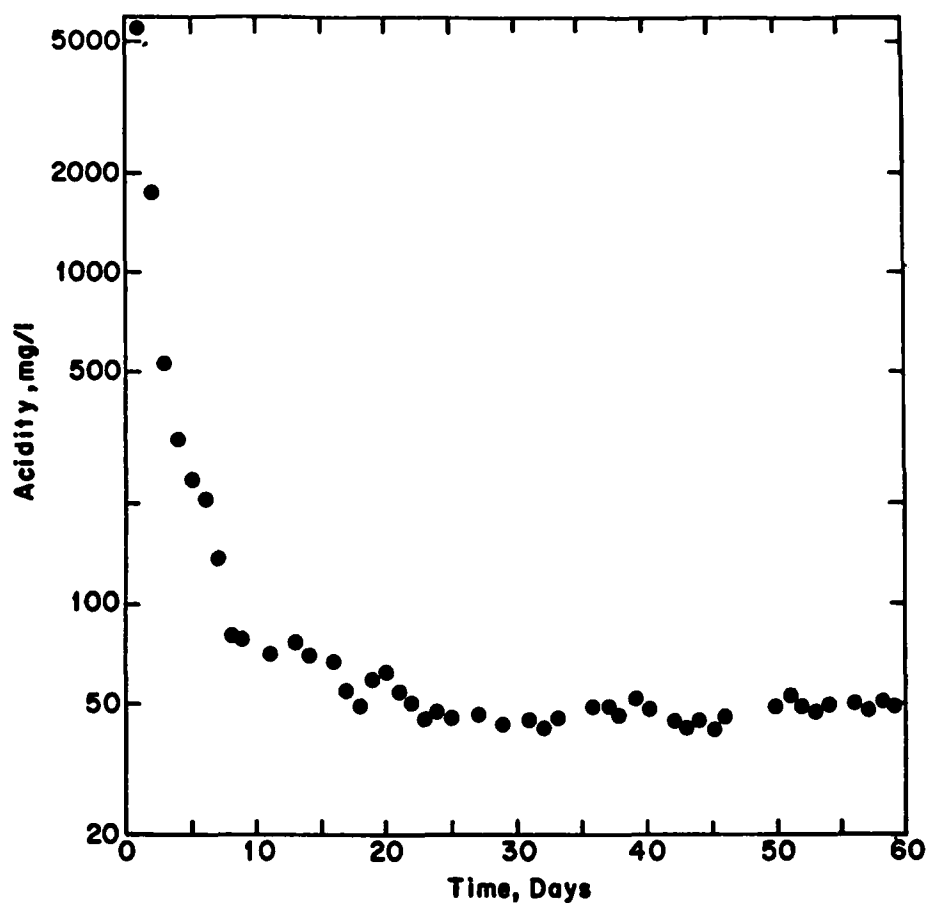


Figure 7. Acidity of Effluent from Aerobic-Seeded Horizontal Reactor

Flow,  $2.3 \text{ D}^{-1}$ , volume water per day  $\pm$  pyrite volume. Shawville pyrite, 625 ml, 3.5 to 7 mesh size. Retention time, 8.7 hours. Influent acidity, 20-25 mg/l.

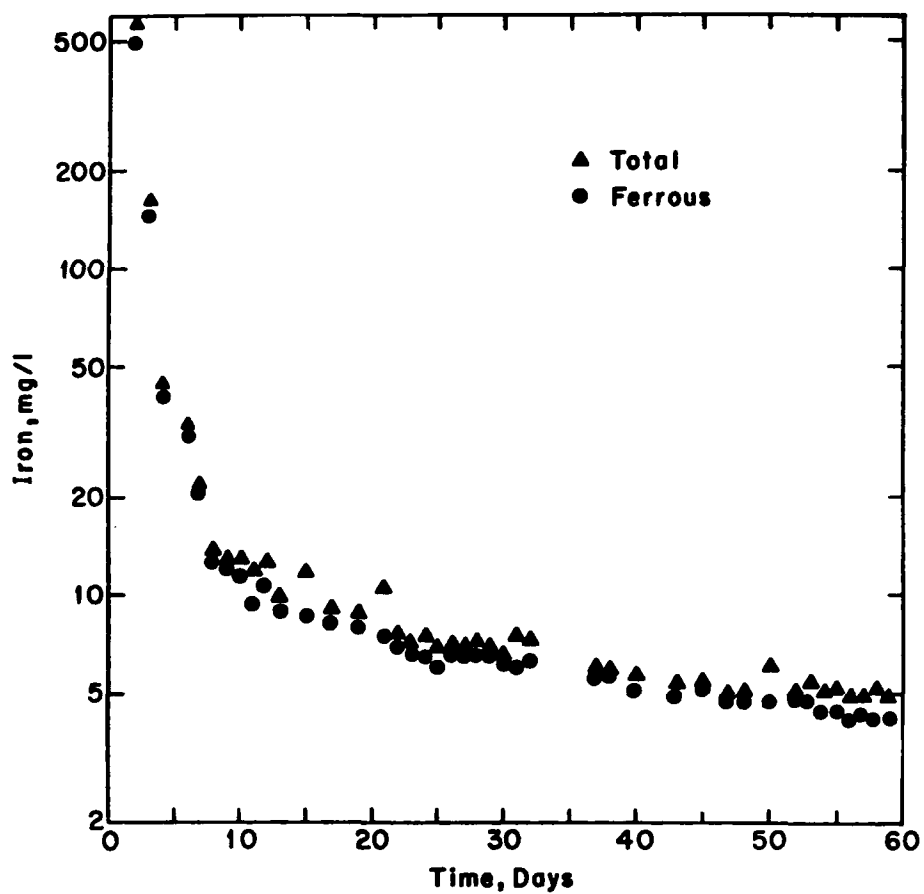


Figure 8. Iron Content of Effluent from Aerobic-Seeded Horizontal Reactor  
Flow,  $2.3 D^{-1}$ , volume water per day  $\div$  pyrite volume. Shawville pyrite, 625  
ml, 3.5 to 7 mesh size. Retention time, 8.7 hours.

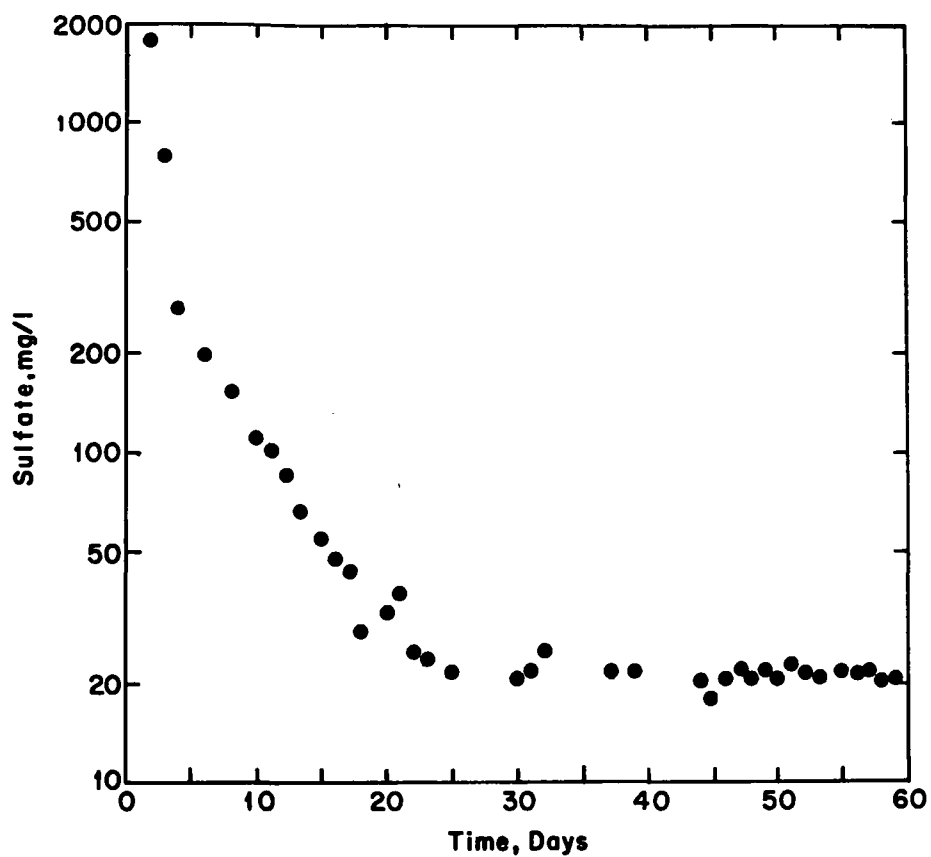
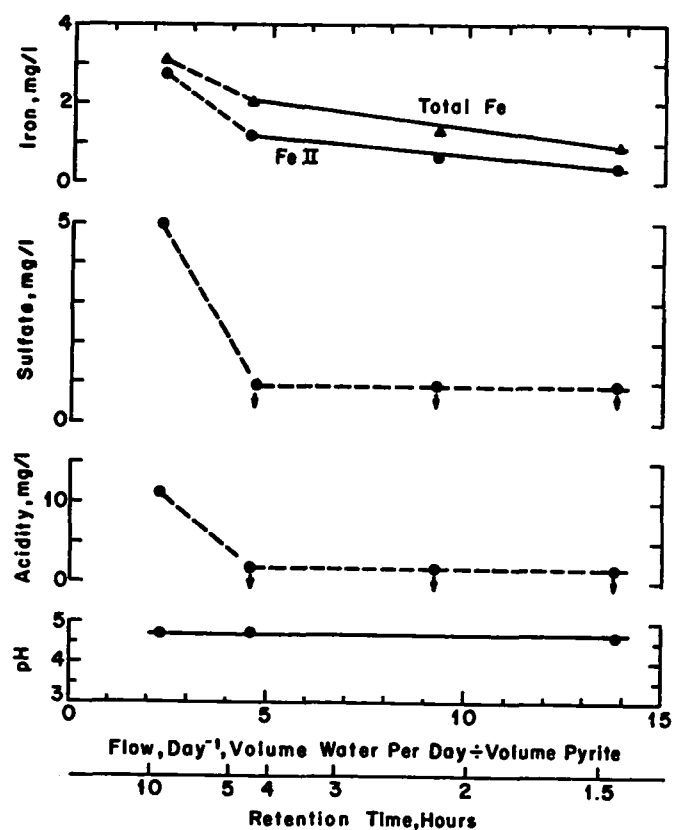


Figure 9. Sulfate Content of Effluent from Aerobic-Seeded Horizontal Reactor

Flow,  $2.3 D^{-1}$ , volume water per day  $\div$  pyrite volume. Shawville pyrite, 625 ml, 3.5 to 7 mesh size. Retention time, 8.7 hours.



**Figure 10. Effluent Characteristics of Nonaerobic-Seeded Horizontal Reactor**  
 Shawville pyrite, 625 ml, 3.5 to 7 mesh size. Dashed lines and arrows indicate analytical limit as concentrations approached zero.

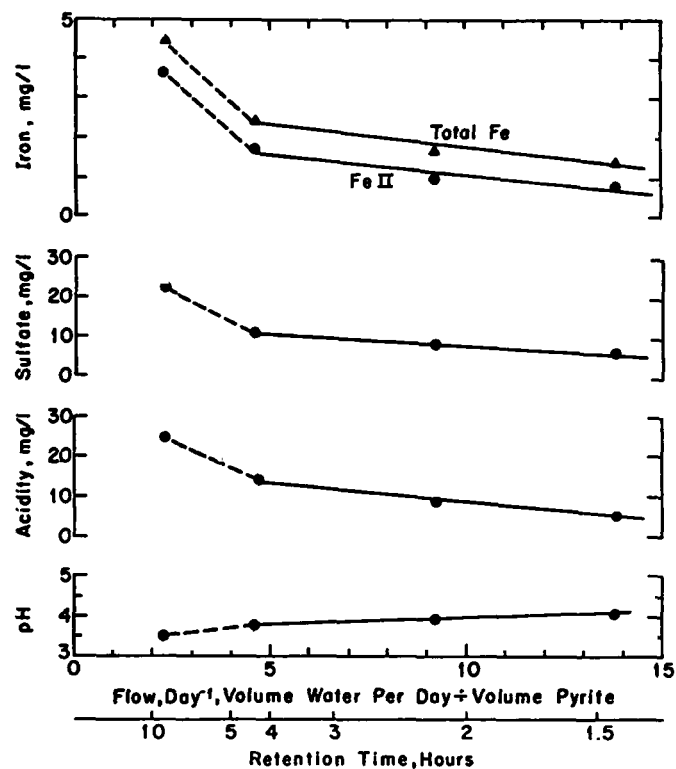
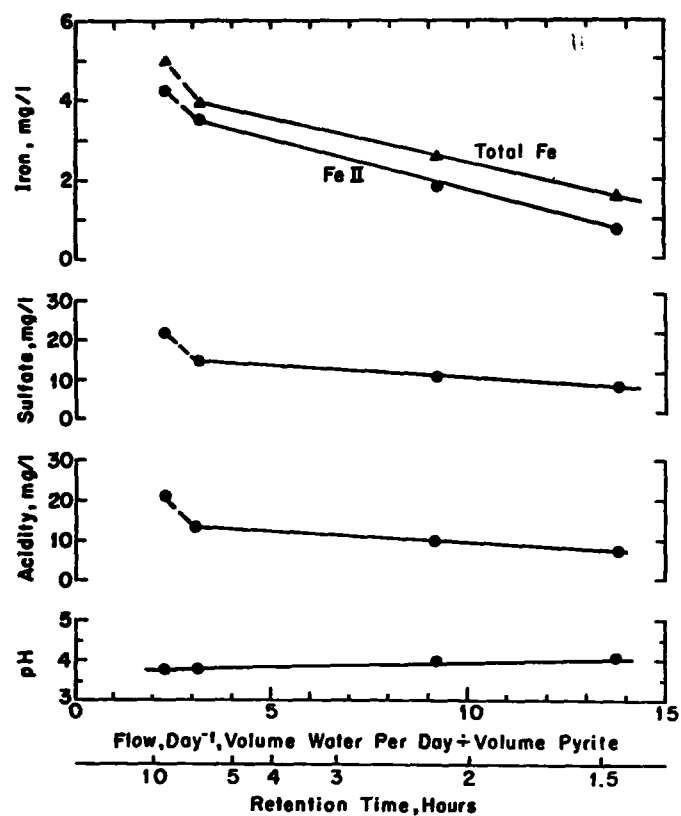


Figure 11. Effluent Characteristics of Aerobic-Nonseeded Horizontal Reactor  
Shawville pyrite, 625 ml, 3.5 to 7 mesh size.



**Figure 12. Effluent Characteristics of Aerobic-Seeded Horizontal Reactor**  
 Shawville pyrite, 625 ml, 3.5 to 7 mesh size.

TABLE 3

Effect of Biological Seed on Horizontal, Flooded-Bed Reactor Effluents  
Under Anaerobic Conditions<sup>a</sup>

	<u>Seeded</u>	<u>Nonseeded</u>
pH <sup>b</sup>	5.5	5.7
Acidity, mg/l	18	18
Ferrous iron, mg/l	2.9	2.9
Total iron, mg/l	2.9	2.9
sulfate, mg/l	10	10

---

<sup>a</sup>Shawville pyrite, 625 ml, 3.5 to 7 mesh size; feedwater flow at  $2.3 \text{ D}^{-1}$ ,  
volume water per day ÷ volume pyrite or 8.7 hours retention

<sup>b</sup>Feedwater pH 4.5



TABLE 4

Characteristics of Horizontal, Flooded-Bed Reactor Effluents Under Varying Operating Modes<sup>a</sup>

Flow		pH			Acidity, mg/l			Total Iron, mg/l			Ferrous Iron, mg/l			Sulfate, mg/l		
L/D <sup>f</sup>	D <sup>-1g</sup>	N-S <sup>b</sup>	A-N <sup>c</sup>	A-S <sup>d</sup>	N-S <sup>b</sup>	A-N <sup>c</sup>	A-S <sup>d</sup>	N-S <sup>b</sup>	A-N <sup>c</sup>	A-S <sup>d</sup>	N-S <sup>b</sup>	A-N <sup>c</sup>	A-S <sup>d</sup>	N-S <sup>b</sup>	A-N <sup>c</sup>	A-S <sup>d</sup>
1.44	2.3	4.68	3.51	3.73	11.7	24.7	21.1	3.1	4.5	5.0	2.7	3.7	4.2	5.0	22.6	21.6
2.16	3.15			3.81			13.5			3.9			3.5			14.6
2.88	4.6	4.76	3.77		~2 <sup>e</sup>	13.7		2.1	2.4		1.2	1.6		~1 <sup>e</sup>	11.3	
5.76	9.2	4.76	3.96	3.99	<2 <sup>e</sup>	9.9	9.8	1.3	1.9	2.5	0.6	1.2	1.9	<1 <sup>e</sup>	8.8	9.8
8.64	13.8	4.76	4.07	4.04	<2 <sup>e</sup>	6.1	7.4	0.9 <sup>e</sup>	1.4	1.7	0.4	0.7	0.8	<1 <sup>e</sup>	6.2	6.9

<sup>a</sup>Shawville pyrite, 625 ml, 3.5 to 7 mesh size; feedwater pH 4.5.<sup>b</sup>nonaerobic-seeded, N-S<sup>c</sup>aerobic-nonseeded, A-N<sup>d</sup>aerobic-seeded, A-S<sup>e</sup>approximations, limit of analytical procedures.<sup>f</sup>liters per day<sup>g</sup>volume water per day ÷ volume of pyrite

which differentiates to indicate that concentration is zero order with respect to ( $D^{-1}$ ). Least squares analyses of the effluent characteristics over the linear range are summarized in Table 5.

All effluents were monitored every 14 days for presence of viable Fe (II)- and  $S_2^{-II}$ -utilizing organisms. Table 6. The effectiveness of gaseous sterilization of the pyrite is readily apparent. Preliminary tests showed that native Fe (II)- and  $S_2^{-II}$ -utilizing organisms were present in the Shawville pyrite but none were found after 84 days of operation of the aerobic-nonseeded reactor. Chemoautotrophs were present in the reactor with a nonaerobic (nitrogen) atmosphere. Tests for both Fe (II)- and  $S_2^{-II}$ -utilizing organisms were positive at 28 days.  $S_2^{-II}$ -oxidizing organisms continued to persist but Fe (II)-oxidizing organisms were not found after 42 days. The latter did not survive in the nonaerobic environment and the seeded Fe (II)-utilizing organisms were either flushed or destroyed. Remaining  $S_2^{-II}$ -utilizing organisms were evidently in an inactive or resting stage based on the effluent characteristics and visual appearance of the pyritic beds.

Biological growth appeared in the aerobic reactors despite a pH 4. The source of seed for this growth is the unsterilized deionized water used to prepare the feedwater. Sterilization of the large quantities of water being used was not practical and biological culturing studies demonstrated that no organisms were present which affected Fe (II) or  $S_2^{-II}$  oxidation. A greyish fungal growth began to appear at the exit end of the reactor after approximately one week. The growth intensified and gradually progressed toward the inlet end until the entire surface was covered. Maximum growth was achieved within 30 to 70 days and then began to diminish in most cases. Phosphate depletion from 30 to 3 mg/l was maximum at approximately 30 days then rapidly decreased to 1 to 2 mg/l after 60 days. At no time did phosphate drop to zero concentration. The diminution of growth in the extended runs is attributed to depletion of the carbon source necessary for the heterotrophic organisms. (Baker and Wilshire, 1968). Oxidation products, ferric hydroxides and sulfates, tended to accumulate in the mycelial mat so that it gradually changed from a grey to yellow-brown color. The "yellowboy" appearance was a function of flow. It was first noticed after 11 days at a flow corresponding to 4 hours residence time but took 34 days to be evident at half this residence time in the aerobic reactor. The mat undoubtedly affected the transport of colloidal-sized reaction products in aerobic operation and reduced their concentration in the effluent. On the other hand, release of  $CO_2$  by the mycelia would be beneficial to the chemoautotrophic organisms if that carbon source were limited.

Microscopic examination and culturing techniques of the mycelial mat indicated a fungus. The mycelia were branched, septate, had fruiting bodies and spores. A *Penicillium* was indicated by growth on Sabouraud dextrose agar. The heterotrophic bacteria isolated were tentatively identified as species of the genus *Pseudomonas* or *Aerobacter*. The presence of chemoautotrophs was confirmed on agar containing Waksman's or Leathen's media.

No mycelial growth developed at any time in the nonaerobic reactors. No sulfuritic or any other characteristic odor was detected

TABLE 5

Least Squares Fit of Horizontal, Flooded-Bed Reactor Effluent Characteristics<sup>†</sup>

	nonaerobic-seeded <sup>a</sup>	aerobic-nonseeded <sup>b</sup>	aerobic-seeded <sup>c</sup>
pH	$(4.72 \pm 0.07) - (0.00 \pm 0.01)D^{-1}$	$(3.64 \pm 0.04) + (0.03 \pm 0.00)D^{-1}$	$(3.75 \pm 0.04) + (0.02 \pm 0.00)D^{-1}$
acidity, mg/l	$< 2^d$	$(17.46 \pm 1.26) - (0.83 \pm 0.13)D^{-1}$	$(15.28 \pm 0.18) - (0.58 \pm 0.02)D^{-1}$
total iron, mg/l	$(2.59 \pm 0.22) - (0.13 \pm 0.02)D^{-1}$	$(2.87 \pm 0.27) - (0.11 \pm 0.03)D^{-1}$	$(4.55 \pm 0.24) - (0.21 \pm 0.02)D^{-1}$
ferrous iron, mg/l	$(1.51 \pm 0.22) - (0.08 \pm 0.02)D^{-1}$	$(2.09 \pm 0.22) - (0.10 \pm 0.02)D^{-1}$	$(4.30 \pm 0.13) - (0.25 \pm 0.01)D^{-1}$
sulfate, mg/l	$< 1^d$	$(13.81 \pm 0.08) - (0.55 \pm 0.08)D^{-1}$	$(16.86 \pm 0.62) - (0.73 \pm 0.07)D^{-1}$

<sup>a</sup> nonaerobic-seeded and nonaerobic-nonseeded results identical; data apply from 2.3 to 13.8  $D^{-1}$ .<sup>b</sup> data apply from 4.6 to 13.8  $D^{-1}$ .<sup>c</sup> data apply from 2.3 to 13.8  $D^{-1}$ .<sup>d</sup> lower limit set by analytical procedure.<sup>†</sup> basic equation of general form  $c = a \pm b (D^{-1})$

TABLE 6

Presence of Viable Sulfide- and Ferrous-Utilizing Organisms in Horizontal, Flooded-Bed Reactor Effluents

Days		0		14		28		42		56		70		84		140	
Environment	Seeding	Fe	S	Fe	S	Fe	S	Fe	S	Fe	S	Fe	S	Fe	S	Fe	S
nitrogen	yes	-	-	+	+	+	+	-	+	-	+	-	+				
air	no	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
air	yes	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

in the exit gases from the reactors or from the effluents.

Samples of the pyrite remaining in aerobic-seeded reactor after 140 days of pilot plant operation were submitted to x-ray diffraction analysis. Debye-Scherrer patterns of the exposed and fresh mineral did not differ and showed only the diffraction lines of pyrite. Thus, surface reaction product accumulation, if any, was too slight to be measured by this spectrographic procedure. There were only small quantities of fines in the reactors after extended operating periods. The individual pyrite particles were essentially unaltered in appearance to the naked eye. Electronmicrographs of the pyrite before and after oxidation show differences. Appendix I. Clusters of unidentified material developed on the pyrite surface during oxidation.

To facilitate comparison of the acid mine drainage release under varying operating modes, the effluent concentrations at each flow rate predicted from the least squares regressions, Table 4, were used to calculate the total mass release of each component.

An equation for relating the mass release in milligrams per day, mg/d, as related to flow expressed as  $(D^{-1})$ , may readily be obtained from the aforementioned concentration-flow equation. The mass release is CQ and hence

$$\begin{aligned} CQ &= aQ - b(D^{-1})Q \\ &= aV(D^{-1}) - bV(D^{-1})^2 \end{aligned}$$

Maximum mass release occurs when  $\frac{d(CQ)}{d(D^{-1})} = 0$

$$\frac{d(CQ)}{d(D^{-1})} = aV - bV(2D^{-1}) = 0 \quad \text{or}$$

Maximum mass release occurs when

$$(D^{-1})_{\max} = \frac{a}{2b}$$

Fig. 13 depicts the pH and acidity ( $H_2SO_4$ ) in mg/d, for each of the three operating modes. There is a slight but uniform increase in pH from a feedwater value of 4.5 to 4.7 in the effluent over the entire hydraulic range for the nonaerobic system. The increase in pH is attributed to release of basic minerals from the pyrite and the absence of oxidative, acid-producing reactions. Correspondingly, acidity approaches that of the feedwater, Fig. 10, over the entire hydraulic range except for a higher value at the very lowest flow rate. This acidity value reflects Fe (II) content. The aerobic-nonseeded reactor effluent characteristics are the result of chemically-promoted oxidative reactions. Fig. 11. The pH increases only slightly from approximately 3.8 to 4.1 over the flow range of 2.3 to 13.8  $D^{-1}$ . Fig. 13. This pH is the result of increased acidity released by pyrite oxidation compared to the nonaerobic dissolution process. The mass release of acidity reaches a maximum at a flow corresponding to approximately 9 to 10  $D^{-1}$ , or 2 hours residence time. Fig. 13. Further increase in flow produces no increase in acidity discharged per unit time suggesting that

the oxidation reaction is rate limited with respect to time. Seeding the aerobic system results in only minor differences in effluent pH from that obtained with a nonseeded aerobic system. Fig. 12. The acidity does vary. With seeding, mass release of acidity is lower at flows  $<10 \text{ D}^{-1}$  and higher at flows  $>10 \text{ D}^{-1}$  than in the case without seeding. Fig. 13. At lower flows, greater residence time, the organisms have an increased opportunity to affect ferrous and sulfide oxidation in the seeded system. Chemoautotrophic metabolic processes utilize acid, Silverman and Lundgren (1959), and Fe (II) which is included in the acidity, is also consumed. These factors would account for the decreased mass release of acidity under seeded compared to nonseeded aerobic operation. At higher flow rates, the rate limiting factors are the rate of pyritic dissolution to release Fe (II) and  $\text{S}_2^{-\text{II}}$  ions and the limited contact time necessary for biologically catalyzed oxidation of these ions.

The mass release of iron under an anaerobic environment is constant at 3.5 mg/d of ferrous and 7.5 mg/d of total iron at flows  $>4.6 \text{ D}^{-1}$ . Fig. 14. The ferrous and total iron concentration under nonaerobic conditions should be identical. Differences, Fig. 10, at a given flow rate are attributed to oxidation during the lengthy sampling period required. For anaerobic operation under equilibrium dynamic conditions a steady-state release of Fe (II) and  $\text{S}_2^{-\text{II}}$  ions occurs even though oxygen is excluded from the system. As noted in the report of the first phase of this research, Baker and Wilshire (1968), mine sealing is practiced to keep air from promoting pyritic oxidation. However, pyritic dissolution takes place without air being present although at a much lower level. Ultimately, the Fe (II) and  $\text{S}_2^{-\text{II}}$  discharged with the effluent into the receiving water body will exert an oxygen demand.

Aerobic systems release significantly greater quantities of Fe (II) and  $\text{S}_2^{-\text{II}}$  ions and their oxidation products than do nonaerobic systems. Biologically promoted aerobic systems further accelerate Fe (II) oxidation over levels obtained by strictly chemical means. The aerobic-nonseeded mass release of total iron approaches a maximum of 12 mg/d at  $10 \text{ D}^{-1}$  flow but then decreases slightly to 14 mg/d at  $14 \text{ D}^{-1}$  flow for the aerobic-seeded system. Fig. 14. Fe (II) release reaches a maximum of 6.8 mg/d at approximately  $10 \text{ D}^{-1}$  and remains constant at higher flow rates for the aerobic-nonseeded system. For the aerobic-seeded system the maximum of 11.3 mg/d at approximately  $9 \text{ D}^{-1}$  is followed by a significant decrease at higher flow rates. At approximately  $14 \text{ D}^{-1}$  the mass release of Fe (II) from the aerobic units is comparable whether or not chemoautotrophic organisms were seeded. The Fe (II) and total iron mass releases from the seeded and nonseeded aerobic units are expected to correspond at an identical high flow rate. In this case, the failure of correspondence is an experimental limitation. At the highest hydraulic rates applied to the reactors, the effluent concentrations of the constituents approached the lower limits of analytical reliability. Very slight deviations in the average effluent concentrations when multiplied by the large volumes would alter the mass release values sufficiently for correspondence. Definition of the exact hydraulic rate for correspondence is of less importance than in the theoretical basis for suggesting that a difference in mass release occurs between the seeded and nonseeded aerobic systems. The number of organisms per unit volume of liquid in the continuously operating system is a function of the availability of Fe (II) and  $\text{S}_2^{-\text{II}}$  ions, their reproduction

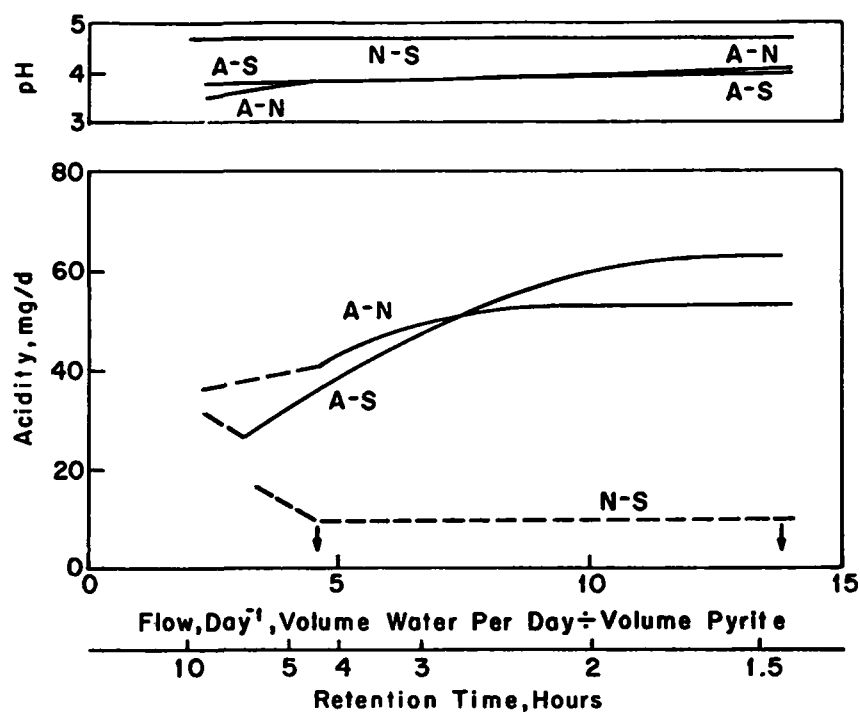
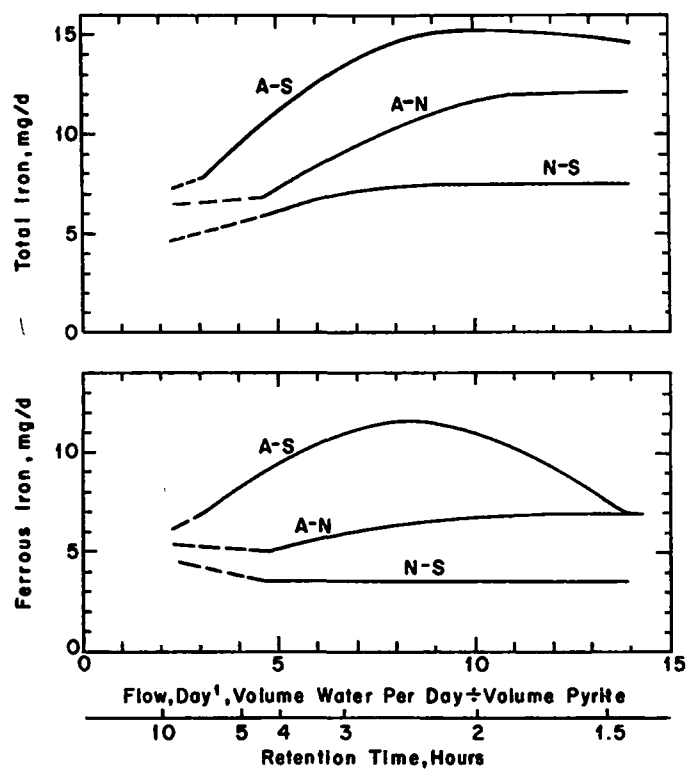


Figure 13. Mass Release of Acidity from Horizontal Reactors Under Varying Operating Modes.

Charged with 625 ml Shawville pyrite, 3.5 to 7 mesh size. Operating mode: Nonaerobic-seeded, N-S; Aerobic-seeded, A-S; Aerobic-nonseeded, A-N. Influent pH 4.5 and acidity 20 to 25 mg/l.



**Figure 14. Mass Release of Iron from Horizontal Reactors Under Varying Operating Modes.**

Charged with 625 ml Shawville pyrite, 3.5 to 7 mesh size. Operating mode: Nonaerobic-seeded, N-S; Aerobic-seeded, A-S; Aerobic-nonseeded, A-N.



rate and their removal from the system. At lower flow rates the reproduction rate is sufficiently great relative to their residence time in the unit so that a dynamic equilibrium is achieved. At higher flow rates residence time becomes limiting and flushout exceeds reproduction. Eventually, residence time is too short for the chemoautotrophs to affect the rate of oxidation and the seeded and nonseeded mass release rates correspond.

An examination of the effluent characteristics from the units operated under various environmental and seeding modes shows agreement with the strictly chemical model of ferrous iron oxidation described by Singer and Stumm (1968). They proposed a cyclical reaction model involving the slow oxidation of Fe (II) to Fe (III) followed immediately by the rapid reduction of Fe (III) by pyrite, which in turn generates more Fe (II) and acidity. The rate determining step is the oxidation of Fe (II). Oxygen is involved only indirectly in pyritic oxidation in their model. Oxygen serves to regenerate Fe (III) which is itself the specific pyritic oxidant. In the biologically seeded aerobic systems the organisms promote Fe (II) oxidation. This increases the Fe (III) concentration leading to greater ferric:ferrous ion ratios and increased acid formation. In the report of the first phase of this study, Baker and Wilshire (1968), it was suggested that chemoautotrophic microorganisms significantly accelerate oxidation of Fe (II) and  $S_2^{-II}$  ions released from the pyrite but apparently do not directly alter the rate of pyrite dissolution in an aerobic environment. The present results tend to support this contention. The effect of the microorganisms raising the ferric:ferrous ion ratio and hence, indirectly affecting the rate of pyritic oxidation is in accord with the original proposition. Further research is necessary to specifically define whether the microorganisms actually take part in the reactions at the pyrite surface.

Sulfate release from nonaerobic systems is essentially nonexistent, Fig. 15, with oxidation during sampling accounting for the minimal quantities measured. Aerobic-nonseeded system mass release of sulfate reached a maximum of approximately 53 mg/d at 13  $D^{-1}$  flow. Further increase in flow does not affect sulfate release. The aerobic-seeded system released more sulfate than the aerobic-nonseeded system over the entire hydraulic range and reached a maximum sulfate release of approximately 61 mg/d near 11  $D^{-1}$  flow then decreased to 56 mg/d at 14  $D^{-1}$  flow. For the particular system being studied, maximum acid mine drainage occurs at a flow of approximately 10  $D^{-1}$  or 2 hours retention time.

One limitation of the pilot plant system being used to make these studies is that an exact material balance cannot be calculated for individual chemical characteristics. The process is being monitored exclusively on the basis of effluent characteristics. Ferric reaction products formed are relatively insoluble and precipitate within the reactor or are incorporated within the mycelial mat. Only those products carried over in the effluent are measured. Carryover is obviously enhanced as flow rate increases. Sulfate often does not account for all of the  $S_2^{-II}$  released from the pyrite. Elemental sulfur has been suggested as a reaction product by Silverman (1967). Pyrite,  $FeS_2$ , dissolution should lead to a Fe (II) to  $S_2^{-II}$  molar ratio of 1.0.

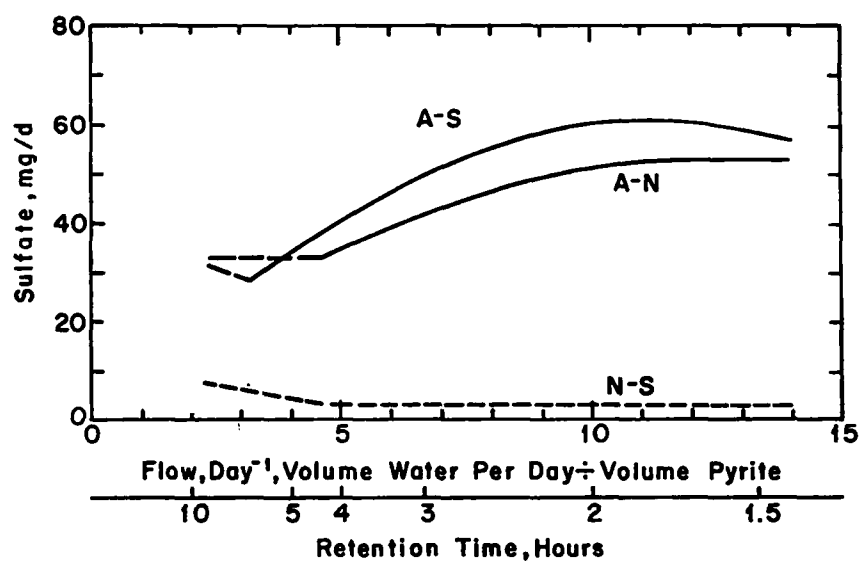
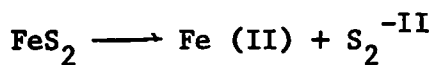
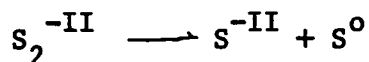


Figure 15. Mass Release of Sulfate from Horizontal Reactors Under Varying Operating Modes.

Charged with 625 ml Shawville pyrite, 3.5 to 7 mesh size. Operating mode: Nonaerobic-seeded, N-S; Aerobic-seeded, A-S; Aerobic-nonseeded, A-N.



and the possible sulfur species of



For the nonaerobic system the total iron to sulfate sulfur molar ratios in the effluent are >7, >4 and >3 at 4.6, 9.2 and 13.8 D<sup>-1</sup> flow rates. Thus, mass release of total iron is in great excess of the mass release of sulfur measured as the sulfate discharged per unit time. For the aerobic-nonseeded system the Fe (II) to S<sub>2</sub><sup>-II</sup> molar ratio changes only slightly from 0.67 to 0.77 over the flow range 2.3 to 13.8 D<sup>-1</sup>. The biologically-seeded, aerobic system produces ratios of 0.79, 0.92, 0.90 and 0.84 for flows of 2.3, 3.15, 9.2 and 13.8 D<sup>-1</sup>. In the aerobic systems the iron content approximates roughly the stoichiometric quantity needed to balance the sulfur. Neither total iron nor sulfur is a measure of the pyrite dissolved. Reaction products precipitated are retained in the aerobic reactors so that effluent concentrations represent minimum rates of pyritic dissolution.

The concentration-flow relationships depicted in Figs. 10 to 12 for the effluents under varying operating modes show a distinct break in slope at approximately 4.6 D<sup>-1</sup> flow or 4.3 hours retention time. Concentrations increase at a greater rate as flow decreases (retention time increases) over that predicted by extrapolation of the zero order relationships at higher flow rates. The point at which this linearity ceases represents a mass transport limitation. At lower flow rates reaction products accumulate in the system because their removal rate is slower than their formation rate. Oxygen transport was by diffusion through a film of water which floods the pyrite surface. The gaseous diffusion processes are slow. It was not possible to devise an internal monitoring probe to be inserted into the closed system to measure dissolved oxygen in the liquid phase. Measurements taken of the dissolved oxygen in the effluent leaving the system were inadequate since reaeration occurred while the sample was collecting at the relatively slow flow rates. Oxygen for the pyritic oxidation was supplied by the feedwater and by diffusion into the liquid phase from the gaseous phase over the reactor surface. Oxygen transfer to the flooded pyrite surface more nearly approximates actual mine conditions than would be the case if forced aeration were practiced to accelerate the oxidative reactions. Mine pyrite surfaces which are alternately flooded then drained will yield higher concentrations of acid mine drainage on a spot basis simply because reaction product accumulation may occur. Smith and Shumate (1970) reported oxygen consumed per mass of pyrite as a function of the water:pyrite ratio in batch experiments. Their results also fall in two distinct regions represented by a common point of transition between zones. The point dividing the mass transport limited regime from that not so restricted is analogous in these studies. However, experimental differences preclude direct comparison.

In all these tests, the effluents from aerobic operation remained in the range pH 3.5 to 4.1 at equilibrium. In no case did pH drop to levels which would inhibit chemoautotrophic activity or significantly raise the solubility of Fe (III), thereby decreasing the potential for Fe (II) oxidation in the sequential model of Singer and Stumm (1969).

Further clarification of the role of the chemoautotrophic microorganisms and the effect of environment is obtained by examining the acidity-ferrous ion interrelationship. Each mole of ferrous ion is equivalent to a mole of sulfuric acid. Conversion of the effluent concentrations of total acidity and ferrous ion to a molar basis permits subtraction to determine the sulfuric acid equivalent of the remaining acidity. For the nonaerobic reactor this difference is negligible confirming that the total acidity consists of the ferrous ion released. For the aerobic systems the effluent acidity concentration corrected for ferrous ion is expressed as a function of flow over the range 3.15 to 14 D<sup>-1</sup> for aerobic-seeded,

$$\text{Acidity (H}_2\text{SO}_4\text{), m moles/l} = [(7.89 \pm 0.05) - (0.14 \pm 0.00)D^{-1}]10^{-2}$$

For aerobic-nonseeded,

$$\text{Acidity (H}_2\text{SO}_4\text{) m moles/l} = [(14.08 \pm 0.90) - (0.67 \pm 0.00)D^{-1}]10^{-2}$$

These least squares regressions are depicted in Fig. 16. The aforementioned claim that the microorganisms consume acid is evident in these results. Since the chemoautotrophic organisms must have a source of hydrogen as well as the carbon to reproduce new cells, it is not unexpected that the hydrogen ion concentration is depleted. It is also apparent that at a flow approximating 14 D<sup>-1</sup> that the seeded and nonseeded aerobic units approach comparable acid release. (The crossover of the regressions at approximately 12 D<sup>-1</sup> is well within experimental error at m molar concentrations.) As flow rates decrease the concentration of nonferrous acidity markedly increases in the nonseeded over the seeded system.

The effluent sulfate content may be considered to consist of sulfuric acid and various sulfates. A calculation was made of the sulfate concentration in the effluent in excess of that necessary to balance the sulfuric acid content depicted in Fig. 16 as a function of flow. For the nonaerobic reactor the quantity is negligible. For the aerobic systems the sulfate in excess of sulfuric acid equivalency is expressed as a function of flow from 3.15 to 14 D<sup>-1</sup>. For aerobic-seeded,

$$\text{Sulfate, m moles/l} = [(9.67 \pm 0.60) - (0.62 \pm 0.07)D^{-1}]10^{-2}$$

For aerobic-nonseeded,

$$\text{Sulfate, m moles/l} = [(0.31 \pm 0.00) + (0.1 \pm 0.00)D^{-1}]10^{-2}$$

These least squares regressions are depicted in Fig. 17. Sulfate concentration in the effluent in excess of sulfuric acid equivalency decreased rapidly as flow rate increases (retention time decreases) for the seeded system. At approximately 13 D<sup>-1</sup> it is comparable to the nonseeded system. The nonseeded system sulfate in excess of sulfuric acid equivalency decreases somewhat as flow decreases. This modest increase in effluent sulfate content of the nonseeded system with increased flow may simply be a flushout of ferric or other sulfates.

The results of this analysis of the corrected acidity expressed as sulfuric acid and sulfate equivalency support the mechanism proposed for role of microorganisms in acid mine drainage formation. They promote

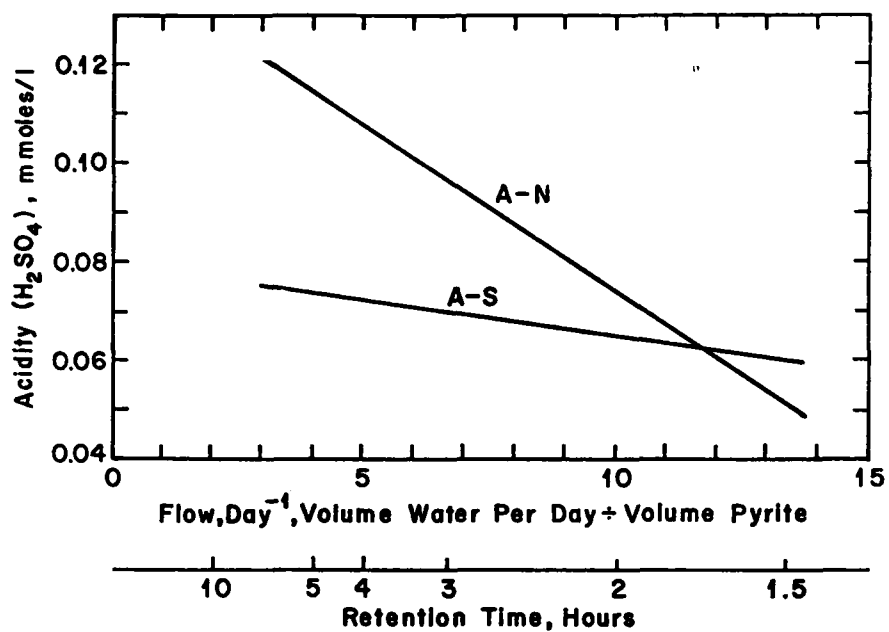
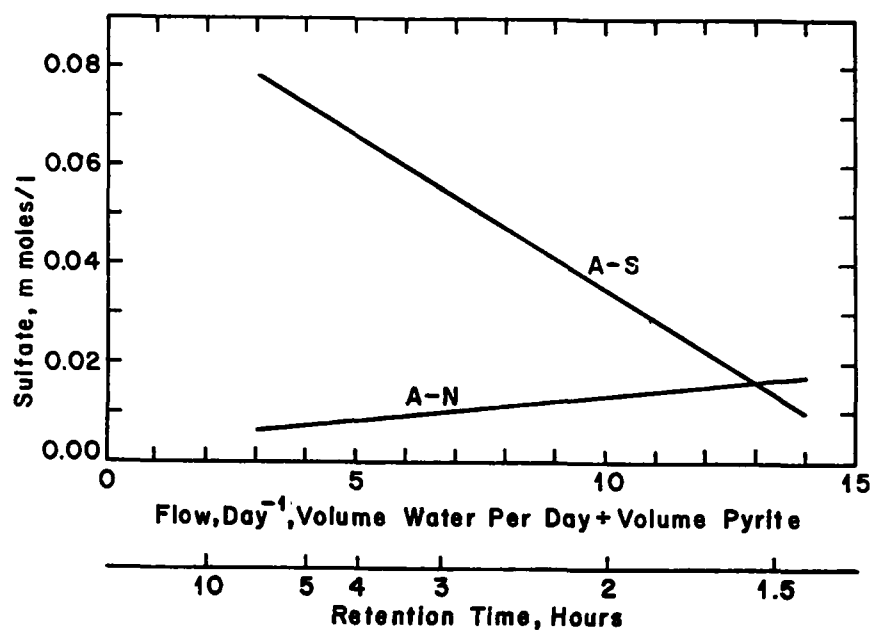


Figure 16. Effluent Concentration of Acidity Corrected for Ferrous Iron Content.

Horizontal reactors charged with 625 ml Shawville pyrite, 3.5 to 7 mesh size. Operating mode: Aerobic-nonseeded, A-N; Aerobic-seeded, A-S.



**Figure 17. Effluent Concentration of Sulfate in Excess of Sulfuric Acid Equivalent of Acidity.**

Horizontal reactors charged with 625 ml Shawville pyrite, 3.5 to 7 mesh size. Operating mode: Aerobic-nonseeded, A-N; Aerobic-seeded, A-S.

Fe (II) and  $S_2^{-II}$  oxidation. The extent to which they affect oxidation is a function of the hydraulic flow rate since microbial regeneration, organism flushout and pyritic dissolution are involved in the dynamic equilibrium.

## 2. Effect of Varying Biological Seed

In the aforementioned sections the effect of biological seeding with a ternary mixture of Fe (II)- and  $S_2^{-II}$ -utilizing organisms was described. It was deemed expedient to test the effect on effluent characteristics of seeding with the individual organisms and to compare the results with those for the ternary seeding. The four horizontal, flooded-bed reactors were used. These were charged with 625 ml of 3.5 to 7 mesh Shawville pyrite. Normally, fresh pyritic charge was used for each test. In this case, the four units were pressed into this service immediately after completion of the aerobic-nonseeded test series to save several months of operating time. Biological culturing tests confirmed that no Fe (II)- or  $S_2^{-II}$ -utilizing organisms were present in the system prior to seeding. Flow through all four reactors was adjusted to  $4.6 D^{-1}$  or 4.3 hours retention time. The effluent characteristics after several weeks equilibration were comparable in concentration and closely approximated values predicted by the least squares equations of Table 4 for aerobic-nonseeded operation.

The reactors were then seeded for 14 days as follows: (1) *Thiobacillus thiooxidans*, TT, (2) *Ferrobacillus sulfooxidans*, FS, (3) *Ferrobacillus ferrooxidans*, FF, and (4) a ternary mixture of all three. After 21 days the four effluent characteristics were not too different. Table 7. Sulfate tended to be slightly higher in the effluent of the *Thiobacillus*-seeded unit as might be expected. The iron content of the effluent from all four reactors was comparable and was less than that predicted from the least squares equations obtained from the analyses of results using fresh pyrite, seeded immediately on start-up of the test series. All other effluent characteristics correspond closely to least squares prediction values for aerobic-seeded operation. The important finding is that the nature of the biological seed made little difference in the effluent composition from the four reactors.

## 3. Effect of Recycle

The tests series in which the environmental and seeding factors were examined utilized once-through flow systems. Reaction products which formed were chiefly precipitated with only soluble products and fine particulates discharged with the effluent. In an actual mining situation the water arriving at a given pyritic site will probably contain previously-formed reaction products. How the chemical and microbiological materials in the water might affect acid mine drainage formation was determined in the smaller horizontal, flooded-bed pilot unit. The unit was charged with 230 ml of 3.5 to 7 mesh Shawville pyrite. (Additional details of the system are given in Section 4, Part B.) Feedwater flow was at  $1.06 L/D$  or  $4.6 D^{-1}$ . Liquid volume in the bed was 103 ml for a retention time of 2.34 hours on a once-through basis. Operation was under an aerobic-seeded mode with seeding of the standard ternary mixture of chemoautotrophic microorganisms for the first fourteen days. The presence of viable Fe (II)- and  $S_2^{-II}$ -utilizing microorganisms was confirmed by making inoculations in Waksman's and Leathen's media every 14 days. Equilibrium was reached in 53 days

TABLE 7

Effect of Biological Seed on Effluent Characteristics of Aerobic Horizontal,  
Flooded-Bed Reactors<sup>a</sup>

<u>Reactor</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
organism	<i>TT</i>	<i>FS</i>	<i>FF</i>	Mix
pH	3.8	4.0	4.0	4.0
acidity (H <sub>2</sub> SO <sub>4</sub> ), mg/l	16	16	16	16
ferrous iron, mg/l	1.4	1.3	1.3	1.1
total iron, mg/l	2.3	2.0	2.4	2.0
sulfate, mg/l	15	11	12	12

<sup>a</sup>seeded after 84 days by *Thiobacillus thiooxidans*, *TT*; *Ferrobacillus sulf-oxidans*, *FS*; *Ferrobacillus ferrooxidans*, *FF*; or a ternary mixture of all three, Mix. Flow at 4.6 D<sup>-1</sup> or 4.3 hours retention.



without the recycle chamber being on-stream. The recycle chamber was then added to determine the effect of effluent composition of the additional retention volume of 190 ml or retention of 260 minutes at the forward flow rate of 1.06 liters per day or  $4.6 D^{-1}$ . A slight decrease in Fe (II) and total iron concentration and a slight increase in sulfate was measured. The units were also operated at 1:1 and 4:1 recycle to forward flow ratios, corresponding to hydraulic rates through the pyrite packed bed of two- and five-fold over the nonrecycled operation. The unit was operated for approximately five weeks at each recycle ratio. Table 8. Effluent characteristics as a function of recycle ratio are presented in Fig. 18. For a fixed forward flow rate of  $4.6 D^{-1}$ , the effluent composition for various recycle rates from 0:1 to 4:1 is predicted by the following relationships expressed in reciprocal time units:

$$\begin{aligned} \text{acidity, mg/l} &= (10.92 \pm 1.21) + (0.88 \pm 0.07) D^{-1} \\ \text{sulfate, mg/l} &= (8.03 \pm 1.40) + (1.07 \pm 0.08) D^{-1} \\ \text{ferrous ion, mg/l} &= (-0.05 \pm 0.02) + (0.32 \pm 0.01) D^{-1} \\ \text{total iron, mg/l} &= (0.21 \pm 0.17) + (0.36 \pm 0.01) D^{-1} \end{aligned}$$

The mass release of these components per day as a function of recycle rate is presented in Fig. 19. The mass release of acidity, Fe (II), total iron, and sulfate increase directly with increased hydraulic flow through the reactor. This is in marked contrast with the mass release of these components when recycle is not used. Figs. 13 to 15. On a once-through basis the mass release reaches a limiting value at a critical flow. Further increase in flow serves only to dilute the maximum concentration. With recycle, the effluent concentration continues to increase proportionally with flow through the bed. Of particular interest is the increased Fe (II) content of the effluent. The higher flow rates through the bed facilitate pyrite dissolution and transport of the Fe (II) from the pyrite surface. The difference between total iron and Fe (II) concentrations is 0.4, 0.6 and 1.1 at 0:1, 1:1 and 4:1 recycle rates. Thus, these effluent Fe (III) concentrations cannot be used to judge the feasibility of increased pyrite dissolution because of increased ferric:ferrous ion ratios. The rate of change of Fe (III) concentration is not proportional to the Fe (II) concentration increase. The effective ferric:ferrous ion ratio has to be that at the mineral surface. Because of Fe (III) reaction product precipitation, it is not possible from these results to estimate the actual surface concentration. Chemically- and biologically-promoted reactions in the liquid phase at some finite distance from the mineral surface most likely dominate the oxidation process.

An examination of the acidity-iron interrelationship facilitates interpretation of these results. The effluent concentrations were converted to a molar basis and the contribution of the ferrous ion subtracted from the total acidity. The relationship of the acidity remaining, expressed as sulfuric acid equivalents, to recycle ratio, expressed as flow through the pyrite bed, is

$$\text{Acidity (H}_2\text{SO}_4\text{), m moles/l} = [(11.23 \pm 0.88) + (0.32 \pm 0.05) D^{-1}] 10^{-2}$$

TABLE 8

Effect of Recycling on Effluent Characteristics<sup>a</sup>

recycle ratio	0:1	1:1	4:1
pH	3.8	3.8	3.6
acidity ( $\text{H}_2\text{SO}_4$ ), mg/l	16	18	31
ferrous iron, mg/l	1.6	2.8	7.5
total iron, mg/l	2.0	3.4	8.6
sulfate, mg/l	15	15	33

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<sup>a</sup> aerobic-seeded reactor charged with 3.5 to 7 mesh Shawville pyrite; forward flow rate 1.06 L/D ( $4.6 \text{ D}^{-1}$ ).

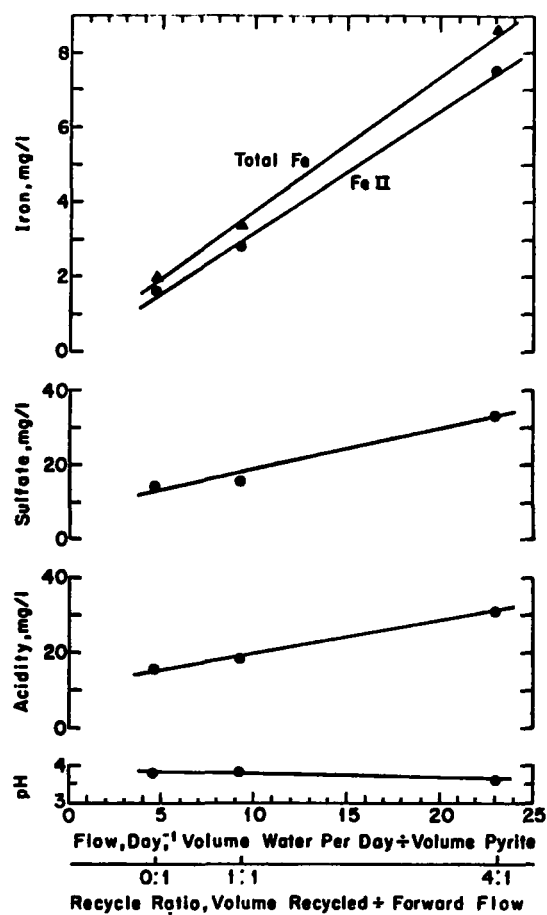


Figure 18. Effluent Characteristics of Acid Mine Drainage with Recycle Shawville pyrite, 230 ml, 3.5 to 7 mesh size. Forward flow 1.06 L/D ( $4.6 \text{ D}^{-1}$ ).

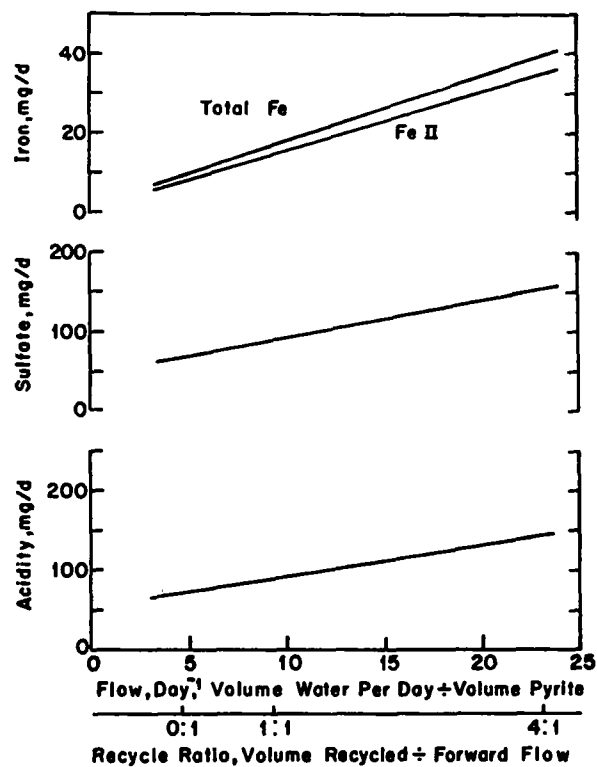


Figure 19. Mass Release of Acid Mine Drainage with Varying Recycle Ratio

Shawville pyrite, 230 ml, 3.5 to 7 mesh size. Forward flow 1.06 L/D (4.6 D<sup>-1</sup>).

The results are presented in Fig. 20 which shows that for the fixed forward flow of  $4.6 \text{ D}^{-1}$  that recycling to increase flow through the pyritic bed serves to increase effluent concentration of acidity corrected for ferrous ion content. This contrasts with a decrease of nonferrous acidity with increasing flow rates on a once-through basis. Fig. 16.

The sulfate concentration in the effluent increases with recycle, Fig. 18. When the sulfate equivalent to the corrected acidity expressed as sulfuric acid is subtracted from the total sulfate concentration, the remainder as a function of total flow through the pyrite bed is

$$\text{Sulfate, m moles/l} = [(-2.87 \pm 0.58) + (0.80 \pm 0.04) \text{D}^{-1}] \times 10^{-2}$$

The results are presented in Fig. 20. The accumulation of sulfate with increased recycle is in contrast to the decrease experienced with increased flow on a once-through basis. Fig. 17. On a once-through basis, the sulfate in excess of sulfuric acid equivalence is not significant. As flow (recycle) increases, the sulfate excess increases. With the recycle system, the chemoautotrophic microorganisms can achieve a higher count per unit volume than would be the case for a once-through operation at the same hydraulic rate through the pyrite bed. Flushout is solely a function of the forward flow rate. Microorganism count represents an equilibrium between regeneration rate and flushout. Hydraulic factors affecting ferric concentration at the surface and similar mass transport considerations also influence the acid mine drainage formation rates.

## B. Vertical, Flooded-Bed Reactors

Supplementary studies of the effect of adding carbon dioxide, forced aeration and varying particle size were examined in vertical, packed-bed, flooded reactors. These units are described in Section 4B.

### 1. Effect of Supplemental Carbon Dioxide

Examination of the effect of environment and seeding took place in clear plastic reactors exposed to light. Algal and heterotrophic as well as chemoautotrophic microorganism growth was favored under aerobic conditions. Mines would not be equally appropriate locations for such symbiotic processes. The proliferation of mycelial growth has already been suggested as a supplemental source of carbon dioxide for the chemoautotrophic microorganisms (Baker and Wilshire, 1968). This supplemental supply could be important if the dissolved carbon dioxide content of the water were rate limiting. This is unlikely. In some tests of many months duration, the effluent characteristics were comparable at the same flow for the periods when mycelial growth was at its peak and after depletion of the available carbon source reduced such growth. A quantification of the symbiotic relationship was beyond the scope of this study. Normally, natural waters in equilibrium with the atmosphere have carbon dioxide concentrations of 0.4 to <1.0 mg/l. Fixation of carbon dioxide by the chemoautotrophic microorganisms might be expected to raise the pH except that their metabolic end products from  $\text{Fe (II)}$ - and  $\text{S}_2^{-\text{II}}$ -utilization are acidic.

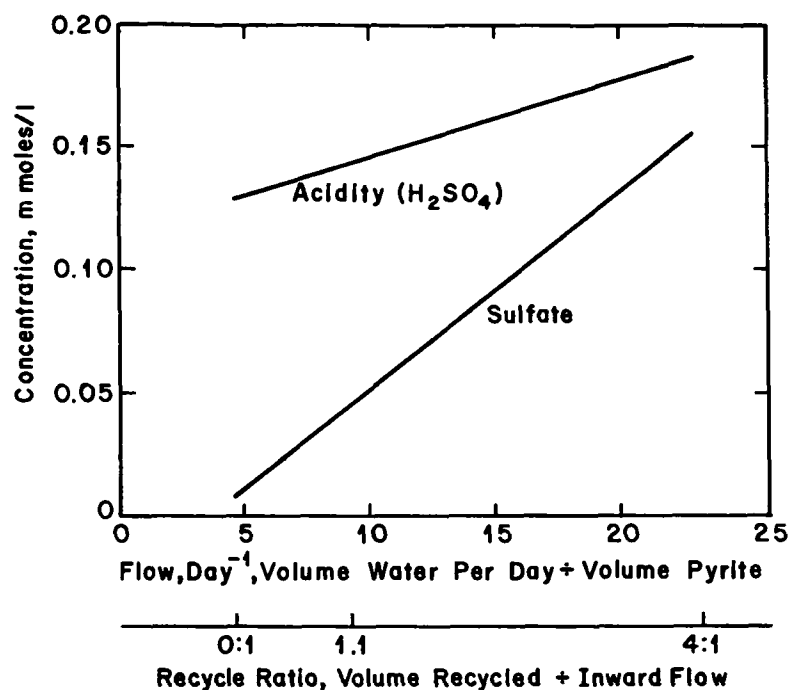


Figure 20. Effluent Concentration of Acidity Corrected for Ferrous Iron Content and of Sulfate in Excess of Sulfuric Acid Equivalent of Acidity.

Aerobic-seeded operating mode. Horizontal, flooded-bed reactor charged with 230 ml of 3.5 to 7 mesh Shawville pyrite.

Whitesell, et.al. (1969) and Borichenski (1967) demonstrated that high levels of carbon dioxide were inhibitory to chemoautotrophic organism cell growth and to iron oxidation. Earlier, Schnaitman and Lundgren (1965) found *Ferrobacillus ferrooxidans* growth to be stimulated when carbon dioxide supplement was added to carefully controlled cultures. The maximum pyruvic acid level of spent media from CO<sub>2</sub>-supplemented and nonsupplemented cultures was the same but the maximum acid level was reached earlier in the CO<sub>2</sub>-supplemented culture. These references suggest that although carbon dioxide may be a limiting factor in pure culture experiments, high cell counts and limited carbon dioxide solubility are more likely to affect such systems. Spot checks of Fe (II)- and S<sub>2</sub><sup>-II</sup>-utilizing microorganism counts in the effluent from the horizontal, aerobic-seeded reactors of this study indicated 10<sup>3</sup> to 10<sup>4</sup> cells per ml under dynamic equilibrium conditions. The cell concentration of 10<sup>4</sup> per ml is identical to that reported by Smith and Shumate (1970) as the population required to supply ferric ions at sufficient rate to support a detectable oxidation of pyrite by ferric ions. Their study of the microbiological effect on oxidation utilized a batch respirometer with recirculation.

The effect of bubbling carbon dioxide through a flooded, vertical bed of pyrite has no relevance to the aforementioned horizontal reactor studies. The experiment only demonstrates the general effect on pyritic dissolution and oxidation of pyrite under aerobic-seeded operating conditions supplemented by carbon dioxide. Two reactors were used. One served as a control while the other was subjected to carbon dioxide gasification at 10 ml/min. Both units contained 925 ml of 3.5 to 7 mesh Shawville pyrite. Feedwater flow corresponded to a rate of 2.3 D<sup>-1</sup> providing 5.6 hours retention time. The units were seeded with the standard ternary mixture of chemoautotrophic microorganisms for the first 14 days. Presence of viable Fe (II)- and S<sub>2</sub><sup>-II</sup>-utilizing organisms in the effluent was confirmed over the entire experimental period of 56 days. The effluent characteristics at equilibrium are presented in Table 9.

Although the effluent pH of the carbonated system differs only slightly, 5.1 versus 5.2 for the control, the increase in acidity is from 6 to 74 mg/l. Fe (II) and total iron concentrations increase three- to four-fold. Sulfate increases only moderately from 7 to 10 mg/l. The molar ratio of Fe (II) to S<sub>2</sub><sup>-II</sup> in the effluents is 1.48 and 3.54 respectively for the control and the carbonated systems. Both show significant excess of total iron to sulfur when compared to the molar ratio of 1.0 in pyrite.

At pH 5.1 the hydroxyl and carbonate equilibrium concentrations are calculated to be 0.8 x 10<sup>-9</sup> and 5 x 10<sup>-11</sup> moles/, respectively. These values and the solubility products

CaCO <sub>3</sub>	4.5 x 10 <sup>-9</sup>
Fe(OH) <sub>2</sub>	1.6 x 10 <sup>-14</sup>
Fe(OH) <sub>3</sub>	1.1 x 10 <sup>-36</sup>
FeCO <sub>3</sub>	3.0 x 10 <sup>-14</sup>

TABLE 9

Effect of Gasification by Carbon Dioxide or Air on Effluent Composition  
from Aerobic-Seeded, Flooded Vertical Columns of Pyrite<sup>a</sup>

<u>Characteristic</u>	<u>Control</u>	<u>CO<sub>2</sub> Added<sup>b</sup></u>	<u>Air Added<sup>c</sup></u>
pH	5.2	5.1	2.8
acidity, mg/l	6	74	208
ferrous iron, mg/l	2.5	9.1	97
total iron, mg/l	3.2	10.5	224
sulfate, mg/l	7	10	866

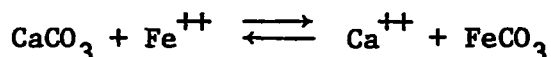
<sup>a</sup> Shawville pyrite, 925 ml of 3.5 to 7 mesh particle size; hydraulic rate 2.3 D<sup>-1</sup> (volume water per day ÷ volume pyrite); retention time 5.6 hours.

<sup>b</sup> carbon dioxide rate, 10 ml/min

<sup>c</sup> aeration rate, 10 ml/min



permit further characterization of the system. Ferrous hydroxide,  $\text{Fe}(\text{OH})_2$ , and ferrous carbonate,  $\text{FeCO}_3$ , cannot exist since their equilibrium concentrations are  $1.4 \times 10^6$  and  $33.5 \text{ g/l}$  respectively. Feedwater calcium concentration is  $76 \text{ mg/l}$  ( $1.9 \times 10^{-3} \text{ moles/l}$ ) and it, too, will remain in solution rather than precipitate as its carbonate ( $3600 \text{ g/l}$  required). Also,  $\text{Fe (II)}$  content would have to be approximately 150 times greater than calcium concentration for ferrous carbonate to form according to



so that its formation is not favored. Singer and Stumm (1970) demonstrated that ferrous bicarbonate concentration relative to ferrous carbonate concentration is insignificant for natural waters of normal alkalinities. Only ferric hydroxide,  $\text{Fe}(\text{OH})_3$ , formation is likely at  $1.2 \times 10^{-5} \text{ mg/l}$ . These results explain why  $\text{Fe (II)}$  released from the pyrite tends to remain in solution until oxidized to the  $\text{Fe (III)}$  state. Biologically-promoted oxidation is not favored at pH 5.1 since the chemoautotrophic organisms are most active at pH 2 to 4.5. Chemical oxidation processes are expected to dominate but are limited since the dissolved oxygen in the aqueous phase is being stripped by the carbon dioxide flow through the bed. Chemical oxidation depends on the dissolved oxygen content of the water in the absence of forced aeration. The bubbling action of the carbon dioxide promotes mixing and transport which also contributes to the increase in effluent concentration of constituents over that in the quiescent control pyrite column.  $\text{Fe (II)}$  accounts for 72% of the total acidity of the control reaction, ( $0.061 - 0.045 \text{ m moles/l}$ ). If the remainder of the acidity is assumed to be sulfuric acid, then the sulfate balance is negative. This occurs because carbon dioxide is oversaturated in the system. Total acidity is consequently artificially increased over that attributable to the  $\text{Fe (II)}$  and sulfuric acid contributions.

## 2. Effect of Aeration

In the horizontal and vertical reactor studies described to this point, only the oxygen dissolved in the aqueous phase was available to chemically- or biologically-promoted oxidation of pyrite. This is the predominant condition existing in mines. A test was also made to determine the dissolution and oxidation of pyrite when forced aeration was practiced. The experiment was made in the same vertical reactors and under identical conditions described in the carbonation study. Air was bubbled through the reactors at  $10 \text{ ml/min}$ . The effluent characteristics are given in Table 9.

There is a decrease of pH from 5.2 for the control to 2.8. Acidity, sulfate and iron content of the effluent is appreciably increased. At pH 2.8, hydroxyl concentration is  $6.3 \times 10^{-12}$ . At these conditions, precipitation of ferrous hydroxide,  $\text{Fe}(\text{OH})_2$ , would require  $2.24 \times 10^{10} \text{ g/l}$  but ferric hydroxide,  $\text{Fe}(\text{OH})_3$ , only  $245 \text{ mg/l}$ . The ferrous iron to  $\text{S}_2^{-\text{II}}$  molar ratio is 0.88 in the aerated reactor effluent which approximates the stoichiometrically necessary quantity of 1.0 for pyrite dissolution. However, the total iron of  $224 \text{ mg/l}$  includes  $97 \text{ mg/l}$  ferrous ion. Hence, either  $\text{S}_2^{-\text{II}}$  oxidation is favored over  $\text{Fe (II)}$  oxidation; or, more likely, ferric hydroxide,  $\text{Fe (OH)}_3$ , rapid formation and precipitation within the bed accounts for the difference.  $\text{Fe (II)}$  accounts for 28% of the total acidity ( $6.2-1.7 \text{ m moles/l}$ ) leaving a great excess which may be attributed to sulfuric acid. There is also an excess of effluent sulfate of  $4.6 \text{ m moles/l}$

over that necessary to balance sulfuric acid acidity of 4.5 m moles/l. All chemical and biological factors favor increased pyrite dissolution and oxidation under forced aerated conditions. These results demonstrate that the reactions in the horizontal-flooded bed reactors were oxygen rate limited.

### 3. Effect of Flow

The effect on effluent composition and mass release of acid mine drainage of varying hydraulic flow rate through the vertical columns of pyrite was determined. The vertical columns, pyrite and operating conditions were identical to those described in 6 B.1. The flow rate was adjusted to 2.15, 4.3, 8.6 and 12.9 liters/day. These flow rates correspond to 2.3, 4.6, 9.2 and 13.8  $D^{-1}$  (volume of water per day  $\div$  volume of pyrite) or 5.6, 2.8, 1.4 and 0.9 hours retention time respectively. The retention time versus  $D^{-1}$  relationship in the case of the vertical units differs from that in the horizontal units described in 6A. Although the depth of liquid film over the pyrite was approximately the same in each case, volume of water over the surface was greater with the horizontal reactors. The surface area for the vertical reactor was very small relative to that of the horizontal bed although the former contained approximately 50% more pyrite. The ratio of volume of liquid to volume of pyrite is smaller for the vertical columns. Actual liquid volumes in the pyrite-containing zones of the reactors were measured. Retention time is based on this volume. Effluent characteristics for the horizontal and vertical units are therefore not comparable at the same volumetric ratio,  $D^{-1}$ .

The effluent composition and corresponding mass release per day of various constituents is tabulated as a function of flow in Table 10. The effluent characteristics at 2.3  $D^{-1}$  agree very closely with those for the control unit operated under identical conditions in the carbon dioxide study, Table 9. This illustrates the reproducibility of the tests. The mass release rates suggest that flow rate was limiting total acid mine drainage production probably because available oxygen was limited. Pyrite dissolution and chemical-biological oxidation rates were sufficiently rapid so that at lower flow rates,  $<9.2 D^{-1}$  or  $<1.4$  hours retention time, there is poor correlation of mass release with flow. Precipitation of reaction products is also enhanced at low flow rates. At higher flow rates insoluble (particulate) carryover is more likely.

### 4. Effect of Surface Area

It is expected that a direct relationship should exist between available pyritic surface area and release of acid mine drainage. This is a reasonable assumption if reaction product accumulation or depletion of reactant are not factors under aerobic conditions. An estimate of the effect of varying surface area on effluent composition under aerobic-seeded conditions was obtained with the vertical reactors. Operating procedures were identical to those described in 6 B.1 except that three reactors were filled with Shawville pyrite of three different sizes. Table 11. The crushed pyrite was first screened for size range. The mean diameter based on a spherical shape and the number of particles making up the reactor volume of 925 ml provided an estimate of mean area for the pyrite surface. These mean area values are uncorrected for contact area. The original particles are so irregular that only a rough estimate of relative

TABLE 10

Effect of Flow Rate on Effluent Composition from Aerobic-Seeded, Flooded Vertical Columns of Pyrite<sup>a</sup>

Effluent	Flow Rate, Volume Water Per Day ÷ Volume Pyrite <sup>c</sup>			
Concentration	2.3	4.6	9.2	13.8
pH	5.2	4.2	4.2	4.1
acidity, mg/l	6	6	7	7
ferrous iron, mg/l	2.5	1.3	0.7	0.8
total iron, mg/l	3.0	1.6	1.0	1.3
sulfate, mg/l	8	2	4	7
Mass Release <sup>b</sup>				
acidity, mg/d	13	26	61	90
ferrous iron, mg/d	5.4	5.6	6.1	10.3
total iron, mg/d	6.5	6.9	8.6	16.8
sulfate, mg/d	17	9	35	90

<sup>a</sup>Shawville pyrite, 925 ml of 3.5 to 7 mesh particle size

<sup>b</sup>mass released, milligrams per day

<sup>c</sup>retention times: 5.6, 2.8, 1.4 and 0.9 hours, respectively

TABLE 11

Relative Surface Area of Pyrite Particles Charged to Vertical Reactors<sup>a</sup>

Size	Mean Dia., mm	Mean Area, mm <sup>2</sup>	Number Particles	Mean Area 10 <sup>3</sup> mm <sup>2</sup>	Relative Surface Area
0.5 - 0.75 inch	15.9	795	139	107	0.17
3.5 mesh - 0.5 inch	9.18	266	760	202	0.32
3.5 - 7 mesh	4.25	56.7	11,137	632	1.00

<sup>a</sup>Shawville pyrite, 925 ml volume

TABLE 12

Effect of Varying Surface Area on Effluent Composition from Aerobic-Seeded,  
Flooded Vertical Reactors Containing Pyrite<sup>a</sup>

Effluent Concentration	Relative Surface Area		
	A <sub>1</sub>	0.32A <sub>1</sub>	0.17A <sub>1</sub>
pH	4.1	4.1	4.2
acidity, mg/l	7	6	4
ferrous iron, mg/l	0.8	0.7	0.5
total iron, mg/l	1.3	1.2	1.0
sulfate, mg/l	7	6	5

<sup>a</sup>Shawville pyrite, 925 ml volume; flow at 13.8 D<sup>-1</sup>, 0.9 hours retention

surface area could be expected. The 3.5 to 7 mesh particles were used in most of these experiments. The surface areas of the two beds containing larger particles are 0.32 and 0.17 relative to the standard particle size. The void space in the bed also varied with particle size and is 6% and 16% greater for the larger particles than for the 3.5 to 7 mesh size pyrite. The results of varying flow through the vertical reactors, Table 10, suggested that the study of the effect of surface area be made at  $13.8 \text{ D}^{-1}$  or 0.9 hours retention time for the 3.5 to 7 mesh particles. Retention time in the other units is 1.0 and 1.1 hours respectively. The reactors were seeded for the first 14 days with the standard ternary mixture of chemoautotrophic microorganisms. The effluent continued to show the presence of viable Fe (II)- and  $\text{S}_2^{-\text{II}}$ -utilizing organisms through the duration of the study, 95 days. The gray mycelial growth observed in the horizontal, flooded-bed reactors also developed in these vertical reactors. In time, it assumed the yellow-brown color typical of pyrite oxidation products.

Effluent characteristics at equilibrium are summarized in Table 12. Effluent pH varies only slightly, increasing from 4.1 to 4.2 as particle size increases. Acidity, Fe (II) and total iron and sulfate decrease as particle size increases (surface area decreases). Although the relative surface area of the pyrite bed with the largest particles is only 17% of that with the smallest particles, the mass release of each of the acid mine contaminants decreases only about 30 to 40%. The increased mass release is not directly proportional to the increased surface areas of 1:2:6. The difference is chiefly attributable to the variances involved in estimating surface area, variation in residence time and the probability that the choice of operating conditions may have been rate limited with respect to oxidation rate and/or transport relationships. A more exact experiment would have to be performed to isolate the surface area effect. However, this study suggests that if reactant (chemical or biological) depletion or reaction product concentration is not rate limiting, the release of acid mine drainage will be proportional to the available pyritic surface area for a given hydraulic rate under flow conditions which are not so slow as to be diffusion rate limited.

## Section 7

### ACKNOWLEDGMENTS

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## Section 8

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## Section 9

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Baker, R. A. and Wilshire, A. G. (1970) Evaluation of Potential Acid Mine Drainage, Water & Sewage Works, 117, No. 6, IW/10-16.

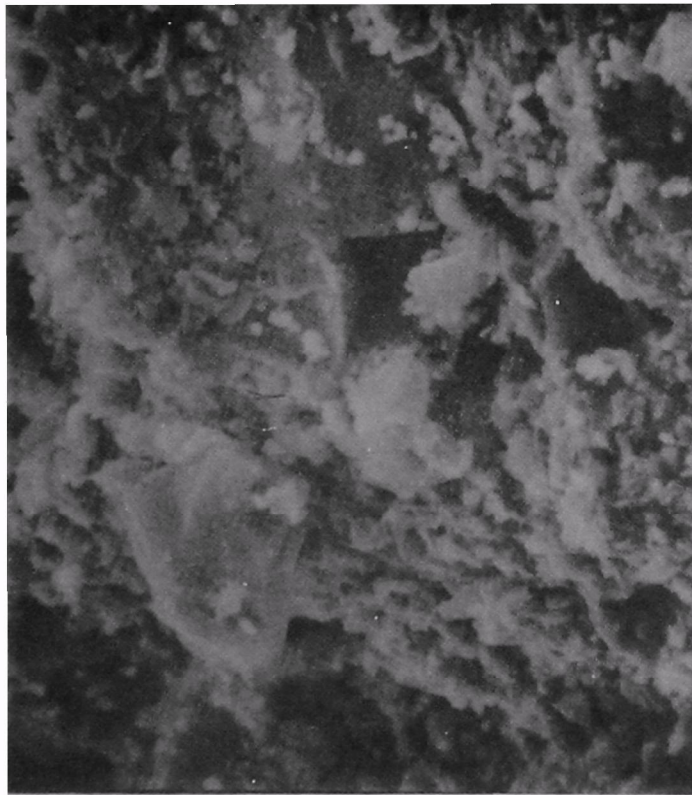
Baker, R. A. and Wilshire, A. G. (1970) Microbiological Factor in Acid Mine Drainage Formation: II. Further Observations from a Pilot Plant Study, Paper to 160th National Meeting of American Chemical Society, Chicago, Illinois, September 13-18.

## Section 10

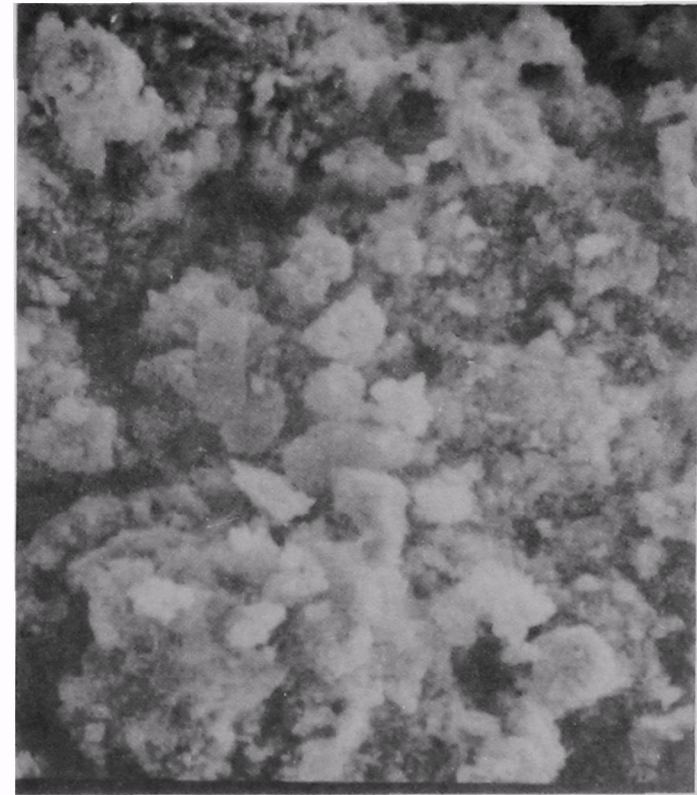
### APPENDIX I

#### PYRITE ELECTRONMICROGRAPHS

The surface characteristics of selected 3.5 to 7 mesh particles of Shawville pyrite were examined by means of a Jeolco scanning electron microscope before and after oxidation. The reacted pyrite has been exposed to an aerobic-seeded environment for 50 days. It was taken from the reactor and air dried. The unreacted pyrite was not freshly-ground but had been stored in a closed container prior to this examination so that some oxidation may have occurred on the mineral surface. The surface appearance at 1000 and 5000 magnifications for the unreacted and reactor pyrite is shown in Figs. 21 and 22, respectively. Although x-ray diffraction spectroscopy failed to detect differences between the unreacted and reactor pyrite, the photomicrographs indicate a significant surface alteration. Clusters which appear as lighter-colored areas developed on the pyrite surface during oxidation. Further elaboration on the nature of these crystal clusters is not possible at this time.



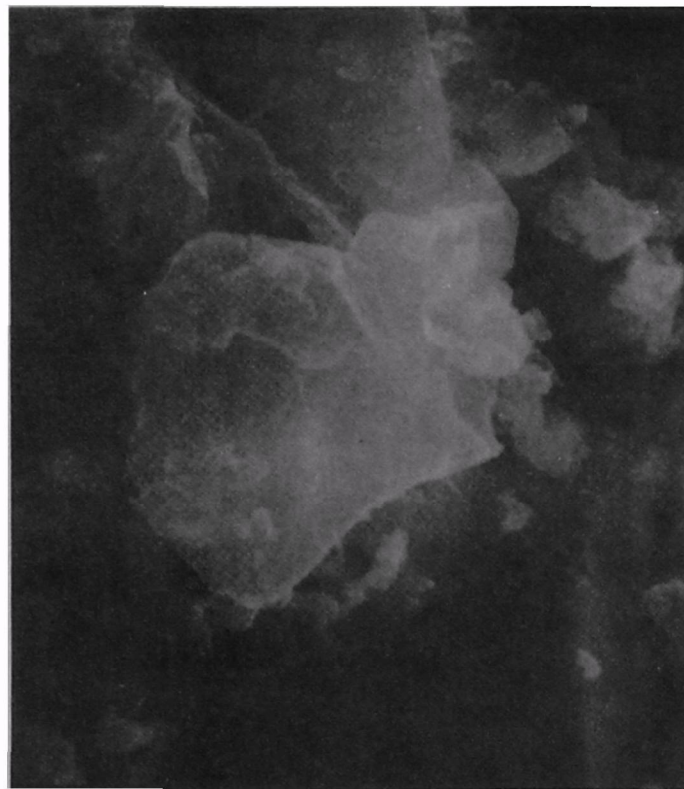
Unreacted 1000X



Reacted 1000X

10μ

Figure 21. Photomicrograph of Unreacted and Reacted Pyrite at 1000 Magnifications.



Unreacted 5000 X



Reacted 5000X

2 $\mu$

Figure 22. Photomicrograph of Unreacted and Reacted Pyrite at 5000 Magnifications.

BIBLIOGRAPHIC: Carnegie-Mellon University; Mellon  
Institute  
Microbiological Factor in Acid Mine Drainage Form-  
ation. FWQA Publication No. 14010 DKN

The role of chemoautotrophic organisms in the  
formation of acid mine drainage from pyritic ma-  
terials associated with coal mining has been inves-  
tigated by pilot plant techniques. Dynamic flow,  
controlled environment units which served as  
models of mines were used.

It was demonstrated that the concentration of  
acidity, ferrous and total iron and sulfate in  
effluent from aerobic-seeded or -unseeded pyritic  
beds is zero order with respect to flow, except  
where mass transport is diffusion limited.

Nonaerobic systems produce acidity consisting  
only of ferrous iron. Total acidity was lower  
from seeded- than nonseeded-aerobic systems at  
retention times exceeding three hours because micro-  
organisms consume acid. For lower retention time,

ACCESSION NO.

KEY WORDS:

Mine Drainage  
Chemoautotrophic  
Microorganisms  
Ferrobacillus  
Thiobacillus  
Pyrite  
Pilot Plant  
Dynamic Equilibria

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retention times exceeding three hours because micro-  
organisms consume acid. For lower retention time,

ACCESSION NO.

KEY WORDS:

Mine Drainage  
Chemoautotrophic  
Microorganisms  
Ferrobacillus  
Thiobacillus  
Pyrite  
Pilot Plant  
Dynamic Equilibria

total acidity is greater in the aerobic-nonseeded effluents. Under aerobic-nonseeded conditions acid discharge reaches a maximum rate at a specific flow. Further increase in flow only dilutes the concentration. Under aerobic-seeded conditions, a dynamic equilibrium exists between the rates at which pyrite dissolves, organisms reproduce and are removed from the system, and a maximum discharge occurs at a specific hydraulic rate.

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<b>1</b>	<b>Accession Number</b>	<b>2</b>	<b>Subject Field &amp; Group</b>	<b>SELECTED WATER RESOURCES ABSTRACTS</b> <b>INPUT TRANSACTION FORM</b>								
<b>5</b>	<b>Organization</b> Carnegie-Mellon University											
<b>6</b>	<b>Title</b> Microbial Factor in Mine Drainage Formation											
<b>10</b>	<b>Author(s)</b> Baker, Robert A. Wilshire, Albert G.	<b>16</b>	<b>Project Designation</b> 14010DKN (Federal Water Quality Administration)									
		<b>21</b>	<b>Note</b>									
<b>22</b>	<b>Citation</b>											
<b>23</b>	<b>Descriptors (Starred First)</b>											
	<table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">*Mine Drainage</td> <td>Bacteria</td> </tr> <tr> <td>*Mine Acid</td> <td>Ferrobacillus</td> </tr> <tr> <td>*Mine Wastes</td> <td>Thiobacillus</td> </tr> <tr> <td>*Mine Water</td> <td>Mine Models</td> </tr> </table>				*Mine Drainage	Bacteria	*Mine Acid	Ferrobacillus	*Mine Wastes	Thiobacillus	*Mine Water	Mine Models
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<b>25</b>	<b>Identifiers (Starred First)</b>											
<b>27</b>	<b>Abstract</b>											
	<p>The role of chemoautrophic organisms in the formation of acid mine drainage from pyritic materials associated with coal mining has been investigated by pilot plant techniques. Dynamic flow, controlled environment units which served as models of mines were used.</p> <p>It was demonstrated that the concentration of acidity, ferrous and total iron and sulfate in the effluent from aerobic, biologically-seeded or unseeded pyritic beds is zero order with respect to flow, except at low flow rates where mass transport is diffusion limited. Algal growth occurred in the acidic, aerobic environment but did not affect acid production.</p> <p>Nonaerobic systems produce acidity consisting only of ferrous iron. Total acidity is lower from biologically-seeded than -nonseeded aerobic systems. Acid mine drainage was not significantly affected by seeding with the individual or a mixture of three different organisms but was increased by recycle of the flow and increased appreciably under forced aeration. The rate is directly related to the available pyrite surface area.</p>											
<b>Abstractor</b> J.M. Shackelford		<b>Institution</b> Federal Water Quality Administration										

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