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BIOLOGICAL TREATMENT OF ACID MINE DRAINAGE

EDWIN E. HERRICKS
Department of Civil Engineering
University of Illinois at Urbana-Champaign

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UNIVERSITY OF ILLINOIS
WATER RESOURCES CENTER
2525 Hydrosystems Laboratory
Urbana, Illinois 61801

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ABSTRACT

Research was conducted to evaluate methods for the biological treatment of acid mine drainage (AMD). Two general approaches were evaluated. The first evaluated treatment of impounded AMD through the addition of a mixed microbial community and carbon sources appropriate for their maintenance. This approach was designed to promote sulfate reduction. Hydrogen sulfide produced by sulfate reduction can potentially react with metals in solution to form metal sulfide precipitates. The process improves pH and generally improves water qulaity. Several approaches were attempted to provide suitable microbial communities, carbon sources, and environmental conditions suitable for continuous microbial activity. Wood dust and typical solid waste materials were shown to support sulfate reduction, but maintenance of sulfate reduction in AMD solutions was limited. In addition to wood dust and solid waste materials, sponge substrates were used to "package" the microbial community in portable units which could be added to AMD impoundments. Tests of sulfate reduction and water quality improvement indicated that microbial communities were limited by "packaging" procedures. Sustained sulfate reduction was not obtained and water quality improvement was minimal. Several mechanisms of AMD quality improvement were evaluated or identified. Most significant was the potential for wood dust improvement of AMD quality when no biological activity was present. To provide adequate treatment of AMD, a second effort was directed to evaluation of a biologically based unit process for AMD treatment. Using an anerobic digestor which provided waste liquors high in organic acids, AMD was mixed with digestor effluent in an anaerobic reactor which maintained sulfate reduction. The end result was production of an effluent with low iron concentrations and no detectable heavy metals with a pH in the range of 6.5 to 8.5. The utility of a pilot plant design incorporating anaerobic digestion, sulfate reduction, aeration, and final clarification and settling was demonstrated. Useful outcomes of the proposed process were the production of metal precipitates in a form which is easily dewatered and potentially valuable, and the production of organic material (from the digestor) which may be considered a useful soil amendment in site reclamation.

KEYWORDS: Acid mine drainage treatment, anaerobic digestion, sulfate reduction, pH neutralization, biological treatment processes

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1. INTRODUCTION

Acid mine drainage (AMD) is considered to be one of the most troublesome industrial pollutants with significant local and regional impact in areas where acid-forming minerals are exposed by mining. The highly mineralized, low pH waters are formed by the oxidation and hydrolysis of sulfide minerals, one of the most common being iron sulfide (FeS). In Illinois, acid mine drainage is a regional water resources problem, generally localized to mine sites in the southern part of the state although AMD does occur in northern and western coal fields. AMD may affect large areas if discharge to streams and rivers occurs because neutralization capacity of the receiving system is typically limited by watershed geology. In general, AMD problems in Illinois may be classified according to location and extent. By far the most significant problem is the reduced water quality of impoundments on active or abandoned mines. The most serious problem occurs in pit lakes which may be quite deep and contain large volumes of contaminated water. The discharge of AMD into streams may severely degrade areas near the discharge and affect downstream water quality over a large area. By and large the most significantly affected stream system in Illinois is the Saline River and its tributaries in southern Illinois.

The treatment of AMD is generally difficult and costly. Typical treatment processes involve neutralization with a base, generally limestone, lime, or other low cost neutralization sources. Neutralization does result in an increase of pH, but numerous secondary problems occur. The most significant problem is the generation of large quantities of

metal hydroxide sludges. These sludges have a high volume, are hard to dewater, and contain large quantities of toxic metals. In addition to sludge, neutralization with limestone, lime, or even strongly basic materials such as sodium hydroxide result in increased total dissolved solids which include large quantities of sulfate. The neutralized AMD with high levels of calcium sulfate (typical of lime or limestone neutralization) has a limited buffering capacity and is subject to pH deterioration if additional AMD loading is encountered.

This project was undertaken to study alternatives to lime or limestone neutralization processes which could be used either directly in impoundments or as a unit process which could be applied to point or collected nonpoint sources of AMD. The treatment scheme adopted depends on microbiological activity, particularly removal of sulfate by sulfate-reducing bacteria. The bacteria reduce sulfate present in the AMD, producing either hydrogen sulfide which is purged and lost from the system, or metal sulfides which are precipitated and collected. The result includes the improvement of pH by neutralization, while altering the chemistry of the effluent through reduction of sulfate and precipitation of metals.

It was anticipated that the characteristics of the resulting effluent would be an improvement over lime neutralization since the sulfate-based buffer system is altered improving the capacity to withstand further degradation if additional AMD sources are discharged into the treated stream. The approach taken in this research included the evaluation of the potential for treatment of AMD on site by enhancing sulfate reduction as well as the design and testing of unit process for AMD treatment.

Research results for methods to treat impounded AMD were discouraging because it was not possible to sustain sulfate reduction for the time necessary to improve water quality. Design of a unit process followed initial experiments and was considered successful because precise control of effluent quality was possible. This report contains results of initial experiments and eventual unit process designs. In addition a brief review is provided of AMD formation and its general characteristics, as well as existing AMD treatment processes.

1.1 ACID MINE DRAINAGE FORMATION AND CHARACTERISTICS

Acid mine drainage is formed by a combination of physical, chemical, and biological processes when surface or groundwater interact with pyritic compounds (FeS_2) in a atmosphere containing oxygen. The material formed is complex, its characteristics determined by the mineralogy of the pyritic compound, surrounding geology and soil characteristics, climate, and the biological and ecological characteristics of the mined watershed. In addition the type and extent of the mining activity play a major role in AMD formation and chemical characteristics. The general reaction which forms AMD involves oxidation of the sulfide mineral and subsequent hydrolysis of the oxidation products, equation 1, (Ohio State University Research Foundation 1971).

$$FeS_2 \text{ (s)} + 3.75 \ O_2 + 3.5 \ H_2O \rightarrow Fe(OH)_3 \text{ (s)} + 2 \ SO_4^= + 4 \ H^+ \quad (1\text{-}1)$$
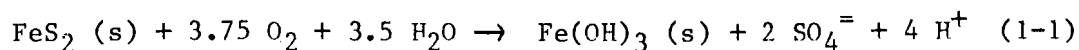
This reaction has been shown to be accelerated through the activity of bacteria from the Thiobacillus and Ferrobacillus groups (Colmer and

Hinkle, 1947; Temple and Delcamps, 1952; Kuznestor 1963). Additional hydrogen ions (H^+) are produced and a significant increase in the acid and electron buffer potential occurs when AMD comes into contact with clay minerals, equation 2 (King, 1974).

$$6\ H^+ + Al_2Si_2O_5(OH)_4\ (s) \rightarrow 2Al^{+3} + 2H_4SiO_4 + H_2O \quad (1\text{-}2)$$
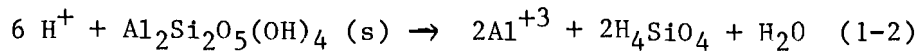
The dissolved aluminium can continue to be hydrolyzed, thus decreasing pH and promoting additional hydrogen ion liberation. The chemical composition of this highly mineralized water inhibits further pH variations as well as shifts in redox potential, equations 3,4,5.

$$Al^{+3} + H_2O \ \underset{\longrightarrow}{\longleftarrow}\ AlOH^{+2} + H^+ \quad (1\text{-}3)$$
$$AlOH^{+2} + H_2O \ \underset{\longrightarrow}{\longleftarrow}\ Al(OH)_2{}^+ + H^+ \quad (1\text{-}4)$$
$$Al(OH)^{2+} + H_2O \ \underset{\longrightarrow}{\longleftarrow}\ Al(OH)_3\ (s) + H^+ \quad (1\text{-}4)$$
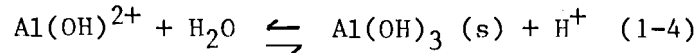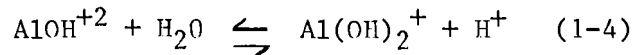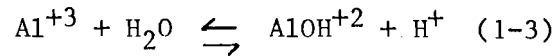
A formidable buffer system is created in AMD which contains high concentrations of aluminium. Additional modification of AMD may occur through dissolution of minerals, including other heavy metals. Neutralization must provide sufficient base (OH^-) to neutralize primary hydrolysis and oxidation products as well as hydrogen ions formed by secondary reactions.

Acid mine drainage is thus characterized as a low pH water with high dissolved sulfate and metals concentration (particularly Fe^{+3} and Al^{+3}).

AMD will typically be low in total organics. High particulate concentrations usually occur and a high redox potential is typical. The following criteria are generally used to identify AMD.

pH	< 6.0
acidity	> 3 mg/l
alkalinity	normally 0
iron	> 0.5 mg/l
sulfate	> 250 mg/l
TDS	> 500 mg/l
TSS	> 250 mg/l
hardness	> 250 mg/l

In the following studies AMD collected from the Will Scarlet Mine near Carrier Mills, Illinois was used. Approximately 850 1 (225 gallons) were collected in August 1976 (batch 1), December 1978 (batch 2), and April 1979 (batch 3). The characteristics of the AMD are contained in Table 1-1.

Table 1-1. AMD Characteristics for the Three Batches Used for Study

	Batch No. 1	Batch No. 2	Batch No. 3
pH	2.1-2.3	2.2-2.3	2.25
conductivity (μmhos)	7000	7000	1940
raw acidity (mg/l as $CaCO_3$)	7400	6200	1880
"hot" acidity (mg/l as $CaCO_3$)	-	7100	-
$[SO_4^{-2}]$, mg/l	6690	10,000	3520
$[Fe^{+2/+3}]$, mg/l	404	855	183
$[Al^{+3}]$, mg/l	365	738	213
$[Zn^{+2}]$, mg/l	22	24	6
$[Ni]$, mg/l	3	-	1.4
$[Cu]$, mg/l	trace	-	0
total solids, mg/l	-	14,367	4661
total dissolved solids, mg/l	-	14,078	4658
total volative solids, mg/l	-	2878	840
total diss. vol. solids, mg/l	-	2829	840

1.2 ACID MINE DRAINAGE TREATMENT

The quality of discharges from mining operations must be controlled to meet effluent limitations specified by National Pollutant Discharge Elimination System (NPDES) permits issued under authority of the Clean Water Act. Because AMD severely degrades water quality, treatment is essential before discharge into surface waters. Research and development of AMD treatment processes have been a major part of the Environmental Protection Agency's program for some time. Activity can be traced back to the early part of the century and a milestone in AMD treatment research was the publication of a comprehensive review of technologies by the Appalachian Regional Commission (1966). An idealized operation for AMD treatment include the following (Bogner et al., 1981):

1) Water collection
2) Flow equalization (storage and surge capacity)
3) Reagent selection, handling, and mixing to effect neutralization
4) Oxidation and aeration
5) Solid/liquid separation
6) Sludge handling and dewatering
7) Sludge disposal

The primary focus of past research has been on reagent selection and neutralization although solids handling and disposal generally present the most significant problems to effective waste treatment. Oxidation and aeration have also received considerable attention, particularly as a pretreatment process prior to neutralization because up to one half of the acidity in AMD arises from the oxidation of iron (II) to iron (III)

(Singer and Stumm 1969).

Neutralization processes can be developed around a number of materials which have suitable properties (availability of OH^-) but are typically limited by cost. Table 1-2, taken from Bogner et al. (1981), summarizes the advantages and disadvantages of the primary neutralization reagents used in AMD treatment processes. The lowest cost options are limestone and hydrated lime. A summary of AMD neutralization processes is provided in USEPA (1973).

In addition to neutralization processes, AMD has also been treated with reverse osmosis and ion exchange (USEPA, 1972; Burns and Roe, 1973). These processes have been shown to be quite effective, but costly. A typical problem of AMD treatment is the design of processes for drainages which may have significantly different compositions. AMD composition is defined by site conditions such as the mineral form of the iron sulfide as well as edaphic characteristics of the site (e.g., clay mineralogy and availability, etc.). Thus AMD treatment must be designed to reflect site specific requirements.

The reduction of sulfates through microbial sulfate reduction and biological treatment of AMD have been the subject of several studies. Using acetic acid as a carbon source, Middleton and Lawrence (1977) studied microbial sulfate reduction. They operated completely mixed flow reactors at various temperatures to evaluate growth coefficients. They estimated that sulfate concentrations below 150 mg/l were growth limiting at $20^{\circ}C$ while concentrations below 30 mg/l were limiting at $25^{\circ}C$ and $31^{\circ}C$. The results of the study include preliminary design equations for a sulfate reduction process. In another study, Smith (1978) evaluated the

Table 1-2

Summary of Advantages and Disadvantages of
Neutralization Reagents for Coal Mine Drainage

Reagent	Advantages	Disadvantages
Hydrated lime $(Ca[OH]_2)$	Relatively low unit cost. Dry--available bulk or bagged. Safe to transport and handle Relatively high neutralizing equivalent/unit quantity. Generally readily available. Does not introduce constituents that are toxic to aquatic organisms.	A water-lime slurry needed for maximum efficiency. May cake in storage shed or feed hopper. Must keep dry until use. Treated water is hard. Forms calcium sulfate sludge.
Quiklime (CaO)	Higher neutralizing equivalent/unit quantity than hydrated lime. Others same as for hydrated lime.	Must be slaked before use. More caustic to handle than hydrated lime. Other disadvantages same as hydrated lime.
Limestone $(CaCO_3)$	Extremely low unit cost. Dry. Extremely safe to transport and handle. Generally readily available. Does not introduce constituents toxic to aquatic organisms. Resulting sludge is more dense than hydrated lime sludge.	Relatively low neutralizing equivalent/unit quantity. Lower solubility than hydrated lime. Finely ground material or some mechanical abrasion system required to ensure that reaction surfaces are kept clean. Forms calcium sulfate sludge. Treated water is hard.
Soda ash (Na_2CO_3)	More soluble than calcium reagents. Dry, usually formed into bruquettes easily dispensed by a gravity feed device. Safe to transport and relatively safe to handle. Treated water is soft. Minimum of sludge formation.	High unit cost. May be more difficult to obtain than calcium reagents. Relatively low neutralizing equivalent/unit quantity. Elevated sodium concentrations in treated water. Must be stored dry.

Table 1-2 (Cont'd)

Reagent	Advantages	Disadvantages
Caustic soda (NaOH)	Stored and dispensed as extremely reactive solution (20-50%). Relatively high neutralizing equivalent/unit quantity. Treated water is soft. Minimum of sludge formation.	Caustic to handle. Must be stored carefully; may develop concentrated slurry in bottom of tank and become crystallized at relatively low temperature. High unit cost. Elevated sodium concentrations in treated water. Possible to overtreat and discharge water of extremely high pH.
Anhydrous ammonia (NH_3)	Same as for caustic soda.	Must be stored and handled very carefully. High unit cost. Possible to overtreat and discharge water of extremely high pH with elevated NH_3 concentrations toxic to aquatic life. Prohibited by law for treating mine drainage in some states.

feasibility of a microbial sulfate-reducing system to remove heavy metals from wastewaters. Primary sewage sludge was used as the carbon source and analytical grade gypsum was used as the sulfate source. The two-stage treatment system included a gas-stripping system for sulfides in the first stage with the hydrogen sulfide used in a second stage for metal sulfide precipitation. He found that organic carbon oxidation was the rate-limiting step in sulfide production (similar to previous studies by Middleton and Lawrence), the pH range of the system was favorable, and the process was technologically feasible. In studies of treatment of high strength acidic wastewater Chian and DeWalle (1977) used a completely mixed anaerobic reactor. Because of reduced growth in anaerobic systems, this study suggested that retention of biological solids independent of wastewater flow would eliminate the potential for biomass wash out.

The feasibility of joint treatment of domestic sewage and AMD was evaluated by Pearson and Nesbitt (1972). The study determined that AMD was a suitable coagulant and that the removal of phosphorus, as well as AMD improvement, was possible. The treatment design depended on the availability of ferrous iron to precipitate phosphorus. As previously stated, solids handling and disposal is often one of the most significant problems in the design of AMD treatment. Depending on the neutralization reagent, the end product of AMD treatment can be large quantities of metal hydroxide flocs producing sludges which are hard to dewater and difficult to dispose of. Although a number of dewatering systems have been evaluated (Akers and Moss, 1973), solids handling still requires space for permanent storage or disposal. The sludges often contain large quantities of toxic materials (particularly heavy metals) and may present a long-term hazard

which must be considered as part of the treatment process.

In addition to waste treatment processes which improve water quality, a number of "natural" AMD treatment processes have been identified. The most straightforward natural AMD treatment process involves the mixing of AMD with water which has sufficient alkalinity and neutralization capacity to improve water quality. This process is dependent on the presence of limestone or other geological formations which can contribute OH^- ions when surface or groundwaters come into contact with rocks (Herricks and Cairns, 1974). Natural treatment can also be evaluated in light of natural restoration processes (Lind and Campbell, 1970; Riley, 1965; Herricks et al., 1975). The critical requirement for natural restoration appears to be the availability of organic carbon. The organic carbon provides the necessary energy source for bacteria which can modify components of AMD. The accumulation of organic materials produces conditions favorable for the proliferation of sulfate-reducing bacteria, particularly Desulfovibrio (Tuttle et al., 1969 a and b). These bacteria reduce sulfate to sulfide. When sulfide is purged from the system or precipitated in the form of metal sulfides, the buffer system in AMD is modified and pH can return to near neutrality. The recovery process has been described by King (1974) as the titration of the acid buffer system by the products of microbial metabolic activity. The rate of this process will be governed by the quality and quantity of the two components (e.i., acid buffer capacity of the AMD and the activity of microorganisms) and regulated by physical, chemical and biological characteristics of the watershed. In studies of final pit lakes of various ages Campbell and Lind (1969) observed a slow recovery of water quality although the

transition period between low pH and neutral pH conditions appeared to be quite short. To enhance this natural recovery process King (1974) recommended addition of organic material to acid lakes which he felt would speed the recovery process.

The treatment processes which are the subject of this report were developed after careful study of neutralization processes and natural AMD water quality restoration. The goal of the research was to develop a treatment process which would reduce neutralization reagent costs and problems associated with solids handling and disposal which were noted with available neutralization processes. In addition the process design should allow more precise control of effluent quality than is available in natural restoration schemes and should effectively treat AMD over short periods rather than the extended time periods observed by Campbell and Lind (1969).

2. TREATMENT OF IMPOUNDED AMD

The initial research approach was to design a treatment system which would be suitable for treatment of large volumes of impounded AMD such as found in final pit lakes or treatment of relatively small volumes of AMD collected in shallow basins on the mine site. The initial design incorporated processes identified by King (1974) but "packaged" the process. The package consisted of a microbial community acclimated to AMD conditions and a carbon source which would support microbial activity, specifically sulfate reduction. The treatment design thus called for a preconditioned microbial community inoculated in the substrate package; the control of the sulfate reduction rate (and AMD water quality improvement) would be achieved by controlling the number or size of the substrates. The design also included provision for precipitation of metal ions as metal sulfides within the package. The goal was containment of any potentially toxic materials and precipitation of metals in a form which might also lead to recovery and recycling of valuable metal resources. The design of the "substrate/biomass" package was the primary focus of the initial research activity. The following discussions summarize the results of research conducted to evaluate 1) substrate materials to support sulfate reduction, 2) "packaging" of carbon sources and active microbial communities for AMD treatment, 3) AMD treatment potential of substrate/biomass packages, and 4) abiotic treatment of AMD using wood dust.

The AMD used in the following studies originated from batch 1 of the Will Scarlet Mine collected in August 1976, Table 1-1.

2.1 INITIAL SUBSTRATE TESTING

The identification of suitable substrates to be packaged was based on two criteria. The first was that the material to be used was either readily available near the mine site or of low enough cost that use on the mine site would not add significantly to reclamation costs. The second criteria was that sufficient carbon sources exist in the material to support microbial populations for the period necessary to effect water quality improvement. The first material selected was wood chips or wood dust. Since many mining operations are located near areas where waste products from wood processing operations are available or clearing of the mine site results in the availability of woody materials, wood dust and wood chips were selected as one of the primary substrates for evaluation. The second source of substrate materials was domestic solid wastes. Although not available on the mine site, solid wastes contain significant quantities of carbon (Pfeffer, 1974) as well as quantities of metals which could assist in sulfide precipitation. Solid wastes typically present disposal problems and their use in AMD treatment might be considered a significant benefit. In addition to wood dust and solid wastes, a media suitable for the culture of sulfate-reducing bacteria supplemented substrate carbon sources. A modified Baar's media was used in initial testing to encourage sulfate reducer growth and assist in establishing a mixed microbial system which would degrade carbon compounds and support sulfate reduction (Cappenberg, 1975; King, 1974).

2.1.1 Substrate Performance

Large culture reactor systems were established to evaluate substrate performance and provide a source of sulfate reducing organisms for later

testing. The reactors consisted of large (19 cm diameter) clear Plexiglas

tubing approximately 90 cm in height. Three sample ports were located

at 25 cm intervals. A sampling vent was located in the top end plate to

provide for gas production measurement gas traps were utilized to prevent

contamination of the anaerobic system with oxygen in the laboratory

atmosphere. Following start-up of initial reactor systems, a modification

was made to allow gas to escape from compacted substrate materials by placing

a perforated tube (9 cm diameter) in the center of the reactor before

addition of substrate materials.

The wood substrate consisted of a mixture of wood dust and wood chips.

Solid waste substrate consisted of shredded solid waste. Both materials

had a maximum diameter of approximately 1.5 cm. Modified Baar's media

was prepared according to Table 3. This media has been shown to provide

a satisfactory growth media for several species of sulfate-reducing

bacteria. A small quantity of iron filings was added to the reactors

containing wood dust to reduce sulfide concentrations through precipitation

of metal sulfides and to reduce the possibility of inhibition of sulfate

reduction.

The reactors were inoculated with material from two sources. Anaerobic

sediments from Crystal Lake, Urbana, Illinois were used as one source of

sulfate reducing bacteria. A second inocula was obtained from an

operating anaerobic digester and included digester sludge and a small

quantity of digester liquor.

Loading of the reactors was as follows:

A one to two liter layer of dry media was followed by addition of 3
to 4 liters of selective media (a sufficient quantity to cover the dry

Table 2-1

Modified Baar's Media for Sulfate Reducers

Compound	Grams or ml/l Water
Sodium lactate (60%)	6.0
Na_2SO_4	1.0
$MgSO_4$	2.0
NH_4CL	1.0
KH_2PO_4	0.5
$CaCl_2$	0.1
Yeast extract	1.0
$Fe(NH_4)_2(SO_4) \cdot 6\ H_2O$	0.1
KOH - pH adjustment to desired pH	(7.2)

material). Following addition of selective media a portion of the inocula was added followed by more dry material and more selective media. The following were the contents of the two reactors:

Wood dust reactor - 10 liters of dry wood substrate with 3 g iron filings, 16 l or selective media, 800 ml inocula.

Solid waste reactor - 10 l dry solid waste material, 16 l selective media, 800 ml inocula.

Results - Qualitative assesments of sulfate-reducing activity were made on the reactors. There was an initial period of high gas production immediately following reactor inoculation. This gas was largely CO_2 associated with aerobic activity in the reactors. After approximately one month, black swirls were apparent in the wood dust and widespread in the solid waste reactor. The appearance of black areas was noted at the same time hydrogen sulfide gas production began. After one month there was a rapid spread of the black areas until the reactors were completely black. Gas production declined through this period but could be increased with addition of Baar's media. These qualitative observations indicate slow growth of sulfate-reducing bacteria and carbon limitation of sulfate reducer growth. In both reactors the substrate tended to float due to trapped gas. Since hydrogen sulfide may inhibit sulfate reduction, the reduced growth may have been due to several factors including poor gas exchange within the reactors and inhibition of sulfate-reducer activity. Total gas produced over a 24 hour period approximately one month following inoculation was 19.6 ml/hr for wood dust and 28.9 ml/hr for solid waste. Analysis of this gas indicated the presence of hydrogen sulfide. Hydrogen sulfide production was estimated at 2 ml/hr. The initial pH value in each reactor was adjusted to 7.2 prior to inoculation. Approximately one month after inoculation the pH in both reactors had

decreased to 5.3. Following the onset of sulfate reduction the pH began to increase with the pH of the wood dust reactor 6.9 and the solid waste reactor 5.8. The redox potential of both reactors decreased with increasing pH. After three months, gas production in the wood dust reactor had significantly decreased and the color of the substrate changed from black to a green-black. The reactor with solid waste continued at a high level of gas production for approximately six months.

Using inocula from the initial reactors it was possible to establish new reactors with a lag of only one week (vs. a one-month lag in initial reactors). This rapid start-up was achieved by allowing anaerobic conditions to develop in a new reactor before anaerobic transfer of inocula was made. Attempts were made to use thioglycollate to reduce oxygen levels, but this procedure inhibited sulfate reducer activity.

Conclusions - From the initial reactor experiments, it was demonstrated that both wood dust and solid waste materials will support sulfate reduction. It was apparent that the use of Baar's media was an essential component of the reactor system because obtaining the balanced microbial community to support sulfate reducers was difficult even under more or less optimal conditions. The results also indicate that solid waste materials supported sulfate reduction for a longer time. This was likely due to higher concentration, a greater variety, or more available carbon sources of this material. It was possible to rapidly establish a microbial community in the materials evaluated although solid waste was somewhat preferable to wood dust.

2.2 SUBSTRATE/BIOMASS PACKAGE TESTING

Following initial experiments designed to generally evaluate
substrate suitability and establish active sulfate-reducing bacteria
communities, a number of substrate/biomass packages were evaluated.
The primary goal of the experiments was testing the capacity for
maintenance of sulfate reduction in an AMD solution. The initial
experiments used both polyurethane and cellulose sponges which were
saturated with selective (Baar's) media and Difco Bactoagar and then
inoculated with supernatant from reactors showing active sulfate
reduction. These experiments included preliminary jar tests as well
as larger scale testing using 20 liter sealed aquaria. A final series
of experiments were performed using wood dust which was inoculated
with sulfate reducer organisms and this system was compared with a
noninoculated (abiotic) reactor.

The use of polyurethane and cellulose materials to provide a
package for maintenance of appropriate microbiological communities was
designed to test the capacity for functional maintenance in various
AMD concentrations. The sponge materials contained open cells which
could retain a significant quantity of selective media (for sulfate
reducers). Inoculating the sponges with material from functional
reactors thus created a portable package which, it was hoped, would
support the development of a mixed microbial community providing
long-term support for sulfate-reducing organisms. This concept was
generated from King's proposal for AMD treatment (King, 1974) and
from studies of Cappenberg (1974a and b) indicating that mixed
cultures of anaerobic microorganisms provided complementary functions.

When viewed in light of AMD treatment, the mixed microbial system provided moderation of harsh environmental conditions (low pH, high concentration of heavy metals, etc) created by the AMD. It was hoped that the sponge substrate itself might provide carbon sources for bacterial degradation which might support further sulfate reduction.

2.2.1 Initial Jar Tests

The initial experiments included use of 24 1-liter jars which replicated substrate type and AMD dilutions. Cubes of polyurethane or cellulose containing 32 cm^3 of material were prepared from commercially available material. All substrates were flushed with deionized water for 24 hours then saturated with 50 ml of selective media. Agar was used to keep selective media in the sponge substrates when they were added to AMD solutions. The biomass packages were placed in two strengths of AMD. The first was 10% AMD diluted with distilled water and a final pH adjusted to 3.0 with sodium hydroxide. The second solution contained 10% AMD adjusted to a pH of 5.0. Half of the jars contained substrate packages inoculated with 10 ml of the supernatant from active reactors. Inoculation was made using a sterile Cornwall syringe attached directly to the culture reactor. Each cube received the inoculum in the center of the agar/selective media substrate. Immediately after inoculation the cube was placed in a jar and sealed. All jars were incubated at 26°C in the dark. Jars were sampled one, two and four months after inoculation.

Results - Generally higher metabolic activity was noted in the AMD solutions adjusted to a pH of 5. In both pH 3 and pH 5 solutions pH and redox showed similar trends through time. The pH and redox

peaked during the first month of incubation but then returned to original conditions. After four months the final pH in the solutions adjusted to 5.0 had increased between 1.8 and 3.3 pH units while the pH in the solutions adjusted to 3.0 had not changed or had increased only slightly (an increase of approximately 0.5 pH units). Polyurethane sponges evidenced slightly better performance (the pH was 0.2 to 0.4 pH units higher in polyurethane sponges than recorded in cellulose sponges. The possibility of pore size as well as substrate chemistry may account for this result.

The experimental procedure had an effect on the AMD solutions only during the first month of incubation, and only in solutions adjusted to a pH of 5.0. It appears that the inoculum was not able to survive a pH of 3.0. Microbial activity also ceased after approximately one month indicating a dependence on suitable carbon sources (probably lactate). The sulfate concentrations in the test were not significantly altered. Neither substrate type produced appreciable change in sulfate concentration.

Conclusions - The results of this experiment indicated that long term maintenance of a viable sulfate reducer population was tied to availability of food resources and suitable environmental conditions. The small sponge cubes did not support an active sulfate-reducing community and maintenance of activity, as indicated by alterations in pH, and redox occurred for only the first month of the experiment. Environmental conditions can also be considered critical. Either sufficient support must be provided to allow establishment of a mixed microbial community which will buffer sulfate reducers against harsh

environmental conditions (such as pH) or general environmental conditions must be modified to allow maintenance of microbiological activity.

2.2.2 Static Reactor Tests

A second series of experiments were conducted using larger static reactors which would accommodate larger sponge cubes. This experiment was designed to evaluate the effect of surface area to volume ratios on microbial activity and water quality changes. Based on the results of the initial jar tests, only polyurethane substrates were used; larger blocks (1000 cm^3 volume) were saturated with selective media and agar and then were inoculated with 60 ml of liquor from a reactor which showed evidence of active sulfate reduction. A modification to the jar test procedure also allowed a 24 hour "acclimation" period for each substrate before it was placed in an AMD solution. AMD concentrations of 0, 10, 50, and 100 percent were used in reactors with a total volume of 15 liters. In addition a reference reactor with an noninoculated substrate was also tested. Reactors were sealed and vented. Periodic analyses were made for pH, redox, sulfide, sulfate, organic carbon, iron and aluminium. Since some stratification was expected in the reactors, substrates were placed on the bottom of each reactor as well as suspended near the surface.

Results - Figures 1-5 illustrate the results of chemical sampling in each of the AMD concentrations. During the first month major changes were noted in pH, redox, and organic carbon in an inverse relationship to AMD concentration. A noticeable stratification and differences in sulfide production were noted in the 0% and 10%
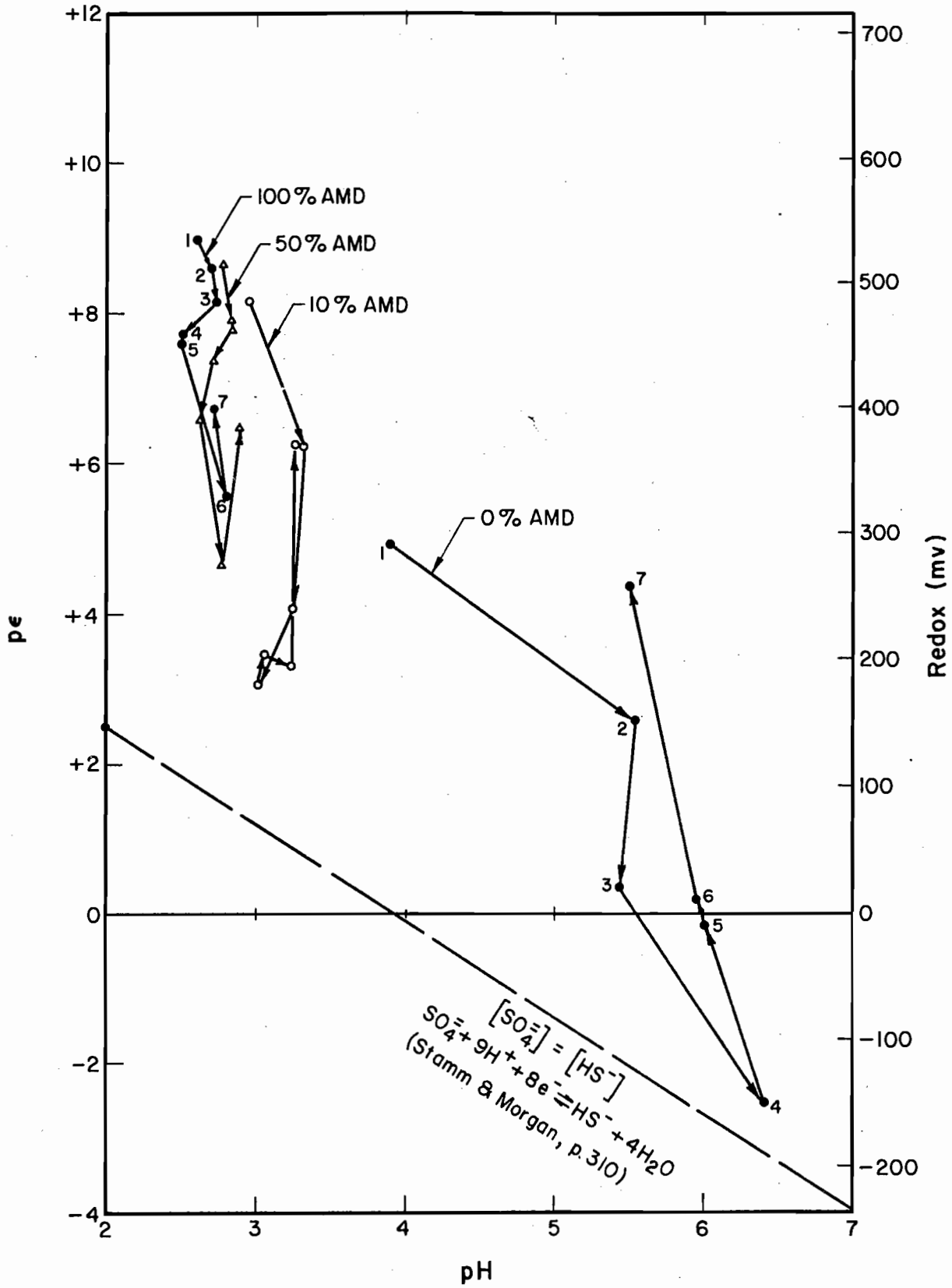
Figure 1. pH/Redox Trends in Bottom of Substrate
Tanks. 4/1 Through 5/16.
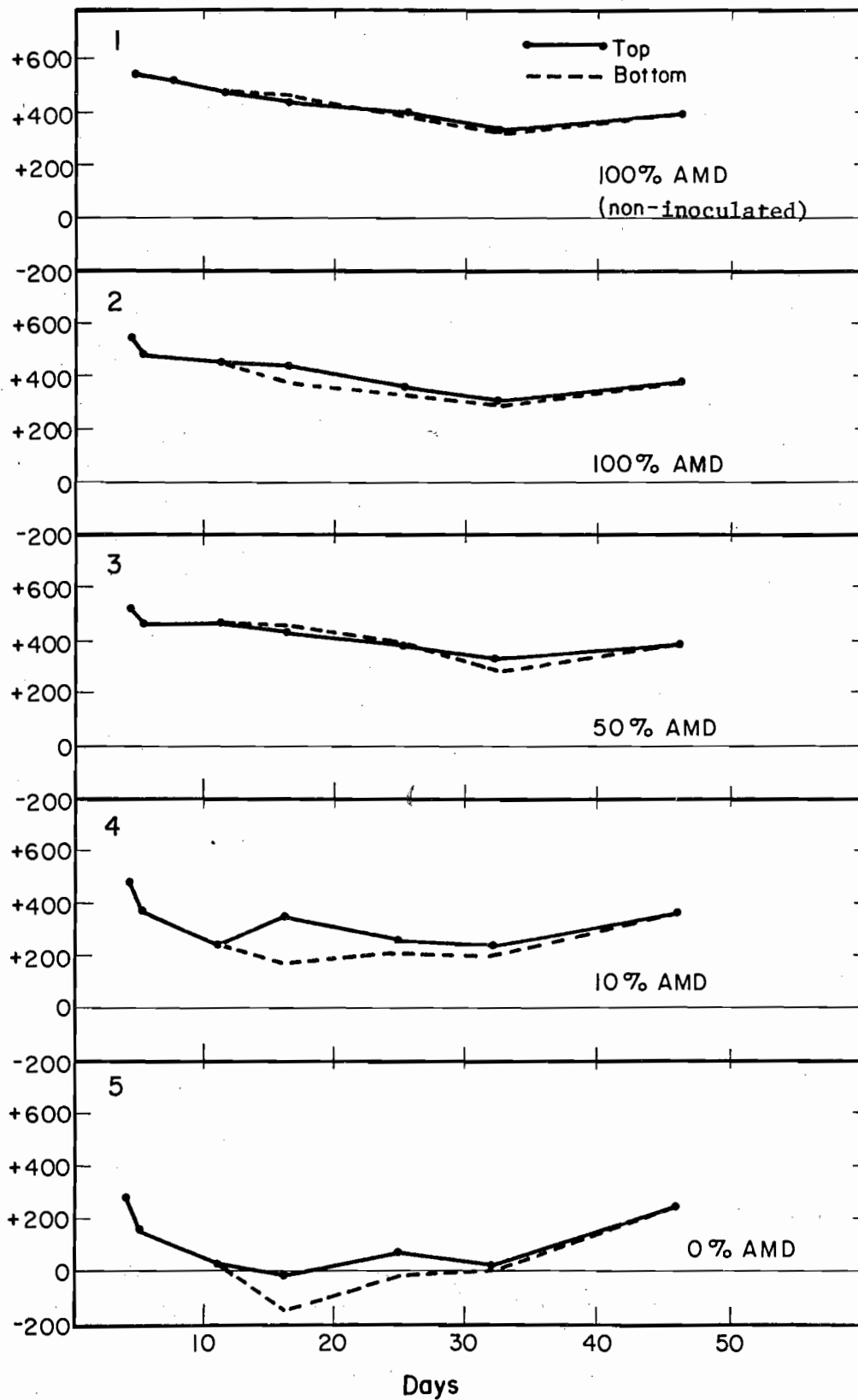
Figure 2.   Redox potentials in the top
             and bottom of static reactors.
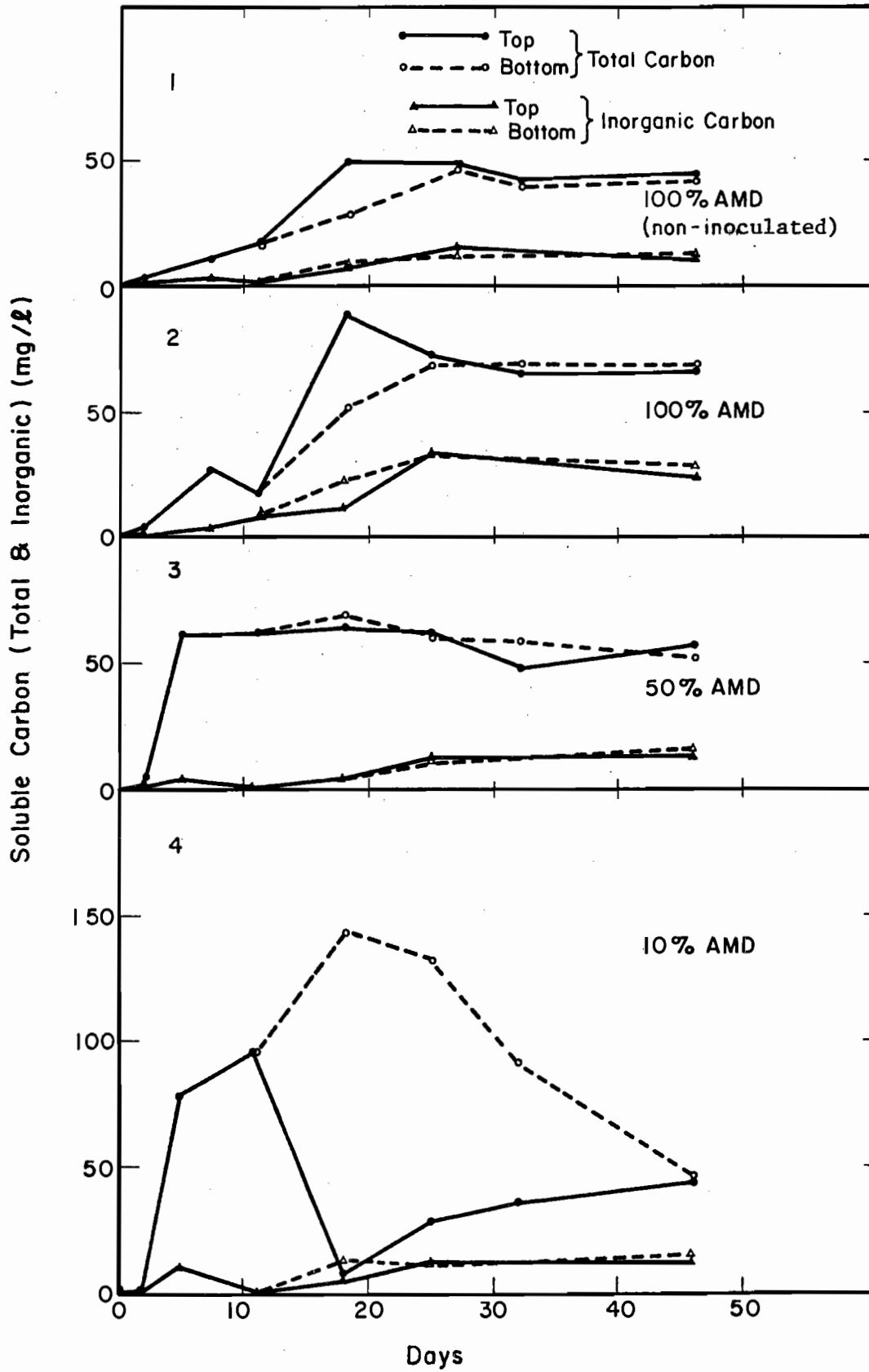
Figure 3.   Carbon concentrations in the top and
bottom of static reactors.
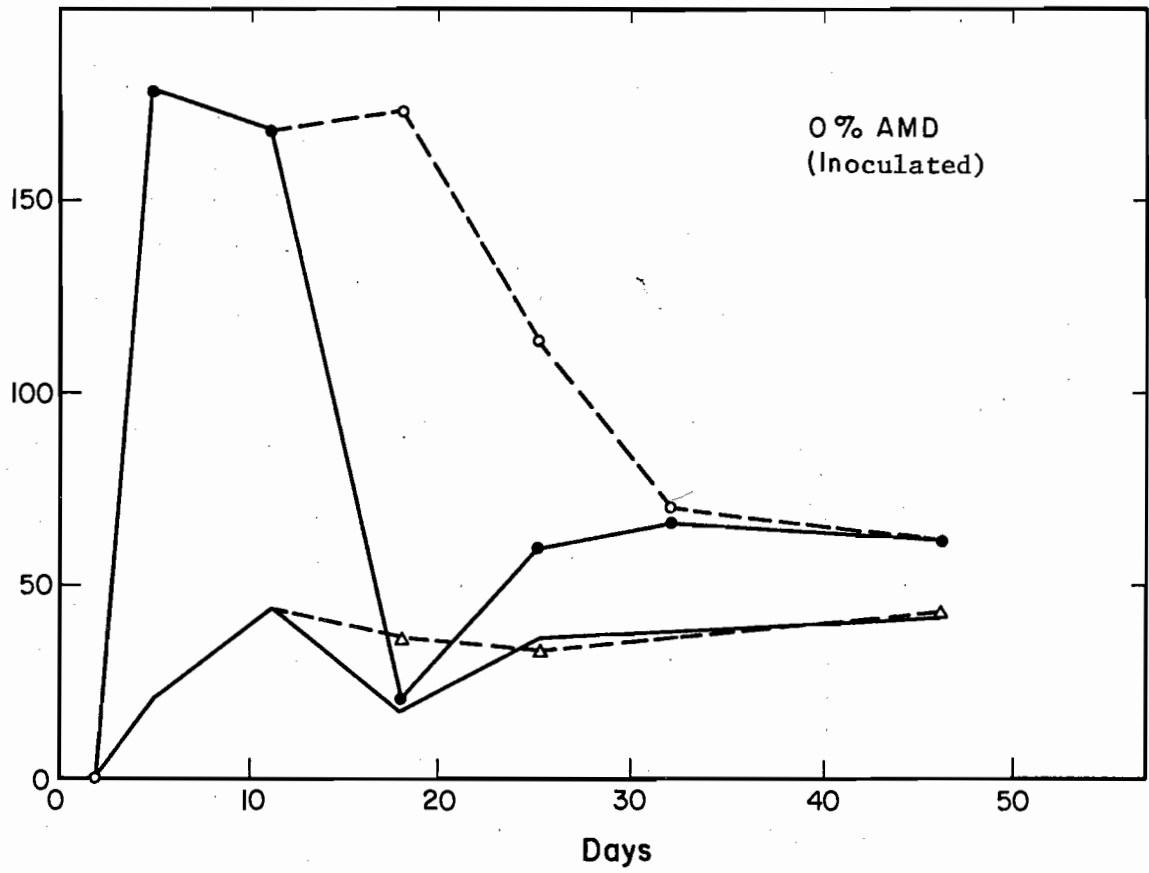
Figure 3. Carbon Concentrations in the top
and bottom of static reactors. (Cont'd)

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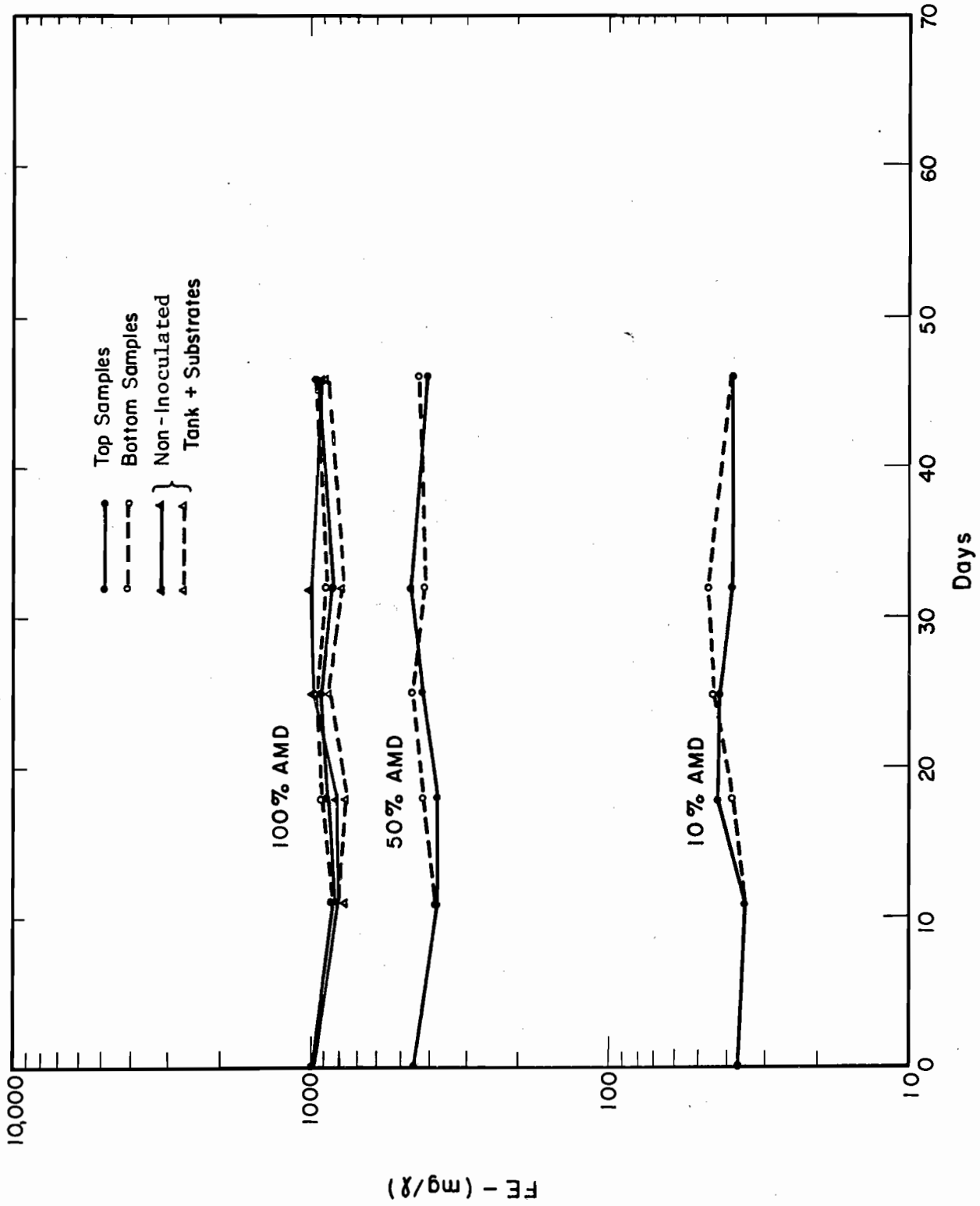
Figure 4. Variation in iron concentration in reactors of 100%, 50%, and 10% AMD, samples drawn from top and bottom of static reactors.
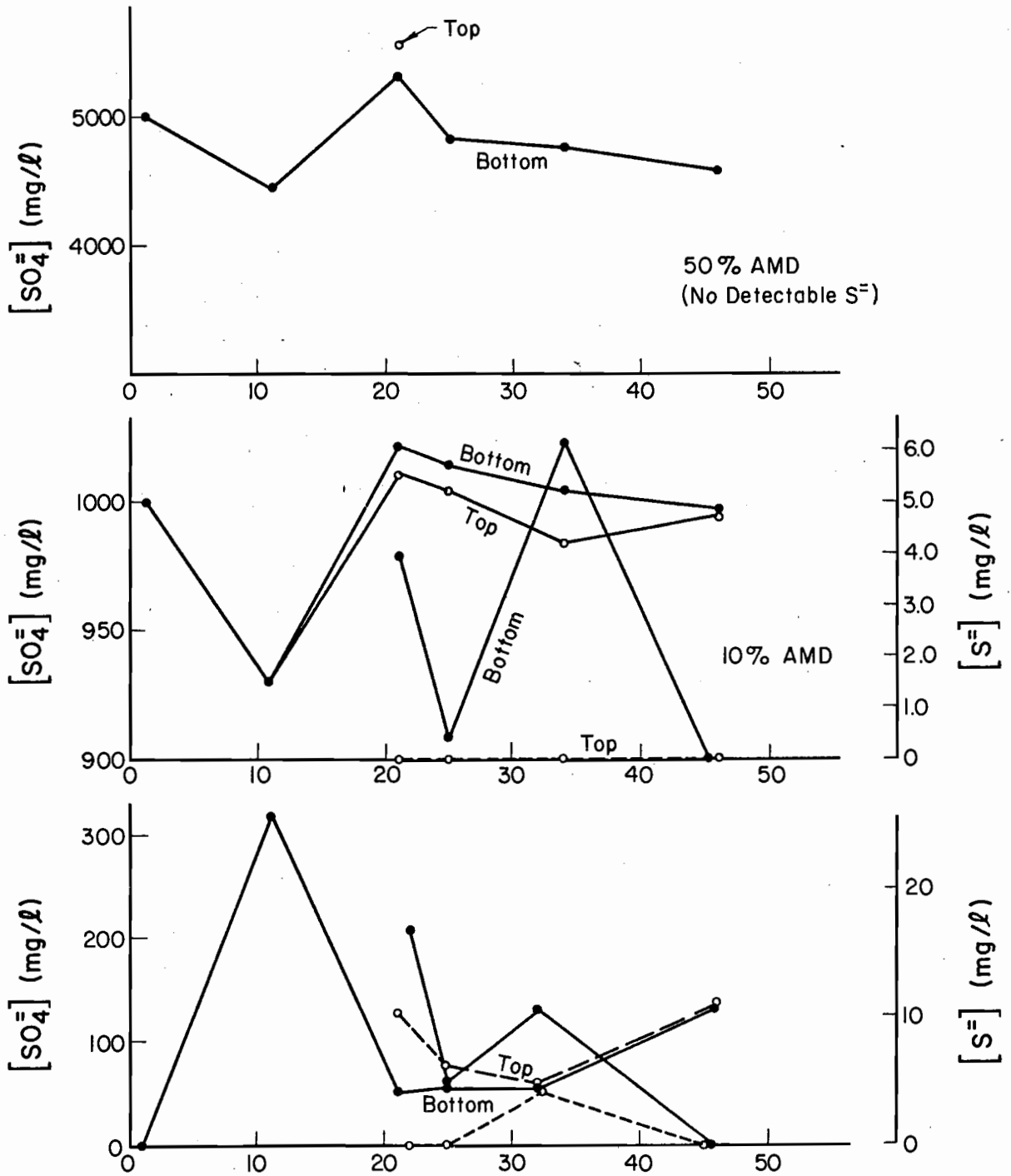
Figure 5. Sulfate concentration in top and bottom of static reactors.

reactors but no sulfide production was noted in 50% and 100% AMD reactors. There was a trend toward increasing organic carbon in the 50% and 100% inoculated, and the 100% noninoculated reactor while organic carbon at first peaked then declined in the 0% and 10% AMD reactors. There was no appreciable change in iron or sulfate concentration at any concentration. Microbial activity as indicated by change in chemical parameters was high in the 0% and 10% AMD reactors during the first month but trailed off rapidly to little change (low microbial activity) at 50 days.

Conclusions – This experiment demonstrated that foam substrates could support a microbial community which included sulfate reducers (based on sulfide production), but total sulfate reduction activity was incapable of reducing sulfate concentrations appreciably in the reactors. The tests also indicated that maintenance of suitable energy sources for the microbial communities desired would be extremely difficult. Although some activity was demonstrated in low concentrations of AMD, virtually no activity was noted in higher concentrations suggesting that an easily portable package such as a foam substrate would be inappropriate for treatment of the typical AMD sources (low pH, high dissolved solids). The rapid loss of microbial activity also indicated that establishment of the appropriate mixed microbial communities would require a different substrate "package" which would provide resources for long-term maintenance of the microbial community.

2.3 ADDITIONAL SUBSTRATE EVALUATIONS

A final series of experiments were performed to evaluate the efficacy of using other materials, particularly wood dust as a substrate for packaging and maintenance of microbial communities. The inital experimental evaluation was made using unsterilized, inoculated and sterilized wood dust in a 20 liter reactor. The experimental design was based on previous studies which indicated that sulfate reduction could be maintained in a large scale reactor. A 10% AMD solution was used in both reactors, the sterilized wood dust intended to provide a control for the experiment. The wood dust in the experimental reactor was not sterilized so that initial microbiological activity would produce an anaerobic environment suitable for introduction of inocula from an actively reducing system. Initial chemical analyses were performed on day 2, and inoculation of the experimental reactor was made on day 3. Analyses were made for pH, redox, sulfate, sulfide, gas production, and sulfate-reducing bacteria populations.

Results - The results of the initial experiment are provided in Figures 6-10. There were dramatic shifts noted in both reactor systems in pH, redox, gas production, and sulfate during the first five days of the experiment. Of particular interest is Figure 9, which indicates similar changes in sulfate in both uninoculated (abiotic) and inoculated (biotic) reactors during the first five days of the experiment. It appears that the simple addition of wood dust reduced sulfate concentrations and may play a significant role in the maintenance of pH within the reactors, Figure 6. The gas production observed in the experimental reactor was expected, based on previous
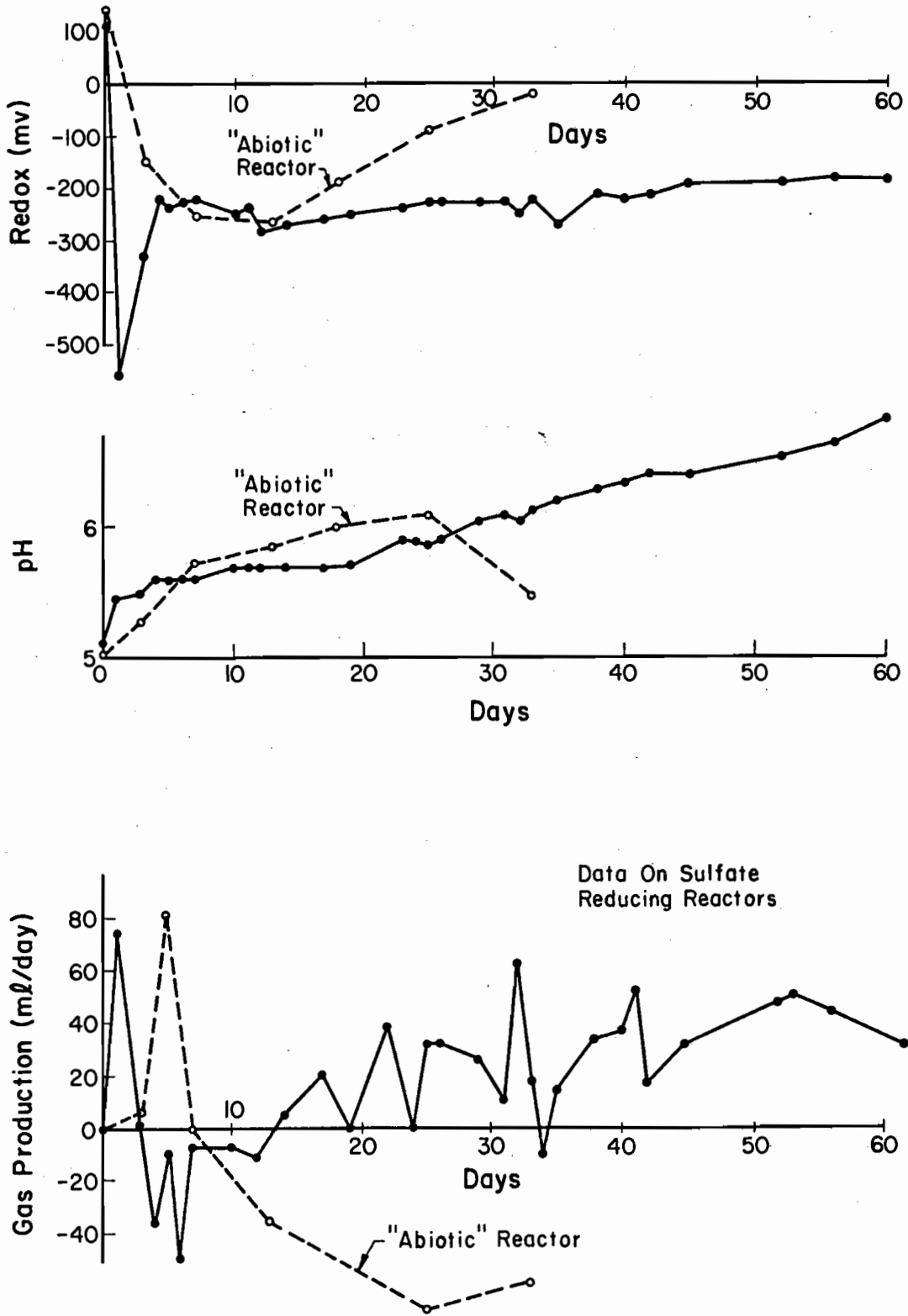
Figure 6. Redox, pH, and gas production from wood dust reactors.
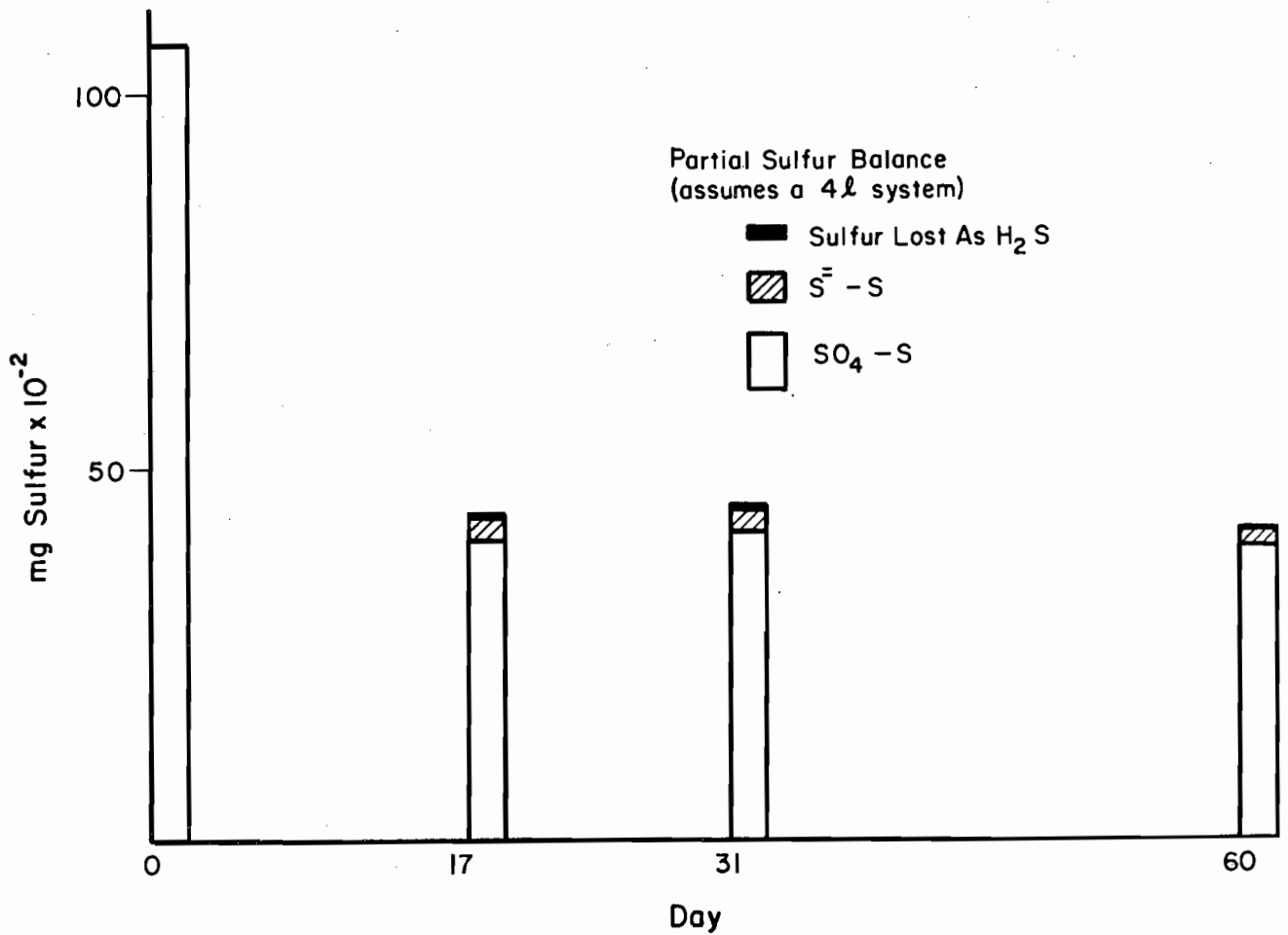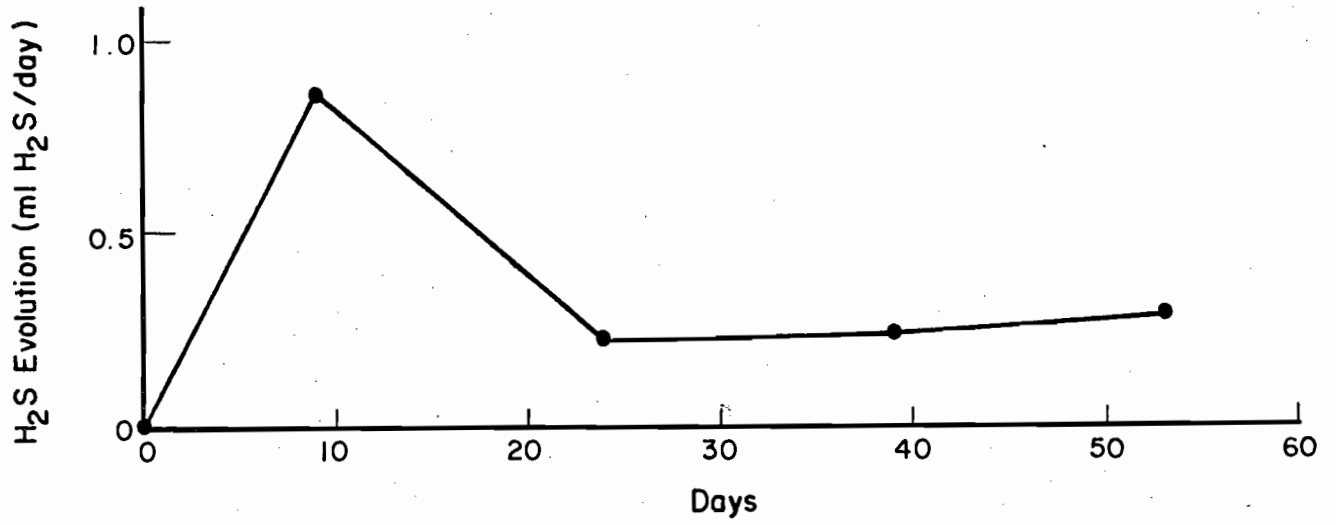
Figure 7. Hydrogen sulfide production and partial sulfur
balance in wood dust reactors.
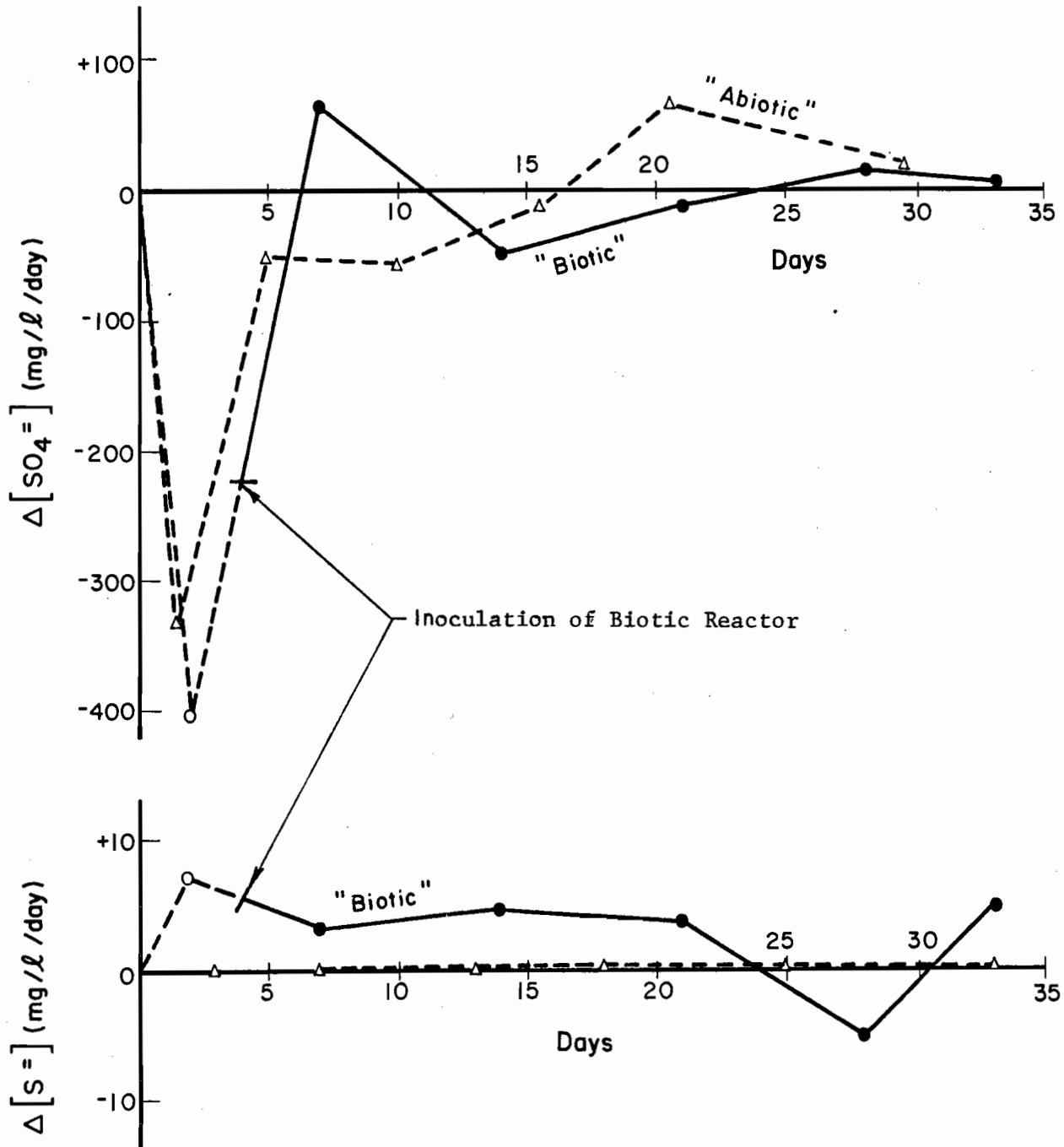
Figure 8.   Sulfide and sulfate concentration trends in wood dust reactors.
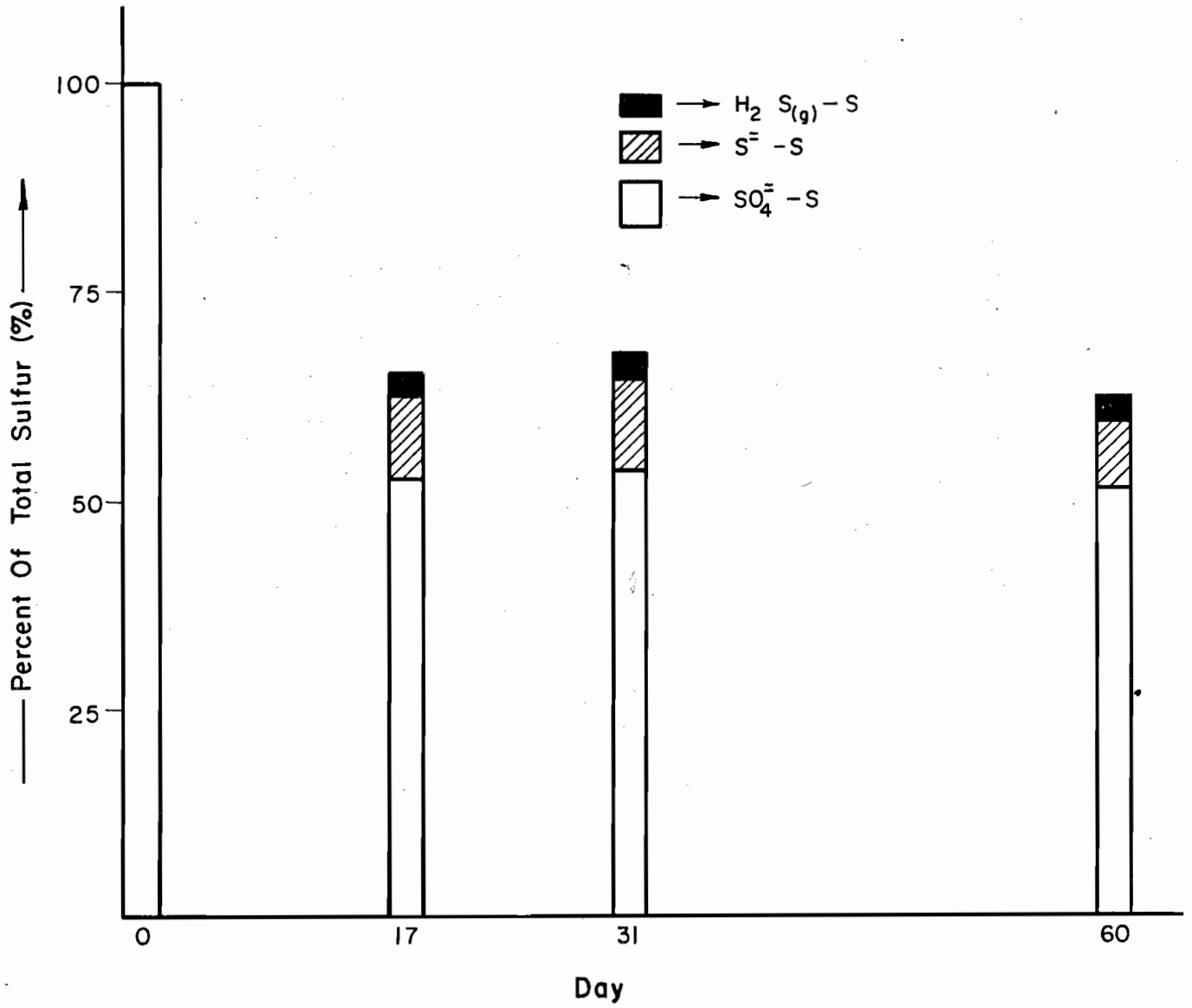
Figure 9. Partial Sulfur Balance in Wood Dust Reactors
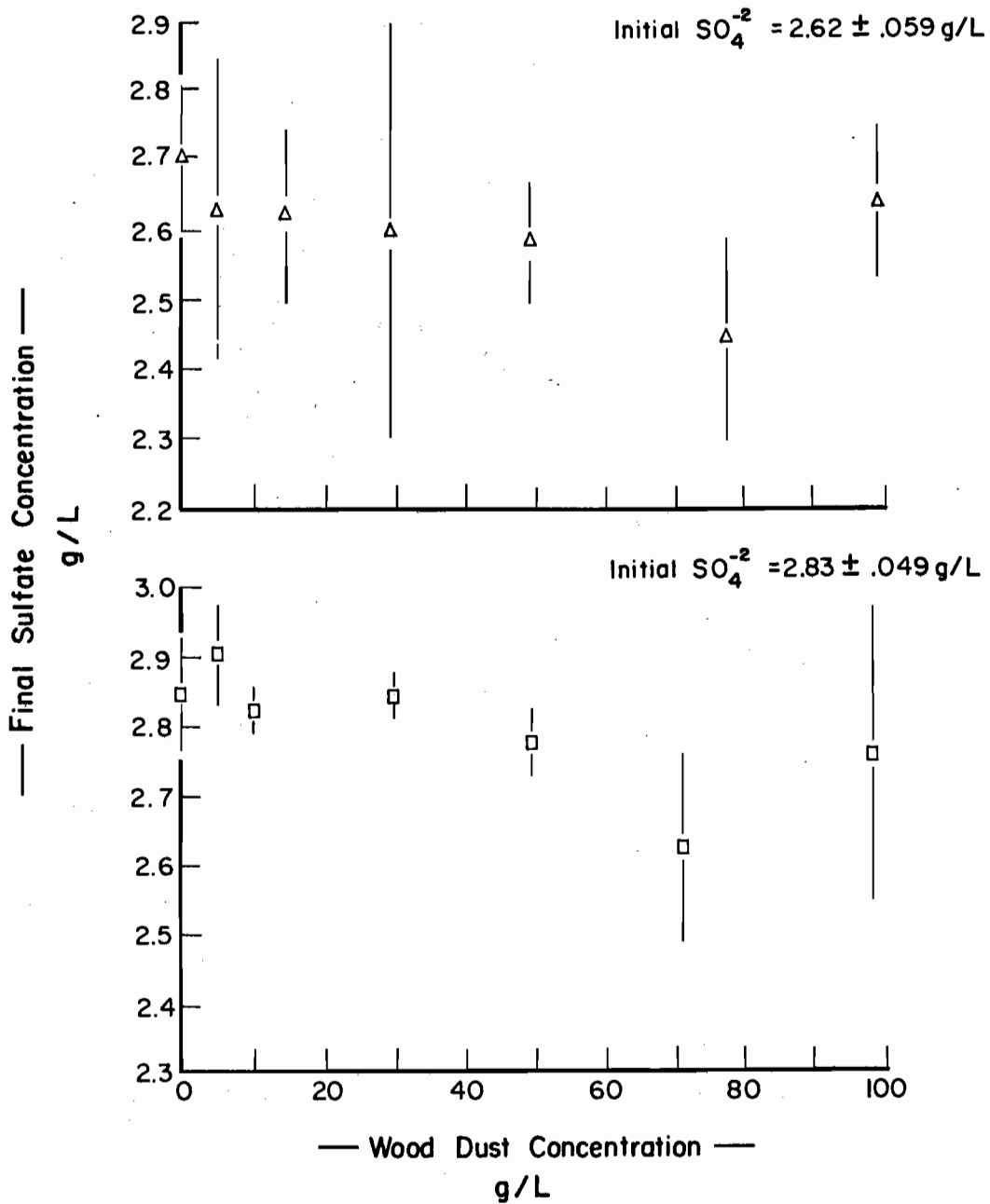
Figure 10. Final Sulfate Concentration as a Function of Wood Dust Quantity, $\square$-SO_4^{-2} Solution ($pH_i = 3$) $\triangle$-SO_4^{-2} Solution ($pH_i = 5$).

experimental results, but the delayed gas production (and drop in redox potential) in the abiotic reactor was unexpected. Although wood dust was sterilized, attempted sterilization of AMD solutions resulted in production of large quantities of metal precipitates. To maintain experimental consistency between reactors, only wood dust, not the entire AMD AMD/wood dust system,was sterilized. The observed changes in the abiotic reactor may be due to delayed microbial activity initiated by bacteria or spores contained in the AMD. The numbers of sulfate reducer organisms in the inoculated reactor showed an increase to a peak on day 10 then dropped off to a relatively constant level from day 25 to termination of the experiment. Hydrogen sulfide production followed the same trend. A calculation of a sulfur balance indicated an initial reduction in sulfate but only small quantities were lost as hydrogen sulfide.

Conclusions - The results of this experiment indicate that wood dust substrates suffer from the same difficulties identified for the foam substrate, loss of microbiological activity after a short time. Although all data point to some improvement in AMD quality, the sustained activity which would significantly improve water quality or modify the sulfate buffer system did not occur. Of particular interest and importance was the initial reduction of sulfate concentration which was observed in both experimental and abiotic reactors. It appears that the possibility exists for a physical-chemical alteration of AMD caused by the substrate alone. This result is of particular importance in planning any remedial treatment of severely degraded water quality and was the subject of further investigation as a part of this research.

2.4 WOOD DUST MODIFICATION OF AMD

The findings of the previous reactor studies indicated a possibility for abiotic modification of AMD. To further evaluate the importance of physical or chemical factors in AMD treatment a final series of experiments was conducted as a part of substrate evaluation. The following discussion of wood dust effects on AMD is based on a special project report prepared by Lubieneicki (1977) based on research conducted as a part of this project.

The effect of wood dust on AMD was evaluated using batch isotherms. A standard batch isotherm methodology was used to create a reaction between react wood dust (sorbent) and a standard volume (100 ml) of AMD (sorbate). From 0 to 10 grams of wood dust were used in 250 ml french-square bottles. Wood dust and AMD were placed in a rotating mixer maintained at 29°C. Wood dust consisted of sawdust from construction lumber, predominantly fir. Wood dust was sifted through a U.S. 14 standard seive (1.168 mm mesh) and retained on a 60 seive (0.0250 mm mesh). Two solutions were tested. A concentrated sodium sulfate solution was prepared with a reagent grade salt and a pH adjusted with sulfuric acid. This provided a reference sulfate solution which was compared with actual mine drainage, the second solution. The wood dust was dried at 110°C for one hour before being weighed. Sterilization of wood dust and bottles was conducted at 121°C for 20 minutes. AMD solutions were not sterilized because of precipitation problems previously noted. Sorbent and sorbate were mixed for eight days on a rotating mixer. After eight days the contents of each bottle was gravity filtered
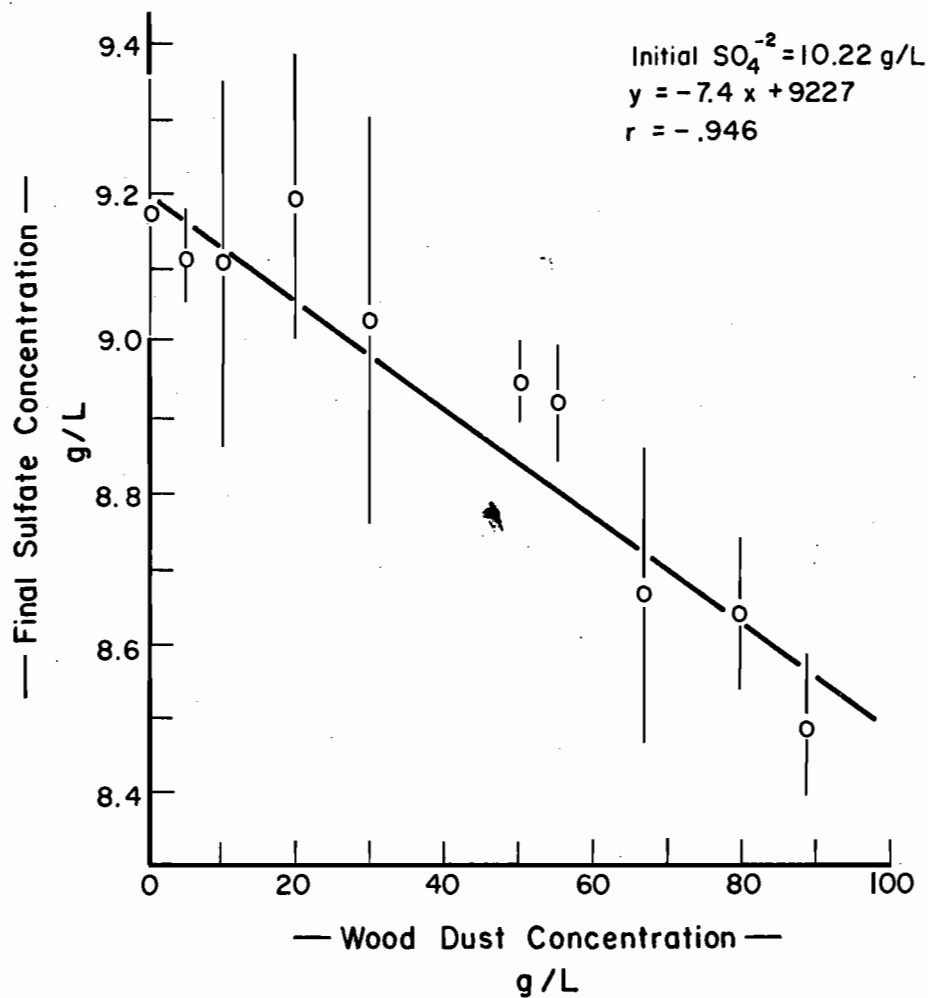
Figure 11. Final Sulfate Concentration of Acid Mine Water as
a Function of Wood Dust Quantity.
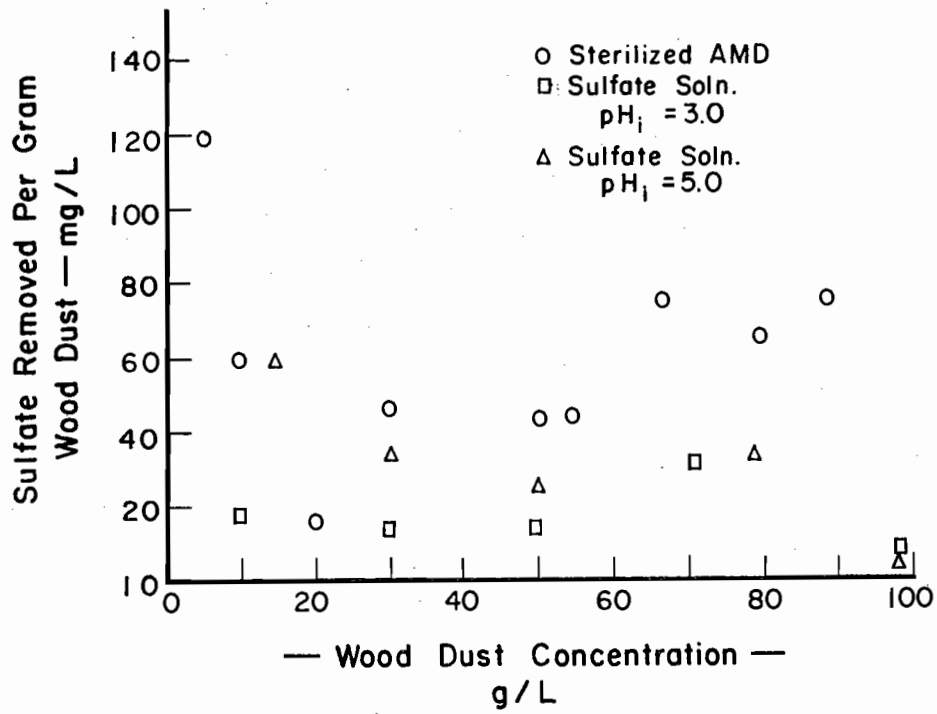
Figure 12. Observed 'Sorption Capacity' of Wood Particles
for Sulfate as a Function of Wood Dust Quantity
for all Solutions Analyzed.

through 4 Watman qualitative paper. Chemical analyses for pH, sulfates, acidity (mineral and total), iron, aluminium and total organic carbon were performed on the filtrate.

Results – The results of the isotherm studies will be discussed separately for each parameter:

Sulfate – The results of the analyses for sulfate for both reference sulfate and AMD sorbates are contained in Figures 10, 11, and 12. There is a direct correlation between increasing wood dust concentration and decreasing sulfates in reference solutions (Figure 10), with the majority of the data points significantly ($<.05$) lower than control values. This general trend is not statistically significant at the 5% level. This is due to the accuracy of the turbidimetric analysis for sulfate concentration which does not allow discrimination of small changes in sulfate ion concentration. Incomplete mixing was observed in the highest (10 g) wood dust concentration. This poor mixing likely caused the decreased sulfate removal at higher sorbent concentrations.

Analysis of sulfate in AMD solutions are illustrated in Figure 11. There is a statistically significant ($p<.05$) reduction in dissolved sulfates with increasing wood dust concentrations confirming observations made in previous studies. Possible mechanisms include chemisorption as negatively charged sulfate ions and nucleophilic carbonyl groups come into contact. In addition a precipitation reaction involving sulfate ions and complex lignin materials may also affect sulfate concentrations. The wood dust-AMD system is extremely complex and major additional studies would be required to

verify actual mechanisms of sulfate reduction. Although data was
scattered (figure 12) an average sorption capacity for the unbuffered
sulfate solutions was calculated at 30 mg sulfate/gram of wood dust.
This value agrees with results obtained from the abiotic reactor in
previous tests (estimated sorption capacity 33 mg sulfate/gram of
wood dust). Sorption capacity for wood dust-AMD solutions were
somewhat higher, 60 mg sulfate/gram of wood dust. This high capacity
may be due to the higher initial sulfate concentration of AMD or
characteristics of the complex AMD solution. Under ideal conditions
it appears that approximately 30% of the sulfate loss observed in
previous studies with wood dust as a substrate may be attributed to
abiotic processes.

Hydrogen Ion Activity (pH) - The effects of wood dust on pH is
shown in Figure 13. The pH of the unbuffered reference solutions
was affected by the wood dust. The wood dust changed the pH from
initial values of 3.5 and 6.5 to approximately 5.8. These changes
were a function of wood dust concentration. The AMD solutions did
not show significant pH alteration. The proposed mechanism for pH
change is hydrolysis of esters or protonation of negatively charged
ions such as the carbonyl oxygen ions. The formation of organic
acids could provide significant buffer capacity in the reference
solutions.

Acidity - Mineral and total acidity decreased with increased
wood dust concentration in AMD solutions, Figure 14. The slope of
the regression line for the reduction of mineral acidity was three
times that of total acidity. Mineral acidity was reduced by more
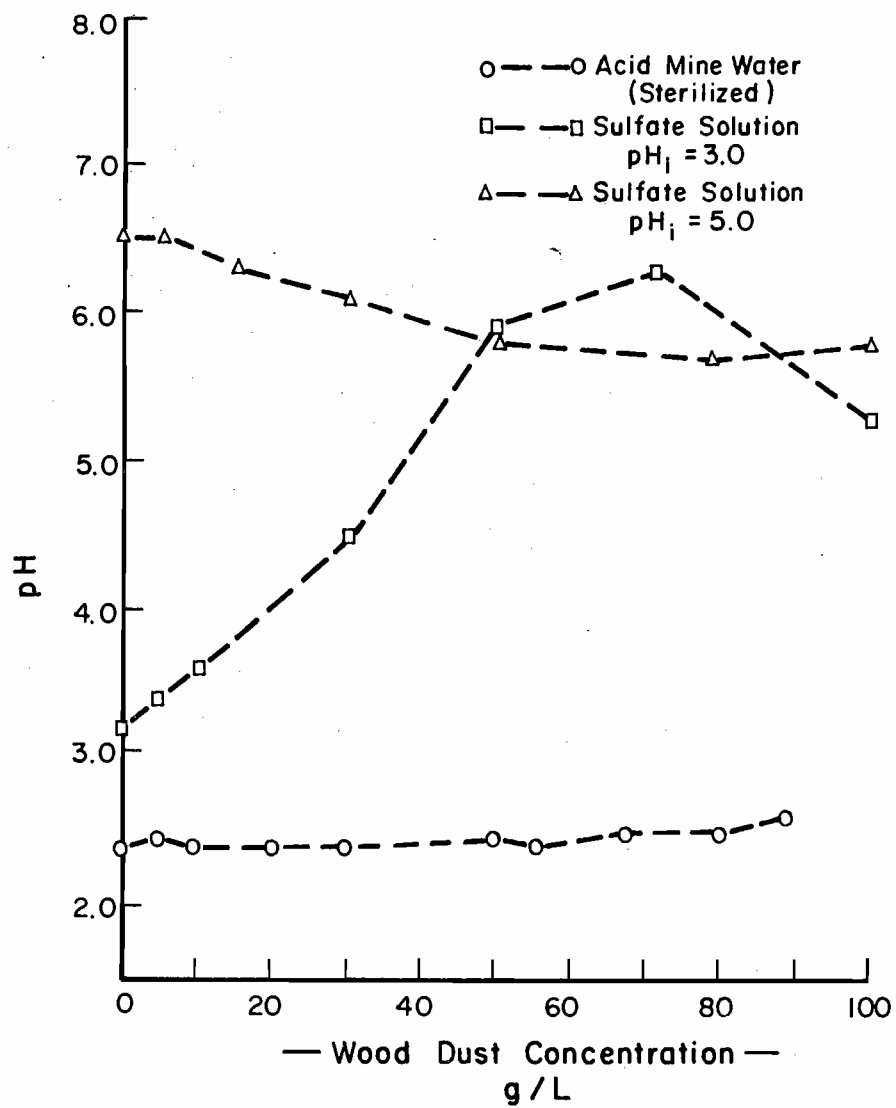
Figure 13. Hydrogen Ion Activity (pH) as a Function of Wood Dust Concentration for all Solutions Analyzed.
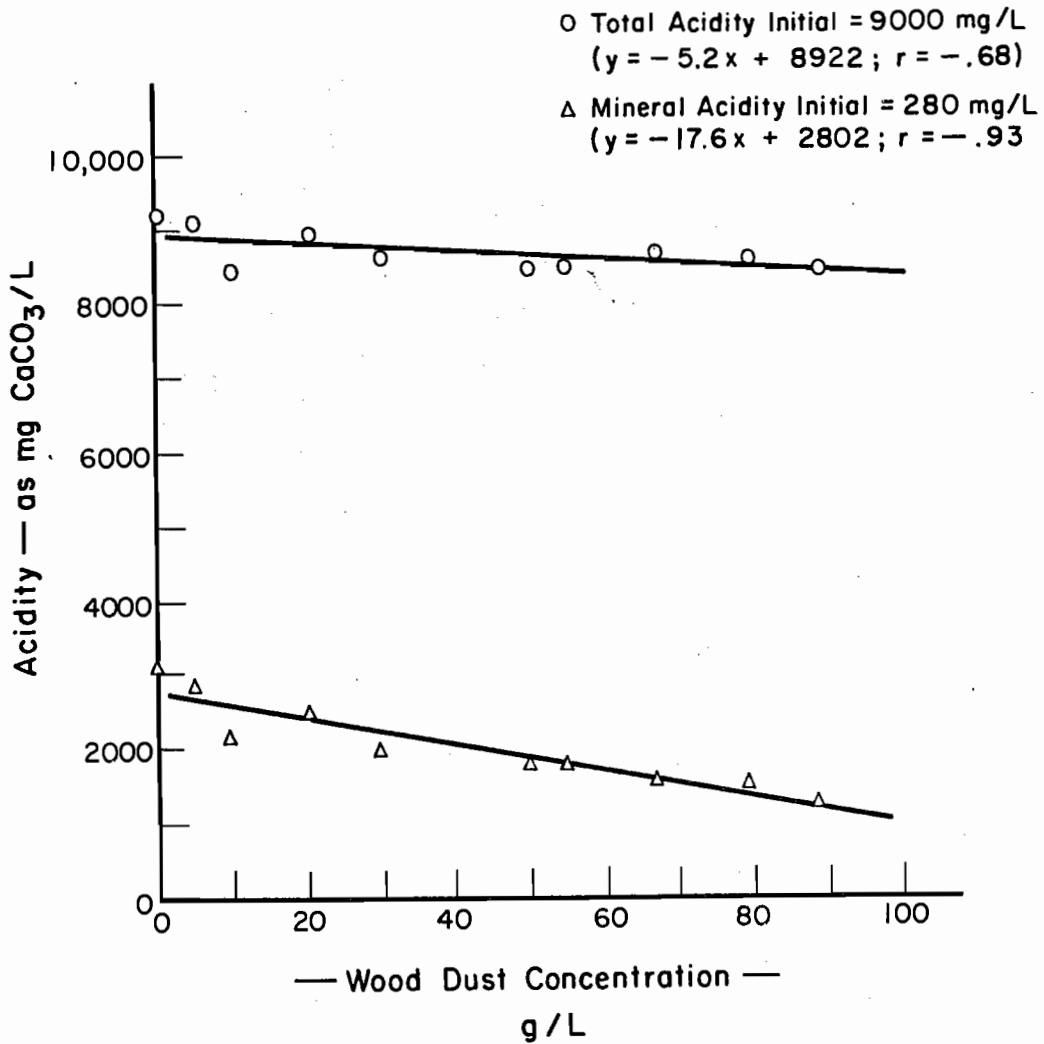
Figure 14.   The Mineral and Total Acidity of Acid Mine Water
as Effected by Varying Wood Dust Concentrations.

than 1800 mg/1 (as calcium carbonate) in the highest wood dust concentration. Figure 15 illustrates the reduction in acidity/gram of wood dust as a function of wood dust concentration. The reduction in acidity is greatest at low wood dust concentrations indicating the capacity for acidity reduction is not reached in higher concentrations of wood dust. If mineral acidity were reduced by 500 mg/g of wood dust, a pH increase of only a tenth of a pH unit would be expected. This calculation agrees well with observed pH increases.

Iron and Aluminium – Iron concentration was reduced with increasing wood dust concentration, Figure 16. As much as 80 mg iron/liter of AMD was removed in the isotherm studies. Possible mechanisms for the observed decrease in iron concentration include electrostatic sorption of charged iron and hydrated iron ions and chelation. Wood dust did not have a significant effect on aluminium concentration, Figure 17.

Total Organic Carbon (TOC) – Total organic carbon increased linearly as a function of wood dust concentration, Figure 18. This relationship is significant at the 5% level for all solutions. Approximately 11 to 14 mg of TOC leach from a gram of wood dust when exposed to the acid solution for eight days.

Conclusion – The presence of wood dust does alter the chemical characteristics of AMD solutions. Wood dust alone appears to reduce sulfate concentration, acidity, and iron concentrations. Although pH changes were not significant in AMD solutions, the effect of wood dust may be significant in AMD treatment. First, reduction in acidity
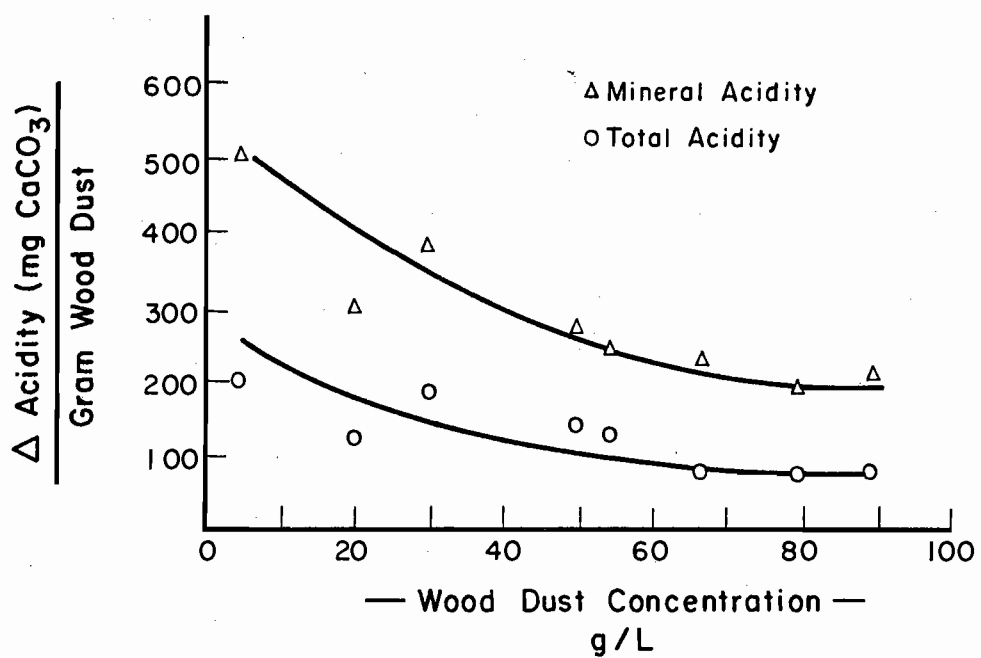
Figure 15. Acidity Reduction per Unit Wood Dust as a Function
of Wood Dust Concentration in Acid Mine Water.
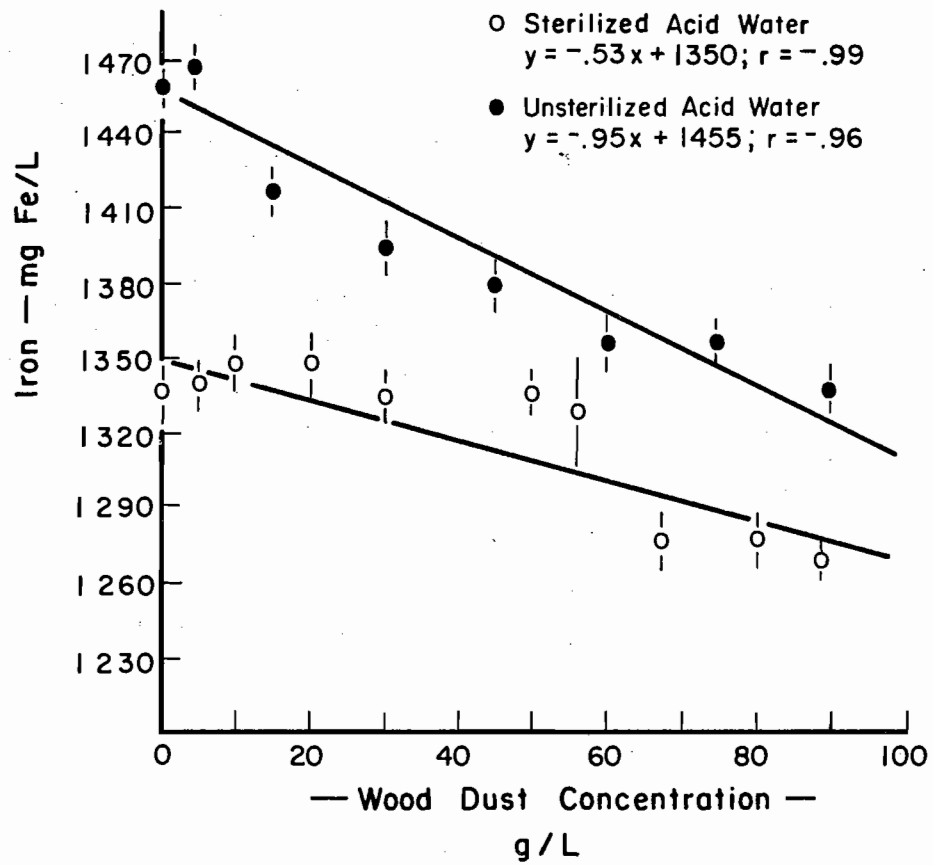
Figure 16. Final Iron Concentration as a Function of Wood Particle Quantity in Acid Mine Water; ●-initial Fe = 1458 ± 9 mg/1, ○-initial Fe = 1272 ± 9 mg/1.
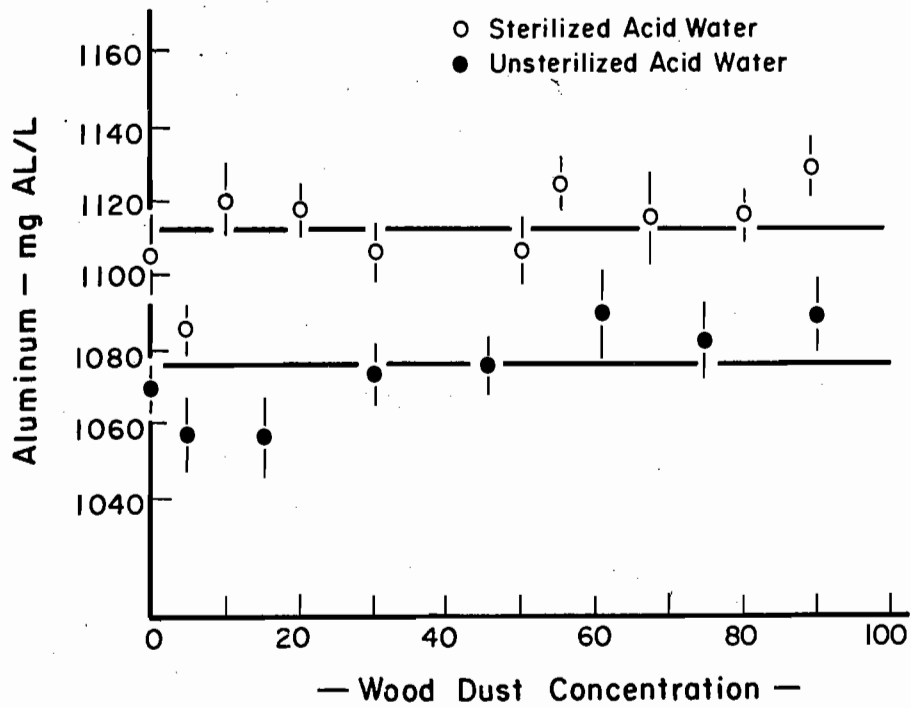
Figure 17. Final Aluminum Concentration as a Function of Wood
Dust Quantity in Acid Mine Water; -initial Al =
1060 ± 14 mg/1,   -initial Al = 1116 ± 10 mg/1.
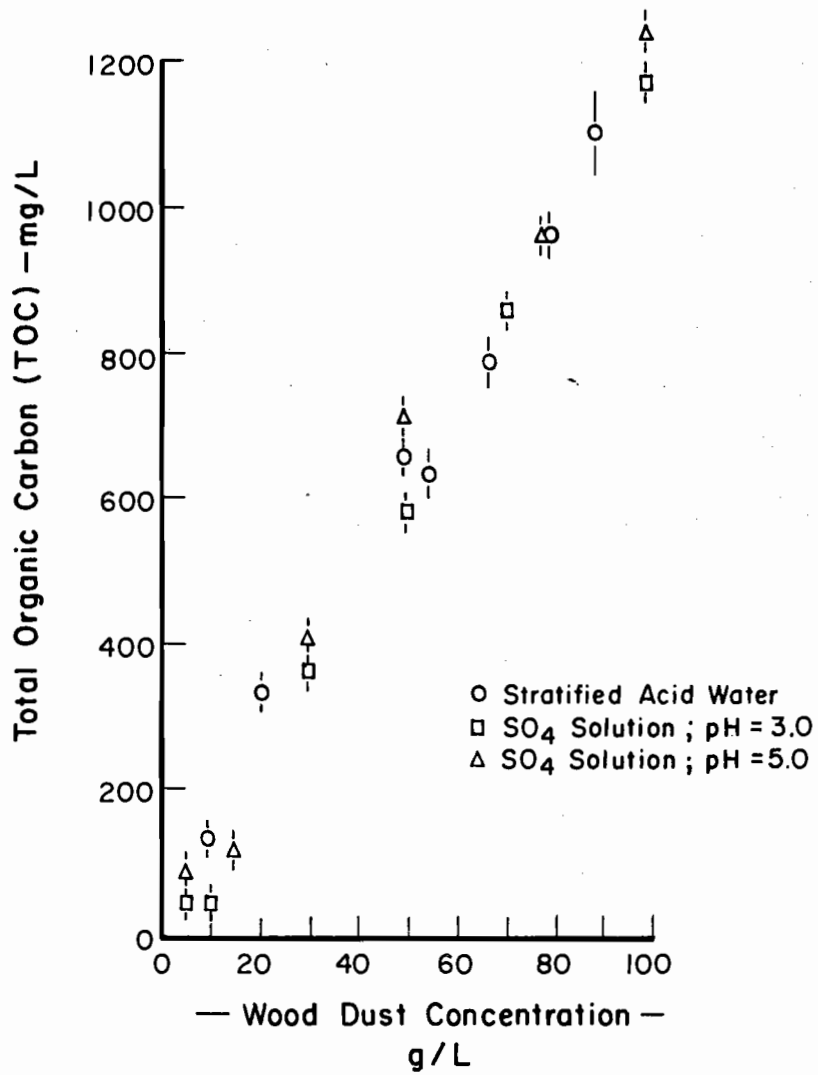
Figure 18.   Total Organic Carbon (mg/1) as a Function of
Wood Dust Concentration for all Solutions Analyzed.

and a slight change in pH, as well as the prospect for creation of a strong buffer system may all contribute to the development of microbial communities. A reduction in iron concentration may also improve water quality. The demonstrated release of soluble organics and the potential for sorption of sulfate onto wood particles may combine to provide energy resources and sulfate availability to enhance sulfate reduction. Thus wood dust alone may serve to improve environmental conditions providing an abiotic treatment of AMD. These results also suggest that the mechanism for pH improvement when wood dust is used is abiotic as well as biotic. The improvements noted in the literature (Tuttle 1969, Dugan 1972, King 1974) are likely due to a combination of mechanisms, including microbial activity.

2.5 CONCLUSIONS

In evaluating the potential for on-site treatment of AMD, a number

of methods were evaluated to determine if microbial activity could be

introduced to impounded AMD and controlled in such a way that water quality

improvement could occur in a relatively short time. A number of substrate

types were evaluated to determine if they could be used to "package"

microbial communities to effect treatment. The conclusions of these

studies were:

1) Mixed microbial cultures which support sulfate reduction
   can be easily established, but suitable energy sources
   for sulfate-reducing bacteria must be provided to
   maintain active cultures.
2) Either wood dust or solid waste material can be used to
   support sulfate reduction, although solid waste appears
   to provide a better support for sulfate reduction.
3) Use of sponge materials to provide "packages" for sulfate
   reduction showed little promise. Energy sources are
   limited, and environmental conditions are affected
   significantly by sponge as well as pore size.
4) Improvement of AMD solutions will require very large
   substrate/biomass packages to support appropriate microbial
   communities and control of environmental conditions in the
   "package" is critical.
5) Where improvement in AMD quality was noted, some of that
   improvement may be attributed to abiotic or
   physical/chemical interactions between the substrate and
   AMD solutions.
6) Wood dust was shown to improve water quality in AMD
   solutions without microbiological activity supporting its
   suitability as a material which might be considered for
   remedial AMD treatment.

Upon final evaluation of these conclusions it was apparent that

development of an AMD treatment system would require greater control of

effluent quality and reduced time for treatment. As a part of the previous

studies, particularly the evaluation of solid waste as a substrate, several

designs were developed to provide a unit process design for AMD treatment.

Preliminary testing indicated that it was not necessary to mix AMD directly

with a solid waste; improvement in water quality could be achieved by
using decant liquors from an anaerobic digester to neutralize AMD. From
this initial finding a unit process design was developed which separated
the anaerobic digestion process from the AMD neutralization process.
Through maintenance of an anaerobic reactor with decant liquors at a high
alkalinity, neutralization of AMD could be achieved. In preliminary
testing it was also determined that sulfides could be used to precipitate
heavy metals. The design which was developed from these initial studies
and the actual pilot evaluation of treatment effectiveness is discussed
in the following section of this report.

3.0 PILOT PLANT DESIGN AND TESTING

The development of a pilot plant design for the treatment of AMD was an evolutionary process initiated during the final stages of substrate analysis. An initial series of tests was conducted with a small anaerobic reactor which produced a liquor which was mixed with small quantities of AMD. This preliminary research conducted by Hays (1978) to partially fulfill his requirements for a Master's of Science in Environmental Science led to the development of a final pilot plant configuration. The testing and analysis of the final pilot plant configuration was conducted by Cohen (1978) and Vriesman (1980) in partial fulfillment of the requirements for a Masters of Science in Environmental Science and Master's of Science in Environmental Engineering respectively. The study conducted by Vriesman was under the direction of Professor John Pfeffer. The following review of the basis for pilot plant design and pilot plant operation is summarized from Vriesman (1980).

The objectives of the pilot plant study included evaluation of the feasibility for operation of a continuous biological treatment process for AMD, determination of operational parameters for the treatment process, evaluation of reductions in sulfate and metals concentrations, evaluation of the potential for improvement of pH, and determination of the solids produced, both the metal bearing sludges and the products of anaerobic digestion.

3.1 BIOLOGICAL TREATMENT OF AMD

The principal objectives of the biological treatment process for AMD include reduction of sulfates to sulfides, precipitation of soluble metals, and pH improvement. Since Desulfovibrio and Desulfotomaculum have been identified as being capable of dissimilatory inorganic sulfate reduction under anaerobic conditions, provision for maintenance of these bacteria is an essential element of the proposed treatment process. A net pH increase and a reduction in soluble metals may simultaneously occur due to microbial sulfate reduction through precipitation of metals in solution which alter the buffer system in AMD.

Postgate (1951) has suggested the following sulfate reduction process:

$$SO_4^= \xrightarrow{H_2} SO_3^= \longrightarrow [unkonwn\ intermediates] \longrightarrow S^= \qquad (3-1)$$
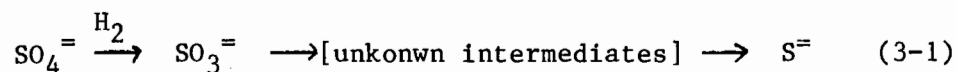
For this reaction to occur a carbon source, such as lactic or acetic acid,is required as a hydrogen doner for sulfate reduction. A number of species are capable of sulfate reduction,and Campbell and Postgate (1965) have classified species on the basis of carbon sources utilized. The species Desulfotomaculum acetoxidans has been associated with acetic acid as an energy source. Since a number of organic carbon sources can be used by the bacteria during dissimilatory reduction of sulfate to sulfide, selection of carbon source materials will affect species composition as well as decomposition end products. For example, in a system which utilizes acetate as the primary carbon source, complete oxidation will result in a carbon dioxide end product. Under favorable operating conditions such a system should not produce a substantial waste organic output. Under the conditions, pH of 5.5 to 7.5, and redox of -150 to

-250 mV, sulfate reduction is expected to occur in the following manner (Widdel and Pfenning 1977):

$$
\begin{array}{ll}
CH_3COOH + 3\ OH^- \rightleftharpoons 2\ CO_2 + H_2O + 5\ H^+ + 8\ e^- \\
6H^+ + SO4^= + 8\ e^- \rightleftharpoons H_2S + 4\ OH^= \\
\hline
CH_3COOH + H^+ + SO_4^= \rightleftharpoons H_2S + 2CO_2 + H_2O = OH^- \\
H^+ + OH^= \rightleftharpoons H_2O \\
\hline
CH_3COOH + 2H^+ + SO_4^= \rightleftharpoons H_2S + 2\ CO_2 + H_2O
\end{array}
\qquad (3\text{-}2)
$$
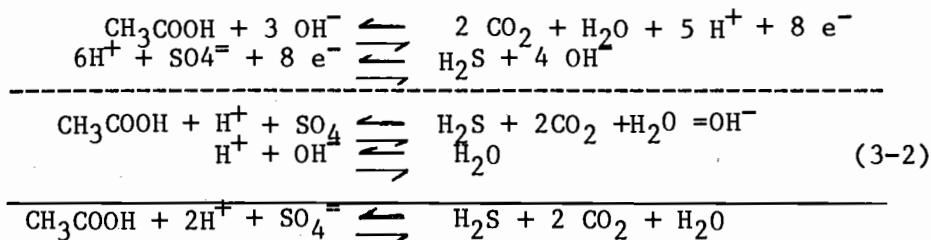
Similar reactions can be expected if other carbon sources are supplied.

Considering the solubilities of various metal sulfide species (Smith, 1978) some of the metals in the AMD may precipitate in a system where hydrogen sulfide is being produced by sulfate reduction. An example of the precipitation reaction using ferrous iron is shown in equation 3.2.

$$
Fe^{++} + H_2S \rightleftharpoons FeS + H^+
\qquad (3\text{-}3)
$$
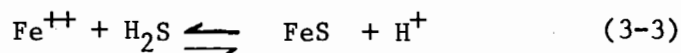
Metal sulfides which precipitate typically produce a distinctive deep black sludge. While many metals precipitate as sulfides, Al^{+3} and Fe^{+3} may precipitate as hydroxide species. From equations 2-1 and 2-2 it can be seen that both sulfate and iron and other heavy metals can be removed from AMD utilizing the biological treatment process. The net reaction is:

$$
\begin{array}{ll}
CH_3COOH + 2\ H^+ = SO_4^= \rightleftharpoons H_2S + 2\ CO_2 + 2\ H_2O \\
Fe^{++} + H_2S \rightleftharpoons FeS + 2\ H^{+2} \\
\hline
CH_3COOH + SO_4^= + Fe^{++} \rightleftharpoons 2\ CO_2 + 2\ H_2O + Fe
\end{array}
\qquad (3\text{-}4)
$$
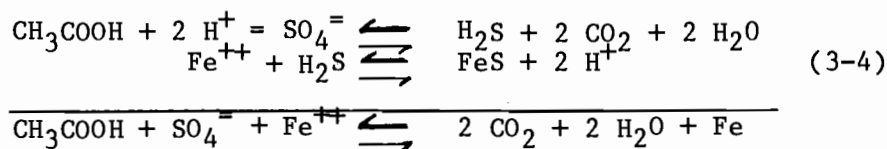
Examining equation 3.3 in detail shows that as long as iron or other metals
are available for precipitation, no net change in pH should occur. A
slight change in pH may be expected, due to dilution effects when AMD
and waste streams are mixed, but no significant modification of AMD
chemistry will occur until the buffer system in the AMD is changed. If
no metals are available for precipitation equation 2-1 suggests that excess
hydrogen sulfide may develop in the system. Although sulfide is not highly
toxic to sulfate-reducing bacteria (Miller, 1950) the following
disadvantages to high sulfide concentrations should be noted:

a) high sulfide concentrations are toxic to fermentative
   bacteria and may affect availability of carbon sources
b) high sulfide concentrations may slow down the rate of
   sulfate reduction
c) high concentrations of hydrogen sulfide affect hydrogen
   ion removal capabilities, and
d) hydrogen sulfide above 10 to 14 mg/1 are hazardous to
   man.

Thus in developing a biological treatment process a number of factors
should be considered. The process should produce sufficient sulfide to
precipitate the soluble metals present in the AMD, but sulfide
concentrations which might inhibit digestor activity should be avoided.
A continuous organic carbon source must be supplied, and suitable
environmental conditions should be maintained to assure high treatment
efficiency.

The form of sulfide present at any one time is primarily a function
of the treatment stream pH, temperature, and partial pressure of the
hydrogen sulfide gas in the headspace of the reactor. The sulfide
concentration will be governed by equilibrium relationships identified

in the following equations:

$$H_2S \text{ (g)} \longrightarrow H_2S \text{ (aq)} \qquad K_H = 1 \text{ X } 10^{-1}$$
$$H_2S \text{ (aq)} \longrightarrow H^+ + HS^- \qquad K_1 = 1.1 \text{ X } 10^{-7}$$
$$HS^- \longrightarrow H^+ + S^= \qquad K_2 = 1 \text{ X } 10^{-4}$$

These equations suggest that methods for removal of excessive

sulfide concentrations from the treatment stream may be required

as part of a treatment process.

In order for sulfate-reducing bacteria to survive, a continuous organic

carbon source must be supplied. An anaerobic fermentor (digestor) can

supply the necessary organic carbon (Metcalf and Eddy, 1979). By using

a high cellulosic content substrate in the digestor, a carbon source in

the form of short chain organic acids should be produced. The primary

microorganisms involved in the production of organic acids are cellulolytic

and acid-forming bacteria. The process of acid formation is shown below

(Bryant, 1979):

Cellulose $\longrightarrow$ Soluble Organics $\longrightarrow$ Low Molecular Weight
$\uparrow$ $\uparrow$ Organic Acids
Cellulolytic bacteria   Fermentative bacteria
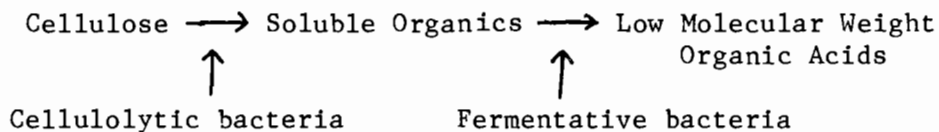
The cellulolytic bacteria act on the highly polymerized cellulosic

substances breaking them down to lower molecular weight soluble organics.

These soluble organics are utilized by fermentative bacteria producing

short chain organic acids. Since biosynthesis is maintained during this

process, necessary growth factors must be provided to maintain continuous

microbial function.

Digestor liquors maintained with high concentrations of organic acids

as well as available sulfide can be mixed with AMD with the expectation

that metals will be precipitated and sulfates reduced by sulfate reduction.

Since the proposed treatment is anaerobic, aeration of the process effluent

will be necessary. In addition, process stream clarification and solids

disposal should be considered as part of the process. Thus a biological

treatment process for AMD should include the following process elements:

1) an anaerobic digestor to provide an organic carbon source
for sulfate-reducing microorganisms
2) a reactor where AMD, organic carbon, and free sulfide are
introduced to precipitate metals, improve pH, and reduce
sulfate concentrations
3) a solids settling tank for effluent clarification
4) an aeration basin to polish the effluent, and
5) a hydrogen sulfide removal system to prevent microbial
inhibition in the anaerobic digestor

3.2 EXPERIMENTAL DESIGN AND ANALYSIS PROCEDURES

The pilot AMD biological treatment results reported in following sections were obtained from a system which was the product of several modifications to an initial design which met the requirements stated in the previous section. The configuration of the pilot plant for the final treatment evaluations is shown in Figure 19. All liquid pumps used were FMI laboratory-scale variable speed pumps. A General Electric portable pressure/vacuum pump was used for gas mixing and partial sulfide stripping. AMD used during the treatment process was pumped directly from a storage tank. To prevent bacterial slime development in supply lines (caused by low flow rates) hoses were periodically flushed with tap water.

The reactor where AMD and digestor liquors were mixed was a stainless steel tank with a 57.2 cm (22.5in) ID and an effective height of 64.8 cm (25.5 in). The total working volume of the reactor was 166 liters (44 gallons). A 7.6 cm (3 in) headspace was maintained and the reactor was partially sealed to assist in maintaining anaerobic conditions. A variable speed mixer was used in the reactor to periodically mix the contents. Mixing was achieved with a paddle with a small surface area. Influent entered at the bottom of the reactor and exited at the top. Recycled sludge was pumped through the reactor cover. All samples taken from the reactor were collected at mid depth using a siphon hose through the cover.

Effluent from the reactor was gravity fed to the settling tank. This tank was a 100 liter (26 gallon) plastic trash barrel with an effective depth of 49.4 cm (19.5 in) providing a working volume of 68 liters (18 gal). Influent entered the settling tank approximately 23 cm (9 in) below the water surface to aid in solids removal. Supernatant flowed from a depth
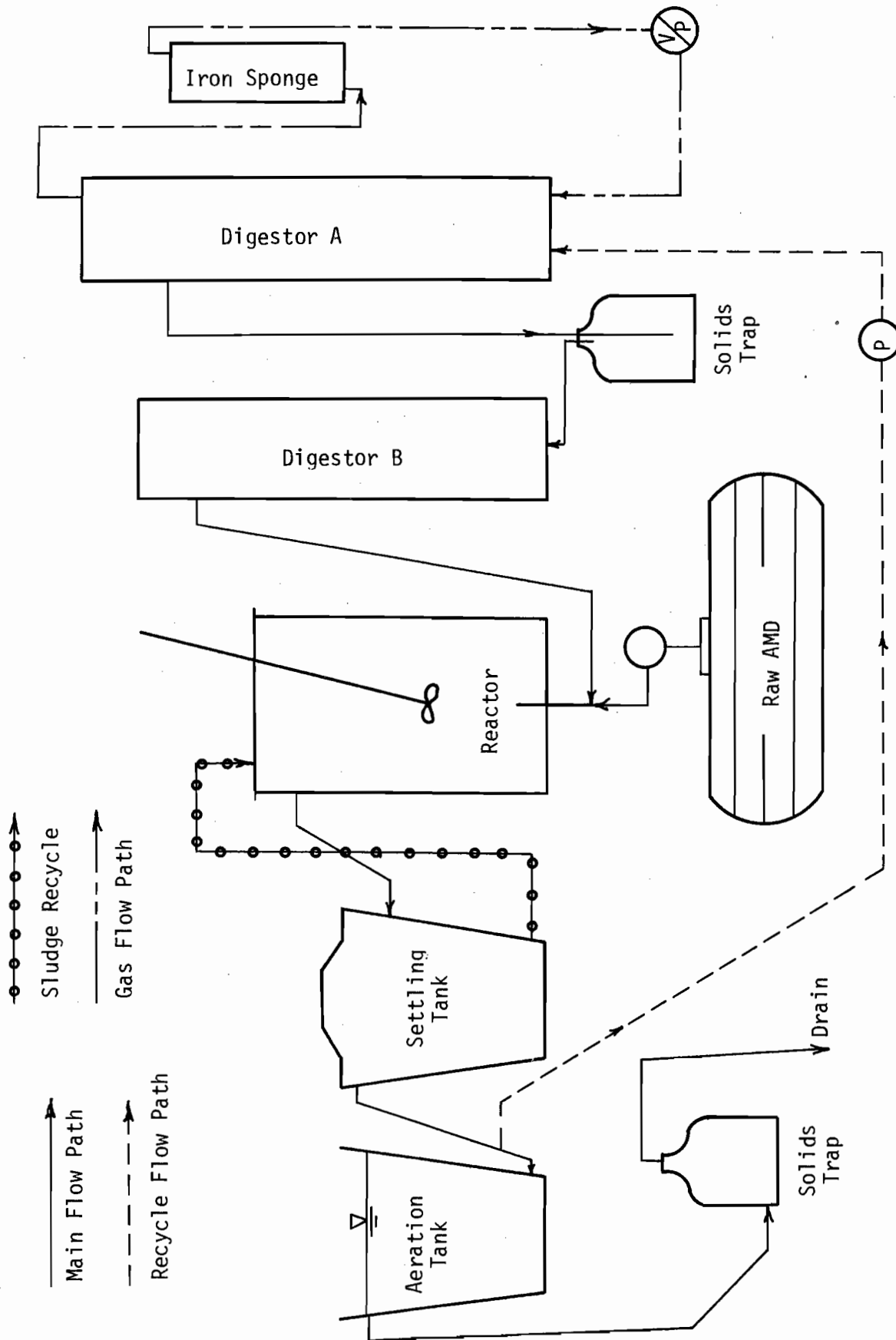
Figure 19. Schematic of AMD Biological Treatment System

of 49.5 cm (19.5 in). An effluent port at the bottom of the settling

tank was used for sludge recycle to the reactor. The settling tank was

covered and no attempt was made to aerate the process waters.

Effluent from the settling tank had two flow paths. Flow was either

directed to an aeration tank or recycled flow to the digestors. Settling

tank effluent was fed to the bottom of the aeration tank by gravity.

The aeration tank was similar to the settling tank but no cover was

provided. Air was supplied from a compressed air line in the laboratory.

The aeration tank was used to polish the effluent by maintaining aerobic

conditions, oxidizing any remaining metals, and mixing precipitated solids

prior to final settling.

Upflow clarification of the aeration tank effluent was obtained by

passing the flow through a glass solids trap with a working volume of

approximately 4 liters (1 gallon). Effluent from the solids trap was

periodically analyzed for solids, sulfate, metals, and pH to evaluate

treatment efficiency. A portion of the settling tank supernatant flow

was used as recycle flow to the digestor.

Two digestors were utilized in the final configuration of the pilot

plant. Digestor A was 19 cm (7.5 in) ID plexiglass, 188 cm (74 in) tall,

while Digestor B was the same diameter and 175 cm (69 in) tall. A FMI

pump was used to circulate process waters through the digestors. The

flow would enter at the bottom of the digestor and exit at a height of

155 cm (61 in) for an effective working volume of 44 liters (11.6 gal)

for Digestor A, and at a height of 145 cm (57 in) for an effective working

volume of 41 liters (11 gal) for Digestor B. A headspace of 33 cm (13

in) was maintained in each reactor. The digestors were filled with

shredded computer paper to provide an organic carbon substrate. The paper expanded when wet; thus retention times based on effective working volumes were less than the empty bed volume. Process waters were pumped into the bottom and withdrawn from the top of the digestors. The digestors were wrapped with electric heat tape and insulated. Temperature was maintained near 60°C (140°F). During final phases of the study Digestor A was equipped for gas stripping and mixing using a combination vacuum/pump. A solids trap was used between Digestor A and Digestor B to prevent solids from clogging the connecting lines. The flow from Digestor B was gravity fed to the reactor.

During the final phase of the study, an iron sponge was used to strip hydrogen sulfide from Digestor A. The sponge was a 10 cm (4 in) ID plexiglass column, 71 cm (28 in) in length packed with wood shavings soaked in a ferric oxide slurry. By using a combination vacuum/pump, gas from Digestor A was stripped of hydrogen sulfide. From time to time the packing in the iron sponge was replaced with fresh material to maintain hydrogen sulfide removal capacity. This process assured that sulfides generated from sulfate reduction in Digestor A would not inhibit microbial activity in Digestor B; thus additional sulfate reduction capacity was provided in the digestors.

3.3 SYSTEM OPERATION AND SAMPLING

At start-up the treatment system was filled with approximately 250
liters (66 gal) of raw sewage. This initial filling of process units
(reactor, digestors, and settling tank) provided a microbial seed as well
as sufficient liquid to provide process waters for the digestors.
Digestor A was filled with 5.25 kg (11.5 lb) of shredded computer paper.
Analysis of this paper indicated 21% fixed solids plus lignin; the
remainder, 79%, was biodegradable. Circulation of liquid in the system
was initiated and AMD was added when the level of organic acids present
in the effluent from Digestor A reached approximately 100 to 200 mg/1
total organic acids (TOA). Three months after Digestor A was established
(with system start-up), Digestor B was connected in the flow stream.

The FMI pumps were calibrated on a regular basis. Flow rates
were measured rather than calculated to minimize error. Analyses
of pH, redox (ORP) and total organic acids were performed daily.
Other parameters were monitored three times a week. Four sampling
locations were established: 1) Digestor B effluent, 2) settling
tank supernatant, 3) reactor contents, and 4) aerator-mixed
liquor. Other locations were sampled periodically to evaluate
specific unit operating conditions.

Parameters selected to evaluate microbial activity included
pH, ORP, TOA, soluble sulfides, and alkalinity. Other parameters
monitored during the study included acidity, soluble metals,
sulfates, and solids. Samples not analyzed immediately were

preserved with nitric acid and stored at $4^{\circ}C$ ($39^{\circ}F$). All analyses
were performed according to the 14th edition of Standard Methods.

pH was measured daily using a Beckman Electromate pH Meter
     with a Sensorex combination glass electrode

ORP was measured daily using an Orion model 407A
     potnetiometer with a combination redox electrode.
     ORP was measured in millivolts (mV) of Standard
     Calomel Electrode units (Ec). No attempt was made to
     correlate Ec values to standard Eh.

Alkalinity was measured by titrating samples to a final
     pH of 4.3. Soluble alkalinity was measured by passing
     samples through a 0.45u milliport membrane filter.

Total Organic Acids were measured daily using the column
     chromatographic separation method. Supernatents were
     analyzed; nitrogen gas was used to purge the eluted
     samples during titration. Because the method did not
     differentiate between various types of short chain
     organic acids a gas chromatographic analysis was made
     of a typical sample.

Soluble Sulfides were measured by the iodine-thiosulfate
     colormetric back titration method. Samples were
     initially coagulated with aluminum chloride. The
     clear supernatent was used for analysis.

Acidity was measured by titrating to a pH of 8.3.
     Both cold and hot (heated to 90+ C) were perforned.

Metals were analyzed using a Perkin-Elmer Model 370
     Atomic Absorption Spectrophotometer. Samples were
     passed through a 0.45u millipore filter. Iron, nickel
     zinc, and coper were determined by direct absorption
     using an air-acetylene flame; aluminium analysis
     required a nitrous oxide flame. Interferences to the
     iron and aluminium analyses were corrected for by
     reacting the samples with thiocyanate ion and passage
     through an ion exchange column.

Sulfates were determined using the turbidimetric method
     and a Bekman Acta III Spectorphotometer. Samples were
     filtered through 0.45u millipore filters and preserved
     with nitric acid; large dilutions minimized
     interferences. Gravimetric analysis was performed to
     evaluate accuracy of the turbidimetric method. There
     was close agreement between the two values.

Solids were determined at 103 C for total solids and 550 C
     for fixed solids.

In addition to the regular monitoring identified above, the
biodegradability test on the computer paper used a concentrated acid
hydrolysis technique. Metal composition of sludges was determined by

atomic absorption analysis following ashing samples at 550 C and redissolving metals in concentrated sulfuric acid. Metals were recorded as percent of total solids analyzed. Settling rate tests followed the procedure outlined in Metcalf and Eddy (1979) using a 1000 ml graduated cylinder with a slow speed mixing device.

3.4 RESULTS AND DISCUSSION

The final configuration of the pilot plant for the biological treatment of AMD was the result of a series of initial studies, in particular, analysis of start-up performance. Although the emphasis in these discussions of results is directed toward monitoring of the final system configuration, a discussion of start-up observations and monitoring of the performance of early configurations of the treatment system will provide insight into conditions necessary for operational success and provide support for design modifications which led to the final treatment system configuration.

The digestor was filled with shredded computer paper and raw sewage and allowed to circulate for approximately 30 days. Initial results indicated cellulose fermentation rates exceeded sulfate reduction rates as total organic acid (TOA) increased to 600 mg/l and the pH dropped from 7.4 to 5.9. An increase in sulfide production was accompanied by a reduction in TOA and an increase in pH indicating the development of a sulfate-reducing population in the digestor. Soon after the onset of sulfide production, the TOA dropped to less than 25 mg/l. At this point (4 days after start-up) the digestor was wrapped with heating tape and insulated and a mesophilic temperature of $32^{o}C$ was obtained. Following heating, TOA increased and pH decreased, and after a short lag period, sulfate reduction again increased and a sulfide concentration of 140 mg/l stabilized. An equilibrium was evidently reached because TOA remained constant between 100 and 200 mg/l. Gas chromatographic analysis indicated only acetic acid as the primary constituent of the digestor effluent. These results suggest that a carbon limitation affected sulfate reduction,

with increases in sulfide lagging behind increases in TOA. Analysis of digestor contents indicated TOA concentrations of 600 mg/l in the middle, while the effluent remained relatively constant in the range of 100 to 200 mg/l. There were no metals in the digestor effluent, the pH remained between 6.3 and 6.5, and alkalinity remained near 300 mg/l. The retention time in the digestor was approximately one day.

It is possible to calculate general digestor performance from the analyses for sulfate, sulfide, TOA, and alkalinity. The sulfate loading at equilibrium was 2.06 x 105 mg/day while the export was 1.04 x 105 mg/day. A net sulfate reduction (accounting for some variability during the analysis period) is 4.67 x 102 mg/l/day or 4.85 m mole/l/day. The average iron loading was 26.6 m mole/day with an export of 0 suggesting a sludge production of 2.3 gm/day as FeS, requiring approximately 19 mg/l/day sulfide.

Because the system was recirculated the input of sulfide was 125 mg/day and a net output of 6.69 x 103 mg/day. This would correspond to a net production of 4.65 m mole/l/day. The average of 4.75 m mole/l/day will be used in the following calculations. This rate agrees well with the semi-batch reactor of Rice and Rabolini (1972) in which digestor sludge and AMD were mixed.

The organic acid input was 0 with an average output of 150 mg/l. From the average sulfate reduction rate of 2.1×10^2 m mole/day and equation 4-1 a net organic acid production of 435 mg/l/day (320 m mole/day) is calculated. In addition it is possible to estimate alkalinity production. Since two equivalents of alkalinity are produced per mole of sulfate reduced (in the absence of iron), a crude alkalinity production

rate of 475 mg/1/day is calculated (neglecting the remaining organic acids and alkalinity lost in iron sulfide precipitation). This is consistent with the observed average effluent alkalinity of 400 mg/1 and is useful in selecting digestor effluent AMD:mixing ratios.

After approximately 30 days of recirculation, AMD was added to the reactor and a series of analyses were performed to evaluate initial reactor performance. The detention time in the reactor was approximately three days. The sulfate concentration in the effluent soon after start-up was 5000 mg/1 but decreased steadily to 3000 mg/1. This indicates there was a lack of significant sulfate reduction in the reactor. Sulfides in the settling tank remained between 2 and 4 mg/1. The reactor depended on the digestor as a source of alkalinity. The AMD contributed 2.74×10 mg/day acidity while the digestor provided 1.67×10^4 mg/day alkalinity. The result was a slow decline in pH dropping below 6.0 on the 23rd day after AMD flow initiation. At this time soda ash was used to provide additional alkalinity. A change in the digestor:AMD flow ratio from 3:1 to 5:1 would also have met the alkalinity demand.

The treatment system demonstrated a significant reduction of metals. The aluminium loading was 3.18×10^3 mg/day; with complete removal, 9.2 g/day of aluminium hydroxide sludge was produced per day. Iron loading was 43.35 m mole/day while the average export (to the aerator) was 35 m mole/day indicating 0.73 gm of iron sulfide sludge was produced. The aerator provided complete removal of iron, producing 1 g/day ferric hydroxide. Zinc, copper and nickel were removed but contributed an insignificant amount to the mass balance of the sludges.

With the results of initial system operation available, another series of experiments was performed. These tests were performed on the system after approximately three and four months of operation with an emphasis placed on those results obtained in the final 20 days of operation when the system was at equilibrium.

During approximately the last 20 days of pilot plant operation it was possible to obtain the highest total organic acid (TOA) concentrations from the digestor. Treatment efficiencies for this 20 day period were compared to a prior test period when TOA concentrations were much lower (Table 4-1). Average flow rates were approximately equal during both periods. These flow rates are 1.0 1/hr (9/20 - 9/25) and 1.04 1/hr (9/27).

Table 4-1 summarizes various treatment parameters for the two test periods (low and high TOA). During both periods the overall metals removal efficiency was determined to be 100% (as calculated within the analytical range examined). Such levels of metal removal were expected for two reasons. First, according to the theoretical reactions which should occur during treatment and the ratio of "possible" sulfide equivalents to metal equivalents in the AMD, more than an adequate supply of sulfides was available to precipitate the metals. Second, "excess" sulfides were actually detected in the settling tank effluent and evidence of metal sulfide sludge production was apparent.

Not given in Table 4-1 is the average pH and soluble alkalinity for the settling tank effluent during the two test periods. For the period 9/20-9/25, the average pH was 6.5 and the average alkalinity was 645 mg/1 as $CaCO_3$. Likewise for the latter period, average pH

Table 4-1. Average Treatment System Characteristics for Two Different Test Periods

Time Span	TOA* (mg/l)	$SO_4^=$ Reduction Rate in Reactor (mg/l·D)	$S^=$ in Reactor Effluent (mg/l)	Ave. ORP in Reactor (mV)	Metals (mg/l) †
9/20 - 9/25	350	189	15	-380	ND
10/27 - 10/13	1490	435	45	-400	ND

*Total Organic Acids in the Digestor Effluent

†Fe, Al, Ni, Cn, Zn in the Settling Tank Effluent

ND = Not Detectable

Table 4-2. Average Solids Concentrations for 9/27/79 to 10/13/79

Flow Stream	Total Solids (mg/l)	Total Dissolved Solids (mg/l)	Total Volatile Solids (mg/l)	Total Dissolved Volatile Solids (mg/l)
AMD	4661	4658	840	840
Final Effluent	1736	1669	248	228
Percent Reduction	63%	64%	70%	73%

was 6.5 whereas the alkalinity averaged 710 mg/l. For both cases the values show a substantial improvement above that of the raw AMD.

When comparing the two test periods on the basis of sulfate removal, the latter case is shown to be 130% more efficient than the first. The higher sulfate removal efficiency during 9/27-10/13 is attributed to the higher TOA concentrations obtained during this period. Table 4-1 shows that for the case of highest sulfate reduction, larger concentrations of sulfides were being carried in the reactor effluent.

Solids removal efficiencies for the treatment system are based on the change in solids content of the raw AMD and the effluent being discharged from the system. Table 4-2 summarizes solids content values computed for the AMD (batch 3) and effluent. Final effluent solids concentrations were calculated after taking complete-mixed aeration tank samples and allowing them to settle for two hours. A large, open top, glass container was used for settling to minimize wall effects. From Table 4-2 it can be seen that a 63% reduction in total solids was obtained whereas a significant increase in suspended solids occurred (3 mg/l to 67 mg/l). Thus, based on the type of solids content in question, the treatment process could be considered either fairly efficient or highly inefficient in these regards.

The metal sulfide/hydroxide sludge produced within the reactor was a very rich, deep black sludge with a damp mud type texture. The sludge had relatively no odor associated with it. The composition of the sludge is shown in Table 4-3. The "organic" fraction is

Table 4-3.   Reactor Sludge Composition

Metal	IRON	ZINC	COPPER	NICKEL	ALUMINUM
Percent of Total Solids*	9.2	0.6	0.03	0.06	17.6
Percent of Fixed Solids	12.4	0.75	0.04	0.08	22.0
Percent of Volatile (Organic) Solids in Sludge - 20%					

*All percentages are given on a dry weight basis.

Table 4-4.   Reactor Sludge Settling Characteristics

Concentration (g/l)	Percent of Initial Concentration*	Settling Velocity V_s (cm/min)	Solids Flux (g·cm/min·l)
2.417	5	2.71	6.56
4.835	10	1.61	7.78
9.669	20	0.53	5.11
14.504	30	0.25	3.68
24.173	50	0.10	2.42
33.843	70	0.04	1.32
48.347	100	0.01(2)	0.58

*Initial Concentration = 48.347 g/l

Note: 1 cm/min = 3.28 x 10^{-2} ft/min; 1 g·cm/min·l = 0.123 lb/ft^2·hr

believed to be made up primarily from paper fiber carried into the reactor from the digestors and from some biological solids. The sludges in the reactor were not only "freshly" precipitated solids but also included recycled solids which had accumulated in the system from previous operation. By knowing metal concentrations in the sludge, the feasibility of metal recovery operations can be closely evaluated. Table 4-3 gives the percentages of various metals in the sludge on a dry weight basis.

The reactor was operated in a complete-mix mode with high solids loadings. Because of this, removal of the solids from the reactor effluent stream was evaluated. Table 4-4 and Figure 4-1 summarize the results of settling tests performed on the sludge. Figure 4-1 shows that the computed solids flux values very closely produce a typical batch flux curve that might be expected based on the work of Dick (1970). Although this curve shows that the reactor sludge behaves in the same general manner as other sludges, it doesn't easily relate the relative ease of thickening of this sludge. Figure 4-2 is presented to give a comparison of this reactor sludge to various other common sludges, their associated concentrations, and their associated settling velocities. It can be seen that the reactor sludge behaves quite similarly to actived sludge.

As this was an anaerobic treatment process, an aeration step was required to impart a dissolved oxygen concentration to the final effluent. Table 4-5 summarizes the influent (raw AMD) and effluent characteristics for the period 9/27/79 to 10/13/79. The effluent has gone through aeration and two hours of clarification before
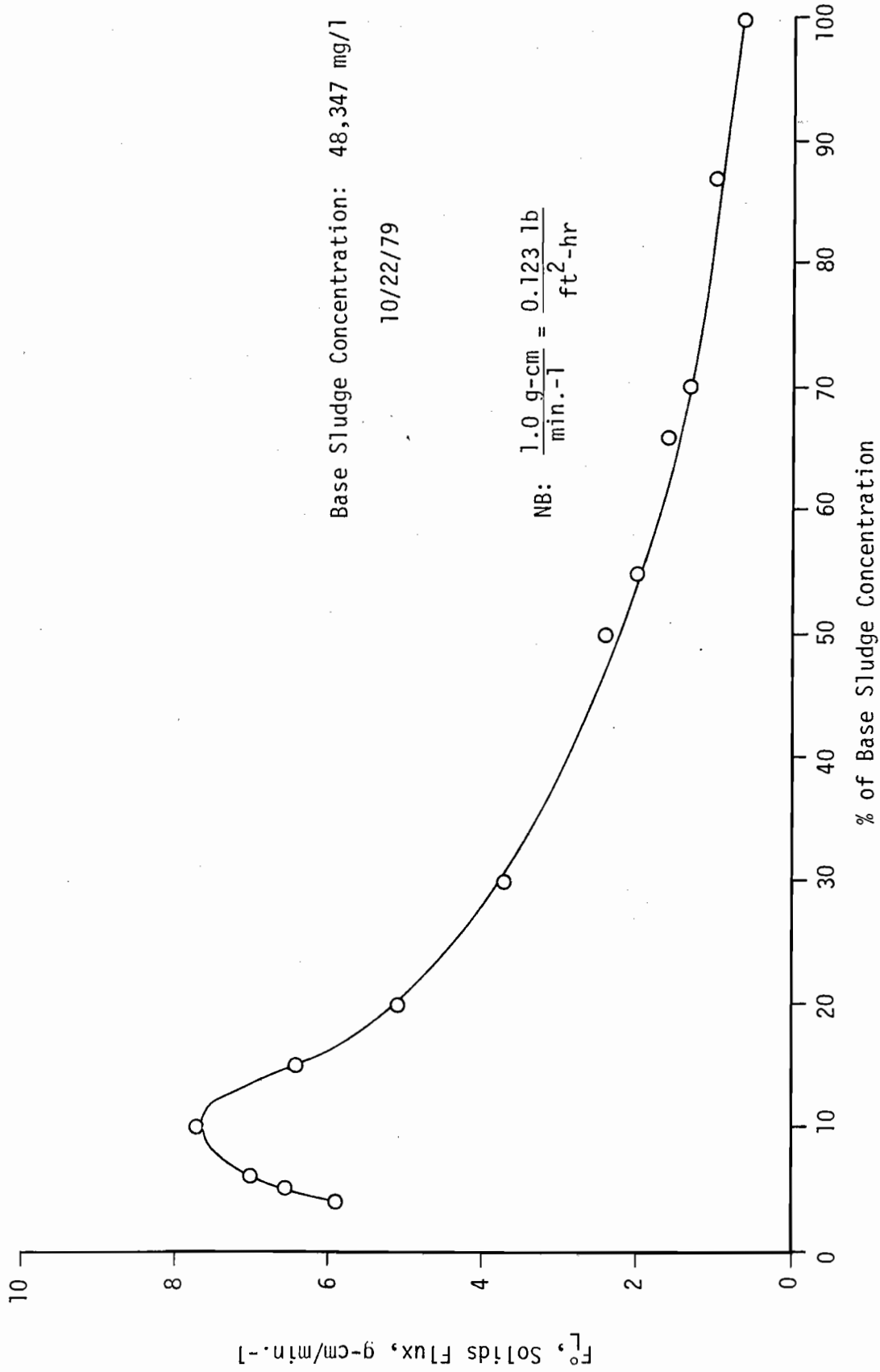
Figure 20. Batch Flux Curve = Reactor Sludge
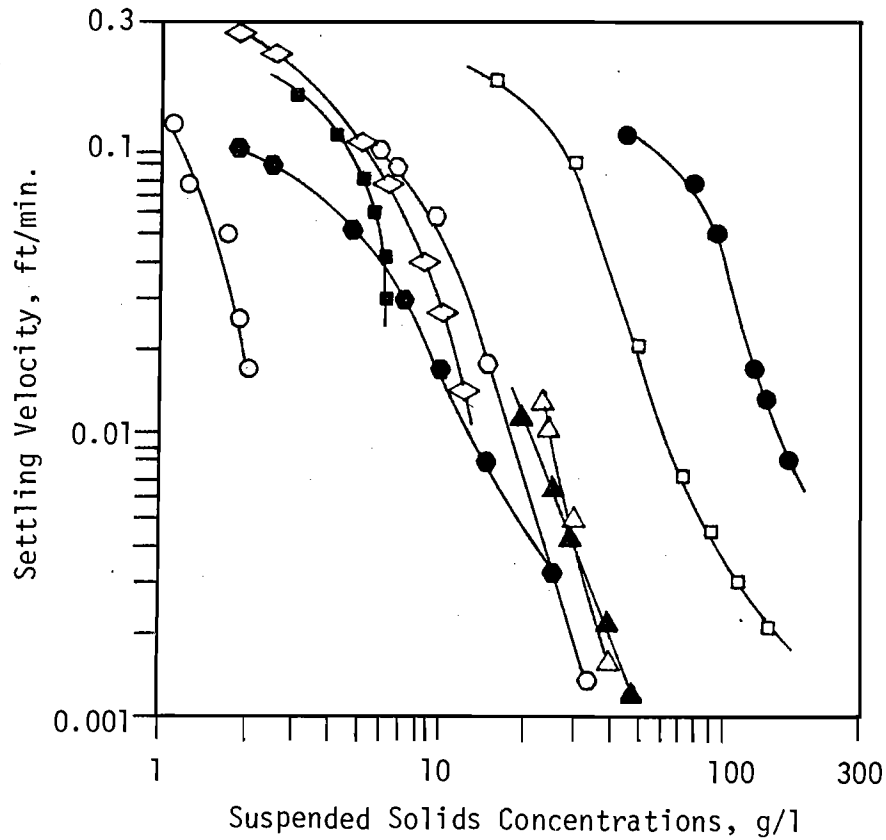
Sources:

● Weber - ceramic casting & glasing waste
○ Dick and Ewing - activated sludge C
■ Dick and Ewing - activated sludge A
□ Weber - lime softening sludge
◇ Javaheri and Dick - activated sludge I
△ Duncan and Kawata - primary sludge
▲ Duncan and Kawate - digested sludge
○ Weber - filter backwash sludge
● Author - metal sulfide/hydroxide sludge

Figure 21.* Settling Velocities of Various Sludges vs.
           the Reactor Sludge

*From Weber, Physiochemical Processes (35).

Table 4-5. Influent-Effluent Characteristics for 9/27/79 to 10/13/79

	RAW AMD	DISCHARGED EFFLUENT
pH	2.2	8.1
Acidity (mg/l as $CaCO_3$)	1940	-
Alkalinity (mg/l as $CaCO_3$)	0	710
$[SO_4^{-2}]$, mg/l	4367	723
[Fe], mg/l	183	ND
[Al], mg/l	213	ND
[Zn], mg/l	6	ND
[Ni], mg/l	1.4	ND
[Cu], mg/l	trace	ND
Total Solids, mg/l	4661	1736
Total Dissolved Solids, mg/l	4658	1669
Total Volatile Solids, mg/l	840	248

ND = Not Detectable

discharge.

It is possible to gain additional insight into the cause of observed conditions as well as the effects these conditions have on treatment efficiency. In addition, a review of the effects of treatment on a parameter by parameter basis may provide insight into the actual operational feasibility of the proposed biological treatment system. The following discussions are provided to review specific treatment results.

Sulfate - The maximum sulfate reduction rate achieved was 435 mg/l of reactor/day as SO_2^-. Although the best sulfate removal for this system proved to be only 67% efficient, it shows that a continuous microbial treatment process for AMD is feasible. It is believed that higher removal efficiencies could be obtained with a more efficient supply of total organic acids (TOA) to the reactor.

Based upon the classification work done by Widdel, et al. (1977), it is believed that the major species of sulfate reducers in this system were Desulfotomaculum acetoxidans. The basis for assuming this is that virtually all the organic acid produced by the digestor was acetic acids. D. acetoxidans are the only known sulfate-reducing species capable of utilizing acetic acid as an organic carbon source. Test cultures to verify their existence were not used. It is anticipated that by verifying the actual identify of the species present, an optimum environment for bacterial growth could be strived for and possibly obtained.

Metals - For the period of 9/27 to 10/13, a 100% reduction of metals in the AMD was obtained. Metal removal occurred through the

formation of either insoluble sulfides or hydroxides. From previous studies and solubility products found in the literature it can be reasoned that the metals precipitated as follows: $Al(OH)_3$, $Fe(OH_3$, FeS, CuS, ZnS, and NiS.

Figure 5-1 below shows the solubility of various metal oxides and hydroxides. For the 6.5 average pH maintained in the reactor, it can be seen that Fe^{+3} and Al^{+3} would easily precipitate as hydroxides. However, a necessary pH to precipitate Fe^{+2} as a hydroxide was never attained. The solubility product for FeS shows that Fe^{+2} most likely precipitated in this manner. Although it is quite likely that the metals precipitated in the forms identified, actual precipitation reactions would be very difficult to determine. The many different anions and cations in the AMD and the various redox reactions which occur within the reactor combine to make a very complex chemical system. It is possible that with different AMD sources, chemical characteristics may require other methods of metals removal.

Stripping and Activity - In some AMD sources sulfide production may significantly exceed metal precipitation requirements.

As given in Equation 3-1, 1 mole of SO^{2-}, yields 1 mole of H_2S in the sulfate reduction process. With an initial concentration of 4367 mg/1 of SO^{2-} in the AMD, a 100% reduction of sulfides would yield 91 meq ofH2=S/1. Assuming that all iron exist as Fe^{+2} and that this is the only major metal species present to react with H_2S, only 3.3 meq/1 of this metal species exist in the AMD. Since 1 meq of sulfide will react with 1 meq of metal species, there is the
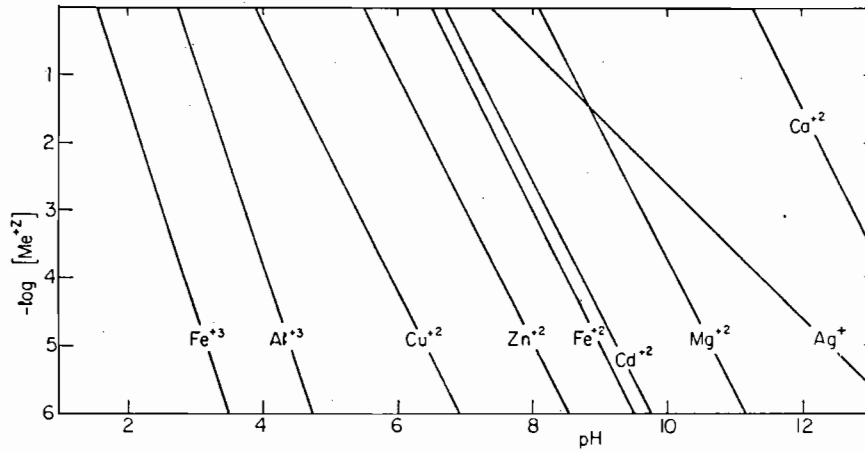
Figure 22.   Solubilities of Various Metal Oxides and
             Hydroxides (30).

possibility of large "excess" concentrations of H_2S being produced. This is true even with only a 67% reduction in SO^{2-}.

Equation 2-2 shows that no net acidity reduction and subsequent pH increase is achieved during metal precipitation. This acidity reduction and pH increase must occur with the formation of excess H_2S in the system. To accomplish such quality improvements the H_2S must be removed from the system. To remove the excess sulfides in this system a sulfide-stripping procedure was designed.

A combined vacuum/pump was connected just prior to Digestor A. A closed loop system was used with the pump to prevent oxygen toxicity to the digestor. Gas, bubbling through Digestor A, stripped sulfides out of the liquid stream and deposited them on the iron sponge. This iron sponge worked well in absorbing the sulfide gas. A net acidity reduction and pH increase is thus obtained. For this system it was even possible to achieve a net positive alkalinity.

When the concentration of metal equivalents is greater than the concentration of sulfide equivalents, no net acidity reduction or pH increase may be realized. This was the likely cause of the pH decline observed in start-up studies.

When the use of the sulfide-stripping operation is required, the economic feasibility of a sulfide/sulfur recovery process should be examined. In the early months of this study when using a countercurrent, gas-liquid, sulfide-stripping tower, oxidation of some sulfide to sulfure had occurred. Although the sulfide stripping in the final configuration occurred prior to flow entering Digestor A, a closer evaluation may show that sulfide stripping

immediately following the settling tank would be a better choice. At this location sulfides which might normally flow into the aeration tank may be recovered, reducing the hazards of hydrogen sulfide in the aeration tank.

ORP-pH Stability - During the period of 9/27 to 10/13, Digestor B to AMD flow ratios averaged 1:1. Even though the AMD had a pH of 2.25 and an associated acidity of 1940 mg/1, it was possible to maintain a pH of 6.5 and an ORP of -400 mV (e_c) in the reactor.

Related to the maintenance of a stable pH in the reactor is the high solids load. Tests showed that total alkalinities were six to nine times greater than soluble alkalinities in the reactor. It might thus be reasoned that a complete mix reactor may possibly better withstand a shock AMD load than would a plug flow type reactor.

Significant pH increases were noticed for the settling tank effluent as it flowed into the aeration tank. Values increased from 6.5 to 8.1. The increase is primarily associated with the stripping of CO_2 out of the waste stream. This CO_2 is an end-product associated with the oxidation of acetic acid during sulfate reduction.

Solids Removal - Removal of 63% total solids, 64% total dissolved solids, and 70% total volatile solids were obtained. High suspended solids concentrations were observed in the final configuration. The 1665 mg/1 of solids was an accumulation from many previous system operations. Most of the solids were from $Fe(OH)_3$ floc that precipitated when the treatment system was operating very

poorly and 100% metals reduction was not obtainable. If only settling tank supernatant from 9/27 to 10/13 had been aerated, the solids content of the aeration tank would have been significantly different.

Sludge Characteristics – The sludge produced within the reactor had a 20% volatile (organic) solids fraction on a dry weight basis. This "organic" fraction is believed to be made up primarily of paper fibers and biological mass. Even with the low flow rates carried through the digestors, small concentrations of digested computer paper fibers managed to flow out. When filtering digestor effluent samples on a 0.45 millipore membrane filters, paper fibers could be seen quite plainly. If anticipated sludge quantities are to be estimated, this paper fiber "carry-over" must be considered. Because of the continual degradation of the computer paper, fresh material would need to be added to the system periodically.

A metals analysis of the sludge showed that it had a total metal content of 30%. Based on this fact it would seem reasonable to expect the sludge to settle quite easily. This was not the case. Figure 4-2 shows the sludge to behave somewhat like activated sludge. Concentrations of suspended solids in the range of 2.5 to 3.3% were obtained in the bottom of the settling tank. With the low settling velocities it has an high underflow concentrations it approaches in the settling tank underflow, this sludge would be uneconomical to thicken in a gravity thickener. Large settling areas would be required to achieve a minimal increase in solids content. The reactor was operated at an average solids concentration of 1.8%.

4.0 CONCLUSIONS

The research conducted to evaluate biological methods for AMD treatment identified a unit process design which was capable of effective water quality improvement and provided opportunities for recycling of metal sludges. In addition, digestor solids are characteristic of materials which are useful soil amendments in site reclamation.

The utility of substrate/biomass packages for the treatment of impounded AMD was not clearly demonstrated. Maintenance of mixed microbial communities was limited by substrate size and the availablity of suitable energy sources to maintain microbial populations. Although high levels of microbiological activity could be maintained for a short time, no significant improvement in water quality was demonstrated in the experiments. Of particular interest in the experiments was the improvement in AMD quality which was associated with wood dust. Previous research had indicated wood dust improved AMD quality. This research demonstrated that physical and chemical reactions occured when wood dust and AMD were mixed, which reduced metal concentration and genrally improved AMD quality in the absence of biological activity.

LIST OF REFERENCES

Akera, D. J., Jr. and E. A. Moss (Feb. 1973). Dewatering of Mine Drainage Sludge, 1973. EPA-R-2-73-169.

Bogner, J. E., J. P. Schubert, and G. M. Kubzynski (1981). Environmental Control Technology Survey of Selected U.S. Strip Mining Sites, Vol. 3, Control Technology. ANL/EMR-2 Vol. 3, Argonne National Laboratory.

Bryart, M. P. (1979). Microbial methane production-Theoretical Aspects. J. Animal Science, 48, 193-201.

Burns and Roe, Inc. (1973). Evaluation of ion exchange processes for treatment of mine drainage waters. Report Project #CR111, p. 181.

Campbell, R. S. and L. T. Lind (1969). Water quality and aging of strip-mine lakes. Journal Water Pollut. Control Fed., Vol. 41, No. 11, Part 1, p. 1943.

Campbell, L. L. and J. R. Postgate (Sept. 1965). Classification of the spore-forming sulfate-reducing bacteria. Bacteriological Reviews, 29, 3.

Cappenberg, Th. E. (1974a). Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. Field observations Antonie van Leeuwenhoek, 40, 285-295.

Cappenberg, Th. E. (1974b). Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. Inhibition experiments Antonie van Leeuwenhoek, 40, 297-306.

Cappenberg, Th. E. (1975). Relationships between sulfate-reducing and methane producing bacteria. Plant and Soil, 43, 125-139.

Chian, E.S.K. and F. B. DeWalle (1977). Treatment of high strength acidic wastewater with a complete mixed anaerobic filter. Water Research, 11, 295-304.

Cohen, E. B. (1979). A biological treatment of acid mine water. Special problem report Department of Civil Engineering, University of Illinois.

Colmer, A. R. and M. E. Hinkle (1947). The role of microorganisms in acid mine drainage: Preliminary Report. Science, 106, 253.

Dick, Richard I. (1970). Thickening. Advances in Water Quality Improvement - Physical and Chemical Processes, Gloyna and Eckenfelder (eds.), University of Texas Press, 358-369.

Dugan, P. R. (1974). Biochemical Ecology of Water Pollution. Plenum Press, 159.

Hayes, R. B. III (1978). A biological treatment system for acid mind waters. Special Problem Report. Department of Civil Engineering, University of Illinois.

Herricks, E. E., V. O. Shanholtz, and D. W. Contractor (1975). Models to predict the environmental impact of mine drainage on streams. Trans. Am. Soc. Agricultural Eng., 18, 4, 657-663 and 667.

Herricks, E. E. and J. Cairns, Jr. (1974). Rehabilitation of streams receiving acid mine drainage. Bulletin 66, Va. Water Res. Center.

King, D. L., et al. (1974). Acid Strip Mine Lake Recovery, Journal WPCF, Vol. 46, No. 10, 2301.

Kuznetsor, S., et al. (1963). Introduction to Geological Microbiology. P. T. Brayeer (translator), McGraw-Hill, New York, N.Y.

Lind, O. T. and Campbell, R. S. (1970). Community metabolism in acid and alkaline strip-mine lakes, Trans. Amer. Fish. Soc. No. 3, 577.

Lubieniecki, E. Jr. (1977). Abiotic Interactions Related to the Biological Treatment of Acid Mine Water. Special Problem Report, Department of Civil Engineering, University of Illinois.

Metcalf and Eddy, Inc., Wastewater Engineering, McGraw-Hill, 2nd ed., 1979.

Middleton, A. C. and A. W. Lawrence. Kinetics of Microbial Sulfate Reduction, Journal Water Pollution Control Federation, 49 (7), July 1977.

Miller, L. P. Tolerance of Sulfate Reducing Bacteria to Hydrogen Sulfide, Contributions from Boyce Thompson Institute, 16 (3), 73-83, 1950.

Ohio State University Research Foundation (1971). Acid mine drainage formation and abatement, U.S.EPA, AST-42 14010 FPR 04171.

Pearson, F. H. and Nesbitt, J. B. (1972). Combined treatment of municipal wastewater and acid mine drainage. Research Publication No. 73, Pennsylvania State University

Postgate, J. R. (1951). On the Nutrition of Desulfovibrio desulfuricans, J. Gen. Microbiol. 9, 440.

Rice, P. A. and Rasolini, F. (1982). Biological treatment of acid mine water, Fourth Symposium on Coal Mine Drainage, Mellon Institute, April 26-27, 293-326.

Riley, C. U. (1965). Limnology of Acid Mine Impoundments, Proc. Symp. Acid Mine Drainage, Res., Mellon Inst., Pittsburgh, Pa, p. 175.

Ross, Lawrence W. Removal of Heavy Metals from Mine Drainage by Precipitation, Washington, D.C.: United States EPA, Research and Monitoring Office, Report No. EPA-670/2-73-080 (September 1973).

Smith, J. R., The Technological Feasibility of a Microbial Sulfate Reducing Reclamation System, Water Resources and Environmental Engineering Research Report, No. 78-5, State University of New York at Buffalo, September 1978.

Temple, K. L. and Delchamps, E. W. Autotrophic bacteria and the formation of acid in bituminous coal mines. Appl. Microbiol. 1, 255, 1953.

Tuttle, J. H., et al. "Microbial sulfate reduction and its potential utility as an acid mine water pollution abatement procedure, Applied Microbiol. 17 (2), 297, February 1969.

Tuttle, J. H., Dugan, P. R., Macmillan, C. B., and Randles, C. I. Microbial Dissimilatory Sulfur Cycle in Acid Mine Water, Journal of Bacteriology, 97 (2), 594, February 1969.

Vriesman, R. R. (1980). A Continuous Biological Process to Treat Acid Mine Drainage Waters, Special Problem Report, Department of Civil Engineering, University of Illinois.

Widdel, F. and Pfennig, N. A new anaerobic, sporting, acetate-oxidizing, sulfate-reducing bacterium, Desulfotomaculum (emend.) acetoxidans, Archives of Microbiology, 112, 119, 1977.