Inhibition of Iron-Oxidizing Bacteria in Wastes From Coal and Hard-Rock Mines Using the Anti-Bacterial Agent Nitrapyrine

by

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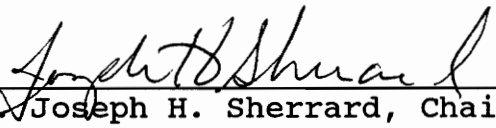

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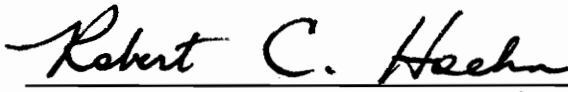

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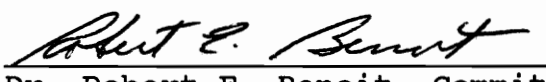

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ABSTRACT

The production of acid mine drainage (AMD) is catalyzed by iron-oxidizing bacteria primarily of the species Thiobacillus ferrooxidans. By inhibiting these bacteria, the production of AMD can be greatly reduced. One compound found to be effective in the inhibition of T. ferrooxidans is nitrapyrine. N-Serve, a product of Dow Chemical, Inc., is the commercially available form of nitrapyrine. This compound has been widely used in agriculture for nitrification inhibition. The purpose of this study was to determine the effectiveness of N-Serve in reducing the production of AMD under simulated field conditions.

A column study was completed using a coal mine waste and a hard-rock mine waste. Eight columns containing 7kg of rock were established for each substrate. Three doses of N-Serve (22% nitrapyrine) were applied once at the beginning of this study: a high dose 2200 mg/kg, a medium dose 220 mg/kg, and a low dose 22 mg/kg. Duplicate columns were included for each N-Serve dose including two untreated columns to serve as a control for each substrate. Beginning the week after treatment, the columns were leached once a week for 29 weeks with deionized, distilled water

(equivalent to 2.5 cm precipitation). Only the highest N-Serve dose produced a column leachate of significantly better quality than that of the controls. The acidity in the high-dose coal mine columns averaged less than 50 percent of the acidity in the control effluent from week 6 through the end of the study.

A monolithic controlled release system utilizing acrylonitrile rubber was successfully developed and tested for use with nitrapyrine. This formulation should withstand the rigors of the environment and with minor modification could produce a variety of release rates.

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I. INTRODUCTION

Acid mine drainage (AMD) is a major source of water pollution worldwide. The U.S. mining industry alone spends over one million dollars a day to treat this problem. The Ohio River, whose watershed is a center for coal mining, receives approximately three million tons of concentrated sulfuric acid annually. Given these factors, it becomes imperative that innovative, cost-effective, and ecologically sound treatment techniques be developed.

The majority of the effort to deal with AMD has focused on treating the acidic leachate from the mine spoils before it enters the receiving water. Although these techniques have greatly improved the water quality leaving the mine site, leachate treatment may have to continue indefinitely at great cost to the mining companies. More recently, several new technologies have been developed which inhibit the actual formation of the AMD.

The reactions that cause the formation of AMD are not strictly of chemical origin but are catalyzed by a group of bacteria, primarily in the genus Thiobacillus. T. ferrooxidans is a chemolithotrophic bacterium that, at low pH, obtains its metabolic energy primarily from the oxidation of ferrous iron. It is the bacterium most often credited as responsible for the catalysis of AMD formation. Most of the techniques being developed to inhibit the

formation of AMD have focused on inhibiting the bacteria that are the cause of the problem. A number of chemicals have been found which have the ability to inhibit T. ferrooxidans, but most of these chemicals, for a number of reasons, are not candidates for field use. In order for a chemical to be considered a for field use in the inhibition of AMD, it must be cost-effective, environmentally safe, and resistant to the effects of weathering and biodegredation.

Antibiotics have not been extensively investigated for AMD control because they are generally expensive, and approval by the U.S. Environmental Protection Agency (EPA) would be extremely difficult for reasons of public health and ecological protection. N-Serve is the commercially available form of nitrapyrine produced by the Dow Chemical Corporation. N-serve (22% nitrapyrine) has been extensively used in agriculture as a nitrification inhibitor. Because N-Serve inhibits the growth of chemotrophic nitrifying bacteria, research was done to determine its effectiveness at inhibiting the chemotrophic T. ferrooxidans. Kavanaugh (1988) found it to be the most effective of 24 antibacterial agents screened for their ability to inhibit cultures of T. ferrooxidans. N-Serve was selected for use in a column study to determine its effectiveness in reducing AMD because it meets all criteria for field use. N-Serve is already EPA approved for application to agricultural lands; therefore, obtaining a permit for mine lands should not pose a problem.

Finally, N-Serve appears to be a relatively inexpensive bactericide costing only six dollars per acre for agricultural application.

OBJECTIVES

The specific objectives of this study were to:

1. Determine the effectiveness of nitrapyrine (N-Serve) in inhibiting acid formation under simulated field conditions for two mine waste materials (coal mine and hard-rock mine wastes).
2. Formulate a controlled release system for use with nitrapyrine and determine nitrapyrine release characteristics.

II. LITERATURE REVIEW

INTRODUCTION

Acid mine drainage (AMD) is a water pollution problem generally associated with the mining of materials high in pyrite content. Pyrite, a crystalline form of FeS_2, is a commonly occurring element in bituminous coals and ores. AMD typically contains iron, metal sulfides, sulfates, acid and other metals. The AMD problem is most commonly linked to coal mining, yet it can occur in strip or subsurface hard-rock mines. Although there is now a good understanding of the chemistry and biology associated with AMD, an adequate solution to controlling the problem has yet to be found.

Many treatment technologies have been researched since the identification of AMD as a major water quality problem. Most treatment practices originally focused on either controlling the hydrology of the mine area to prevent water from entering the system or treatment of the acid and metals rich effluent from the mine site. Although these practices have improved the quality of the water leaving the mine site, they fail to take advantage of present knowledge of the chemistry of acid formation. New technologies have been developed to interrupt chemical reactions and prevent acid production. By inhibiting the production of acid initially,

the need to treat the effluent can be reduced or eliminated. This could significantly reduce the costs of treating AMD.

CHEMISTRY

During mining operations, pyrite (FeS_2) is exposed to air and water. The overall reaction describing pyrite oxidation is written as:

$$4\ FeS_2 + 15\ O_2 + 14\ H_2O \rightarrow 4\ Fe(OH)_3 + 8\ H_2SO_4 \qquad [1]$$

The actual pathway for this reaction involves a series of reactions and a number of intermediates. The following stoichiometric reactions may characterize the oxidation of pyrite (Stumm and Morgan, 1981):

$$FeS_2 + 3\tfrac{1}{2}\ O_2 + H_2O \rightarrow Fe^{2+} + 2\ SO_4^{2-} + 2\ H^+ \qquad [2]$$

$$Fe^{2+} + \tfrac{1}{4}\ O_2 + H^+ \rightarrow Fe^{3+} + \tfrac{1}{2}\ H_2O \qquad [3]$$

$$Fe^{3+} + 3\ H_2O \rightarrow Fe(OH)_3(s) + 3\ H^+ \qquad [4]$$

$$FeS_2 + 14\ Fe^{3+} + 8\ H_2O \rightarrow 15\ Fe^{2+} + 2\ SO_4^{2-} + 16\ H^+ \qquad [5]$$

The two main oxidizing agents acting on pyrite are oxygen and ferric ions. Equation [2] initiates the process as Fe^{2+} is released from iron pyrite either by oxidation by oxygen, or by simple dissociation of the pyrite (Singer and Stumm, 1970).

Ferrous iron is oxidized to the ferric form in Equation [3]. Under natural conditions, this is a very slow reaction, and becomes the rate determining step. (Stumm and Morgan, 1981). In 1985, Backes, Pulford and Duncan found that at high levels of pyrite, the rate determining step is the oxidation of ferrous ions to ferric ions. At low pyrite levels, the rate determining step becomes the oxidation of pyrite by ferric ions.

At pH values above 3.5, the ferric ions produced will precipitate out as ferric hydroxide, $Fe(OH)_3$. At pH values less than 3.5, the ferric ions will act to rapidly oxidize the pyrite, as in Equation [5] (Garrels and Thompson, 1960). In the absence of carbonate rock, the acid produced in Equation [2] will eventually keep the pH below 3.5. As the pH continues to decrease, the Fe^{3+}:Fe^{2+} ratio will increase and continue to accelerate the reaction shown in Equation (4) (Kleinmann, Crerar and Pacelli, 1981). Thus, a cyclical reaction occurs in which ferric ions that had been oxidized by bacteria are used to oxidize pyrite. The cycle can produce large amounts of acidity (H^+) in a short amount of time. This reaction produces ferrous ions which will again be oxidized by the bacteria, as shown in Figure [1].

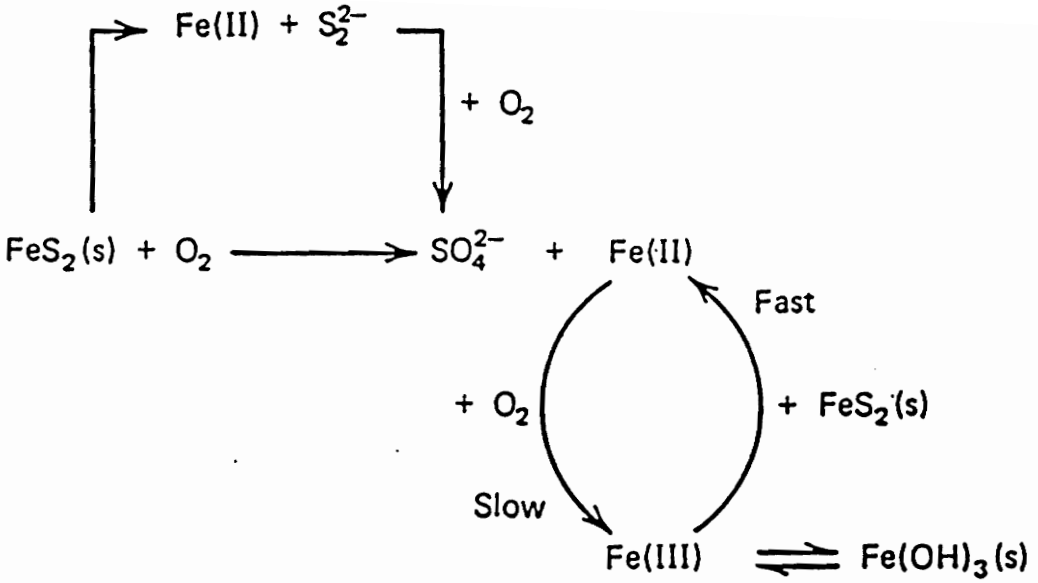

Figure 1. Model for the oxidation of pyrite (from Stumm and Morgan, 1981).

ROLE AND CHARACTERISTICS OF THE BACTERIA

Thiobacillus is a genus of sulfur oxidizing autotrophic bacteria. Included in this genus is a group of iron oxidizing bacteria. The first iron oxidizing bacteria discovered was T. ferrooxidans, a gram negative, microaerophilic rod which can utilize ferrous iron as its sole energy source (Colmer, Temple and Hinkle, 1959). Other iron oxidizers, including Ferrobacillus ferrooxidans and Ferrobacillus sulfoxidans have since been discovered. Silver (1978) suggested that because of nutritional and taxonomic similarities, these three bacteria should all be included under the name T. ferrooxidans. T. ferrooxidans, initially cultured on ferrous iron, is also capable of chemolithotrophic growth on inorganic sulfur compounds including thiosulfate, tetrathionate, trithionate, or elemental sulfur in liquid media. These bacteria, conditioned to grow on inorganic sulfur, are still capable of returning to ferrous iron as their energy source (Tuovinen and Kelly, 1974). Despite the more recent findings indicating the existence of some minor metabolic differences, much of the literature still refers to all three species under T. ferrooxidans.

T. ferrooxidans plays a key role in the production of AMD. Singer and Stumm (1970) determined that direct oxygenation by air of pyrite was too slow to generate the

amount of acidity found at mine sites. They suggested that oxidation by the aqueous ferric ion was catalyzed by T. ferrooxidans, resulting in a significantly faster pyrite oxygenation rate. In the presence of the iron oxidizing bacteria T. ferrooxidans, the rate of this step can be increased by 10^6 (Singer and Stumm, 1970). Therefore, if this step can be controlled, AMD can be controlled.

T. ferrooxidans is widely distributed in nature, but is dependent upon the presence of ferrous iron, sulfur, or inorganic sulfide in an acidic environment (Razzel and Trussell, 1963). This species is extremely acid tolerant and can withstand pH levels as low as 0.8. Growth can occur as low as pH 1.6, with an optimal pH range from 2.5 to 5.5. Lundgren and Tano (1978) suggested that these bacteria were able to operate in such an acidic environment by utilizing a cellular envelope. The envelope somehow establishes a microenvironment in which appropriate oxidases are allowed to function in the acidic surroundings.

Tuovinen, Niemela and Gyllenberg (1971) investigated the effects of mineral nutrients on T. ferrooxidans. They found the essential limiting nutrients to include ammonium-nitrogen, phosphorous, sulphate and magnesium. Microbial iron oxidation can occur in the presence of very low nitrogen and phosphorous concentrations. Phosphate can be taken out of solution by precipitation and can become

limiting at low concentrations. Nitrate (NO_3^-), in concentrations greater than 500 mg/L, has been found to be inhibitory. Sulphate is important in the role of binding ferrous iron to the cell envelope, thus making it available for oxidation. Magnesium (Mg^{2+}) is essential for CO_2 fixation but is rarely limiting because natural waters normally contain sufficient concentrations.

The majority of work with iron oxidizing bacteria refers to these species as obligate chemolithotrophic. Organic material was found to inhibit iron oxidation in several early studies (Tuttle and Dugan, 1976; Torma and Itzkovitch, 1976). More recently, Wakao et al. (1983) established organic substances as promoters of pyrite oxidation. The addition of cell-free extracts of both chemolithotrophic thiobacilli and heterotrophic bacteria enhanced iron oxidation. It was also determined that protein, nucleic acid, yeast extract, peptone and surface active agents all showed a similar promoting effect. The stimulating effects of the surfactants were believed to be due in part to surface active effects on the pyrite oxidation. Shafia and Wilkinson (1969) established that F. ferrooxidans could, after a short adaptation period, grow on glucose, mannitol, several other sugars, and several amino acids in absence of an oxidizable iron source. However, there is a strong posibility that these investigators began

with a mixed bacterial culture because no other publication has supported their conclusions regarding organotrophic growth. A new species of bacteria designated T. cuprinus has been isolated from mine waste materials and is capable of facultative organotrophic growth at low pH (Huber and Stetter, 1990). This species is also capable of metals mobilization similar to other thiobacilli, but is unable to oxidize ferrous iron. Organotrophic growth occurred on yeast extract, peptone, Casamino Acids, and meat extract.

TREATMENT TECHNOLOGIES

There are two main philosophies on the management of mine sites for AMD. The first is to treat the AMD waters before they leave the site and the second is to prevent the formation of the acidity in the first place. Both can be accomplished by either controlling the hydrology of the site or by inhibiting the iron oxidizing bacteria that cause the problem.

Effluent Treatment Techniques

Some of the more popular methods for treating AMD include: lime, limestone, or soda ash neutralization; microbial oxidation; chemical oxidation/neutralization; foam separation of metals; and ion exchange treatment. Nakamura (1988) utilized sulfur oxidizing bacteria in a continuous

flow system to precipitate metals out of solution. One of the more recent innovations has been the use of wetland systems to 'naturally' treat the waters. All of these techniques can be very effective in reducing the AMD pollution problem. The main disadvantage in the philosophy of treating the effluent is that the treatment does nothing to reduce the production of the pollutants. Thus, the treatment period can extend indefinitely. This fact alone has inspired volumes of research into the prevention of AMD formation which could reduce or eliminate the costs of treating the water that leaves the mine site.

AMD Prevention Techniques

Early efforts in AMD prevention were focused on controlling the hydrology of the mine site. By preventing water from coming into contact with the substrate a critical element in the reaction which produces AMD is eliminated. Another common practice involves sealing the mine with concrete so that water can not leave the mine. Ackman and Jones (1988) described a process called stream sealing which can eliminate stream water infiltration into underground mines by constructing a polyurethane barrier several feet beneath the streambed. The geology of mine areas works against both of these practices. Hard-rock and coal mining areas are often characterized by fractured rock systems. It

is impossible to completely halt the movement of water through these innumerable pathways. Thus, physical means of controlling the formation of mine drainage have not been very successful.

Water functions as a principle reactant in the oxidation of pyrite, as a reaction medium, and as a transport medium for oxidation products. Therefore it will always be beneficial to gain knowledge of mine site geo-hydrology. The importance of understanding hydrologic processes within mine spoil/backfill materials was summarized by Caruccio (1988). Efficient and cost effective abatement requires development of site-specific characterizations of the hydrology such as knowledge of sources of spoil water recharge, zones of acid production, and movement of water through acid producing zones. This information can measurably increase the effectiveness of a treatment plan for mine site reclamation.

Much of the recent work on AMD prevention has centered on inhibiting the bacteria that catalyze pyrite oxidation. Several naturally occurring inhibitors have been found which can effectively reduce acid production. Shearer et al. (1969) reported that certain strains of Caulobacter were inhibitory to growth of acid-producing bacteria in laboratory coal pile effluents. They also determined that when antibiotic-producing strains of the bacterium

Streptomyces aurofaciens were adapted to grow in acid/mine environments, they could reduce acidity yields from acid-producing coal piles.

Bactericides. The use of bactericides plays a key role in today's AMD abatement programs. By inhibiting bacterial oxidation of pyrite, the costs of treating mine waters can be reduced or eliminated entirely. Rastogi and Sobek (1986) point to three major segments of the mining industry which could potentially benefit from the use of bactericides: (1) Active Operations - an active surface mine or active refuse disposal site; (2) Final Reclamation of a Mining Operation - a surface mine site or refuse disposal area; and (3) Abandoned Mine Lands (AML) Reclamation. By inhibiting the formation of AMD, bactericides can reduce the costs of normal site maintenance reclamation practices including: topsoil coverage, lime treatment, creating borrow areas, and future site maintenance, in addition to a reducing effluent water treatment costs.

Although effective over a wide variety of conditions, bactericide application rates and methods are site-specific and dependent on five factors (Rastogi and Sobek, 1986):

1. Site material - pyrite content, sulfur forms, net neutralization potential, refuse, spoil, mine tailings.

2. Site topography - steepness of slopes, compactness, permeability, hollow fill, above-ground pile.
3. Hydrology - surface water flow, ground water sources and quality, proximity to bodies of water.
4. Activity of iron-oxidizing bacteria.
5. Site operations - active mining, reclamation with and without soil cover, quantity and quality of soil cover, soil substitute.

Bactericides not only inhibit the bacteria that catalyze AMD formation, but also provide an environment conducive to the growth of heterotrophs which can enhance the growth of vegetative cover. A strong vegetative cover for three years can break the cycle of acid production because the root system competes with the Thiobacilli for oxygen and water (Tuttle et al., (1977). The respiration of the root system and heterotrophic bacteria also increases CO_2 levels in the spoil creating adverse conditions for the aerobic acid-producing bacteria. By enhancing vegetative growth, the soil biological processes will produce organic acids which can further inhibit bacterial oxidation of pyrite and acid formation (Rastogi and Sobek, 1986). Rastogi, Bohac and Horowitz (1987) reported that in many cases, the use of bactericides could reduce the costs of reclaiming a mine site by $2,500 to $8,000 per acre.

Organic Acids. Several researchers have resolved that organic acids can effectively disrupt iron oxidation in *T. ferrooxidans* cells. Tuttle, Dugan and Apel (1977) reported cellular leakage in *T. ferrooxidans* when exposed to 10^{-2} to 10^{-1} M concentrations of propionic, butyric, valeric, hexanoic, and oxalacetic acids. Organic acids are valuable as inhibitors in AMD situations because they are not toxic to other organisms at the concentrations applied. Onysko, Kleinmann and Erickson (1984) established benzoic and sorbic acids as also inhibitory at low concentrations (5 to 10 mg/L). It is believed that organic acids inhibit *T. ferrooxidans* by reacting with cations in the cell envelope and cell membrane allowing H^+ ions to enter the cell.

Simple Organic Compounds. The effects of organic compounds on autotrophic iron-oxidizing bacteria have been debated at length. On reviewing the literature, it is evident that under certain conditions, simple organic compounds can be either inhibitory or stimulatory. In flasks containing a pyrite suspension, the growth of *T. thiooxidans* was enhanced by the addition of yeast extract, peptone, nucleic acids and protein (Wakao *et al.*, 1983). Tuovinen *et al.* (1971) have found that both peptone and yeast extract, as well as fructose and lactose, cause complete inhibition of *T. ferrooxidans*.

In the laboratory T. ferrooxidans has been adapted to utilize organic compounds as an energy source (Shafia and Wilkinson, 1969). Under natural conditions, however, it is likely to remain autotrophic (Tuovinen et al., 1971). Everson (1970) concluded that organic matter is quickly oxidized by Fe (III), which is reduced to Fe (II) in the process. Because Fe (III) is a critical reactant in the oxidation of pyrite, increased organic matter in the system will slow the reaction by reducing Fe (II) concentrations. Organics will also benefit the heterotrophs which can crowd out the growth of iron bacteria. For this reason, organics are often incorporated into the acid producing areas of the mine site to assist in the remediation process. Tuovinen et al., (1971) concluded that organic compounds including lactose, fructose, tryptone, peptone, yeast extract, and meat extract in 0.5 percent w/v concentrations all inhibited iron oxidation for a 14 day study period.

Surfactants. Surfactants have recently become one of the leading bactericides used in treating AMD problems. Many theories exist on how surfactants cause inhibition in T. ferrooxidans. Torma and Itzkovitch (1976) observed that organic solvents often had an inhibitory effect on iron bacteria. Because these solvents reduce the surface tension of the aqueous phase, they hypothesized that the inhibition

was due to surface active effects. The solvents adsorb to the mineral surface, thus preventing intimate contact of the bacteria with the mineral surface.

By using the surfactant Tween 20, Wakao et al. (1984) discovered that surfactants could also enhance pyrite oxidation. Their conclusions were that the iron oxidizing activity of bacteria was inhibited when cells were adsorbed to pyrite surfaces. It was postulated that the surfactant prevented bacteria from becoming adsorbed and enhanced oxidation of pyrite.

Sodium Lauryl Sulfate (SLS) is probably the most commonly used surfactant for AMD control today. Kleinmann et al. (1981) applied SLS in two field tests on coal piles. In both cases controlled release systems were utilized because SLS is extremely soluble and would wash away with the first rainfall. In both tests the acidity of the treated pile was substantially reduced.

Watzlaf (1988) indicated that the effectiveness of anionic surfactants was inversely related to the amount of weathering that the substrate had undergone. In slightly weathered coal the bacteria were partially inhibited, but the amount of acidity produced was not significantly reduced. In heavily weathered materials neither bacterial metabolism or acidity production was inhibited. Because lime is often used to neutralize mine refuse, and SLS is

only effective at low pH, Dugan (1986) suggested using a mixture of SLS and benzoic acid to keep the pH low. In simulated field conditions, the combination of SLS and benzoic acid inhibited pyrite oxidation for 2 to 5 weeks in the presence of lime.

In 1985, Erickson, Kleinmann and Onysko summarized the surfactant work conducted by the U.S. Bureau of Mines at that time. Acidity was reduced in the field by 60 to 90 percent using SLS. They referred to Hugo (1965) in explaining how surfactants inhibit microbial cells. Hugo determined that surfactants caused disaggregation of the cell wall. In the case of T. ferrooxidans, the cell wall is critical in maintaining a neutral environment inside the cell. Thus, any damage to the cell wall or cell membrane could allow H^+ ions to enter and destroy the cell.

Antibiotics. Pichuantes et al. (1986) tested the effectiveness of five antibiotics toward inhibiting T. ferrooxidans. At pH 3.2, greater than 62 ppm of the antibiotic was required for inhibition. Four of the five antibiotics required greater than 250 ppm for inhibition. In the project leading to this study, Kavanaugh (1988) tested 22 antibiotics and two antibacterial agents for their ability to inhibit T. ferrooxidans. The results showed 7 of the antibiotics to be inhibitory, but required

concentrations greater than 200 mg/L. Because antibiotics are expensive, and high concentrations were required to achieve the desired effects, it is likely that antibiotics would not be economically practical for field application.

N-Serve (nitrapyrine). N-Serve, a product of Dow Chemical Company U.S.A., is the most widely used nitrification inhibitor in the agricultural industry. The active ingredient in N-Serve, nitrapyrine [2-chloro-6-(trichloromethyl) pyridine], works by inhibiting the autotrophic bacteria responsible for nitrification. Nitrapyrine is a sparingly soluble white crystalline solid. N-Serve contains 22 percent active ingredient, 2.5 percent related chlorinated pyridines, and 75.5 percent inert ingredients (from a Technical Information Document provided by Dow Chemical Corporation). The nitrapyrine is dissolved in a xylene range aromatic solvent which makes up most of the inert ingredient portion of N-Serve.

N-Serve is used to delay nitrification in soil by controlling the nitrification process. Nitrification inhibition is important because as ammonium is converted to the more soluble nitrate, it is easily lost from the field as runoff with the first storm event making it unavailable to crops, or lost to the atmosphere thru denitrification. N-Serve inhibits the autotrophic bacteria responsible for

the oxidation of ammonium to nitrite. Inhibition is believed to be the result of chelating the copper components of enzymes involved in ammonia oxidation (Hooper and Terry, 1973). N-Serve is thought to be more bacteriostatic than bactericidal because although iron oxidation is inhibited, CO_2 utilization is not inhibited (Rodgers and Ashworth, 1982). It has been observed that N-Serve becomes more bactericidal in soil cultures vs. liquid cultures (Jones and Morita, 1984). These investigators found that 10 μg/L N-Serve inhibited ammonia oxidation, while 100 μg/L was necessary to completely inhibit CO oxidation.

Soil provides bacteria significant protection from inhibition and requires approximately one order of magnitude higher dosage than in liquid cultures (Powell and Prosser, 1986). This difference is attributed to adsorption and inactivation of the inhibitor by organic matter, pH, and temperature effects. Hendrickson and Keeney (1979) also established that effectiveness of nitrapyrine decreased in organic soils. They indicated that loss by volatilization was also a significant factor in the reduction of bactericidal effectiveness. Relative inhibition was increased as soil pH increased. Inhibition was very limited at a soil pH of 4.7, and almost 100 percent effective at pH 7.4. Similarly, Strayer et al. (1981) determined that nitrapyrine caused only limited inhibition in soils treated

with simulated acid rain water (pH 3.2). These trends were due in part to the fact that nitrification proceeds very slowly in soils with low pH.

Bremner et al. (1978) defined hydrolysis of nitrapyrine to the less effective 6-chloropicolinic acid as a potential problem associated with the use of N-Serve. Powell and Prosser (1986) also indicated that hydrolysis of nitrapyrine could be a factor in longer studies because the rate of hydrolysis in aqueous solutions was quite rapid. Nitrapyrine had a half-life of about 27 hours under these conditions, and the rate was independent of nitrapyrine concentration. It is likely that the rate of hydrolysis in the field would be substantially lower due to adsorption to organic soil particles. Briggs (1975) proposed that the relatively short half-life of nitrapyrine in the field was due largely to volatilization. The half-life of N-Serve incorporated to a 3 cm soil depth was 28 days in soil low in organic matter and 50 days in soil high in organic matter. In broadcast application of fertilizer coated with N-Serve to the soil surface, 80 percent of the nitrapyrine was volatilized in the first 24 hours on wetted soils, and 60 percent volatilized on dry soils in the same period of time. After the first day, very little additional volatilization was observed.

Because N-Serve so effectively inhibited autotrophic nitrifying bacteria, it seemed likely that it could also inhibit iron oxidizing bacteria. In the study that led to the present study, Kavanaugh (1988) determined N-Serve to be the most effective of 11 compounds at inhibiting T. ferrooxidans. N-Serve inhibited ferrous iron oxidation at concentrations as low as 0.1 mg/L. Nitrapyrine inhibited oxidation just as well as the N-Serve, indicating that it is the active ingredient within N-Serve which is responsible for the inhibition of to T. ferrooxidans.

Controlled Release. Bactericides have traditionally been applied by simply spraying a solution of the bactericide and water onto the surface of the mine spoil. This has been shown to be effective in inhibiting bacteria for short periods of time. In most cases, the bacterial agent must be reapplied a number of times each year. This is expensive, time consuming, and makes the establishment of vegetative cover all but impossible because bacterial agent application is usually unable to penetrate topsoil. Controlled release of bactericides offers a long-term solution by providing a continuous dose of the chemical for a sufficient period of time for establishment of healthy vegetative cover. A typical controlled release system would consist of a

physical blend of the bactericide in a polymer matrix, from which the bactericide would slowly diffuse.

Baker (1987) suggested a monolithic controlled release system for use with hydrophobic or low melting point organic compounds. Halogenated polymers were determined to be most suitable for use with chlorinated pesticides. An acrylonitrile-polybutadiene rubber was selected for use in this project. By varying the acrylonitrile content of the compound, the physical characteristics of the device can be controlled. As acrylonitrile content increases:

a) Oil and solvent resistance improves
b) Tensile strength increases
c) Hardness increases
d) Abrasion resistance increases
e) Permeability decreases
f) Heat resistance improves
g) Low temperature resistance becomes poorer
h) Resilience decreases

(Lufter, 1964).

Technology is now available to produce controlled release systems that will be effective for 5 to 7 years (Kleinmann, Crerar, and Pacelli, 1981). Some specific factors that must be taken into account when developing a controlled release system were reviewed by Fox and Rastogi (1983) and can be found in Table I. A disadvantage to using controlled release, is the long time period required to release the minimum effective dose required to control the problem. Kleinmann and Erickson (1981) solved this problem

by proposing an initial spray application of the bactericide along with application of the controlled release system. Thus, as the spray application would normally be beginning to lose its effectiveness, the controlled release system maintains a dose sufficient to inhibit bacteria.

The initial spray application required is dependent on the amount of chemical required to kill bacteria, as well as the capacity of the overburden to adsorb the chemical. Success of this technique requires good infiltration of rainfall to allow for transport of the bactericide to lower layers of the spoil (Kleinmann and Erickson, 1981).

Table I. Specific factors which must be considered to develop a controlled release system.

- Chemical Environment
  - pH, acidity, sulfates, iron
  - Moisture content
  - Pyrite content

- Physical Environment
  - Particle size distribution
  - Soil cover
  - Slope
  - Accessibility (application)
  - Degree of vegetation
  - Type of overburden material
  - Spoil age - stage of acid production

- Climatological
  - Precipitation (average, events)
  - Temperature (average and extremes)

- Hydrologic
  - Percolation rates
  - Runoff rates
  - Transpiration rate

SUMMARY

Based on the review of the literature, it is clear that N-Serve has the potential for effective AMD control. A column study would be the next logical step to evaluate its potential for field application. Development of a reliable controlled release system for use with N-Serve would also be recommended in order that treatment duration and effectiveness could be increased. Future study should include field application of N-Serve to mine waste piles under a variety of conditions to determine application rates for full-scale use.

III. MATERIALS AND METHODS

To analyze the effectiveness of nitrapyrine (N-Serve) at inhibiting AMD, it was necessary to simulate field conditions. Toward this purpose, a column study was conducted. At the beginning of the study period, sixteen columns, eight containing 7 kg coal mine waste and eight containing 7 kg hard-rock mine waste, were treated with varying doses of the antibiotic N-Serve. Beginning the following week, the columns were leached weekly with water. The majority of the laboratory work for this study involved the analysis of the the column effluent. Physical, chemical and biological characteristics of the column effluent were monitored to determine the effects of N-Serve on the production of AMD from the columns. In a second study, a controlled release device for applying the antibiotic was developed. This chapter describes all of the materials and methods that were used in conducting these studies.

COLUMN STUDY

Two different solid substrates were used for the column study: 1) an unweathered coal waste rock from recently unearthed refuse piles at a mine in Northwestern North Carolina, and 2) a gold, silver, and copper mine waste material, originated from a hard-rock mine in British

Columbia, Canada. This material had a high pyrite content and was not processed by the mine because it was below ore grade.

Both substrates were treated identically for this study. The mine wastes were received in large (3 - 30 cm) pieces. The material was processed through a Deco Jaw Crusher, which reduced the particle size to less than 3 cm. The material was then seived with a No.8 mesh sieve (2.38 mm). Thus, the particle size of the material used in the column study ranged from 2.38 mm to 30 mm. To obtain a random distribution of the material for each column, the entire supply of each substrate was coned and quartered (Peele, 1988).

The columns were five-gallon, plastic buckets. Two 7/16-inch holes were drilled in the bottom of each column to allow complete drainage of water. The holes were then covered with a plastic screen with openings of approximately 1 mm. This screen allowed fine materials to pass out of the column, and prevented blockage of the holes in the buckets by larger particles. Seven kilograms (kg) of the waste rock was placed in each column. The columns were then placed on a level, elevated, wooden-support structure with one smaller bucket placed beneath each column to collect the effluent from that column.

N-Serve Dosage Determination

Sixteen columns were prepared for this study, eight contained the coal substrate and eight contained the hard-rock substrate. Two columns of each substrate were used as controls and were not treated with N-Serve. Another two columns received a low dosage of N-Serve, two others received a medium dosage, and two received a high dosage for each of the two substrates. The experimental design is summarized in Table II.

The high-dose used in this study was 480 mg nitrapyrine/kg waste material or 2200 mg N-Serve/kg. The moles of nitrapyrine in the high-dose was equivalent to the moles of Sodium Lauryl Sulfate (SLS) in the high-dose of 600 mg SLS/kg waste material used by Watzlaf (1988). The medium dose was 48.1 mg nitrapyrine/kg or 220 mg N-Serve/kg, and was the molar equivalent of the low dose of 60 mg SLS/kg used by Watzlaf. The low dose 4.8 mg nitrapyrine/kg or 22 mg N-Serve/kg substrate.

Table II. Experimental design for the column study.

Column Number	Substrate	N-Serve Dose (g/kg)
1	Coal Mine Waste	Untreated Control
2	"	"
3	"	Low (0.022 g/kg)
4	"	"
5	"	Medium (0.22 g/kg)
6	"	"
7	"	High (2.2 g/kg)
8	"	"
9	Hard-Rock Mine Waste	Untreated Control
10	"	"
11	"	Low (0.022 g/kg)
12	"	"
13	"	Medium (0.22 g/kg)
14	"	"
15	"	High (2.2 g/kg)
16	"	"

N-Serve Application Procedure

At the beginning of the study, N-Serve was applied to the columns as an emulsion in distilled water. The N-Serve dose for each column was emulsified for 30 minutes using a E/MC Model 250 ultrasonic bath. Each emulsion was then diluted enough that when applied to the column, it would just cover all of the substrate in that column. Concurrently, the untreated control columns received an application of distilled water sufficient to completely submerge the substrate in those columns. The holes in the bottom of the columns were temporarily plugged so that the emulsion could stay in contact with that substrate for a period of 24 hours, after which the holes were unplugged. The water and any N-Serve that was not adhered to the substrate or the column was allowed to flow freely from the column.

Weekly Leaching Procedure

Beginning one week after the N-Serve treatment, each column was leached with the equivalent of 2.5 cm (1 inch) of precipitation based on the surface area of the column. This leaching process then continued once per week, each week thereafter. Deionized-distilled water (1.4 liters) was applied per column per week. The water was applied to the columns using a common plastic watering bucket with

perforations at the outlet to allow an even distribution of water over the entire surface of the substrate. The leachate was collected and prepared for analysis within eight hours of leaching. The leachate collection buckets were cleaned by scrubbing in hot soapy water immediately after sample collection for use the following week.

Analytical Measurements

Acidity. The acidity of the effluent was measured each week according to the method listed in Standard Methods For The Examination Of Water and Wastewater (APHA, 1985). Because concentrations of reduced metals in the samples were often high, the hot peroxide method was employed in preparing the samples. Each sample was adjusted to a pH less than 4.0 with sulfuric acid (H_2SO_4), and treated with five drops of 30 percent hydrogen peroxide (H_2O_2) to oxidize the reduced metal forms. This process was accelerated by boiling the samples for from two to five minutes. The samples were then titrated to the phenolthalene endpoint (pH 8.3) using sodium hydroxide. Acidity was recorded as mg $CaCO_3$/L.

pH. The pH of the column leachates was measured with a Fisher Model 601A pH Meter within eight hours after collection.

Metals. The metal concentrations monitored in the column study included: total iron (Fe), manganese (Mn), magnesium (Mg), calcium (Ca), and aluminum (Al). A sample of the effluent from each column was taken each week for metals analysis. These samples were then acidified to a pH less than 2.0 using nitric acid (HNO_3). The metal concentrations were analyzed with a Perkin-Elmer Model 703 Atomic Absorption Spectrophotometer, with the exception of aluminum, which was analyzed on a Perkin-Elmer Model MHS 20 Atomic Adsorption Spectrophotometer. The spectrophotometer was standardized after every six samples to increase accuracy.

Sulfate. Samples for sulfate (SO_4^{2-}) analysis were collected each week, filtered to remove large particultes, and immediately refrigerated at 4°C. The analyses were performed within one week after collection. The sulfate concentrations (ppm) were found using a Dionex Model 2010i ion chromatograph.

Ferrous Iron. Ferrous iron (Fe^{2+}) was determined by potassium dichromate titration with sodium diphenylamine sulfonate as the end-point indicator (Koltoff and Sandell, 1952). One standard, one spike, and one duplicate analysis were performed with each set of ten Fe^{2+} determinations.

Aliquots of ferrous ammonium sulfate were used for standards and spikes.

Bacterial Counts. The number of T. ferrooxidans cells/liter in the column effluent was monitored using the five-tube, most probable number (MPN) method with 9K medium, as described in Standard Methods For The Examination Of Water and Wastewater (APHA, 1985). MPN tests were performed on Week 1 and Week 29, the beginning and end of the study period.

CONTROLLED RELEASE STUDY

A controlled release system containing nitrapyrine was developed for the sole purpose of determining release-rate characteristics of the system. The recipe for the monolithic controlled release system used in this study is as follows (Baker, 1987):

Polybutadiene-acrylonitrile copolymers (22 to 44% acrylic)	100.0 parts
Sulfur	1.5 parts
Tetramethyl thiazam disulfide	0.4 parts
Zinc oxide	3.6 parts
Lauric acid	3.0 parts
Carbon black	40.0 parts

Cure for 20 minutes at 150°C.

This recipe can be used to prepare formulations containing five to fifteen percent nitrapyrine. Higher concentrations could interfere with the physical properties of the elastomer, in this case Hycar (Baker, 1987). A personal communication with Mr. Keith Riew of the Elastomers Department at B.F. Goodrich Corp. in Brecksville, OH yielded a sample of Hycar VT 355, 35 percent acrylic, which the consultant indicated would be most suitable polymer for this project.

Two controlled release formulations, each with a nitrapyrine content of 15 percent, were prepared for the study. The first device contained 10 percent carbon filler, and the second device contained 25 percent carbon filler. Because no proper rubber-mixing device (rubber mill or Banberry rubber mixer) was available, the controlled release formulations were mixed on a hand-operated, hydraulic press, max. 20,000 psi. Rubber formulations with carbon contents above 25 percent were extremely difficult to handle using the hydraulic press. Filler, accelerator, vulcanizing agent and nitrapyrine were mixed together, folded, and pressed into the polymer repeatedly (15,000-20,000 psi) until the compound appeared to be homogeneous. The result was a circular sheet of rubber which remained pressed between two sheets of steel with C-clamps until vulcanization was complete. After vulcanization the rubber sheets averaged

1.3 mm thickness and ranged from 1.1 to 1.7 mm. Because no extrusion device was available, the sheet of rubber was cut into 1.0 cm X 1.5 cm strips for nitrapyrine release-rate determinations.

The U.S. Bureau of Mines has developed an extraction proceedure to evaluate release rates from a polymer matrix under simulated field conditions (Kleinmann and Erickson, 1981). However, this test is time consuming and inconvenient for screening materials (Fox and Rastogi, 1983). To more efficiently determine release characteristics for the controlled release formulations, a quick extraction procedure was developed.

Nitrapyrine release rates were determined as follows. One gram of the rubber strips was placed in a 50-mL beaker containing 10 mL Milli-Q water. The water was poured off and collected daily and the rubber in the beaker was then reimmersed in 10 mL of Milli-Q water. The water sample was extracted with 0.20 mL methylene chloride, 2 μL of which was injected directly into a Tracor 560 gas chromatograph for comparison against standard solutions of nitrapyrine in methylene chloride. This method of determining release rates proved suitable for this study.

IV. RESULTS

The two objectives of this study were to determine the effectiveness of N-Serve in inhibiting the formation of AMD in columns, and to develope a formulation for the controlled release of nitrapyrine. In the first section of this chapter, the data collected in the column study from January through August 1989 will be presented. The second section summarizes the results of an investigation of relative release rates for two, monolithic, controlled release formulations developed for nitrapyrine application.

COLUMN STUDY

Two substrates, a coal mine waste and a hard-rock mine waste, were used in the column study with the results from the two substrates presented separately in this section. The results summarize the chemical and biological characteristics of the column effluents. For both the coal and hard-rock substrates there were four sets of duplicate columns including: two control columns, two low N-Serve dose columns, two medium N-Serve dose columns, and two high N-Serve dose columns. All data presented in this section represent averaged values for the duplicate columns.

Before application of distilled water (Week 0) the columns were treated with varying doses of N-Serve in the form of an emulsion. The emulsion was left in contact with the substrate for 24 hours, after which time it was allowed to drain. The effluent from this treatment procedure was analyzed to determine how much of the N-Serve had either been retained by the column or been volatilized. The results of this test are presented in Table III. In all of the coal waste columns, the percentage of N-Serve that was either retained by the columns or volatilized was greater than 97 percent. The percentage of N-Serve retained or volitilized in the hard-rock mine waste columns varied with dosage. In the low-dose columns containing hard-rock mine waste, an average of 80 percent of the N-Serve was either retained or volatilized, the medium and high-dose columns averaged 94 and 99 percent retention-volatilization respectively.

Data concerning column effluent acidity, pH, iron, manganese, magnesium, calcium, aluminum, and sulfate for each sampling week are listed in Tables A-1 through A-29 in the Appendix.

Table III. Percentage of applied N-Serve volume retained by the columns and/or lost to volitilization.

Substrate	Initial N-Serve Dose	Percentage of N-Serve Adsorbed/Volitilized
Coal Mine Waste	Low Dose (0.022g/kg)	97.8%
"	Medium Dose (0.22 g/kg)	98.3%
"	High Dose (2.2 g/kg)	98.4%
Hard-Rock Mine Waste	Low Dose (0.022 g/kg)	79.8%
"	Medium Dose (0.22 g/kg)	94.3%
"	High Dose (2.2 g/kg)	99.3%

Coal Mine Waste. Figures 2-10 summarize the effluent characteristics of the coal mine waste columns. The trends in effluent acidity for the coal mine waste columns are shown in Figure 2. At the beginning of the study, all of the columns produced very little acidity, ranging from 57 to 64 mg/L as $CaCO_3$. The acidity trend in the control-, low-dose, and medium-dose columns then increased during the remainder of the study period. The highest average acidity of leachate from the control columns was 1351 mg/L as $CaCO_3$ during Week 28. After Week 1, both the low-dose and medium-dose columns consistently produced more acidity than the control columns. The medium-dose columns produced the most acidity, with the highest average value approaching 1800 mg/L $CaCO_3$ in Week 25. The high-dose columns produced considerably less acidity than the control columns throughout the study period. From Weeks 1 through 24 the average acidity in the high-dose columns remained below 415 mg/L $CaCO_3$, after which the acidity began to increase and peaked at 692 mg/L $CaCO_3$ on Week 29.

Figure 3 illustrates the variations in pH of the leachate from the coal waste columns. In Week 1, all columns (control-, low-dose, medium-dose, and high-dose) produced effluent with pH values between 3.7 and 3.9. After Week 1, the effluent pH from all columns decreased. In the control, low-dose, and medium-dose columns, the average pH

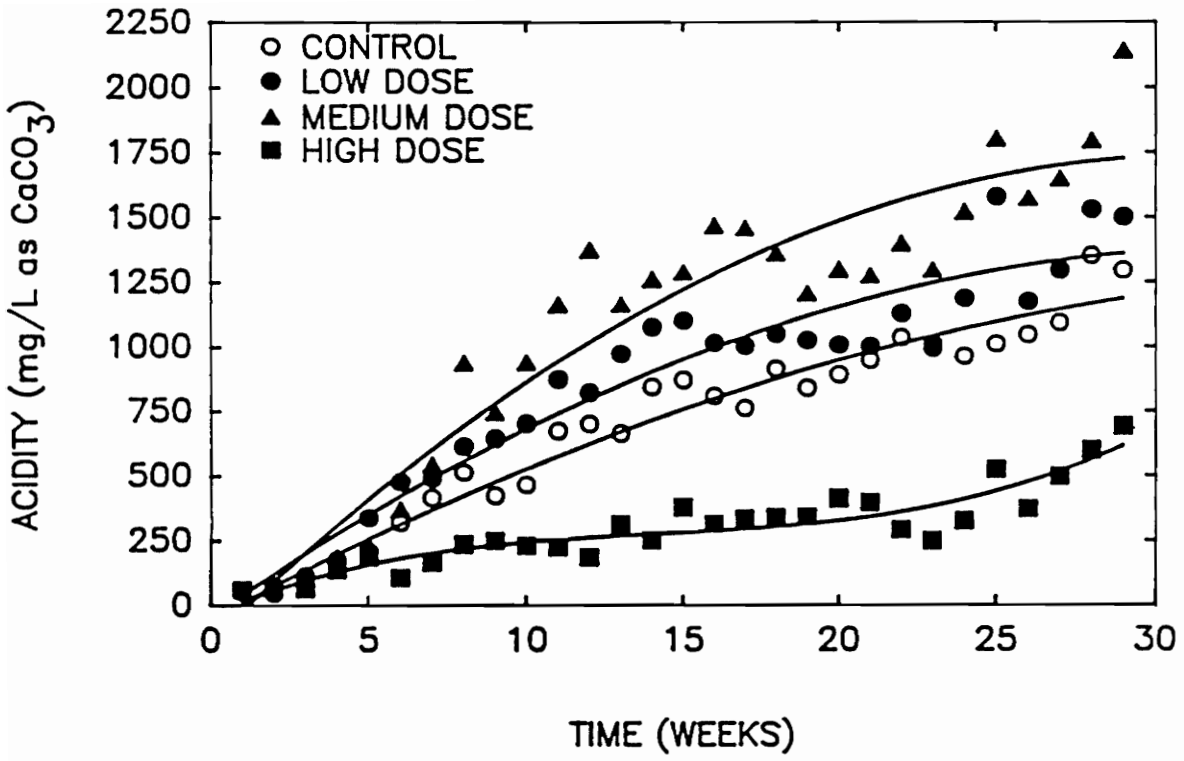

Figure 2. Concentration of acidity vs. time for coal mine waste with different doses of N-Serve.

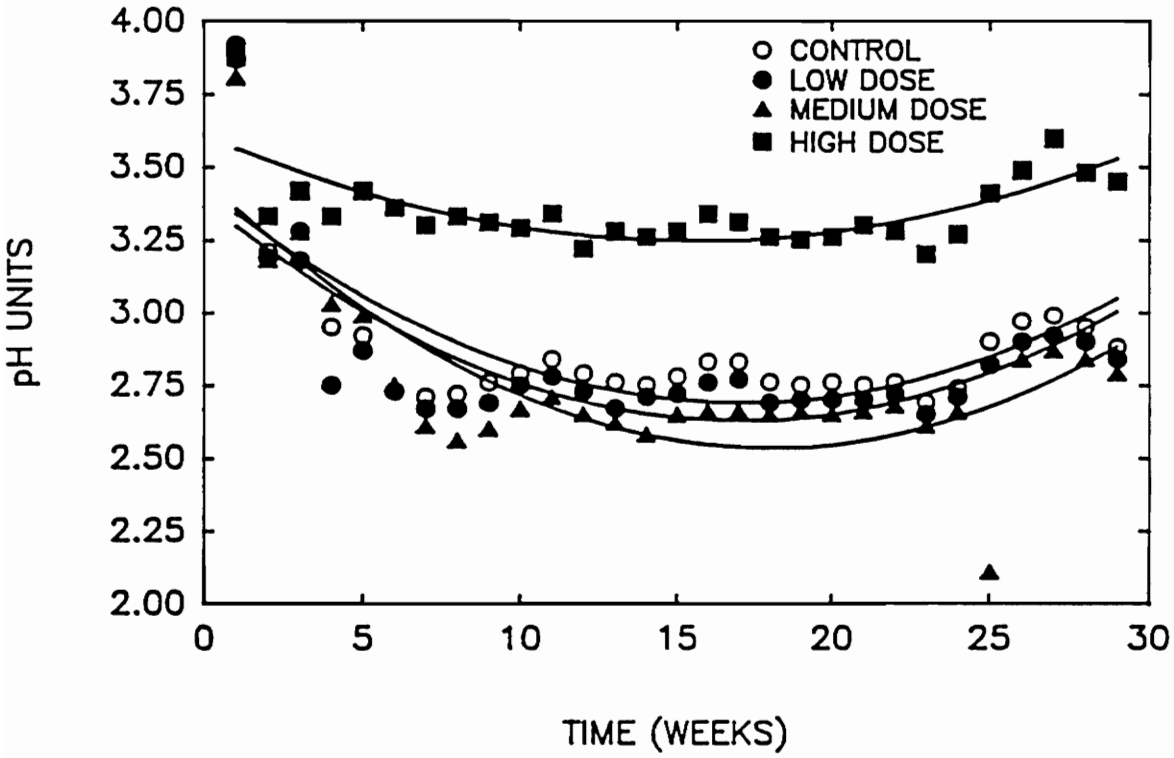

Figure 3. pH vs. time for coal mine waste with different doses of N-Serve.

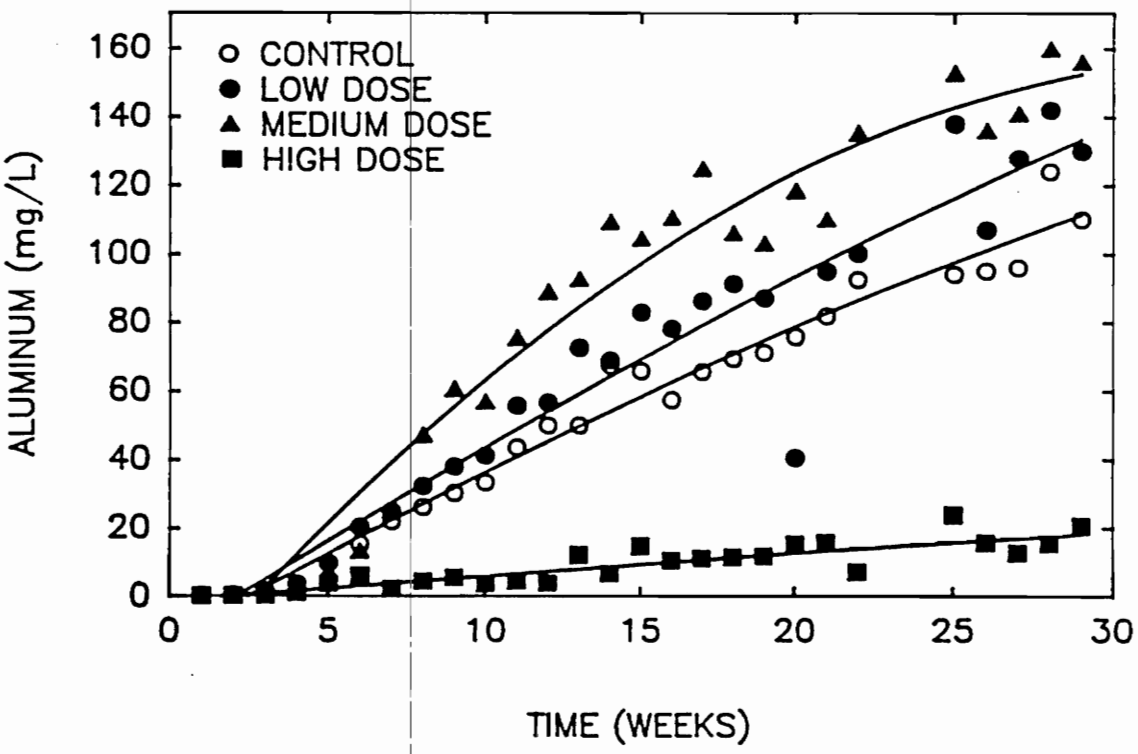

Figure 4. Aluminum concentration vs. time for coal mine waste with different doses of N-Serve.

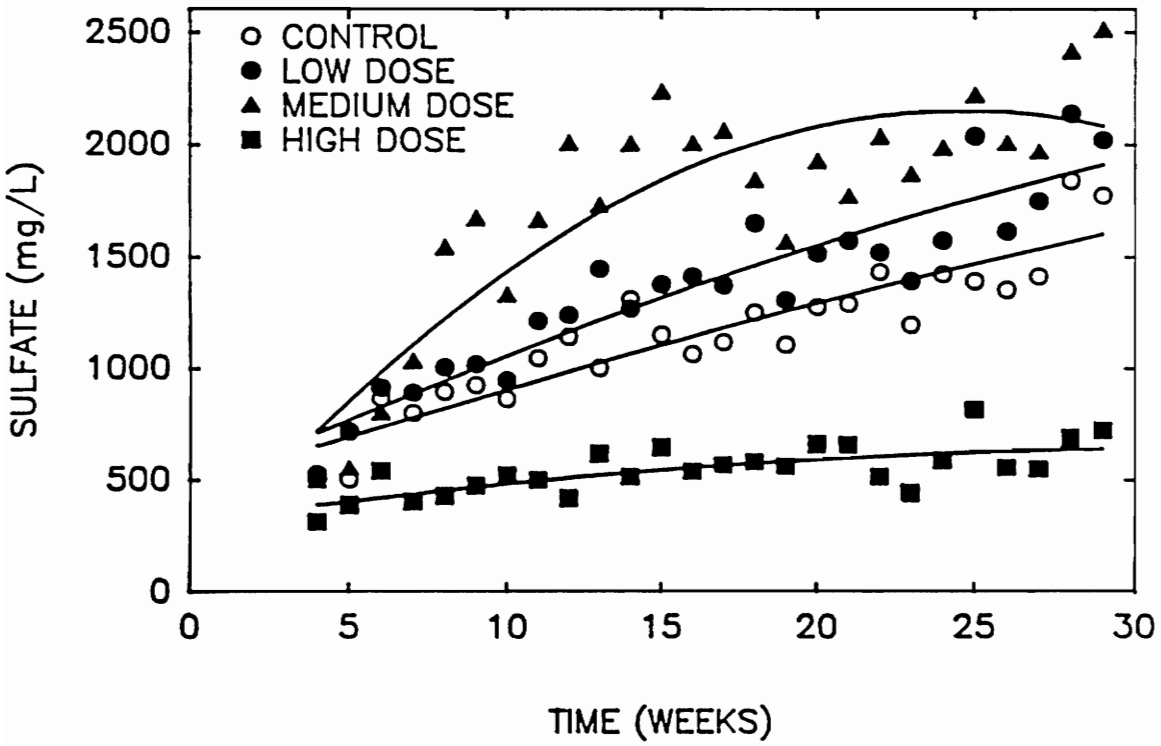

Figure 5. Sulfate concentration vs. time for coal mine waste with different doses of N-Serve.

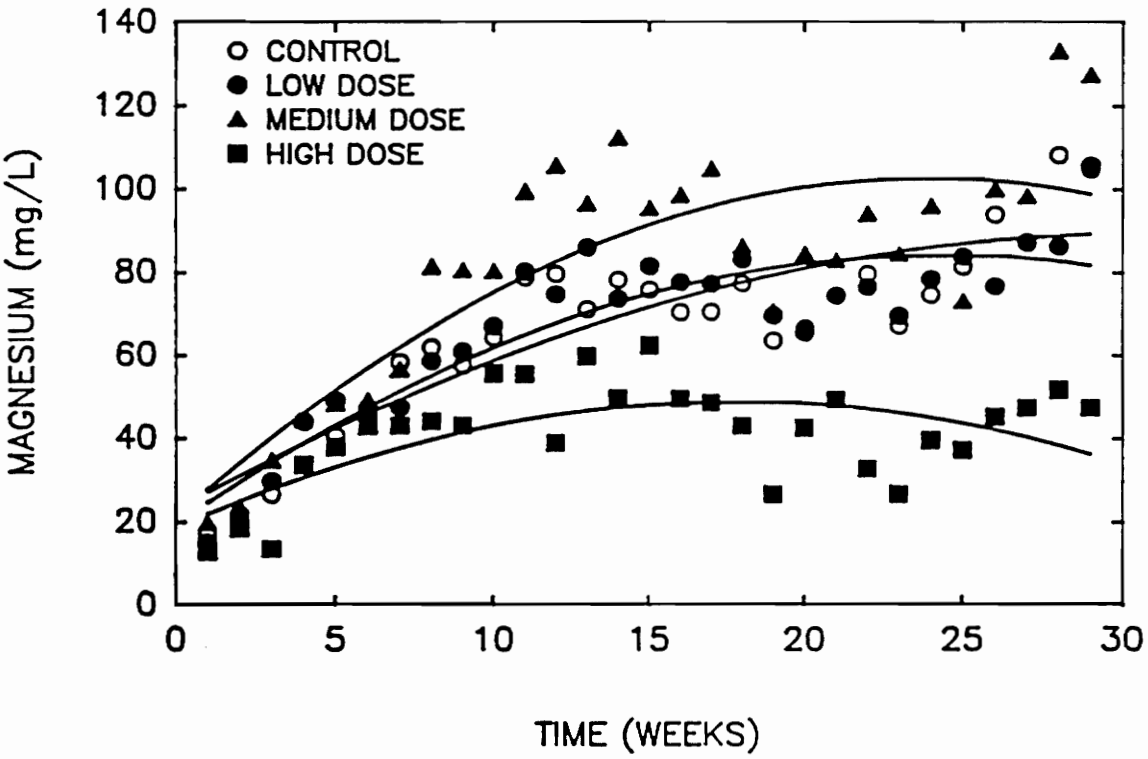

Figure 6. Magnesium concentration vs. time for coal mine waste with different doses of N-Serve.

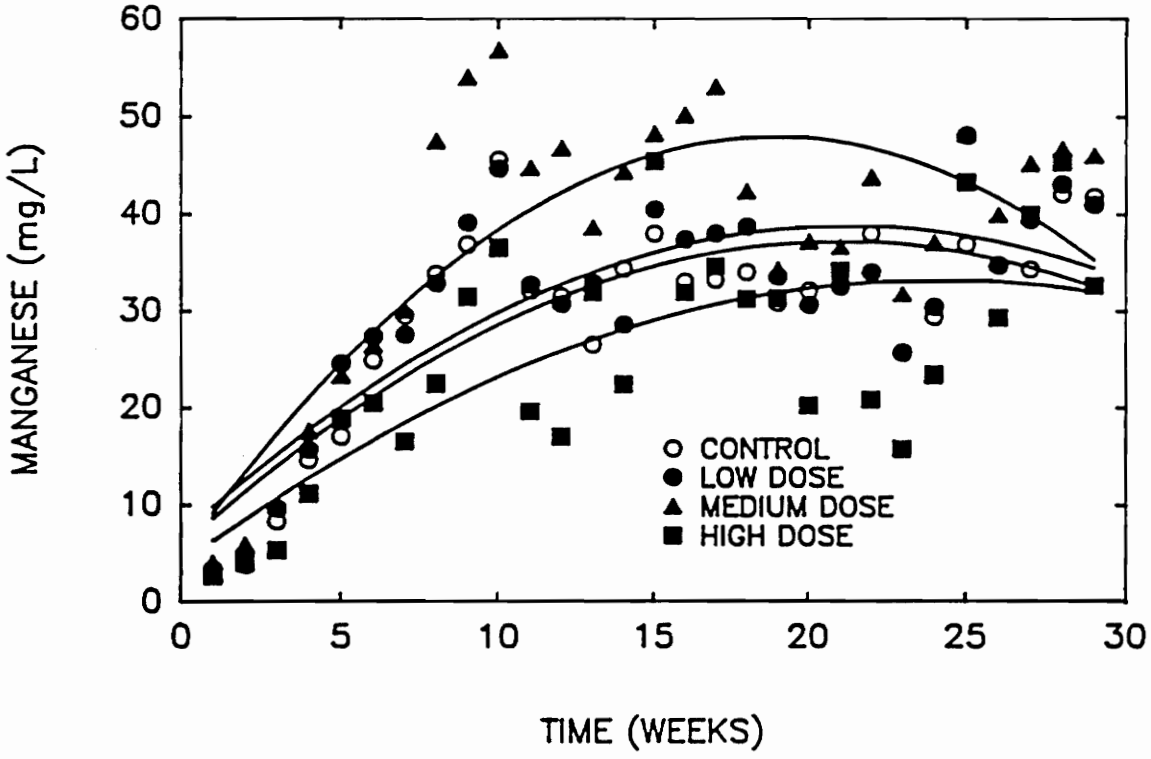

Figure 7. Manganese concentration vs. time for coal mine waste with different doses of N-Serve.

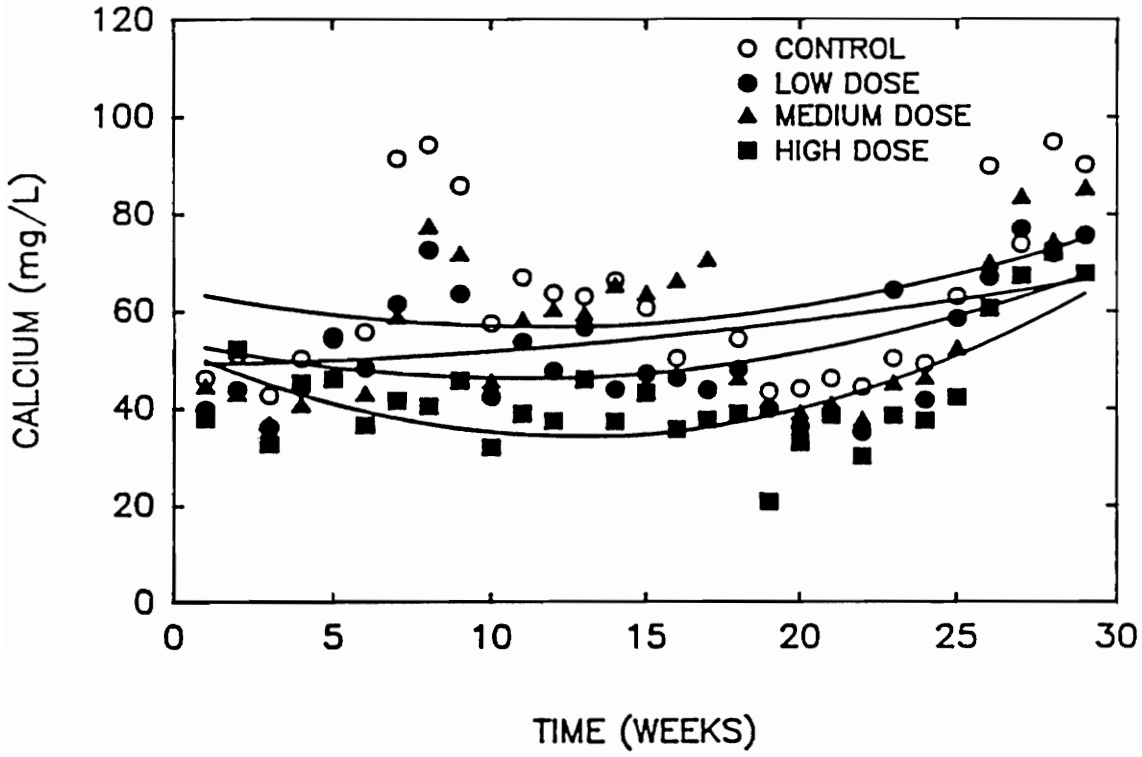

Figure 8. Calcium concentration vs. time for coal mine waste with different doses of N-Serve.

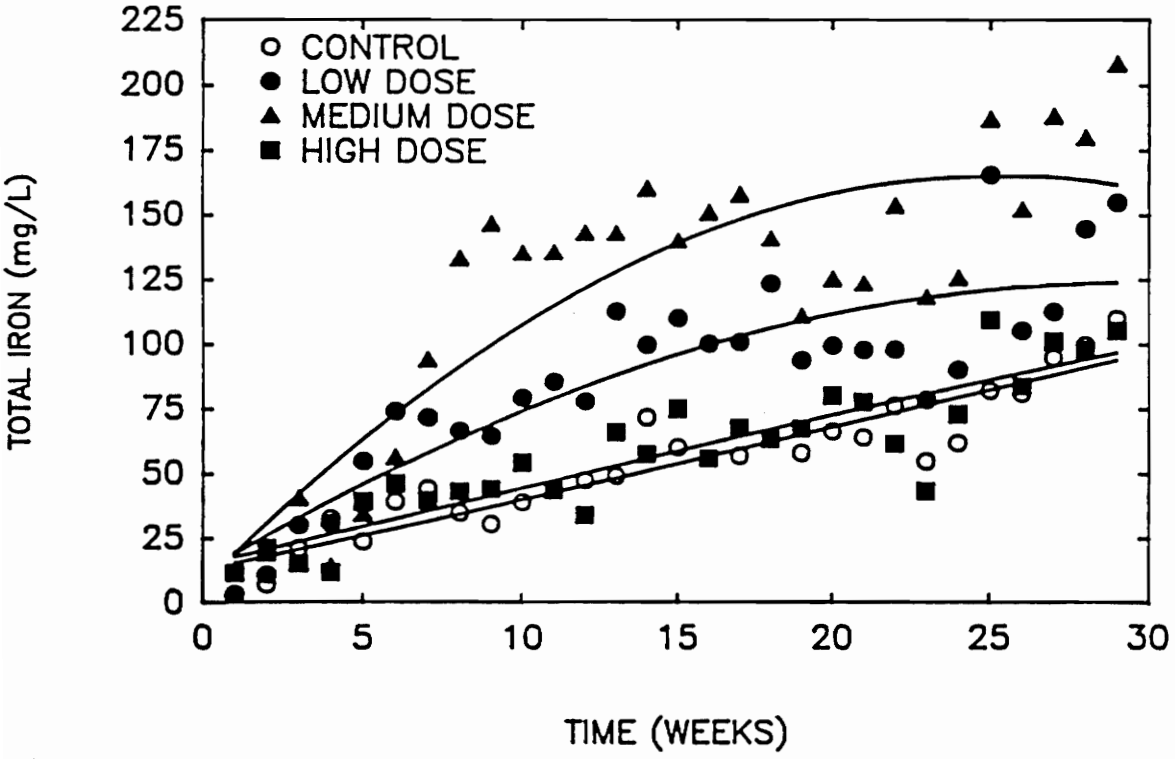

Figure 9. Total iron concentration vs. time for coal mine waste with different doses of N-Serve.

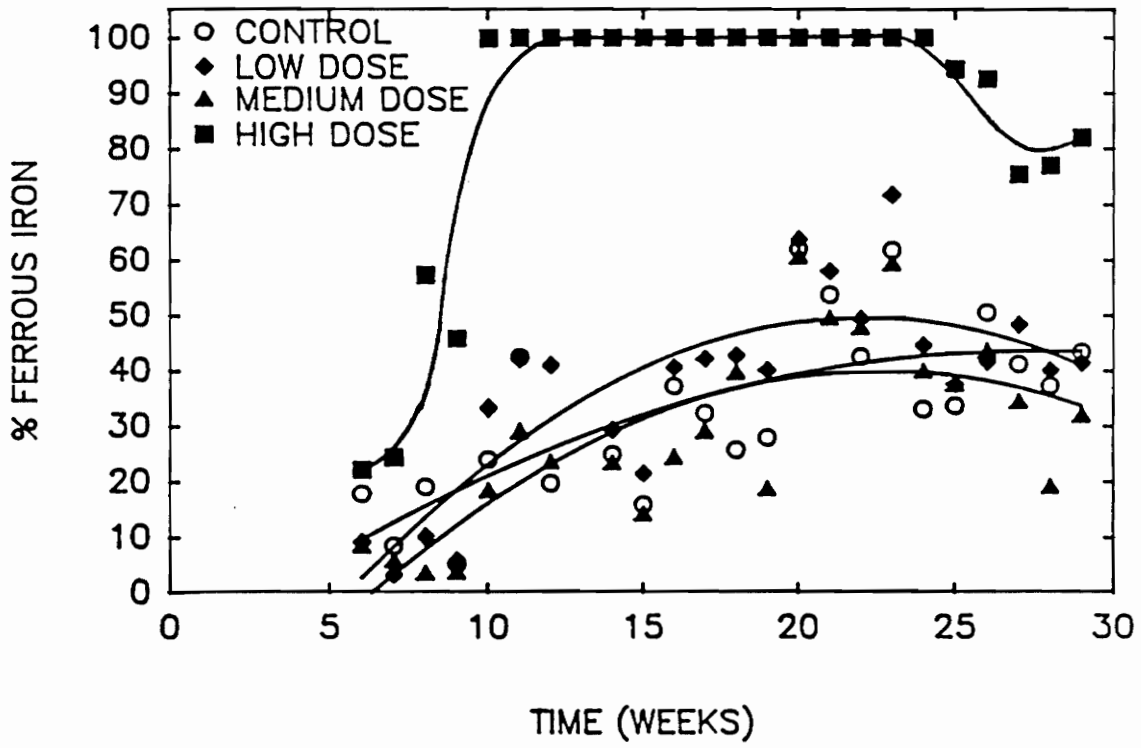

Figure 10. Percent of total iron in ferrous state vs. time for coal mine waste with different doses of N-Serve.

began to stabilize at about Week 6. From Week 6 through the end of the study these columns produced an effluent with a pH in the range of 2.5 to 3.0. The pH of the high-dose column effluent stabilized immediately after the first week and averaged between 3.4 and 3.6 during the remainder of the study period.

The trends in average aluminum, sulfate, and magnesium concentrations vs. time (Figures 4, 5, and 6 respectively) very closely followed the trends in acidity of the coal mine refuse columns. In each case, the control columns produced less aluminum, sulfate, and magnesium than either the low or medium-dose columns but produced considerably more aluminum, sulfate, and magnesium than the high-dose columns.

The average aluminum concentrations in effluents from the control columns peaked at 124 mg/L, while the highest average aluminum concentration in the high-dose columns registered only 24 mg/L. The highest average aluminum concentration (160 mg/L) occurred on Week 28 in the effluent from the medium-dose columns.

Sulfate was not analyzed until Week 4 because the ion chromatograph was disabled from Week 1 to Week 4. Average sulfate concentrations in the efluent from control columns were 501 mg/L on Week 4, and increased to a peak value of 1839 mg/L on Week 28. Both the low and medium-dose columns produced more sulfate than the controls throughout the

study, with the peak value of 2416 mg/L occurring on Week 28 in the medium-dose columns. The high-dose columns produced less sulfate than the controls, and never exceeded the value of 814 mg/L recorded on Week 25.

The magnesium levels (Figure 6) in all of the columns were very similar during Weeks 1 through 7; thereafter the medium-dose columns produced more magnesium than any of the others. The control and low-dose columns continued to produce comparable amounts of magnesium. After Week 7, only the high-dose columns consistently produced lower average magnesium concentrations than the control. The highest magnesium level in the high-dose columns occurred during Week 28 at 108.2 mg/L, while the highest average magnesium level produced by the high-dose columns was 62.5 mg/L during Week 15.

The trends for manganese (Figure 7) and calcium (Figure 8) were difficult to distinguish from one another. Although there was scatter in the data, the overall trends in manganese concentration were increasing with time through approximately Week 10, after which the scatter was too great to interpret trends. All of the columns produced fairly consistent amounts of calcium through Week 25 after which the trend was increasing with time for all the columns.

The average total iron concentration (Figure 9) in the leachates form all columns increased throughout the study

period. The control and high-dose columns produced comparable amounts of iron throughout the study. The low-dose columns produced more iron than either the control- or high-dose columns after Week 5. The high-dose columns produced more iron than any of the columns from Weeks 7 through 29.

Ferrous iron determinations began on Week 6 of the study and continued through Week 29. Ferrous iron results are presented as percentage of effluent total iron in the ferrous state. The data for percent ferrous iron vs. time from the coal waste were presented earlier in Figure 10. The percent ferrous iron in the control-, low-dose, and medium-dose columns increased with time through the study period but remained at less than 70 percent of the total iron concentration at all times. In effluents from the high-dose columns, all of the iron in the effluent was in the unoxidized ferrous state from Week 10 through Week 24, after which time the percentage of iron in the ferrous state decreased slightly.

Bacteria counts were completed at the beginning and end of the study period (Week 1 and 30). The results are presented as averaged data from each set of duplicate columns in Table IV. On Week 1, the MPN tests for the coal waste indicated greater than 10^6 cells/100 mL in all of the columns. On Week 30, T. ferrooxidans numbers (1.8×10^7

Table IV. MPN test results for Week 1 and Week 30 indicating the number of T. ferrooxidans cells/100 mL of column effluent.

Substrate	N-Serve Dose	Cells/100 mL Week 1	Cells/100 mL Week 30
Coal Mine Waste	Control	$> 10^6$	1.8×10^7
"	Low Dose	$> 10^6$	1.7×10^3
"	Medium Dose	$> 10^6$	6.3×10^4
"	High Dose	$> 10^6$	1.3×10^3
Hard-Rock Mine Waste	Control	$> 10^6$	4.5×10^6
"	Low Dose	$> 10^6$	1.5×10^8
"	Medium Dose	$> 10^6$	2.4×10^5
"	High Dose	$> 10^6$	2.0×10^4

cells/100 mL) were highest in the control column effluent. T. ferrooxidans numbers (1.3×10^3 cells/100 mL) were lowest in the high-dose columns.

Hard-Rock Mine Waste. Figures 11-19 summarize characteristics of the effluents from the hard-rock mine waste columns. The trends in effluent acidity in the hard-rock mine waste columns are illustrated in Figure 11. The acidity produced by the hard-rock mine waste was low for the entire study period, never exceeding an average value of 161 mg/L $CaCO_3$ in any of the columns. Acidity concentrations decreased with time throughout the study period for the control-, medium-dose, and high-dose columns. The trend for the low-dose columns was scattered but remained consistently between 100 and 150 mg/L $CaCO_3$. The regression lines plotted through the data show clearly that the lowest acidity effluent was produced by the high-dose columns, followed in order of increasing acidity production by the medium-dose, control-, and low-dose columns. These trends persisted for the entire study period.

Figure 12 illustrates the pH trend in effluents from the hard-rock mine waste columns. During the first week, all of the columns (control-, low-dose, medium-dose, and high-dose) produced an average effluent with pH of between 3.9 and 4.1. By Week 2, the pH decreased to its lowest

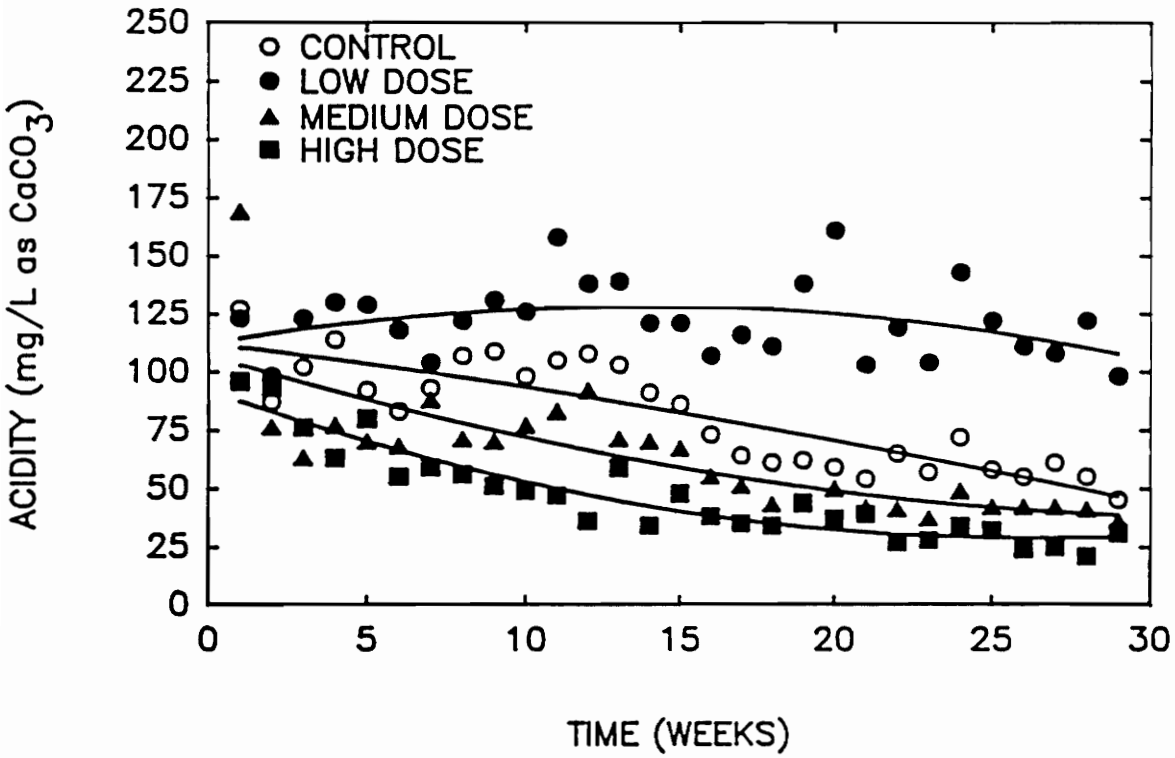

Figure 11. Concentration of acidity vs. time for hard-rock mine waste with different doses of N-Serve.

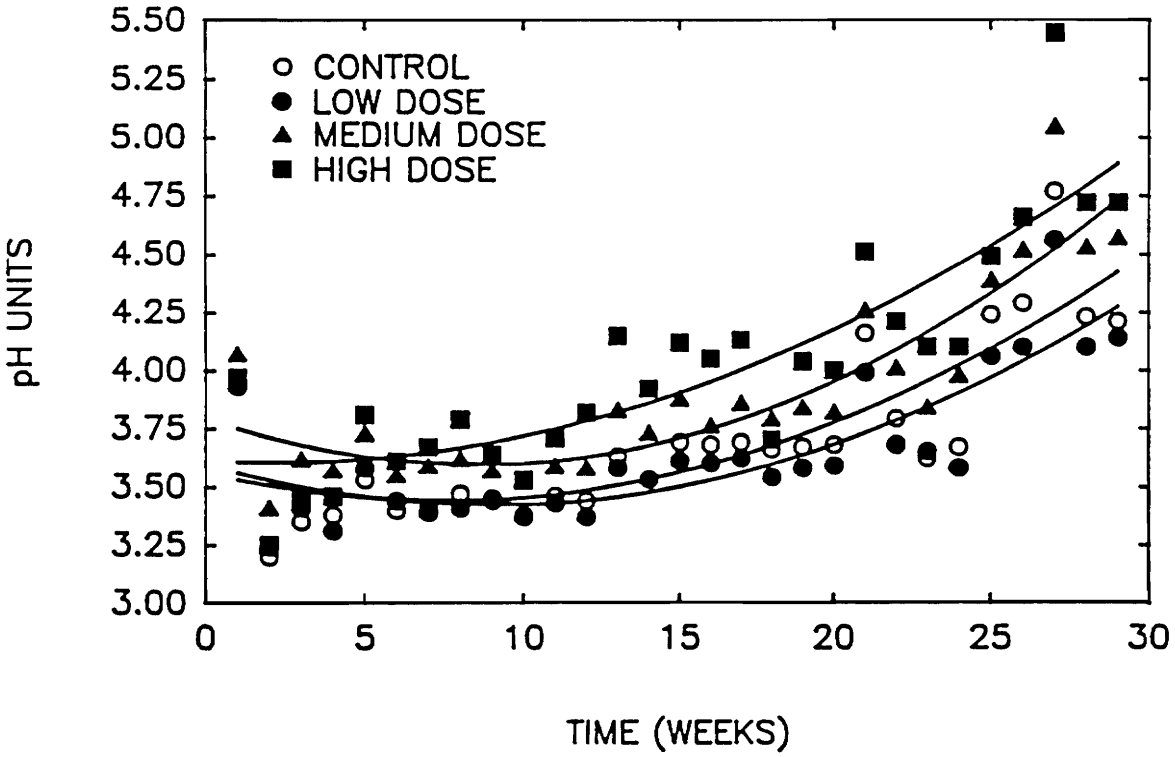

Figure 12. pH vs. time for hard-rock mine waste with different doses of N-Serve.

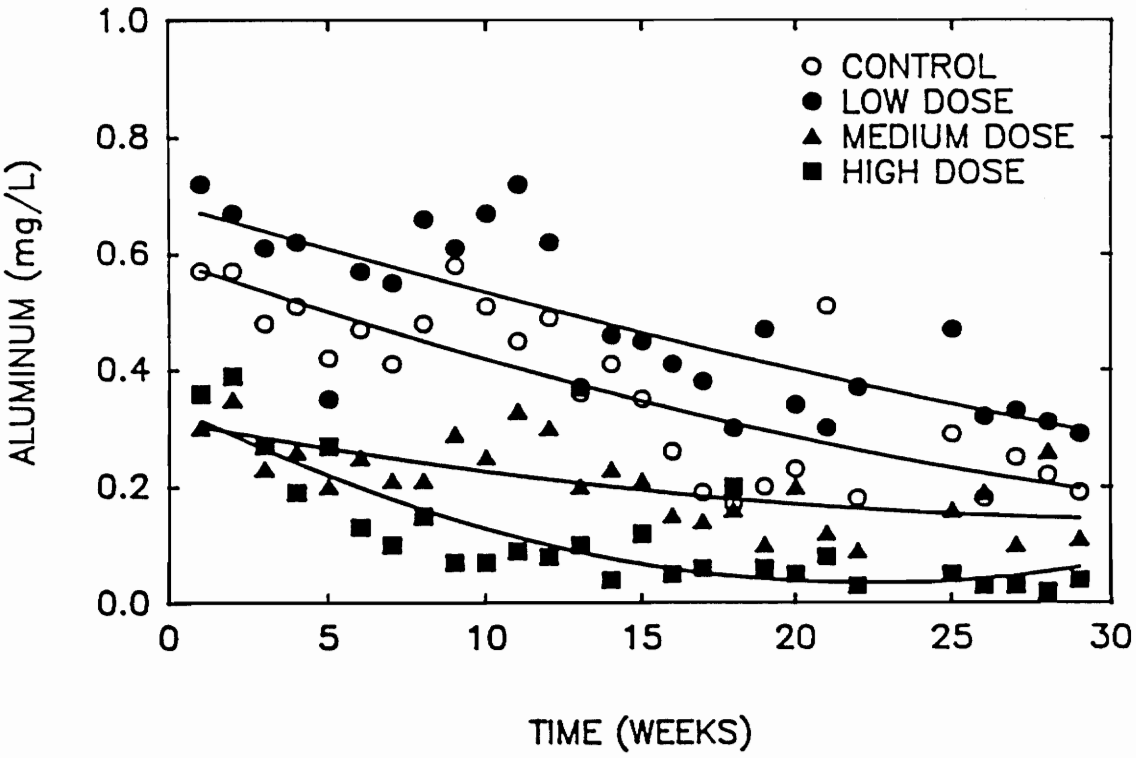

Figure 13. Aluminum concentration vs. time for hard-rock mine waste with different doses of N-Serve.

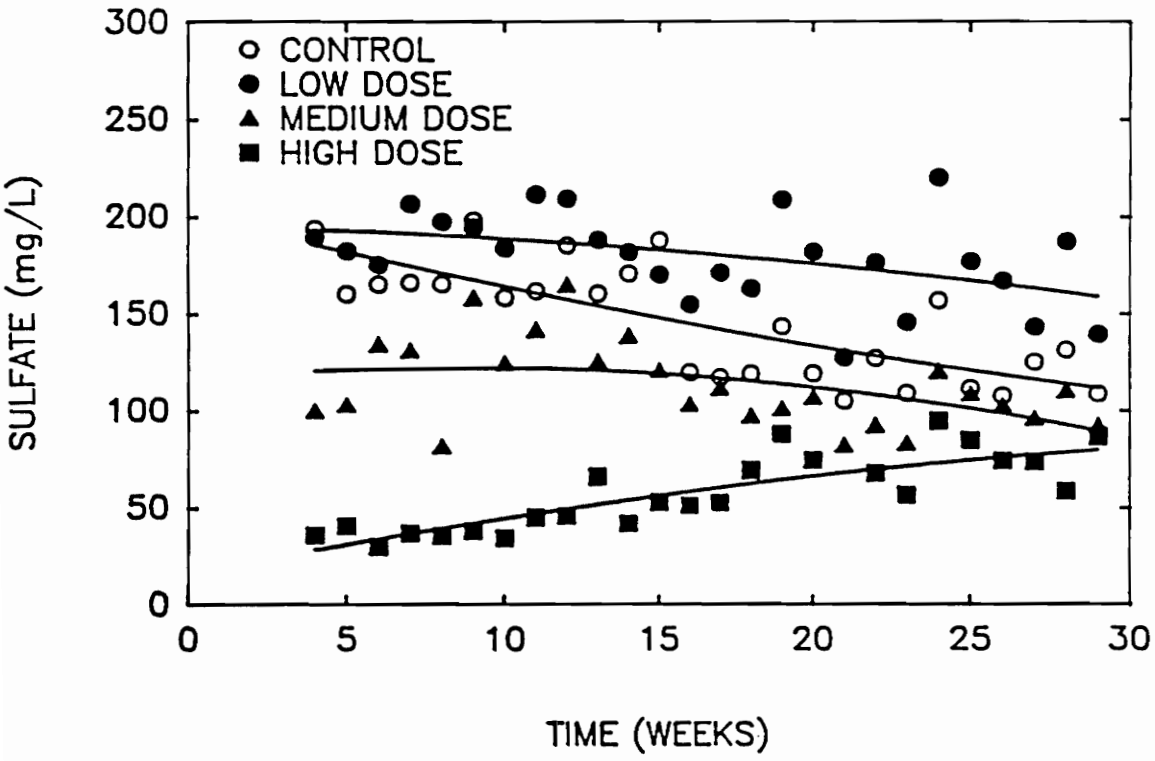

Figure 14. Sulfate concentration vs. time for hard-rock mine waste with different doses of N-Serve.

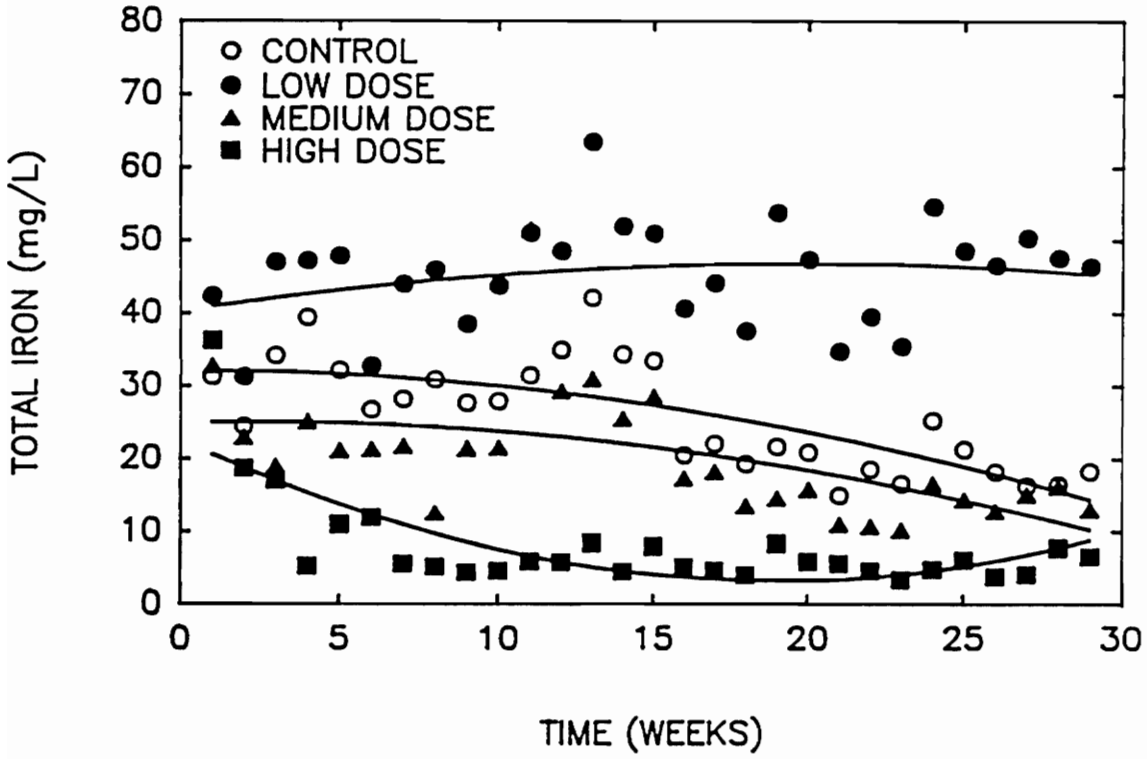

Figure 15. Total iron concentration vs. time for hard-rock mine waste with different doses of N-Serve.

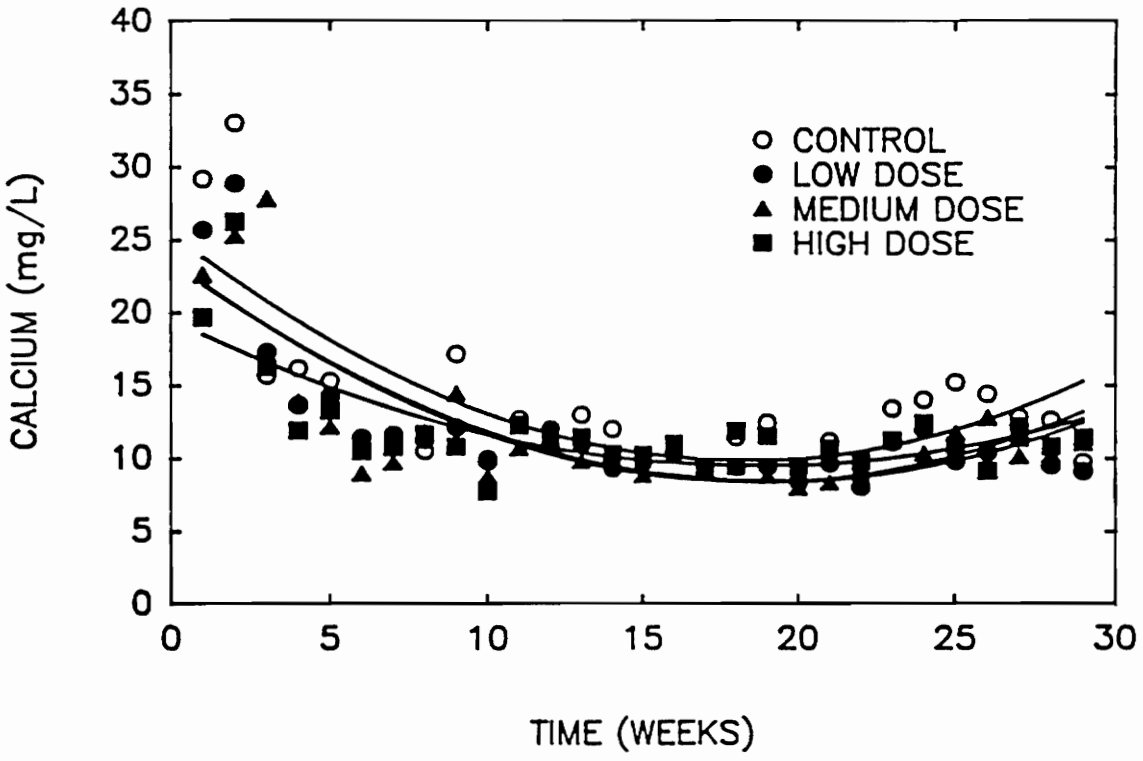

Figure 16. Calcium concentration vs. time for hard-rock mine waste with different doses of N-Serve.

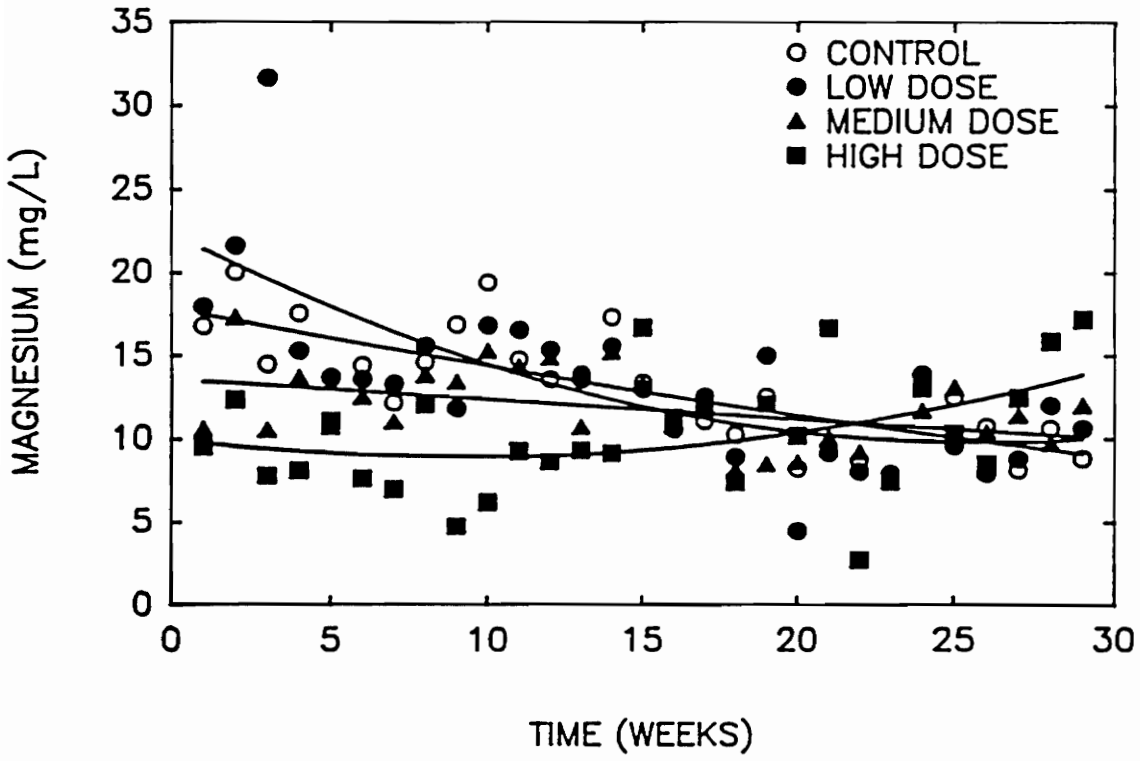

Figure 17. Magnesium concentration vs. time for hard-rock mine waste with different doses of N-Serve.

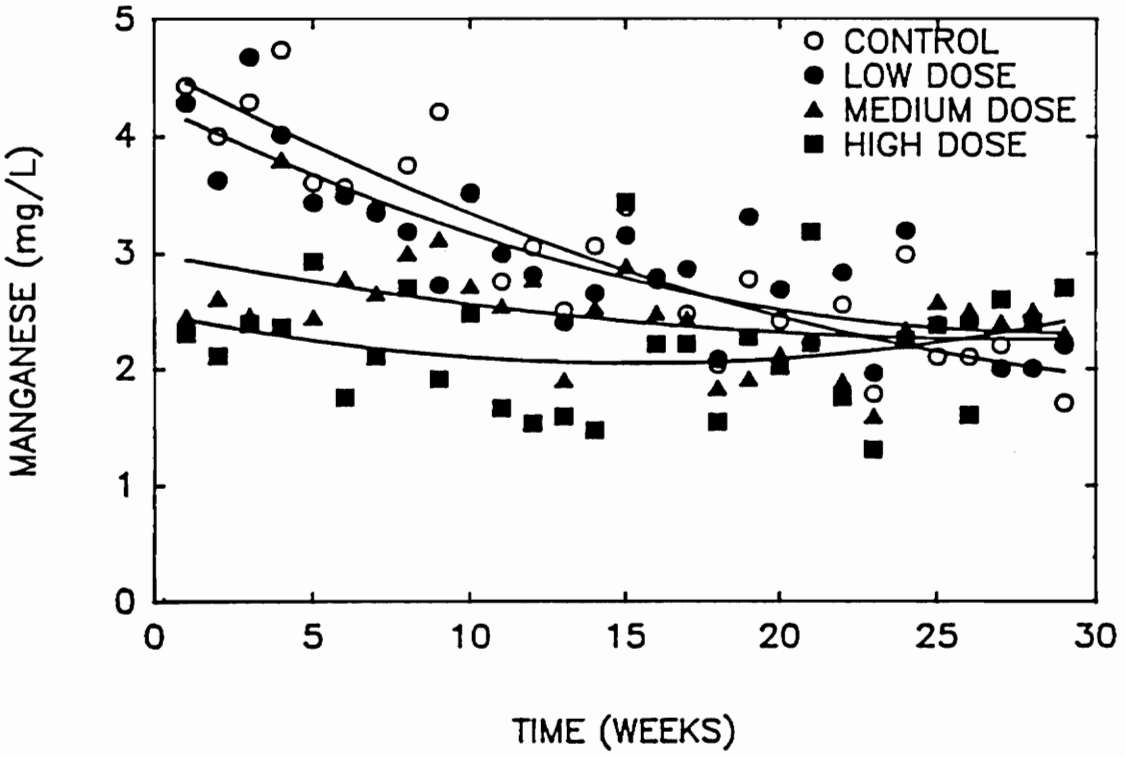

Figure 18. Manganese concentration vs. time for hard-rock mine waste with different doses of N-Serve.

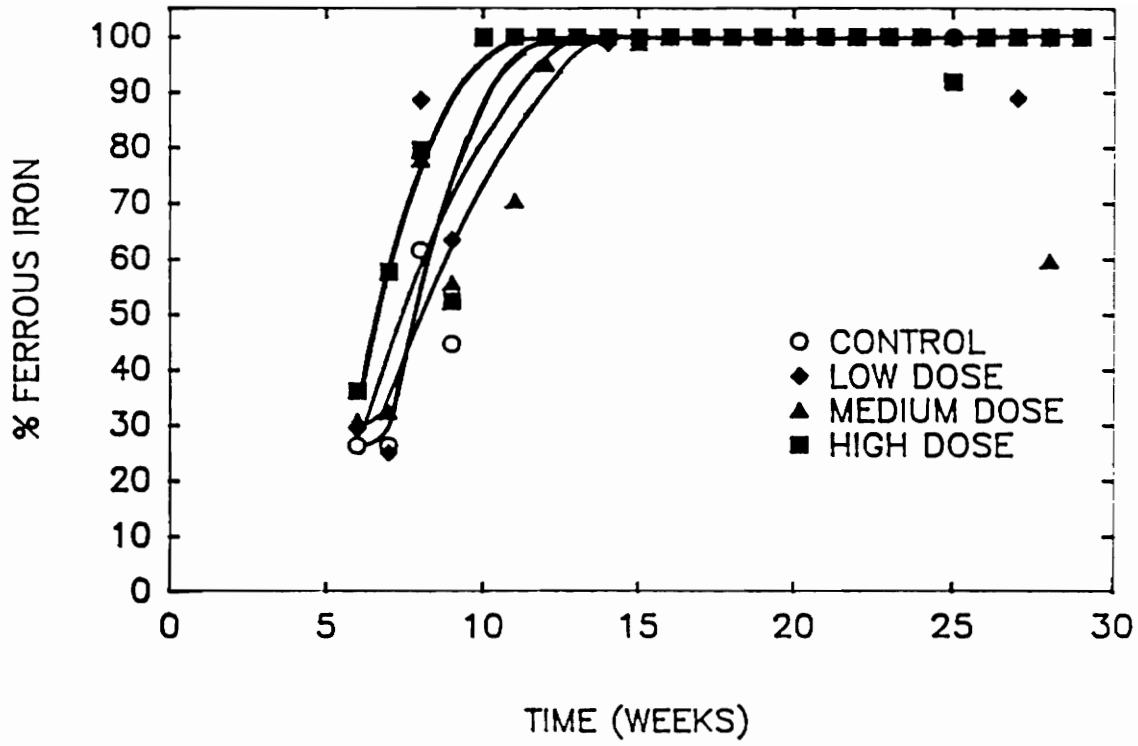

Figure 19. Percent of total iron in ferrous state vs. time for hard-rock mine waste with different doses of N-Serve.

level in all columns, ranging between 3.2 and 3.4. After Week 2, the trend in pH was increasing in all of the columns. The regression lines (see Figure 12) indicate that from Week 6 through 29, the low-dose columns yielded the lowest effluent pH, followed, in order of increasing pH, by the control columns, the medium-dose columns, and the high-dose columns.

The trends in aluminum, sulfate, and total iron (Figures 13, 14, and 15, respectively) followed very closely the trends in acidity in effluents from the hard-rock mine refuse columns. In each case, the control columns produced less acid than the low-dose columns, while outproducing both the medium-dose and high-dose columns in aluminum, sulfate, and total iron.

The average aluminum levels from the control columns peaked at 0.58 mg/L, while the highest aluminum concentration in effluents from the high-dose columns was only 0.39 mg/L. The high-dose columns produced effluent aluminum levels greater than 0.1 mg/L only on two occasions after Week 9. The highest aluminum value from all the hard-rock mine columns (0.72 mg/L) occurred on Week 11 in effluents form the low-dose column pair.

The columns exposed to the high-dose of N-Serve produced the lowest sulfate levels in every week of the study, never exceeding 100 mg/L during all but two weeks of

the study. The low-dose columns produced the highest sulfate values, peaking at 220 mg/L on Week 24.

The high-dose columns also produced the least amount of total iron in Weeks 2 through 29. Average total iron levels from the high-dose columns did not exceed 10 mg/L after Week 6. The highest levels of total iron in the hard-rock mine waste columns were consistently recorded by the low-dose columns, peaking at 63.5 mg/L in Week 13.

The effects of different N-Serve dosages on calcium, magnesium, and manganese output (Figures 16, 17, and 18, respectively) were difficult to interpret because the concentrations in the effluents were highly variable.

Ferrous iron results are presented as percentage of effluent total iron in the ferrous state. The temporal variation of ferrous iron in effluents from the coal waste columns are presented in Figure 19. The percentage ferrous iron in all of the column effluents increased from approximately 30 percent during Week 6 to 100 percent by Week 13. All of the column effluents remained near 100 percent ferrous iron in the effluent in Weeks 6 through 29, indicating little or no bacterial iron oxidation through this period.

The column effluents were analyzed for silver on Week 29. The results indicated that silver was present in all

effluents from the hard-rock columns at concentrations near the detection limit of 1 μg/L.

Bacterial counts were determined on Weeks 1 and 30, the beginning and end of the study period (Table IV). On Week 1, the MPNs were greater than 10^6 cells/100 mL in all of the column effluents. On Week 30, MPNs in the effluent from the low-dose column were the highest (1.5×10^8 cells/100 mL). The lowest MPNS (2.0×10^4 cells/100 mL) were observed in the high-dose columns.

CONTROLLED RELEASE STUDY

Two monolithic, controlled release formulations were prepared for this study, the only difference between the two being the carbon content of the formulations. The nitrapyrine release rates for these two formulations were monitored for 60 days, and the data are presented in Figure 20. The release rate for the formulation containing 10 percent carbon was slower than the release rate for the formulation containing 25 percent carbon. The release rate in the 10 percent carbon formulation remained below 5 ug/day for the first 25 days of the study period. After day 25, the release rate increased and began to stabilize after day 35. The release rate from the 10 percent carbon formulation averaged 12 ug/day over the period from day 35 through day 60. The release rate form the 25 percent carbon formulation

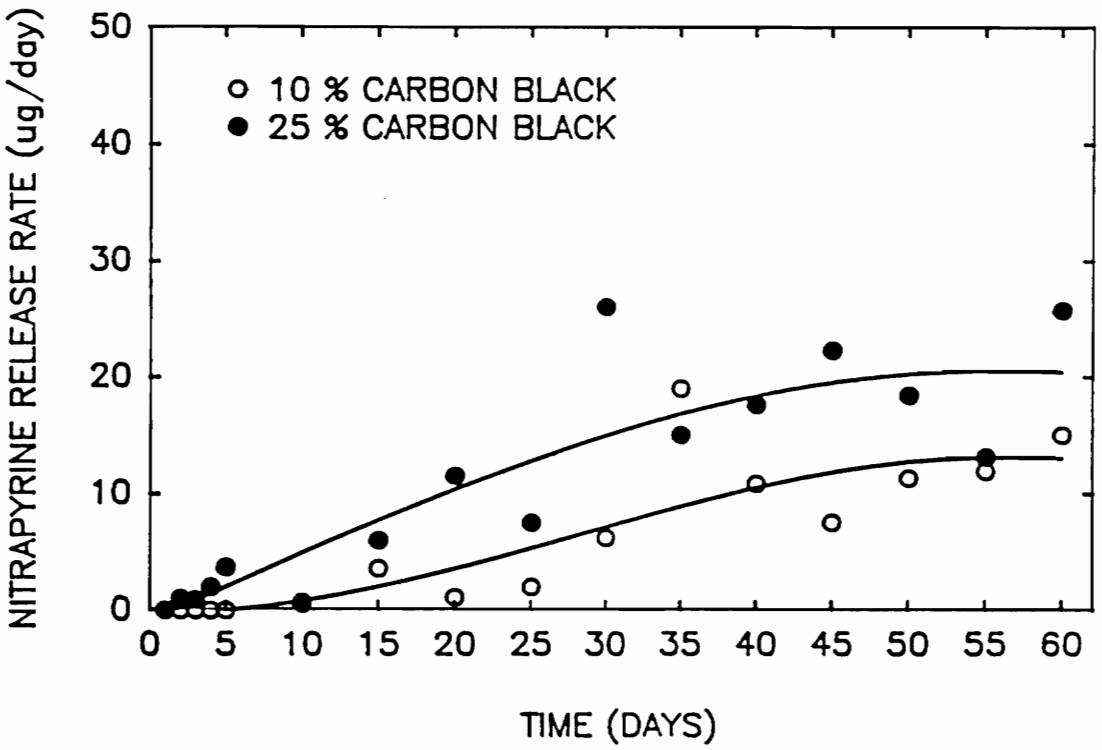

Figure 20. Nitrapyrine release rate vs. time for two monolithic controlled release formulations with varying carbon content.

remained below 5 ug/day through day 10. After day 10, the release rate increased and began to stabilize after day 30. The release rate for the 25 percent carbon formulation averaged 20 ug/day over the period from day 30 through day 60.

V. DISCUSSION

Iron-oxidizing bacteria have been identified as the catalyst in AMD formation. By suppressing their activity, the costs for treating the water leaving a mine site can be reduced or eliminated. Nitrapyrine (2-chloro-6 (trichloro) methylpyridine) is the active ingredient in the agricultural nitrification inhibitor N-Serve. N-Serve has recently been found to be inhibitory to T. ferrooxidans in culture studies (Kavanaugh, 1988). Research was initiated to determine the effectiveness of N-Serve in inhibiting iron-oxidizing bacteria in a simulated field environment. A column study was completed using waste materials from a coal mine and a hard-rock mine. N-Serve was applied to the columns which were then leached with water each week after the treatment. The effluent from the weekly leachings was then analyzed for metals, ions, pH, and acidity as a measure of the effectiveness of N-Serve.

Controlled release has become a popular method for application of AMD inhibitors because application frequency can be reduced. A controlled release system for nitrapyrine was therefore developed and tested for its potential for field use. The controlled release portion of this study was completely seperate from the column study. Two controlled

release formulations were analyzed for release rate characteristics, and were not applied to the columns.

COLUMN STUDY

Watzlaf (1988) indicated that the results of weekly leaching tests, as were used in this study, should not be extrapolated to predict results in field use. Because none of the water applied to a column was lost to evaporation or runoff as it would have been in the field, the columns likely exhibited accelerated AMD production. The water was applied to the columns each week over a very short period of time; therefore, it is possible that more of the bactericide was flushed from the column than would be lost under field conditions. Thus, the duration of treatment effectiveness in the columns is likely shorter it would be in the field.

The effectiveness of the three different doses of N-Serve can be evaluated by a comparison of the effluents form the N-Serve dosed columns with the effluents from the control columns containing both the coal and hard-rock substrates. In this section, the results from the coal mine and hard-rock mine substrates will be discussed separately. The results from the coal waste and hard-rock mine waste will then be compared.

Coal Mine Waste. The effectiveness of N-Serve in inhibiting the formation of AMD was determined by monitoring the water quality of the column effluent. Figures 2-10 summarize the effluent characteristics from the coal mine waste columns. A comparison of the effluents from the control columns with that from columns dosed with N-Serve, shows that only the highest dose used in this study (60 mg/kg) effectively and consistently produced a better effluent water quality than the control columns. The average, effluent concentrations of acidity, aluminum, sulfate, magnesium, manganese, and calcium (Figures 2, 4-8) were considerably lower in effluents from the high-dose columns than in the controls throughout most of the study. Effluent pH and percent ferrous iron were consistently higher in the high-dose column effluents than in the controls (Figures 2 and 10). Of the parameters monitored, only total iron (Figure 9) did not exhibit improved water quality conditions in the high-dose columns versus the control columns. Trends in total iron concentration in the high-dose columns were not distinguishable from those in the controls throughout the study.

Effluents from the coal-waste, low-dose columns were of slightly lower quality than those of the controls. The control column effluent quality was better in terms of acidity, pH, aluminum, sulfate, and total iron (Figures 2-5

and 9). Magnesium and manganese concentrations in the low-dose columns were similar to the control columns, while calcium concentrations were higher in the controls than in the low-dose columns (Figures 6-8). The overall difference in effluent water quality between the low-dose columns and the controls was not very large. The variation between the control and low-dose column effluents was not great enough to substantiate the hypothesis that the difference was due to the effects of N-Serve. The difference was small enough that variability of the substrate would mask any difference or similarity between the control columns and the low-dose columns. Because only two columns received each N-Serve dose, it is statistically impossible to rule out substrate variability as the cause of any trends identified during this study. If only one of the columns in a given column set developed a particularly active or inactive bacterial population, the difference in effluent water quality could be substantial. In a similar AMD column study, Watzlaf (1988) found that "although basic trends were fairly consistent...large discrepancies in contaminant concentration were observed" under controlled conditions almost identical to this study.

The medium-dose columns produced an effluent water quality considerably worse than that produced by the control columns containing coal mine waste. Figures 2-7 and 9

clearly demonstrate this difference. Calcium (Figure 8) was the only characteristic monitored that did not reflect the lower water quality in the medium-dose columns. The trends in efluent calcium may be due to variability in the substrate or to the complex chemistry of calcium solubility. The possibility that the medium-dose of N-Serve actually had a stimulatory effect on AMD production will be investigated later in this chapter.

Hard-rock Mine Waste. Because the hard-rock mine waste columns produced very little acidity, it was difficult to determine the inhibitory effectiveness of N-Serve. There is some evidence, however, to support the conclusion that the highest N-Serve dose inhibited AMD formation. Figures 11-15 clearly indicate that the high-dose columns consistently produced the highest effluent water quality as indicated by their effluents being lowest in acidity, highest in pH, and lowest in sulfate, aluminum, and iron levels. Results from several of the effluent metals analyses were inconclusive regarding the effects of N-Serve on the concentration in the hard-rock column effluents. The effluent magnesium and manganese concentrations in the high-dose columns were lower than the controls in Weeks 1-20. After Week 20 the effluent water quality did not differ appreciably with respect to these two metals (Figures 17-18). The calcium

concentrations (Figure 16) did not differ substantially between the control and high-dose columns throughout the study period.

The medium-dose columns produced an effluent water of only slightly higher quality than that of the controls. This trend is reflected in Figures 11-15, which include the trends in acidity, pH, sulfate, aluminum, and iron. As with the high-dose columns, effluent magnesium and manganese concentrations were slightly lower in the medium-dose columns than in the controls in Weeks 1-20, after which time they were not distinguishable, and calcium trends were similar to the controls throughout the study (Figures 16-18). Because the difference in water quality between the control columns and medium-dose columns was not large, it was difficult to determine whether it could be attributed to the inhibitory effects of N-Serve. It is possible that the water quality difference could simply be the result of variability in the substrate.

The two low-dose columns containing hard-rock mine waste produced a substantially degraded water quality from that of the controls. This lower water quality was indicated by higher acidity, aluminum, sulfate, and total iron concentrations in the low-dose column effluent (Figures 11 and 13-15). The effluent water quality was not degraded substantially with respect to pH, calcium, magnesium, and

manganese (Figures 12 and 16-18). Because acidity production was low in all of the hard-rock columns, it is difficult to determine whether the difference between the control column and the low-dose columns was due to a stimulatory effect of the N-Serve, or to the variability of the substrate.

Coal Mine Waste vs. Hard-Rock Mine Waste. The hard-rock mine waste produced very little acidity compared to the coal substrate. Figure 21 clearly illustrates the difference in acidity production as demonstrated by the control columns for both substrates. Both substrates began the study period producing less than 130 mg/L as $CaCO_3$ of acidity. However, while the coal waste control columns increased acidity output throughout the study peaking at 1351 mg/L in Week 28 of the study, the effluent acidity in the control columns for the hard-rock mine waste decreased through the study period producing only 45 mg/L acidity on Week 29. On the last week of the study the acidity produced by the coal waste control columns was almost 30 times the amount produced by the hard-rock mine controls. Because the hard-rock mine columns were not actively producing acidity, it is very difficult to interpret the results from these columns.

The MPN tests conducted at the beginning and end of the study seem to indicate that bacterial numbers were not the

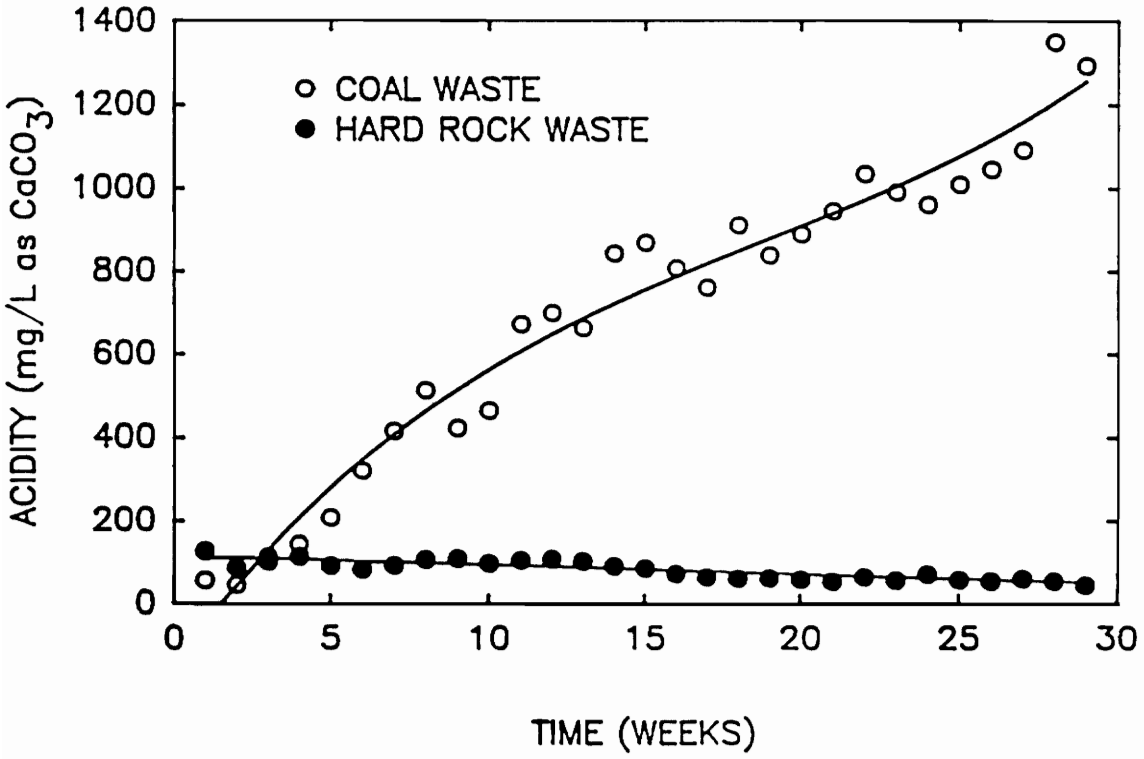

Figure 21. Comparison of acidity production vs. time for the coal and hard-rock control columns.

cause of the difference in acidity production; the control columns for both substrates had greater than 10^6 iron-oxidizing bacterial cells/100mL in the column leachate (see Table IV). Because both ferric iron and sulfate are byproducts of pyrite oxidation, and because bacteria increase this reaction rate 10^6 times (Singer and Stumm, 1970), these ions are good indicators of the bacterial activity in the substrate (Watzlaf, 1988). In analyzing the ferrous iron data for the hard-rock mine waste columns (Figure 19), it should be noted that after Week 12 virtually all of the iron in the column effluent remained in the unoxidized ferrous state. Any small amount of iron oxidized to the ferric state by the bacteria was likely utilized as an oxidant in the pyrite oxidation process. Thus, it appears that the bacteria were present, but not actively oxidizing the available iron in any of the hard-rock waste columns after Week 12. The iron-oxidizing bacteria present in the columns were probably inhibited or rendered dormant by some factor in their environment. T. ferrooxidans do not respire while resting and, therefore, are able to withstand long periods of dormancy (Apel and Dugan, 1978). Because the source of the hard-rock mine waste was a gold, silver and copper mine, the concentration of silver in the column effluent was determined on Week 29. Silver has been found to be toxic to T. ferrooxidans at concentrations as low as

11 μg/L Ag^+ (Tuovinen et al., 1985). Trace levels of silver (approximately 1 μg/L) were detected in the column effluent. Metals concentrations in the column effluents are substantially more dilute than the concentrations in the column substrate between leachings. Silver concentrations may have been high enough in the substrate to cause low microbial activity in the hard-rock mine columns.

N-Serve Effectiveness. There is a strong indication that a dose of 60 mg N-Serve/kg did at least partially inhibit the production of AMD in the columns. In columns containing both substrates, the high N-Serve dose (60 mg/kg) improved the quality of the effluent water considerably. From Week 6 thru the end of the study the high-dose columns produced an effluent with an average acidity 2.6 times lower than the acidity produced by the control column set in the coal mine waste. The graph of percent ferrous iron in the effluent (Figure 10) clearly indicates that the high N-Serve dose strongly inhibited iron oxidation from Weeks 10 through 24, after which the effectiveness decreased slightly through Week 29. In the hard-rock mine waste columns, the acidity produced by the high-dose columns was 2.1 times lower than that produced by the controls during this same period. The problem in interpreting the hard-rock waste data is demonstrated in the graph of percent ferrous iron in the

effluent (Figure 19). This graph indicates that the bacteria in the N-Serve treated columns as well as in the untreated controls were inhibited from oxidizing iron after Week 10 in the hard-rock waste.

Both substrates also showed some indication that a lower dose of N-Serve may actually stimulate the production of AMD. In culture studies with acid-producing Thiobacillus bacteria, Kavanaugh (1988) found that N-Serve either inhibited or had no effect on the bacteria. At no time did the N-Serve have a stimulatory effect. However, some organic compounds have been found to have a stimulatory effect on acid-producing bacteria. Wakao et al. (1983) found that bacterial pyrite oxidation was actually enhanced by addition of certain organic substances. They hypothesized that this effect was not due to direct utilization of the organics, but rather to certain physio-chemical or surface active effects. The assumption is that surface active effects could make the pyrite more available for oxidation, or could increase the accessibility of the pyrite to the bacteria. Watzlaf (1988) hypothesized that surface active effects could simply increase contaminant flushing from the substrate, thus having nothing to do with bacterial stimulation.

Watzlaf (1988) did a similar column study to determine the effectiveness of sodium lauryl sulfate (SLS), benzoate,

and sorbate on reducing AMD. SLS was determined to be the most effective of these three chemicals. In the 7 kg columns, 600 mg (0.0021 moles) SLS/kg substrate kept acidity below 50 percent of the controls for 343 days. A molar equivalent dose of nitrapyrine 481 mg (0.0021 moles)/kg or 2200 mg N-Serve/kg kept the acidity in the coal mine waste columns below 50 percent of the controls for all but three samples after day 35 in the 203 day study period (Figure 2). N-Serve does therefore appear to be comparable to SLS as a means of reducing AMD. Cost analysis for the use of N-Serve would be difficult at this point because of the great variability between column and field conditions.

CONTROLLED RELEASE STUDY

Controlled release is becoming a popular method for applying AMD inhibitors to acid producing mine sites (Kleinmann, 1981). The objective of this portion of the study was to attempt to find a controlled release system that might be effective for use with N-Serve (nitrapyrine). A review of the literature revealed that monolithic systems are easiest to manage. In monolithic controlled release systems, the active agent is simply incorporated into a polymer matrix by physical mixing. For this study, a monolithic system was developed using a polymer from B.F. Goodrich called Hycar. Two different formulations were

examined for their ability to incorporate and subsequently release the nitrapyrine. The only variable in the formulations was the amount of carbon filler incorporated. The carbon content was varied to determine its effects on nitrapyrine release rates. The first formulation contained 10 percent carbon and the second contained 25 percent carbon.

The results of this study, presented in Figure 20, indicate that this type of formulation does have potential for use with nitrapyrine. The acrylonitrile polymer produces a controlled release system resistant to the effects of weather and abrasion (Lufter, 1964). Nitrapyrine was easily incorporated into the polymer matrix, and the release rate appears to be controllable by varying the carbon content of formulation. By increasing the amount of carbon filler, the nitrapyrine release rate appears to increase. Because the controlled release formulations were mixed on a hydraulic press instead of a proper rubber mixing device, no specific conclusions pertaining to release rates should be drawn from this study.

Any future study of this controlled release method should be carried out utilizing proper rubber mixing equipment, specifically a rubber mill or Banberry mixer. The use of an extrusion device would also be recommended to produce a more uniform pellet size thus increasing accuracy

in estimating release rates. It is important to note that the inconsistencies resulting from lack of access to a rubber mill and extrusion device could have been responsible for any difference in release rate trends between the 10 percent and 25 percent carbon formulations.

V. CONCLUSIONS

From the results of the research described in this investigation, the following conclusions seem warranted:

1. Nitrapyrine was, for the first time, found to be inhibitory to autotrophic bacteria other than nitrifiers under simulated field conditions.

2. The highest dose of N-Serve applied to he columns (2200 mg/kg) reduced AMD for at least 200 days. N-Serve therefore appears to have good potential for field use in the control of AMD and should be studied further toward this end.

3. Lower doses of N-Serve may have stimulated AMD formation in columns containing coal or hard-rock mine wastes.

4. The controlled release method developed in this study is effective at gradually releasing nitrapyrine. The nitrapyrine release rate appears to be regulated by varying the amount of carbon black in the controlled release formulation.

VII. SUGGESTIONS FOR FUTURE STUDY

Research into the use of N-Serve for inhibition of iron-oxidizing bacteria has produced some encouraging results. The potential for full-scale mine site application appears to be strong. Before full-scale application is undertaken, a number of preliminary studies should be completed. It was unclear whether the highest dose used in this study represented the full inhibitory potential of N-Serve. A second column study using an order of magnitude higher N-Serve dose would therefore be recommended. Using three or more replicate columns would help to decrease variability in the results, and produce statistically verifiable conclusions. The next logical step would include a field study using N-Serve on several small unweathered mine refuse piles. A small-scale field study would assist in the determination of full-scale application rates. Investigation into the possibilities of a controlled release system for use with N-Serve instead of pure nitrapyrine should be carried out because N-Serve is a more economical form of the chemical. Any future controlled release system should be developed using a rubber mill and an extrusion device to produce a more consistent product.

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APPENDIX A

RAW DATA FILES: COLUMN STUDY

TABLE A-1. Raw data for Week 1 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al
1	52	3.90		3.4	17.8	48.8	
2	62	3.84	3.6	3.3	15.8	43.4	0.39
3	47	3.96	9.6	2.2	13.1	35.1	0.31
4	59	3.88	6.3	3.6	16.9	44.4	0.76
5	70	3.84	15.7	4.5	19.9	46.1	0.65
6	59	3.79	8.7	3.6	19.2	42.9	0.52
7	60	3.85	12.0	2.6	13.3	36.6	0.32
8	62	3.92	10.9	2.7	12.2	38.8	0.29
9	145	4.04	26.5	4.3	15.9	28.7	0.52
10	110	3.86	35.9	4.6	17.6	29.6	0.63
11	99	4.03	32.2	3.7	14.1	24.1	0.52
12	148	3.83	52.6	4.8	21.8	27.3	0.93
13	103	4.04	31.2	2.1	9.9	21.7	0.32
14	132	4.11	34.2	2.8	11.2	23.5	0.28
15	99	3.98	37.8	2.5	10.2	19.7	0.30
16	93	3.96	34.8	2.1	8.8	19.7	0.42

TABLE A-2. Raw data for Week 2 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al
1	61	3.11	12.7	4.9	24.2	51.8	0.58
2	31	3.31	2.0	2.7	16.3	50.2	0.24
3	66	3.18	6.6	5.1	23.8	46.7	0.69
4	62	3.20	15.3	4.8	19.6	40.9	0.58
5	76	3.17	18.3	5.8	23.1	40.1	0.82
6	85	3.19	20.3	5.9	23.9	45.9	0.97
7	65	3.34	22.4	3.9	18.8	53.7	0.40
8	67	3.33	20.3	4.2	18.4	50.6	0.44
9	82	3.23	28.0	4.0	19.2	31.0	0.56
10	93	3.18	20.8	4.0	20.8	35.0	0.58
11	104	3.24	28.0	4.0	23.2	30.6	0.63
12	92	3.25	34.5	3.2	20.0	27.2	0.72
13	68	3.39	23.2	1.8	11.3	21.4	0.28
14	85	3.43	22.5	3.4	23.3	29.1	0.42
15	84	3.26	15.8	2.1	10.8	22.4	0.30
16	102	3.25	21.5	2.1	14.0	30.1	0.49

TABLE A-3. Raw data for Week 3 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al
1	80	3.20	29.2	13.3	30.6	38.6	1.4
2	57	3.36	13.1	6.3	22.5	46.6	0.61
3	107	3.19	27.8	8.5	29.6	38.1	1.7
4	121	3.17	32.4	10.7	30.0	34.0	1.7
5	72	3.32	36.4	9.9	28.3	33.8	1.7
6	72	3.25	45.1		41.2	39.7	2.4
7	67	3.43	15.3	5.8	14.5	35.7	0.5
8	62	3.42	15.4	4.9	12.3	29.4	0.38
9	97	3.37	37.9	4.5	15.7	15.2	0.49
10	107	3.34	30.4	4.0	13.2	16.2	0.47
11	124	3.41	42.0	3.8	12.9	16.1	0.46
12	123	3.41	52.0	5.6	18.8	18.5	0.76
13	64	3.61	24.7	2.0	8.9	12.8	0.21
14	63	3.64	13.1	3.0	12.1	15.1	0.75
15	68	3.49	17.0	2.3	6.7	15.7	0.20
16	84	3.40	17.0	2.4	8.8	16.7	0.34

TABLE A-4. Raw data for Week 4 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	183	2.80	28.1	17.9	47.2	46.5	3.6	543.6
2	104	3.13	37.6	11.4	41.1	53.9	1.4	458.8
3	153	2.87	37.8	12.5	39.2	44.9	3.1	438.4
4	195	2.64	23.7	18.9	48.7	43.7	4.6	613.9
5	187	2.98	18.9	19.2	46.6	45.8	3.7	537.9
6	186	3.09	9.8	16.0	42.3	35.8	3.7	455.0
7	164	3.34	11.6	13.5	37.5	47.8	1.5	
8	117	3.33	12.2	8.4	29.9	42.6	0.87	313.3
9	125	3.41	38.7	4.7	17.3	17.4	0.56	194.7
10	103	3.35	40.1	4.8	17.8	15.0	0.47	192.8
11	124	3.30	39.3	3.5	12.6	14.3	0.53	170.2
12	137	3.33	55.1	4.5	17.9	13.0	0.71	208.9
13	70	3.55	20.2	2.3	10.6	12.5	0.22	99.7
14	85	3.59	30.0	3.9	16.8	15.2	0.31	
15	69	3.50	11.3	3.0	10.3		0.19	37.1
16	58	3.42	9.2	1.7	5.8	11.9	0.17	34.5

TABLE A-5. Raw data for Week 5 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	280	2.78	36.3	21.0	43.6	53.2	8.3	581.4
2	134	3.07	11.4	13.1	37.4	55.0	2.1	421.6
3	155	2.98	19.4	16.5	38.8	47.4	5.4	452.7
4	521	2.76	90.5	32.6	59.3	62.3	14.4	983.6
5	265	2.92	30.6	25.8	50.4	50.0	6.1	596.1
6	198	3.06	39.0	20.8	46.5	42.2	5.2	508.2
7	210	3.41	45.0	22.7		44.9	3.2	411.9
8	166	3.43	33.8	15.1	38.1	47.1	1.8	370.4
9	79	3.52	29.2	3.1	12.1	14.4	0.34	146.1
10	106	3.55	35.0	4.1	15.2	16.2	0.37	174.1
11	121	3.50	43.8	3.2	12.2	13.0	0.47	170.3
12	137	3.57	51.9	3.6	15.2	15.5	0.53	87.4
13	63	3.68	17.5	2.0	9.1	12.5	0.17	88.8
14	77	3.79	24.6	2.8	12.2	11.8	0.22	117.1
15	87	3.81	11.4	3.4	13.6	14.0	0.19	36.1
16	74	3.82	10.6	2.4	8.5	12.6	0.36	45.1

TABLE A-6. Raw data for Week 6 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	429	2.69	54.9	31.7	52.5	60.3	21.0	1110.5
2	210	2.80	23.8	18.0	42.2	51.1	9.8	627.7
3	261	2.84	24.0	20.0	36.6	47.1	12.4	618.5
4	690	2.62	124.4	34.7	54.7	49.4	28.4	1211.0
5	371	2.71	47.8	25.3	44.7	40.9	12.8	743.5
6	371	2.79	65.5	27.6	53.6	45.1	14.4	865.5
7	116	3.36	48.4	21.3	43.0	36.4	6.2	550.4
8	99	3.36	44.7	19.7	43.1	36.5	6.4	534.3
9	75	3.42	22.9	3.0	12.4	10.4	0.46	144.6
10	91	3.38	30.4	4.1	16.4	12.2	0.49	185.8
11	139	3.39	29.8	4.0	15.4	11.9	0.48	180.1
12	98	3.49	35.6	2.9	11.8	10.9	0.66	170.5
13	67	3.51	18.3	2.3	10.0	7.4	0.18	115.1
14	70	3.59	24.1	3.3	15.1	10.4	0.33	153.7
15	51	3.65	5.3	1.8	8.9	9.6	0.12	29.2
16	59	3.57	18.5	1.7	6.3	11.3	0.15	30.7

TABLE A-7. Raw data for Week 7 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	530	2.69	54.5	35.8	67.8	95.6	29.2	915.8
2	301	2.73	34.2	23.2	48.8	87.2	14.8	686.1
3	288	2.76	29.4	21.9	41.4	61.2	17.0	637.5
4	691	2.58	114.1	33.2	53.6	61.8	32.8	1146.5
5	520	2.63	78.7	29.8	56.0	61.2	24.6	965.4
6	569	2.60	109.9	30.3	57.4	56.8	25.8	1102.9
7	199	3.31	49.4	20.2	49.2	43.0	3.0	469.9
8	138	3.29	30.2	12.8	36.8	40.2	1.6	338.5
9	81	3.43	26.4	3.0	10.3	10.9	0.37	152.1
10	106	3.40	29.9	3.7	14.0	12.3	0.45	180.1
11	103	3.37	40.5	3.1	12.8	9.7	0.46	189.6
12	106	3.41	47.4	3.5	13.8	12.8	0.64	223.8
13	92	3.55	16.6	2.1	8.9	9.2	0.15	105.5
14	84	3.63	26.7	3.2	12.1	10.2	0.28	157.1
15	65	3.70	6.2	2.7	9.6	11.7	0.10	39.3
16	54	3.64	4.9	1.5	4.3	9.8	0.10	34.1

TABLE A-8. Raw data for Week 8 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	618	2.69	47.0	39.2	69.4	84.6	33.4	1003.4
2	408	2.76	22.9	28.5	54.4	104.0	19.0	790.9
3	405	2.74	28.2	28.3	55.4	82.4	24.8	811.2
4	821	2.61	105.1	37.4	61.8	63.0	39.8	1189.7
5	834	2.59	101.7	44.9	74.2	80.6	41.4	1341.1
6	1039	2.53	165.3	50.2	88.8	74.6	53.0	1747.6
7	293	3.33	53.1	29.1	53.2	43.4	6.2	516.9
8	179	3.33	33.3	15.9	35.0	37.4	2.8	341.7
9	89	3.50	28.3	3.4	13.3	10.2	0.53	153.3
10	125	3.44	33.4	4.1	15.9	10.8	0.43	177.5
11	124	3.39	50.5	3.9	15.7	10.4	0.64	206.8
12	121	3.44	41.4	3.3	15.5	12.1	0.68	188.1
13	73	3.57	19.5	2.6	13.1	10.9	0.31	121.7
14	69	3.68	5.4	3.4	14.6	12.7	0.12	41.3
15	60	3.81	4.9	3.4	14.7	12.8	0.16	36.1
16	52	3.77	5.4	2.0	9.5	10.5	0.15	35.0

TABLE A-9. Raw data for Week 9 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	554	2.72	44.4	49.2	63.8	86.9	38.2	1094.6
2	290	2.81	16.8	24.6	51.5	84.9	22.2	757.4
3	389	2.75	26.4	35.0	58.3	78.8	26.8	824.8
4	900	2.63	102.8	43.2	63.6	48.4	49.2	1214.7
5	789	2.63	102.6	53.8	77.9	67.3	53.6	1408.2
6	695	2.57	191.2	54.4	83.2	76.6	67.6	1937.8
7	269	3.30	44.0	29.4	34.3	48.2	6.2	498.9
8	228	3.33	44.2	33.6	51.7	43.3	5.0	448.4
9	97	3.48	23.6	3.6	13.3	16.7	0.57	170.8
10	121	3.41	31.7	4.8	20.5	17.6	0.60	225.4
11	136	3.44	41.7	2.9	10.2	9.8	0.61	190.4
12	127	3.47	35.4	2.5	13.4	14.4	0.62	199.3
13	73	3.57	18.0	3.0	13.2	14.6	0.25	142.7
14	68	3.58	24.8	3.2	13.6	14.3	0.34	174.1
15	60	3.63	4.5	2.6	6.0	13.6	0.09	44.9
16	43	3.65	4.3	1.2	3.4	7.9	0.06	31.1

TABLE A-10. Raw data for Week 10 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	563	2.75	54.6	48.6	64.9	56.1	41.8	981.6
2	366	2.83	23.6	42.6	63.8	58.7	24.8	746.8
3	440	2.81	33.0	42.4	60.3	49.7	28.6	722.2
4	962	2.69	125.6	47.0	73.7	35.0	53.6	1169.6
5	866	2.69	111.6	57.6	81.4	52.8	55.6	1276.6
6	1008	2.66	159.0	56.2	79.2	38.3	58.0	1380.6
7	205	3.32	51.4	38.8	52.1	30.8	3.4	481.1
8	253	3.27	58.0	34.4	59.4	33.0	4.0	560.4
9	88	3.42	25.6	3.3	17.4	9.3	0.49	145.5
10	108	3.35	30.1	3.7	21.4	10.6	0.54	171.2
11	123	3.35	43.6	3.5	15.4	10.1	0.68	182.6
12	130	3.39	43.9	3.5	18.3	9.5	0.66	185.2
13	74	3.52	19.6	2.5	13.6	7.6	0.19	112.7
14	80	3.52	23.3	2.9	16.9	9.9	0.32	136.1
15	47	3.55	3.7	2.6	8.5	7.6	0.07	32.1
16	52	3.52	5.5	2.3	4.0	7.8	0.08	36.3

TABLE A-11. Raw data for Week 11 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	563	2.79	60.2	37.8	90.4	67.3	58.0	1256.6
2	366	2.90	27.4	26.4	67.1	66.7	29.0	834.9
3	440	2.85	35.6	25.0	65.1	56.5	37.8	863.9
4	962	2.72	135.4	40.4	95.3	50.8	73.6	1560.3
5	866	2.73	109.4	43.8	95.5	61.2	71.0	1564.7
6	1008	2.69	161.8	45.6	103.4	55.4	80.2	1766.5
7	205	3.35	41.6	18.6	49.3	39.2	4.8	471.7
8	253	3.33	45.4	20.6	61.6	38.7	4.2	526.1
9	88	3.49	31.4	2.6	14.5	12.1	0.43	155.2
10	108	3.43	31.5	2.9	15.0	13.1	0.47	168.1
11	123	3.40	51.1	2.9	16.5	12.4	0.68	208.7
12	130	3.46	50.9	3.0	16.6	12.2	0.77	214.6
13	74	3.55	22.0	2.0	12.0	8.9	0.34	122.6
14	80	3.63	29.5	3.1	16.7	12.5	0.32	161.6
15	47	3.76	4.7	1.7	9.2	12.2	0.10	40.2
16	52	3.66	7.1	1.6	9.3	12.3	0.09	50.0

TABLE A-12. Raw data for Week 12 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	966	2.73	67.8	38.4	93.9	67.5	68.4	1439.3
2	432	2.85	27.2	24.6	65.3	59.8	31.4	844.3
3	577	2.78	38.8	27.8	72.8	56.1	45.6	1034.5
4	1066	2.68	117.2	33.6	76.6	39.2	67.6	1441.0
5	1149	2.68	113.6	42.8	93.9	60.7	74.2	1706.2
6	1600	2.62	173.2	50.8	117.5	60.1	103.8	2312.4
7	175	3.23	31.8	15.2	33.9	39.4	3.8	404.0
8	195	3.21	36.2	18.8	43.8	35.2	4.0	432.4
9	104	3.47	34.4	3.0	14.6	24.9	0.55	180.1
10	112	3.41	35.5	3.1	12.5	23.1	0.44	190.9
11	145	3.35	50.9	2.8	15.0	23.8	0.66	217.4
12	132	3.40	46.2	2.8	15.7	19.8	0.58	201.4
13	97	3.53	29.8	2.7	15.5	24.6	0.31	167.7
14	87	3.64	28.6	2.8	14.2	23.5	0.29	162.6
15	35	3.89	5.2	1.7	9.6	24.0	0.08	45.1
16	38	3.76	6.4	1.3	7.7	20.2	0.08	46.4

TABLE A-13. Raw data for Week 13 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	865	2.71	68.8	30.4	79.0	60.3	65.2	1189.9
2	460	2.82	29.6	22.6	62.9	65.8	34.0	811.8
3	625	2.74	50.2	26.0	72.2	59.6	48.8	1009.9
4	1315	2.60	175.4	39.8	99.9	53.5	96.4	1881.8
5	1135	2.63	124.6	39.6	100.5	64.5	90.8	1719.7
6	1185	2.62	161.6	37.6	92.4	54.6	94.6	1738.8
7	312	3.31	60.6	29.4	55.7	44.2	11.4	574.1
8	313	3.25	72.0	34.6	64.0	47.7	13.2	664.8
9	98	3.62	39.8	2.5	13.6	13.5	0.43	156.5
10	108	3.64	44.4	2.5	14.1	12.4	0.30	164.3
11	137	3.51	60.0	2.3	12.4	12.0	0.38	179.8
12	141	3.65	67.0	2.5	14.7	9.9	0.37	196.6
13	66	3.74	26.1	1.5	8.8	8.6	0.17	101.0
14	76	3.92	35.8	2.3	12.5	11.0	0.24	149.6
15	58	4.22	8.2	1.6			0.10	61.0
16	61	4.09	8.8	1.6			0.23	71.4

TABLE A-14. Raw data for Week 14 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1146	2.70	96.6	40.6	79.0	65.1	89.8	1610.3
2	540	2.81	47.0	28.2	77.7	67.5	44.8	1009.7
3	1022	2.78	47.6	22.6	60.7	47.1	47.6	922.7
4	1129	2.65	152.0	34.6	86.5	40.7	90.2	1610.8
5	1109	2.66	137.6	44.8	111.5	63.1	103.0	1859.8
6	1409	2.50	183.4	44.0	113.3	67.8	116.0	2150.9
7	277	3.27	59.6	25.0	52.4	38.7	8.6	537.6
8	223	3.26	55.6	19.8	46.9	35.6	5.0	489.8
9	83	3.52	30.2	2.6	15.1	11.5	0.42	152.2
10	99	3.55	38.5	3.5	19.6	12.4	0.40	189.5
11	127	3.48	53.8	2.5	13.4	8.9	0.42	178.9
12	116	3.58	50.1	2.8	17.7	9.7	0.50	184.8
13	65	3.65	23.1	2.3	14.0	10.2	0.25	126.3
14	76	3.81	27.7	2.8	16.5	10.4	0.21	151.2
15	35	3.98	4.6	1.5	9.6	10.2	0.04	42.0
16	33	3.86	4.4	1.5	8.7	10.4	0.04	41.6

TABLE A-15. Raw data for Week 15 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1134	2.73	82.2	44.0	85.1	58.7	87.2	1398.3
2	605	2.84	38.0	32.0	66.4	61.4	44.2	899.2
3	758	2.77	52.4	31.6	65.6	48.4	57.2	994.0
4	1441	2.67	168.0	49.4	97.5	45.8	108.6	1757.2
5	1059	2.72	106.6	42.8	82.1	50.6	88.4	2381.2
6	1514	2.59	173.6	53.8	108.7	76.8	120.4	2090.1
7	406	3.30	76.6	45.6	63.8	42.2	14.6	649.5
8	352	3.26	74.0	45.4	61.2	44.0	15.2	648.3
9	81	3.70	31.9	3.3	12.5	10.3	0.44	134.3
10	92	3.68	35.0	3.5	14.2	10.0	0.27	242.0
11	137	3.55	55.8	3.4	13.6	10.2	0.50	184.2
12	105	3.67	46.1	2.9	12.3	9.1	0.41	156.1
13	70	3.82	28.8	3.0	14.2	8.9	0.22	121.6
14	65	3.95	28.2	2.8	12.3	8.7	0.21	119.6
15	50	4.19	7.0				0.13	54.9
16	47	4.06	9.1				0.12	51.2

TABLE A-16. Raw data for Week 16 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1029	2.78	78.8	38.6	79.0	48.7	73.6	1275.6
2	586	2.89	33.0	27.4	61.6	51.7	41.0	849.8
3	876	2.80	64.4	35.6	76.3	53.2	69.4	1272.9
4	1150	2.73	136.0	39.2	79.0	39.2	86.8	1548.3
5	1268	2.74	114.0	45.2	88.0	52.8	98.0	1711.3
6	1667	2.59	188.4	55.2	109.1	79.9	123.4	2297.0
7	350	3.35	62.0	35.6	53.0	35.0	12.4	575.5
8	277	3.33	49.8	28.2	46.0	36.1	8.8	498.3
9	77	3.68	18.7	3.1	12.3	11.9	0.31	129.8
10	70	3.69	22.1	2.5	9.3	10.0	0.22	109.4
11	120	3.54	46.6	3.1	11.3	11.4	0.43	170.7
12	94	3.67	34.6	2.4	9.8	9.1	0.40	139.0
13	58	3.70	15.7	2.4	11.1	9.8	0.16	100.5
14	52	3.83	18.7	2.5	10.3	11.7	0.14	105.5
15	47	4.16	6.2			12.2	0.06	61.0
16	30	3.95	4.1	1.4	6.7	9.8	0.05	41.4

TABLE A-17. Raw data for Week 17 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	979	2.79	74.0	35.6	76.3	43.8	81.4	1293.6
2	542	2.87	39.8	30.8	64.5	44.0	49.6	938.4
3	937	2.80	70.0	35.8	75.6	49.5	75.4	1225.7
4	1065	2.74	131.8	40.2	79.9	37.8	97.0	1518.6
5	1119	2.76	121.2	45.4	84.9	48.0	98.8	1553.0
6	1786	2.56	195.0	60.8	125.0	93.7	151.0	2568.9
7	365	3.33	68.8	37.0	50.4	35.4	13.0	585.1
8	302	3.30	66.8	32.2	46.6	39.6	9.4	546.3
9	60	3.69	21.7	2.2	10.6	7.8	0.21	112.1
10	68	3.70	22.3	2.7	11.5	10.6	0.17	122.1
11	138	3.55	51.9	3.2	14.0	9.0	0.43	193.0
12	95	3.70	36.4	2.5	11.1	9.6	0.33	149.7
13	66	3.76	22.0	3.0	15.1	9.7	0.18	135.1
14	36	3.96	11.3	1.8	9.6	9.2	0.11	88.4
15	39	4.19	4.2	2.5	14.2	9.9	0.06	57.7
16	31	4.08	5.1	1.9	9.5	8.6	0.06	47.4

TABLE A-18. Raw data for Week 18 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1194	2.72	88.2	40.0	88.9	53.0	90.4	1501.9
2	629	2.81	39.4	28.0	65.8	55.4	48.2	996.3
3	990	2.73	71.8	32.6	72.8	49.5	72.6	1335.3
4	1107	2.65	175.2	44.8	93.3	46.4	110.0	1963.8
5	1253	2.69	111.0	42.0	85.4	48.8	102.0	1782.1
6	1464	2.62	170.8	42.6	87.1	43.6	110.0	1897.9
7	419	3.26	76.6	38.4	51.9	39.2	14.4	688.8
8	259	3.27	50.0	24.0	33.9	38.5	8.8	473.5
9	62	3.67	20.8	1.9			0.20	116.3
10	61	3.65	17.7	2.1	10.2	11.4	0.14	121.5
11	139	3.47	46.8	2.4	9.9	9.5	0.34	189.7
12	84	3.62	28.3	1.8	7.9	9.2	0.26	136.4
13	47	3.68	14.8	2.0	8.8	8.6	0.21	99.3
14	40	3.90	12.1	1.6	7.4	10.1	0.12	94.6
15	36	3.74	3.4	1.5	7.8	12.3	0.06	65.2
16	32	3.66	4.6	1.5	6.9	11.4		73.6

TABLE A-19. Raw data for Week 19 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1017	2.71	77.0	34.0	68.4	41.1	87.0	1284.6
2	660	2.79	39.0	27.6	58.5	45.5	55.2	925.2
3	884	2.73	65.0	32.0	67.3	42.5	75.4	1141.1
4	1162	2.67	122.6	35.0	71.7	37.2	98.6	1467.6
5	1144	2.70	96.4	35.6	69.3	42.2	96.0	1414.6
6	1262	2.62	125.6	33.0	71.7	37.6	110.0	1714.2
7	415	3.25	77.4	37.2	49.5	37.4	15.0	649.5
8	267	3.26	57.4	25.4	33.4	33.9	8.6	467.9
9	59	3.66	22.3	2.4	11.4	12.0	0.24	137.4
10	66	3.68	21.0	3.1	13.6	12.9	0.17	149.6
11	145	3.52	58.5	3.4	15.7	8.8	0.46	221.1
12	131	3.64	49.1	3.2	14.3	10.0	0.48	196.6
13	53	3.75	18.4	2.3	10.0	8.4	0.13	119.4
14	33	3.94	10.7	1.6	6.9	9.1	0.07	82.2
15	40	4.12	6.5	2.2	11.9	11.0	0.05	84.6
16	48	3.96	10.3	2.3	12.3	12.0	0.07	90.8

TABLE A-20. Raw data for Week 20 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1113	2.71	87.2	36.6	72.6	42.2	92.6	1469.1
2	666	2.81	45.6	27.6	60.3	45.8	59.0	1080.6
3	792	2.74	59.4	24.0	51.5	34.3	66.8	1137.6
4	1219	2.66	139.6	37.2	79.4	37.8	114.0	1891.4
5	1236	2.69	110.4	39.2	82.5	39.8	113.0	1827.0
6	1359	2.61	140.4	35.2	86.5	38.3	124.0	2025.8
7	421	3.27	84.2	36.8	44.2	33.0	16.6	692.3
8	405	3.25	76.6	33.6	40.9	33.0	14.2	632.0
9	56	3.68	21.1	2.3	8.0	8.7	0.29	114.9
10	62	3.69	20.5	2.5	8.4	9.2	0.17	122.9
11	157	3.51	60.6	3.3	11.8	9.0	0.42	223.1
12	166	3.67	34.1	2.0	7.1	7.7	0.26	140.9
13	53	3.75	17.1	1.9	8.0	7.6	0.30	106.8
14	47	3.90	14.4	2.3	9.1	8.2	0.11	106.9
15	43	4.14	5.8	2.6	10.1	10.2	0.05	82.1
16	32	3.87	5.9	1.4	7.3	8.7	0.05	66.5

TABLE A-21. Raw data for Week 21 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1159	2.72	84.4	36.2	79.9	41.6	96.0	1454.4
2	732	2.79	43.6	29.6	68.9	50.6	67.6	1122.1
3	839	2.74	70.2	29.0	68.0	38.9	79.8	1268.7
4	1172	2.67	125.4	36.0	80.5	39.6	110.0	1766.1
5	1231	2.71	106.0	37.2	83.6	42.2	103.0	1597.7
6	1313	2.62	141.0	36.0	82.1	39.4	118.0	1940.0
7	412	3.31	81.6	35.4	51.5	38.5	15.8	684.4
8	376	3.30	73.6	33.0	47.1	38.3	15.8	627.6
9	54	4.10	16.8	2.1	8.4	11.5	0.80	113.1
10	54	4.22	13.1	2.3	10.7	10.8	0.22	96.6
11	123	3.93	41.1	2.3	9.1	10.4	0.36	138.2
12	84	4.06	28.3	2.1	9.2	8.8	0.25	116.4
13	41	4.23	10.1	2.0	8.5	7.7	0.12	72.9
14	44	4.30	11.6	2.4	11.7	8.9	0.13	91.2
15	35	4.65	3.5	2.7	15.2	10.8	0.04	
16	43	4.37	7.6	3.6	18.1	10.6	0.12	

TABLE A-22. Raw data for Week 22 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1229	2.74	93.6	38.4	78.1	37.8	102.0	1510.0
2	839	2.79	59.0	38.8	81.2	50.8	83.0	1352.2
3	889	2.77	71.2	30.4	67.3	34.3	85.4	1263.5
4	1366	2.68	124.8	32.6	85.6	35.6	115.0	1775.6
5	1303	2.73	121.4	41.8	88.4	37.8	118.0	1782.2
6	1493	2.63	186.2	45.8	99.9	37.4	153.0	2292.6
7	283	3.33	62.6	20.2	32.6	27.3	6.8	508.4
8	297	3.27	60.6	21.4	32.8	32.8	7.6	517.5
9	74	3.78	22.5	2.7	14.3	8.0	0.24	138.7
10	56	3.81	14.5	2.4	11.5	9.3	0.12	115.3
11	154	3.60	50.6	3.1	13.5	7.7	0.46	205.8
12	84	3.76	28.5	2.5	11.9	8.4	0.28	147.2
13	49	3.93	12.6	2.2	10.4	9.5	0.11	105.9
14	33	4.11	8.7	1.5	8.2	8.9	0.07	78.7
15	31	4.26	3.3	1.7	10.2	9.8	0.02	67.5
16	23	4.16	5.9	1.8	10.0	9.7	0.05	67.8

TABLE A-23. Raw data for Week 23 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1228	2.67	68.2	25.6	67.3	42.9		1231.9
2	752	2.72	41.2	25.8	66.9	57.4		1154.9
3	865	2.67	58.8	23.4	66.0	43.8		1218.1
4	1150	2.63	98.2	27.8	72.8	42.5		1561.6
5	1253	2.66	98.0	31.6	81.6	46.6		1682.9
6	1336	2.57	138.4	31.8	86.9	43.8		2049.4
7	269	3.22	45.2	16.2	28.2	38.3		468.7
8	224	3.20	41.0	15.2	24.9	38.5		410.4
9	58	3.59	18.8	1.7	7.5	13.4		115.0
10	57	3.65	14.3	1.8	8.0	13.3		102.7
11	131	3.47	43.1	2.3	9.2	10.7		170.1
12	78	3.65	27.8	1.6	6.7	11.5		121.2
13	41	3.74	10.0	1.6	6.8	11.2		85.1
14	34	3.95	10.3	1.6	7.9	11.2		80.7
15	26	4.13	2.7	1.2	6.9	12.3		57.7
16	31	4.07	4.0	1.4	7.8	10.1		55.0

TABLE A-24. Raw data for Week 24 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1159	2.71	82.4	31.6	81.8	44.9		1538.8
2	761	2.78	41.2	27.2	67.1	53.2		1303.4
3	928	2.74	64.0	25.2	33.7	39.6		1280.1
4	1443	2.68	116.2	35.6	90.2	43.6		1863.5
5	1354	2.72	102.2	36.8	91.5	46.4		1720.8
6	1689	2.61	149.4	37.4	100.5	46.2		2250.5
7	308	3.28	73.4	22.8	38.1	39.4		585.2
8	344	3.27	73.0	24.0	41.1	35.4		592.9
9	63	3.61	23.7	2.5	11.0	15.3		146.4
10	81	3.73	26.8	3.4	16.8	12.6		167.4
11	169	3.47	63.3	3.7	15.2	11.5		241.6
12	117	3.70	46.0	2.7	12.4	12.5		198.8
13	39	3.90	17.8	2.3	11.0	10.7		140.4
14	60	4.00	15.2	2.4	12.3	9.9		100.5
15	42	4.15	4.9	2.9	17.6	14.7		119.4
16	26	4.05	4.7	1.6	8.7	10.1		70.1

TABLE A-25. Raw data for Week 25 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1206	2.88	104.8	39.4	88.6	54.9	113.0	1542.6
2	813	2.93	59.4	34.4	74.3	71.2	76.0	1234.8
3	1522	2.87	141.2	47.8	77.2	52.3	129.0	1924.5
4	1634	2.78	189.4	48.4	90.4	64.7	148.0	2150.0
5	1392	2.87	119.8	40.2	61.5	46.7	117.0	1668.0
6	2214	2.70	254.2	56.4	84.3	58.1	190.0	2772.8
7	622	3.42	127.6	51.0	38.2	45.5	31.2	949.3
8	428	3.41	91.0	35.6	36.0	38.9	17.8	678.7
9	68	4.22	25.6	2.1	10.5	15.1	0.41	117.6
10	48	4.26	16.8	2.1	14.6	15.4	0.18	105.0
11	144	4.01	54.5	2.5	10.5	9.3	0.44	188.2
12	102	4.12	42.6	2.2	8.8	10.4	0.50	165.8
13	50	4.29	13.2	1.8	11.9	11.9	0.14	92.5
14	35	4.50	15.4	3.3	14.3	11.6	0.18	124.6
15	31	4.56	5.7	2.3	12.3	12.8	0.10	87.9
16	33	4.43	6.6	2.5	8.4	8.8	0.01	80.9

TABLE A-26. Raw data for Week 26 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1230	2.94	102.2	37.0	10.2	87.6	114.0	1499.4
2	859	3.00	59.8	32.6	87.6	92.2	76.8	1201.5
3	986	2.94	82.4	30.6	66.6	63.3	92.0	1371.5
4	1359	2.86	128.0	38.8	86.7	70.8	122.0	1851.7
5	1262	2.92	111.2	37.0	89.8	59.9	115.0	1648.2
6	1879	2.77	193.0	42.8	110.3	80.5	158.0	2364.4
7	450	3.49	99.0	35.2	42.9	60.1	20.6	659.6
8	292	3.49	68.2	23.4	47.5	61.4	10.8	447.8
9	63	4.30	21.3	2.1	8.2	13.3	0.24	117.0
10	48	4.29	14.9	2.1	13.2	15.6	0.13	97.9
11	128	4.04	53.8	2.8	9.1	10.0	0.37	186.5
12	95	4.16	40.1	2.1	6.8	10.8	0.27	147.4
13	42	4.37	13.1	2.0	9.4	12.5	0.14	91.8
14	43	4.67	12.4	3.1	11.4	13.2	0.24	113.0
15	29	4.78	4.0	2.0	8.3	10.0	0.04	88.6
16	19	4.54	3.7	1.1	8.8	8.2	0.03	59.4

TABLE A-27. Raw data for Week 27 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1264	2.97	125.4	35.4	95.5	59.4	111.0	1585.6
2	918	3.02	64.3	33.2	78.4	88.2	82.0	1234.6
3	1123	2.98	136.0	33.6	80.3	74.9	114.0	1584.4
4	1465	2.86	89.5	45.3	94.3	79.1	142.0	1911.0
5	1335	2.95	147.2	39.9	90.6	75.3	121.0	1672.8
6	1962	2.80	229.1	50.6	106.1	92.4	162.0	2261.2
7	340	3.60	113.3	45.5	48.5	70.0	14.4	565.9
8	655	3.61	89.2	34.5	46.2	64.9	11.2	529.7
9	56	4.78	17.6	2.8	7.7	11.9	0.35	117.8
10	66	4.76	14.9	1.7	8.6	13.7	0.15	132.0
11	118	4.49	54.7	2.0	10.5	11.5	0.38	148.8
12	99	4.63	45.9	2.0	7.2	13.0	0.28	137.5
13	50	4.83	15.3	2.2	11.4	11.2	0.12	103.2
14	34	5.27	14.9	2.6	11.4	9.1	0.09	88.2
15	29	5.55	4.3	2.8	14.8	11.6	0.02	70.9
16	21	5.36	4.0	2.4	10.3	11.2	0.04	76.1

TABLE A-28. Raw data for Week 28 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1538	2.93	120.9	44.4	120.0	88.6	139.0	1992.2
2	1164	2.97	78.6	39.8	96.4	101.2	110.0	1685.6
3	1397	2.96	161.1	36.7	78.5	68.5	134.0	1962.6
4	1662	2.85	128.1	49.5	93.9	75.4	150.0	2308.1
5	1691	2.91	150.3	37.3	103.1	72.3	151.0	2233.1
6	1900	2.77	209.4	56.1	163.3	76.7	169.0	2599.1
7	383.3	3.49	104.7	52.1	55.1	72.8	17.6	651.6
8	816.7	3.47	91.2	38.7	48.6	71.8	13.4	722.3
9	54	4.25	18.2	2.0	9.5	12.0	0.29	122.5
10	57	4.22	14.7	2.1	11.8	13.3	0.16	139.9
11	140	4.04	48.8	2.3	12.7	8.5	0.35	208.2
12	105	4.16	46.2	1.7	11.4	10.6	0.28	166.5
13	45	4.36	16.8	2.1	8.4	10.0	0.09	107.5
14	37	4.71	15.5	3.0	11.1	10.4	0.43	113.2
15	20	4.77	8.6	2.8	18.2	11.9	0.02	60.6
16	22	4.67	7.0	2.0	13.6	9.8	0.03	56.2

TABLE A-29. Raw data for Week 29 of the column study. All data presented as mg/L with the exception of acidity (mg/L as $CaCO_3$) and pH (-log $[H^+]$).

COLUMN #	ACIDITY	pH	Fe	Mn	Mg	Ca	Al	SO4
1	1456	2.86	135.2	43.0	111.5	81.0	129.0	1959.6
2	1133	2.91	84.4	40.6	97.7	99.2	92.8	1585.2
3	1118	2.90	117.4	35.2	93.3	72.4	102.0	1664.0
4	1882	2.79	192.2	46.8	117.9	78.8	158.0	2378.0
5	1887	2.87	125.2	37.2	95.9	79.9	114.0	1887.6
6	2396	2.71	291.8	54.8	158.9	91.1	199.0	3139.0
7	515	3.45	132.2	41.0	59.2	70.6	26.0	875.1
8	869	3.45	78.8	24.2	35.5	65.1	13.4	570.1
9	47	4.24	20.4	1.7	8.6	7.5	0.25	112.5
10	44	4.18	16.0	1.7	9.1	12.0	0.14	104.6
11	108	4.09	51.0	2.4	11.3	7.3	0.29	147.4
12	88	4.20	41.7	2.0	9.9	10.9	0.29	131.5
13	46	4.44	17.9	2.5	11.4	10.0	0.16	98.9
14	26	4.70	7.9	2.2	12.6	12.1	0.07	85.4
15	31	4.77	5.5	2.7	17.9	13.1	0.04	94.8
16	32	4.67	7.7	2.7	16.6	9.3	0.05	78.7

VITA

Philip Scott Strobel was born on August 26, 1964, in Grand Rapids, Michigan. He attended Wittenberg University in Springfield, Ohio, where he received his Bachelor of Arts Degree in Biology in 1986. In 1988 he began to pursue a Master of Science Degree in Environmental Sciences and Engineering and completed the requirements toward that degree in 1990. He is a member of the North American Lake Management Society, Michigan United Conservationists Clubs, and Trout Unlimited. Currently, he is likely to be found fishing in a trout stream somewhere in the Midwestern United States.