

# BIOGEOCHEMISTRY AND BEHAVIOR OF ARSENIC AND CHROMIUM IN A WET SUBSTRATE, ANAEROBIC BIOREACTOR DOMINATED BY SULFATE-REDUCING BACTERIA<sup>1</sup>

Toshisuke Ozawa, Ronald R.H. Cohen, and Ronald W. Klusman<sup>2</sup>

**Abstract:** A pilot scale, anaerobic, passive mine drainage treatment system, dominated by sulfate-reducing bacteria, was utilized to investigate the removal rates as well as removal processes of arsenic and chromium in a wastewater and/or acid mine drainage. A computer modeling code, MINTEQA2, modified from MINTEQA2, was utilized for the inverse chemical modeling of the bioreactor. Ninety to over 99% of the arsenic and 86 to 94% of the chromium were removed from the feed solution. Cadmium, copper, iron, lead, and zinc were also removed. There are several mechanisms that could account for arsenic and chromium removal. We believe that the primary mechanism is microbial sulfate reduction resulting in production of high concentrations of hydrogen sulfide and bicarbonate ion. Experimental evidence and inverse modeling with MINTEQA2 confirmed that most (90 to 95%) of the removal of arsenic and chromium occurred in the first quarter volume of the bioreactor. Additional removal of target metals could still occur in the remaining volume of the bioreactor. It also supported the contention that removal was the result of the reduction of Cr(VI) to Cr(III) by hydrogen sulfide, followed by precipitation of chromium hydroxide [Cr(OH)<sub>3(s)</sub>], and reduction of As(V) to As(III), followed by precipitation of arsenic sulfides (As<sub>2</sub>S<sub>3</sub> or AsS). The use of a passive mine drainage treatment system was effective for wastewater and acid mine drainage with elevated concentrations of arsenic and chromium.

**Additional Key Words:** bioremediation, biotreatment, metals treatment, metal sulfides precipitation

## Introduction

### Passive Mine Drainage Treatment (PMDT) Systems

Passive, anaerobic bioreactors dominated by sulfate-reducing bacteria (SRB) have been developed from constructed wetlands in order to treat acid mine drainage (AMD) more effectively (Cohen and Staub, 1992; Staub, 1992). An advantage of the passive mine drainage treatment (PMDT) system is that it requires no power source other than hydraulic head; therefore, it can be located in remote areas without electricity, which is an important consideration at many remote mine sites (Cohen and Staub, 1992; Staub, 1992).

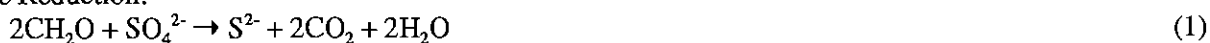
The bioreactor utilizes an organic substrate as the treatment media. Composted livestock manure with an amendment of hay has been determined to be the most effective organic substrate to act as a carbon source for SRB and produce the highest rate of sulfate reduction (Reynolds et al., 1991; Bolis et al., 1992; Dvorak et al., 1992). Approximately 12 genera of SRB have been found in PMDT systems. However, the two dominant genera responsible for sulfate reduction are identified as a Gram-positive, obligate anaerobic, spore-forming bacterium known as *Desulfotomaculum* and a heterotrophic non-sporulating Gram-negative bacterium known as *Desulfovibrio* (Batal, 1988). When heavy metals are present along with sulfide species, they will precipitate as insoluble sulfides, as represented in the following generic reactions (Cohen and Staub, 1992):

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<sup>2</sup>Toshisuke Ozawa is a doctoral candidate, Ronald R.H. Cohen is Professor of Environmental Science and Engineering, and Ronald W. Klusman is a Joint Professor of Chemistry and Geochemistry and Division of Environmental Science and Engineering, Colorado School of Mines, Golden, CO 80401.

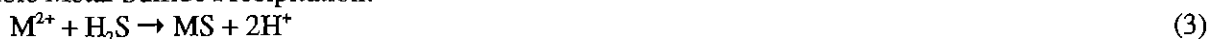
\*Sulfate Reduction:



\*Hydrogen Sulfide and Bicarbonate Production:



\*Insoluble Metal Sulfide Precipitation:



The bioreactor reduces an oxidized incoming solution to below -150 mV. The bicarbonate ion primarily is responsible for the buffering of acidic mine water and neutralizes the pH of the incoming solution from a range of 2 to 5 to approximately 7. The substrate, which has a pH greater than 8.0 acts as a secondary buffering mechanism by exchange reaction. In reaction (3),  $\text{M}^{2+}$  represents a univalent cation or one equivalent of a multivalent cation (Cohen and Staub, 1992; Staub, 1992).

Headspace  $\text{H}_2\text{S}$ , dissolved  $\text{HS}^-$ ,  $\text{S}^{2-}$ , and solid acid volatile sulfides (AVS) are prevalent in a PMDT system; therefore, most of the sulfide produced is available to react with ions in solution (Herlihy et al., 1987). The metals will complex with the  $\text{S}^{2-}$  and, under proper conditions, the metal sulfide will form insoluble amorphous metal mono-sulfides that precipitate (Mills, 1985).

Previous workers have used pilot scale and demonstration scale PMDT systems that are optimized for sulfate reduction with the goal to promote metal retention and alkalinity generation with treatment efficiencies of 95% to near 100% for cations, such as aluminum, cadmium, copper, iron, lead, nickel, and zinc (Cohen and Staub, 1992; Dvorak et al., 1992; Staub, 1992). Successful metal removal also occurred at ambient temperatures less than the optimal growth temperature for SRB. However, no investigations on removal of oxyanions, such as As(V) or Cr(VI) by the PMDT system have been reported. Both arsenic and chromium have received little attention although they are classified as carcinogenic (Federal Register 1985, Title 50, Part 219) and contribute to serious environmental problems in AMD (Wewerka et al., 1978) and several kinds of industrial wastewater (U.S. EPA, 1980). Therefore, this paper focuses on the biogeochemistry and removal processes of arsenic and chromium in a wet substrate, anaerobic bioreactor dominated by SRB.

## Geochemistry of Arsenic and Chromium

Arsenic has four different oxidation states: -3, 0, +3, and +5. However, only trivalent arsenic [As(III)] and pentavalent arsenic [As(V)] are important under most Eh-pH conditions in natural waters (Honeyman et al., 1984). Based on available thermodynamic data, As(V) is present predominantly as the  $\text{H}_2\text{AsO}_4^-$  species and is stable under most environmental conditions. Under oxidizing conditions, arsenic is pentavalent, with  $\text{H}_2\text{AsO}_4^-$  and  $\text{HAsO}_4^{2-}$  being the most abundant species at near-neutral pH; the  $\text{H}_3\text{AsO}_4^0$  species is predominant in AMD (Honeyman et al., 1984).

Chromium can exist in oxidation states ranging from Cr(-II) to Cr(VI). The most frequently found chromium species in the environment, and the most important are trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)] (Ecological Analysts, Inc., 1982; Honeyman et al., 1984). Based on available thermodynamic data, Cr(III) is expected to be stable under moderately reducing and oxidizing conditions (Ecological Analysts, Inc., 1982). In the natural environment, Cr(VI) is likely to be rapidly reduced by dissolved sulfide, organic matter, or ferrous iron to Cr(III) and eventually immobilized as a form of hydroxide (Schroeder and Lee, 1975). Sulfide species, such as  $\text{S}^{2-}$ ,  $\text{HS}^-$ , and  $\text{H}_2\text{S}$  are produced during decomposition of organic matter, by bacterial sulfate reduction, and can be present in the discharge of certain industrial waters. The produced Cr(III) then is likely to be adsorbed by suspended solids and removed from solution (Schroeder and Lee, 1975).

## Materials and Methods

### AMD and Synthetic Solutions Containing Arsenic and Chromium

Untreated AMD was obtained from the Big Five Tunnel, Idaho Springs, Colorado. This high sulfate AMD was used to condition the bioreactor and establish populations of SRB.

A 100 mg/L As(V) stock solution was prepared by dissolving 0.42 g of sodium arsenate dibasic heptahydrate, ( $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ ), in deionized water and by adding deionized water to make one liter. A 100 mg/L Cr(VI) stock solution was prepared by dissolving 0.28 g of potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ), in deionized water and adding deionized water to make one liter.

The solution of synthetic AMD without sulfate was made based on the existing data on concentrations of dissolved metals in a mine drainage from Big Five Tunnel (Table 1). Cadmium 0.6 mg, copper 30 mg, iron wire 1 g,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  10.80 g, lead 1 mg,  $\text{ZnCl}_2$  0.42 g,  $\text{CaCl}_2$  19.42 g, KCl 0.38 g, and  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  25.09 g were dissolved in a mixture of 100 mL concentrated  $\text{HNO}_3$ , and 50 mL concentrated HCl. The solution was diluted and the pH was adjusted to 2.8 with 3N-NaOH. Finally, distilled water was added to bring the total volume to 20 liters. No precipitation of solids was observed.

**Table 1. Concentration of dissolved elements (mg/L) and possible insoluble form of each dissolved metal used for MINTEQAQ modeling.**

| Dissolved Element | Concentration (mg/L) | Source   | Possible Insoluble Form of Each Dissolved Metal  |
|-------------------|----------------------|--|--|
| As                | 5.0                  | $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ | Orpiment ( $\text{As}_2\text{S}_3$ )<br>Scorodite ( $\text{FeAsO}_4 \cdot 2\text{H}_2\text{O}$ ) |
| Cr                | 5.0                  | $\text{K}_2\text{Cr}_2\text{O}_7$                    | Chromium Hydroxide [ $\text{Cr}(\text{OH})_3$ ]<br>Chromite ( $\text{FeCr}_2\text{O}_4$ )        |
| $\text{SO}_4$     | 2100.0 <sup>a</sup>  | AMD  |  |
| Cd                | 0.03 <sup>a</sup>    | AMD  | Greenockite (CdS)  |
| Cu                | 1.6 <sup>a</sup>     | AMD  | Covellite (CuS)  |
| Fe                | 50.0 <sup>a</sup>    | AMD  | am-Iron Sulfide (FeS)<br>Siderite ( $\text{FeCO}_3$ )  |
| Mn                | 150.0 <sup>a</sup>   | AMD  | Rhodochrosite ( $\text{MnCO}_3$ )  |
| Pb                | 0.01 <sup>a</sup>    | AMD  | Galena (PbS)   |
| Zn                | 10.0 <sup>a</sup>    | AMD  | am-Zinc Sulfide (ZnS)  |
| Ca                | 370.0 <sup>a</sup>   | AMD  | Gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ )   |
| K                 | 9.2 <sup>a</sup>     | AMD  |  |
| Mg                | 150.0 <sup>a</sup>   | AMD  |  |
| Na                | 46.0 <sup>a</sup>    | AMD  |  |

<sup>a</sup> Source: Wildeman and Laudon 1989.

### Bench Scale Bioreactor and Substrate

The column type bioreactor was constructed of clear plexiglass of a 5 mm wall thickness with an inner diameter of 10 cm and a total height of 90 cm (Figure 1). There were 1 cm diameter ports on the top and bottom of the bioreactor, and four 1 cm diameter ports on the side wall of the reactor. These ports on the wall were 21.5 cm, 42.0 cm, 62.0 cm, and 82.5 cm from the bottom of the bioreactor. The bioreactor was filled with a mixture of composted livestock manure and hay in a ratio of 3:1 by volume to a height of 82.0 cm. Total volume of the substrate in the column was 6,440 mL. The bioreactor operated in an upflow configuration with a plug flow hydraulic regime. The AMD was pumped through the bioreactor for two months in order to condition the column by saturating the negative sites in the substrate with cationic elements as well as to enhance the activity of SRB.

This enabled the experiments to focus on the effect of SRB while avoiding the uncertainties caused by adsorption and substrate conditioning.

Cohen and Staub (1992) investigated the optimum flow rates among empty bed hydraulic residence times of 280, 140, 70, and 35 hours in terms of metal removal efficiency. Based on Cohen and Staub's suggestions, the hydraulic residence times of 38.3 hours and 73.2 hours were chosen for this experiment, based on taking samples from effluent #3, the second highest effluent port in the bioreactor. Based on those hydraulic residence times, each flow rate was calculated as 2.1 mL/min and 1.1 mL/min, respectively.

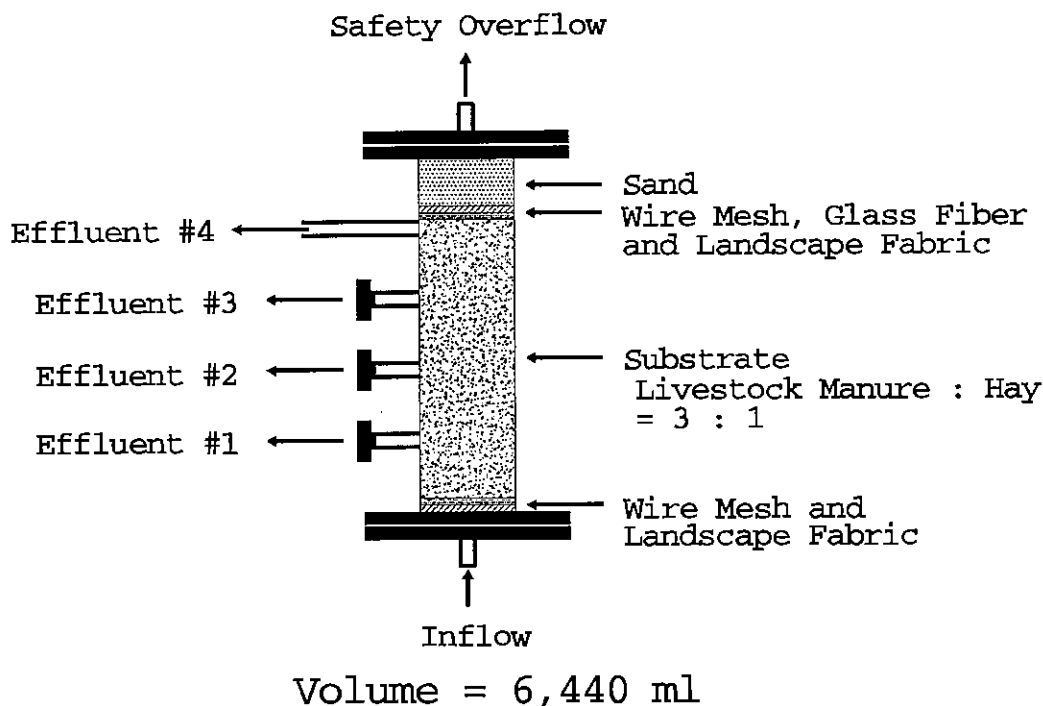


Figure 1. Schematic design of the bench scale bioreactor.

### Experimental Design

Investigation of the geochemistry and behavior of arsenic and chromium was performed with a series of experiments shown in Table 2. There were 9 different experiments, one per week. Following Experiment 6, a 25% HCl solution was pumped through the bioreactor with a flow rate of 2.1 mL/min for three weeks in order to dissolve gypsum, other forms of sulfate, and solid metal sulfide out of the bioreactor. The decision was made to strip the substrate of metals in the bioreactor rather than putting a new substrate in the column for the following reasons: (1) most of the unknown elements which were originally in the composted livestock manure would be stripped out, so that there would not be too many unknown interactions occurring in the substrate during the investigations of non-sulfate reduction processes; (2) the following experiments required no bacterial sulfate reduction; therefore, using a 25% HCl solution would be appropriate to destroy most of the bacteria in the bioreactor and prevent their growth. The process occurring during the treatment is:



where  $M^{2+}$  represents a divalent cation.

**Table 2. Experimental procedure and used solutions, hydraulic residence times, and sample analysis of each experiment.**

| Exp. Number  | Solution Used  | Hydraulic Residence Time (hr) | Sample Analytes  |
|--|--|-------------------------------|--|
| 1.   | As(V)+SO <sub>4</sub>                                | 73.2                          | As, SO <sub>4</sub>  |
| 2.   | Cr(VI)+SO <sub>4</sub>                               | 73.2                          | Cr, SO <sub>4</sub>  |
| 3.   | As(V)+Cr(VI)<br>+AMD                                 | 73.2                          | As, Cr, SO <sub>4</sub> , Cd, Cu, Fe,<br>Mn, Pb, Zn, Ca, Mg, Na. |
| *Add only AMD and increase the flow rate gradually to 2.1 mL/min.  |  |                               |  |
| 4.   | As(V)+SO <sub>4</sub>                                | 73.2                          | As, SO <sub>4</sub>  |
| 5.   | Cr(VI)+SO <sub>4</sub>                               | 73.2                          | Cr, SO <sub>4</sub>  |
| 6.   | As(V)+Cr(VI)<br>+AMD                                 | 73.2                          | As, Cr, SO <sub>4</sub> , Cd, Cu, Fe,<br>Mn, Pb, Zn, Ca, Mg, Na. |
| *Stripping all the sulfide forms in the bioreactor by adding HCl through the bioreactor for three weeks. |  |                               |  |
| *Saturate the negative sites in the substrate with synthetic AMD without SO <sub>4</sub> for one week.   |  |                               |  |
| 7.   | As(V)+Cr(VI)<br>+AMD w/o SO <sub>4</sub><br>(pH=2.5) | 38.3                          | As, Cr, SO <sub>4</sub> , Cd, Cu, Fe,<br>Mn, Pb, Zn, Ca, Mg, Na. |
| 8.   | As(V)+Cr(VI)<br>+HCl (pH=2.5)                        | 38.3                          | As, Cr, SO <sub>4</sub> .  |
| *Add NaCO <sub>3</sub> to the bioreactor to increase buffer capacity of the substrate.                   |  |                               |  |
| 9.   | As(V)+Cr(VI)<br>+HCl (pH=5.5)                        | 38.3                          | As, Cr, SO <sub>4</sub>  |

Synthesized AMD without sulfate was pumped through the bioreactor with a flow rate of 2.1 mL/min for about ten days. This conditioned the substrate in a fashion similar to that of the previous experiments in terms of saturating the protonated negative sites with cations. The stripping process was followed by Experiment 7 and 8. The effluents were sampled seven days after the experiments were started.

Cr(VI), iron, and sulfate were analyzed by ion chromatography. Flow injection hydride flame atomic absorption method was used for the analysis of arsenic, and the flame atomic absorption method was used for cadmium, copper, manganese, lead, zinc, calcium, sodium, and magnesium analyses. Detection limits for the elements were: arsenic 0.03 mg/L; Cr(VI) 0.5 mg/L; iron 0.3 mg/L; copper, manganese, zinc 0.05 mg/L; lead 0.02 mg/L; and cadmium 0.01 mg/L, and these detection limits were determined as 2σ in the blank.

Inverse modeling of the bioreactor with MINTEQAK, which was modified from MINTEQA2 by Klusman (1993), was performed for Experiment 3 as a final procedure to evaluate the possible removal processes for arsenic and chromium. The tested solid forms for removal of each dissolved metals are shown in Table 1. The pH, Eh, and each metal concentration of the effluents, hydraulic residence time, and the room temperature of the experiment were used as input parameters. The estimated rate of sulfate reduction as the input to the model was based on the decrease of aqueous sulfate as water passed through the bioreactor over a period of time.

## Results and Discussions

### **Observation of the Bioreactor**

A week after the inception of AMD flow through the reactor, an odor like rotten eggs (probably hydrogen sulfide,  $H_2S$ ) was detected. Also, black precipitates (probably iron sulfide,  $FeS$ ) in the bioreactor and the effluent tubing was observed about two weeks after the initial start-up. Those observations are usually indicative of SRB growth and activity in the bioreactor (Staub, 1992).

### **pH and Eh**

The highest pH and lowest Eh were observed from effluent port #3 compared to effluent ports #1, #2, and #4 (data not shown). Unfortunately, the effluent pipe of #4 was not set inside of the substrate but on the top surface of the substrate, which is between the substrate and layers of geomembrane and hay. One of the possible reasons for increasing the Eh and lowering the pH of the effluent port #4 is that the hay layer was not completely anaerobic, and oxidizing bacteria in the hay layer oxidized the treated water from the substrate as well as lowered the pH. Consequently, it was determined that effluent #3 should be used for analysis of metal concentrations as a final effluent of the bioreactor. All the hay in the top layer was taken out and sand was added to the top of the bioreactor.

Figure 2 shows the pH and Eh of influent water and effluent #3 during each experiment. The pH and Eh of the effluent #3 measured between 6.8 and 7.1, and between -150 mV and -350 mV, respectively during experiments 1 through 6. Since 25% HCl solution was applied for the stripping process after Experiment 6, the pH of the effluent #3 decreased significantly; the Eh rose to a positive value. Finally, since  $NaCO_3$  solution was added to the bioreactor after Experiment 8 to increase the buffering capacity, pH and Eh of the effluent #3 increased and decreased, respectively.

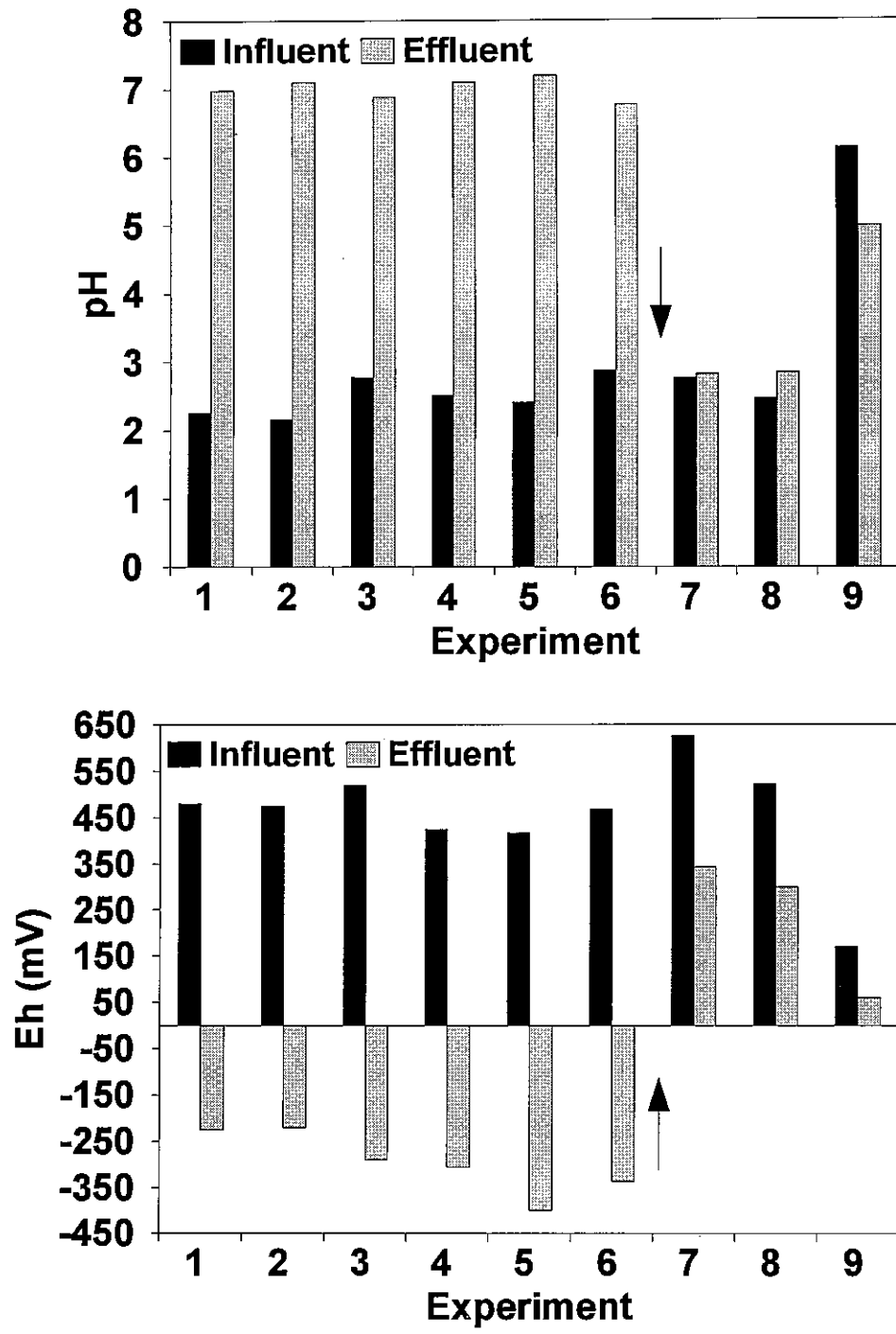


Figure 2. pH and Eh of influent and effluent #3 from the bioreactor during the experiments. An arrow shows the stripping process of the bioreactor.

### Concentrations of the Dissolved Metals and Removal Rates

Figure 3 shows the metal concentrations of influent and effluent #3 during the experiments. During experiments 1 through 3 with a hydraulic residence time of 73.2 hours, influent As(V) of 4.95 mg/L and 0.35 mg/L were reduced to below the detection limit of 0.03 mg/L. The As(V) removal rates of 91% to over 99% or more were attained and were below the U.S. EPA regulation limit (MCL) of 0.03 mg/L. Influent Cr(VI) of 4.97 mg/L and 4.71 mg/L were both reduced to 0.3 mg/L. A removal rate of 94% for Cr(VI) was achieved; however, the outflow concentration of Cr(VI) was over the MCL of 0.1 mg/L. The removal rates of over 90% were achieved for copper, iron, and zinc. Influent cadmium of 0.02 mg/L and lead of 0.04 mg/L were both reduced to below the detection limit of 0.01 mg/L. Actual removal rates for cadmium and lead are probably over 90% based on performance of other PMDT systems (Cohen and Staub, 1992; Staub, 1992). Removal of manganese could not be demonstrated. Previously adsorbed manganese may have been displaced from the system by more strongly adsorbed cations.

During Experiment 4 through 6 with a hydraulic residence time of 38.3 hours, influent As(V) of 4.68 mg/L and 1.55 mg/L were reduced to below detection limit and the MCL of 0.03 mg/L. The removal rate of greater than 99% for As(V) was accomplished even under a flow rate of 2.1 mL/min. Influent Cr(VI) of 4.70 mg/L and 5.01 mg/L were reduced to 0.6 mg/L and 0.5 mg/L, which were over the MCL of 0.1 mg/L. The removal rate was 87% to 91%, which was lower than that of previous experiments. Removal rates of over 90% were accomplished for copper and zinc. Again, actual removal rates for cadmium and lead are probably over 90% based on other PMDT systems. Higher concentrations of iron and manganese from effluent #3 than the influent were observed. This suggests that there may have been desorption of those elements from the organic substrate. It is known that the removal rates for iron are over 90% when the hydraulic residence time is over 50 hours; however, it decreases to 50% when the hydraulic residence time drops to 30 to 50 hours (Cohen and Staub, 1992; Staub, 1992). The removal rates for manganese are about 20 to 40% with a hydraulic residence time of 100 hours (Cohen and Staub, 1992; Staub, 1992). Keeping that in mind, the results from this experiment demonstrated that a hydraulic residence time of 38.3 hours is not sufficient for the PMDT system to treat AMD except for removal of As(V), cadmium, copper, lead, and zinc.

During Experiment 7 through 9, which is after the stripping process, removal rates of arsenic significantly decreased. During Experiment 7 and 9, arsenic concentration of the effluents were higher than that of influents. During Experiment 8, 4.30 mg/L of incoming As(V) was reduced to 1.6 mg/L, or 63% removal. That suggests that sorption sites for As(V) were probably saturated in this experiment. On the other hand, incoming Cr(VI) was approximately 100% removed during all experiments after the stripping process. During Experiment 7, removal rates of over 60% were observed for cadmium, copper, and lead while that of zinc were low. Removal rate of iron was larger than that of previous experiments.

In addition, sulfate concentration of effluent was higher than that of the influent (data not shown). That suggests that there was still residual sulfate in the bioreactor after stripping the metal sulfides and gypsum from the system with hydrochloric acid.



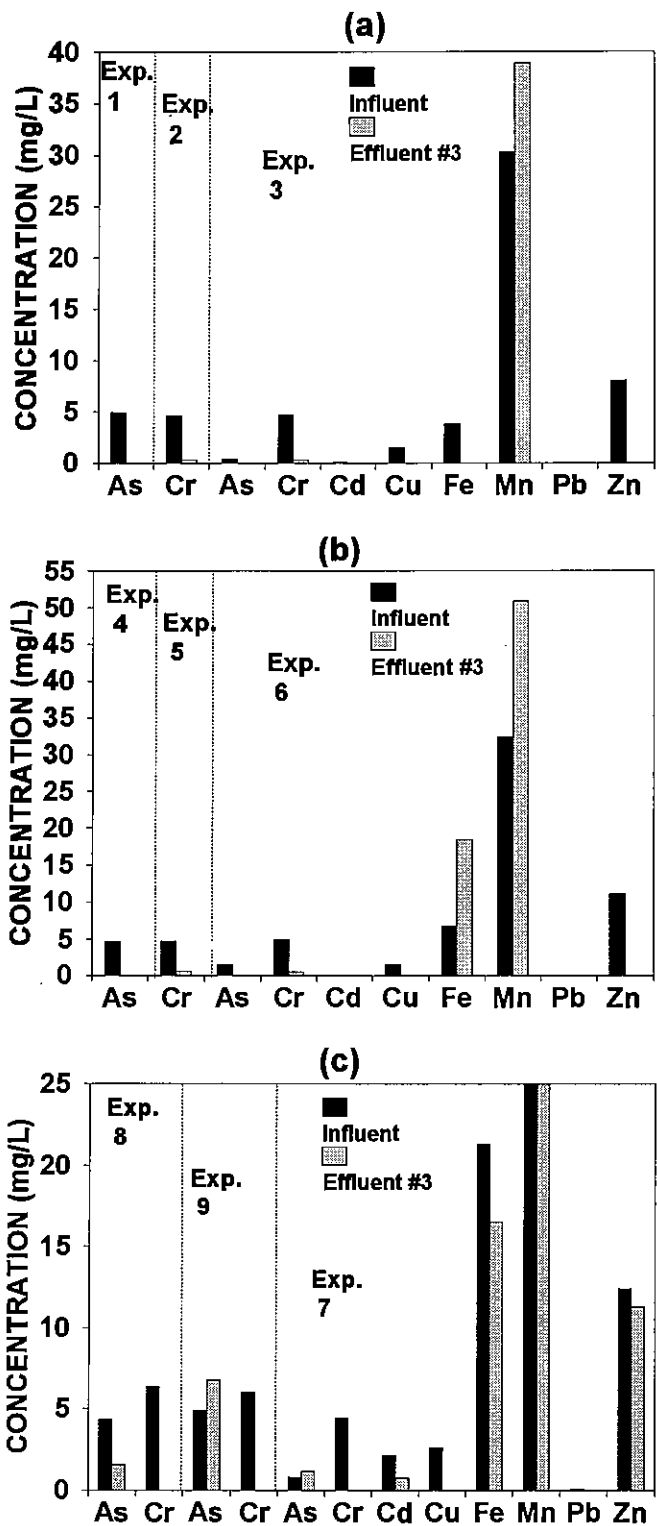


Figure 3. Comparison of removal efficiencies of major heavy metals among different experiments--(a)removal process was sulfate reduction with flow rate=1.1 mL/min and hydraulic residence time=73.2 hr, (b)removal process was sulfate reduction with a flow rate=2.1 mL/min and hydraulic residence time=38.3 hr, and (c)removal process was non-sulfate reduction process with flow rate=1.1 mL/min and hydraulic residence time=73.2 hr.

## As and Cr Removal Processes

Arsenite sorption on the clay minerals, kaolinite, and montmorillonite increases steadily from pH 4 to 9 (Frost and Griffin, 1977). The pH's in the second and third stages in the bioreactor were over 6.5. Thus, the arsenic removal process in this experiment could be adsorption. However, the negative sites on the substrate in the bioreactor had been saturated with cations before the experiments were started.

Also, for the modeling for arsenic compounds with MINTEQAQ, orpiment ( $\text{As}_2\text{S}_3$ ) and scorodite ( $\text{FeAsO}_4 \cdot 2\text{H}_2\text{O}$ ) were input as possible insoluble forms but not both in the same modeling run. When MINTEQAQ was given the option to form scorodite, the model output was an error message and the model did not work. A possible explanation was that reducing conditions were input to the model and therefore, an As(V) mineral would not be expected to form or be stable. When MINTEQAQ was given the option to form orpiment in the bioreactor, the model worked and predicted that arsenic would, in fact, form orpiment. Therefore, arsenic was probably reduced to As(III) and removed by sulfide formation instead of adsorption. The predicted arsenic sulfide could be amorphous. The behavior of arsenic, described above in the wet substrate probably is :

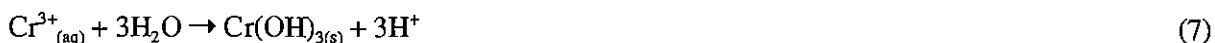


The chromium removal rate for the bioreactor during experiments 1 through 6 was from 86 to 94%. Cr(VI) could be removed in other than sulfate reduction processes. Cr(VI) oxyanions are not adsorbed to any great degree by inorganic materials, such as clays, ferric hydroxide, or ferric and manganese oxides; however, it seems to have a strong affinity for organics, which tend to be reducing agents (Callahan et al., 1979). Griffin et al. (1977) found that 30 to 300 times more Cr(III) than Cr(VI) adsorbed to clays. Although the above study demonstrated that clay adsorbs Cr(III), surface adsorption for chromium results in a weak complexation process (Morel and Hering, 1993) and probably does not account for most of the chromium removal.

Chromium hydroxide [ $\text{Cr}(\text{OH})_{3(s)}$ ] and chromite ( $\text{FeCr}_2\text{O}_4$ ) were entered into MINTEQAQ as possible forms of chromium. When MINTEQAQ was forced to precipitate chromium as chromite, the model output did not agree with measured levels of chromium and other dissolved elements in the bioreactor. Chromite is also highly unlikely in this type of environment. However, when MINTEQAQ was given the option to form chromium hydroxide in the bioreactor, the model predicted that chromium would, indeed, precipitate as the hydroxide. The measured amount of chromium and other metals removed in the bioreactor agreed with the model prediction. Therefore, the primary removal process is, most likely, probably formation of the insoluble hydroxide. The equilibrium reaction between Cr(VI) and Cr(III) forms is (Manahan, 1989):



Since chromium does not form a stable aqueous sulfide, reduction to Cr(III) is likely to be followed by rapid precipitation as hydroxide. The formation of insoluble chromium hydroxide by hydrolysis is:



Eighty-six to 94% of the chromium was removed in experiments 1 through 6. After the bioreactor was acid stripped, chromium was removed to below detection limits. The processes that may have removed Cr(VI) from the solution after the stripping and reactor reconditioning process are: (1) adsorption of Cr(VI) by the organic substrate; (2) reduction of Cr(VI) to Cr(III) and either formation of chromium hydroxide or adsorption of Cr(III) to the organic substrate; (3) formation of organic chromium compounds; (4) other processes, such as chelation. However, formation of hydroxide is probably less likely to occur during experiments 7 through 9 because the bioreactor at that time was highly oxidized (315 mV) with a low pH (2.82). Consequently, most chromium in the reactor would remain as Cr(VI) under this pH-Eh condition. Thus, the possibility that Cr(VI) forms Cr(III), and subsequently chromium hydroxides, in the reactor during experiments 7 through 9 was very low. Some process(es) which do not require the reduction of Cr(VI) to Cr(III), such as adsorption of Cr(VI) on the organic substrate in the reactor are more likely to occur. We were unable to confirm the adsorption process

from the data of the post-stripping experiments. It seems however, that adsorption based removal of Cr(VI) can last only as long as there are adsorption sites available. Machemer (1992) showed that similar organic substrates showed evidence of metal saturation of the sites within approximately 30 days.

### **Coprecipitation of Arsenic in the Influent Solution**

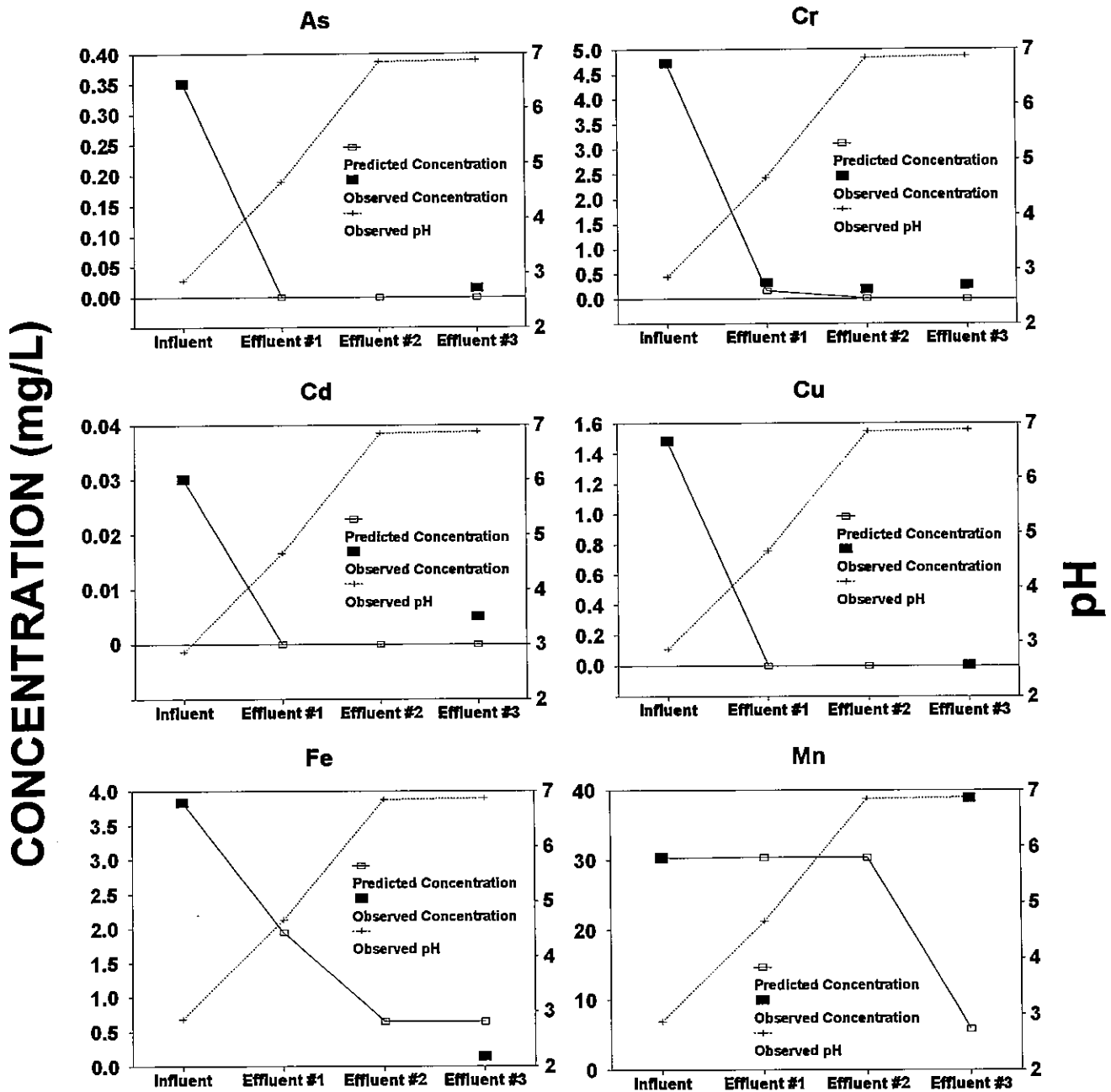
As(V) concentrations in the influent comprised of AMD and added arsenic were always low in the solution although enough As(V) was added to the AMD to yield a concentration of 5 mg/L As. Arsenic chemistry in oxidized solutions is believed to be controlled by adsorption-desorption mechanisms (Livesey and Huang, 1981). According to previous investigations (Aggett, 1986; Mok et al., 1988), both adsorption of the arsenic species onto the existing oxide surfaces of iron oxides in the sediments and the coprecipitation of As(V) into the hydrous iron oxides are main factors affecting the distribution of the arsenic species in the environment. An orange precipitate was observed on the bottom of the influent bottle during those experiments. It was speculated from the discussion on the above that coprecipitation of As(V) with Fe(III) might have occurred while the solution was stored in the influent bottle and only some of the precipitate was pumped into the reactor.

### **Measured Concentrations from the Influent and Effluent Ports #3, Compared to Predicted Values by MINTEQAK.**

Figure 4 shows sequential, actual mean changes in field parameters, such as pH and concentrations of arsenic, chromate, cadmium, copper, iron, manganese, lead, zinc, calcium, magnesium, sodium, and sulfate, from the influent and effluent #3, as well as the predicted concentrations of those dissolved elements by MINTEQAK during Experiment 3. Experiment 3 was chosen because the removal rates of toxic heavy metals were higher during Experiment 3 than in Experiment 6. Concentrations of the influent and all effluents were determined only for chromium and sulfate.

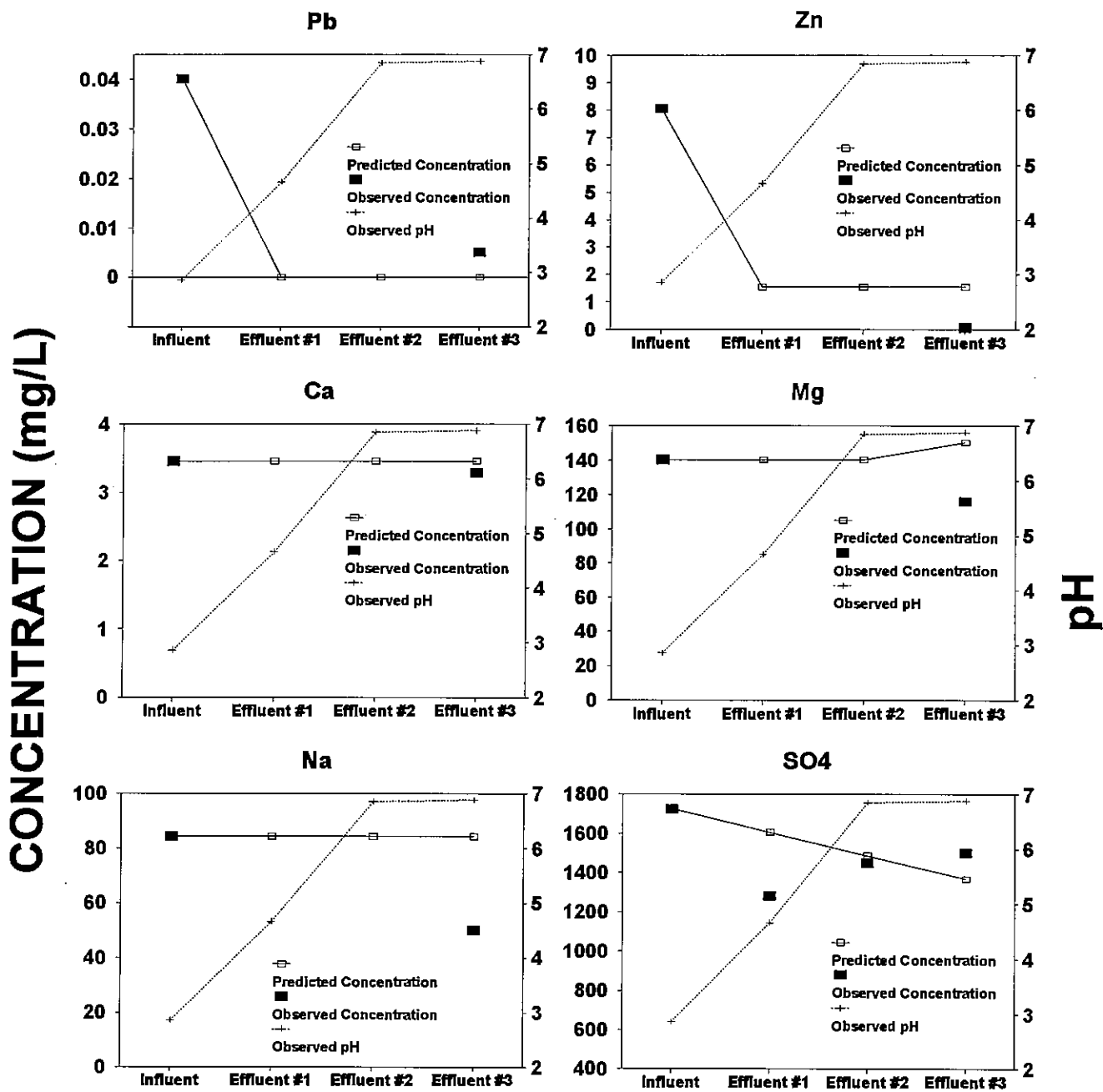
During Experiment 3, the pH increased from 2.86 to 6.85 by the second stage in the bioreactor. The profile of concentrations of dissolved elements predicted with MINTEQAK in effluent #3 for arsenic, chromium, cadmium, copper, iron, lead, zinc, calcium, and sulfate matched the measured concentrations very well. However, the predicted concentrations of manganese, magnesium, and sodium did not match measured concentrations. The computer modeling predicted that alkali and alkaline earth metals, such as calcium, magnesium, and sodium would not be removed by the bioreactor. It also predicted that most of the removal processes for arsenic, chromium, cadmium, copper, lead, and zinc will occur in the first stage in the bioreactor. However, it also predicted that iron and manganese will be transported further down gradient in the bioreactor than port #2 before being removed. MINTEQAK predicted that the decrease in sulfate concentrations was controlled by reduction of sulfate and production of sulfides due to SRB activity as well as by formation of gypsum. Measured sulfate concentration varied in the different stages. That is probably due to the dissolution of previously formed gypsum in the bioreactor. However, it was also known from previous measurements that at such high concentrations of sulfate, small changes in the large concentrations of sulfate is difficult to detect. Therefore, we could not define the decrease of sulfate.

We have confidence that the inverse modeling using MINTEQAK is useful for the following reasons: (1) the predicted chromium removal rate was similar to the measured removal rates; (2) the concentration profiles of metals concentrations during Experiment 3 were very similar to that of the measured concentrations. Therefore, the predicted solid forms for each dissolved metal presented in Table 1 are probably correct, including the sulfide form of arsenic ( $As_2S_3$ ) and hydroxide form of chromium [ $Cr(OH)_{3(s)}$ ].



## STAGE IN THE BIOREACTOR

Figure 4. Results from measured pH and concentrations of the dissolved elements and sulfate, from the influent and effluent #3, as well as the predicted concentrations of those elements with MINTEQAQ during Experiment 3 (0.35 mg/L As(V), 4.71 mg/L Cr(VI), and AMD, flow rate=1.1 mL/min, and hydraulic residence time=73.2 hr). The stage in the bioreactor represents sequential quarter volume of the substrate. The dotted line indicates the pH change in the bioreactor by stage; the solid line indicates the predicted metal concentrations in the bioreactor by MINTEQAQ modeling; and the filled squares indicate the measured concentrations of the metals.



## STAGE IN THE BIOREACTOR

Figure 4. Continued.

## Summary and Conclusions

1. Bacterial sulfate reduction is an important process in removing arsenic and chromium from both wastewater and acid mine drainage.
2. Ninety to over 99% arsenic removal and 86 to 94% chromium removal by sulfate reduction processes of SRB were observed.
3. Possible solid form for arsenic was orpiment ( $\text{As}_2\text{S}_3$ ) and that of chromium was chromium hydroxide [ $\text{Cr}(\text{OH})_{3(s)}$ ].
4. The hypothesized arsenic and chromium removal processes when SRB were functioning in the bioreactor were as follows: (1) reduction of As(V) and Cr(VI) to As(III) and Cr(III) by hydrogen sulfide which is produced by the SRB; (2) near neutral pH in the substrate was attained by bicarbonate ion production by SRB; (3) formation of arsenic sulfide ( $\text{AsS}$  or  $\text{As}_2\text{S}_3$ ) or an amorphous arsenic sulfide was predicted to be formed due to high concentration of sulfide ion in the substrate; and formation of chromium hydroxide [ $\text{Cr}(\text{OH})_{3(s)}$ ] was predicted to be formed due to the neutral pH in the bioreactor.
5. Approximately 90 to 95% of arsenic and chromium removal occurred in the first quarter volume of the bioreactor.
6. More than 38.3 hours of hydraulic residence time is required for the PMDT system to keep removal rate greater than 90% for both arsenic and chromium as well as other toxic heavy metals.
7. The use of a PMDT system as a bioreactor was an effective treatment mechanism for wastewater and AMD with elevated concentrations of arsenic and chromium.

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