

# Use of microbes for cost reduction of metal removal from metals and mining industry waste streams

Ronald R.H. Cohen\*

*Colorado School of Mines, Division of Environmental Science and Engineering, Golden, CO 80401, USA*

Received 17 June 2004; received in revised form 26 October 2004; accepted 28 October 2004

Available online 10 August 2005

## Abstract

Acid-rock drainage (ARD) – also known as acid-mine drainage (AMD) – results from the exposure of sulfide minerals, particularly pyritic and pyrrhotitic minerals, to atmospheric oxygen and water. AMD directly impacts tens of thousands of kilometers of streams, lakes and estuaries throughout the world. The impacted water bodies tend to have elevated concentrations of metals in the water column or sediments, and are also stressed by significant inputs of hydrogen ions.

There are several conventional treatment technologies available. The most common is chemical precipitation using lime or other basic substances. These systems produce large volumes of wet sludge that often require drying facilities to concentrate the metal hydroxide sludge. Wetland treatment systems have also been used for several decades to treat AMD. Recent developments and improvements have resulted in construction of bioreactors that have a smaller footprint, and treat the metals and acidity more effectively.

Many studies have demonstrated that the primary removal mechanisms for the metals are sulphate-reducing bacteria (SRB). These microbes facilitate the conversion of sulphate to sulphide. The sulphides react with metals to precipitate them as metal sulfides, many of which are stable in the anaerobic conditions of the treatment system.

Plants have been shown to remove metals by uptake or oxidative precipitation near the roots. Plants seem to account for only a small percentage of the metal removal capacity of the wetland treatment systems. Adsorption of metals to the organic substrates of the treatment systems can result in metal removal, but adsorption capacity is saturated in short periods of time.

High oxygen, low pH waters often enter the treatment systems. The SRB are obligate anaerobes which prefer conditions between pH 5 and 8. Thus, the input water characteristics could impact the efficiency and life expectancy of the treatment systems. The most important characteristic of input waters seems to be pH. Low oxygen of the influent waters did not enhance treatment capabilities. Low pH waters do reduce the capacity of the treatment systems to treat metals effectively.

Oxyanions such as chromate and arsenate can be removed using the wetland treatment system (passive bioreactor) technology. Arsenic is removed as an arsenic sulfide compound and chromate is reduced to Cr(III) and precipitated as a hydroxide.

The passive bioreactor – wetland treatment system – offers a less expensive alternative to the conventional chemical precipitation technologies. There still are problems of system hydraulics and useful life to be addressed.

© 2005 Published by Elsevier Ltd.

*Keywords:* Acid-mine drainage (AMD); Acid-rock drainage (ARD); Sulphate-reducing bacteria (SRB); pH; Mining

## 1. Introduction

### 1.1. Acid-rock drainage

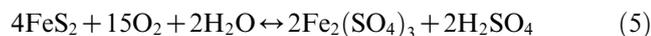
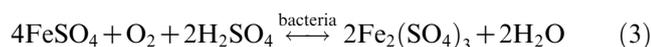
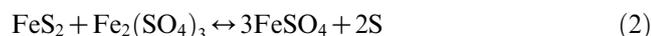
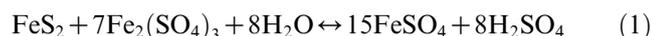
Acid-rock drainage (ARD) – also known as acid-mine drainage (AMD) – results from the exposure of sulfide minerals, particularly pyritic and pyrrhotitic

\* Tel.: +1 303 273 3613; fax: +1 303 273 3413.

E-mail address: [rcohen@mines.edu](mailto:rcohen@mines.edu)

minerals, to atmospheric oxygen and water. An oxidizing environment is established in which the subsequent biological and chemical reactions generate sulfuric acid and mobilize heavy metals associated with the particular ore, waste rock, and/or tailings from mining operations. Both operational and abandoned mine works contribute to ARD.

Upon exposure to the atmosphere, sufficient oxygen and water are present to initiate the cycle of ARD generation. Acid-rock drainage derives part of its acid character from hydrated iron(III). The oxidation of pyrite occurs according to the following process (as modified from Stumm and Morgan [1]):



Eqs. (3) and (4) have “bacteria” as “catalyst”, or accelerator. The bacteria, which are usually strains of *Thiobacillus ferrooxidans* and other iron oxidizers, utilize the sulfur present as their source of energy. At pH 3.5 or less, bacteria such as *T. ferrooxidans* accelerate the rate of conversion of  $\text{Fe}^{++}$  to  $\text{Fe}^{3+}$ . Such bacteria may accelerate reactions (3) and (4) by orders of magnitude [2].

Infiltration of snowmelt and rainfall into mine workings and tailings perpetuates the contamination of downstream aquatic environments. High concentrations of sulfate and ferrous iron and additional base metals such as lead, zinc, copper, cadmium and manganese may result. This aquatic impairment poses an immediate threat to the fauna and flora of the region but if left unchecked, can impact deleteriously upon human activities such as recreation, irrigation, industry and livestock watering for the duration of ARD generation.

The iron and steel manufacturing industries may also generate effluents similar to ARD. After removal of scale from metals rods, bars and sheets in acid baths, metal laden, acid solutions might be produced.

## 2. History and impacts

Pollution control in the U.S. mining and metals industry currently is mandated. This was not always the case. Public demand for action resulted in mining legislation to prevent damage to mined lands. Today, there is federal legislation, and nearly every state has statutes and regulations concerning mining and mined land reclamation. To conform to these regulations, the mining industry has developed and continues to develop pollution control measures and treatment strategies.

AMD directly impacts nearly 7000 km of streams and rivers and over 23,000 ha of lakes and reservoirs throughout this country, while indirectly contributing to contamination of 20,000 km and 70,000 ha, respectively [3].

The predominant source of AMD in the eastern United States is coal mining. The oxidation of pyrite and marcasite found in and around anthracite coal deposits has acidified thousands of kilometers of streams and rivers draining the Appalachian coal fields [4]. The results are waterways with pH less than 7 and a characteristic red–orange color, signifying the presence of oxidized iron (Fig. 1). The predominant AMD-generating source in the western United States is metal mine workings, the drainage from which often contains metals such as cadmium, lead, nickel, copper and zinc [5].

In the Rocky Mountain region, most of this contamination is the result of metal, or “hard-rock”, mining. As the ore veins are mined, the mineral surfaces become exposed to oxygen and water, especially in the waste rock and tailings piles that are discarded outside each mine portal. When the mine becomes flooded with infiltrating groundwater, additional shafts may be constructed to facilitate drainage or to lower the water table in order to expose deeper deposits. More acid-producing minerals are then exposed to oxidation [6,5]. The resultant drainage is typically acidic with higher than normal concentrations of metals, and ultimately discharges into the closest surface and groundwater drainage systems.

It is important to note that the AMD problem is not exclusive to the U.S.A., but is endemic to North America, South America, Africa, Europe and throughout the world. The emphasis on American mine drainage problems is due to the location of the author’s home and work.

## 3. Metal wastes pollution control

Nearly US\$1 million is spent each day on acid-mine drainage prevention and abatement by mining companies throughout the U.S. [7]. The typical AMD wastewater treatment processes employed by active



Fig. 1. AMD discharging from California Gulch, in Leadville, CO. The red–orange material is called “yellow-boy”.

mines are composed of three possible phases [8]: (1) chemical oxidation, (2) lime/caustic soda softening, and (3) sulfide addition, though the last method is infrequently used. The chemical oxidation phase primarily targets iron, manganese, and aluminum in an attempt to precipitate these metals as hydroxides through the addition of oxidizing reagents such as chlorine and potassium permanganate. Sedimentation and filtration are required in order to remove the solid phase precipitates in the wet sludge prior to discharge into receiving waters. The addition of lime/caustic soda raises the pH to nearly 10.5, where metal solubility tends to be the lowest, thus facilitating the nearly 100% removal of dissolved cadmium, copper, lead, nickel, and zinc as hydroxides and carbonates. Again, a secondary sedimentation or filtration step is necessary. The introduction of sulfides, typically in the form of hydrogen sulfide ( $H_2S$ ), at a pH of 8.5, results in heavy metals combining with the aqueous bisulfide ion and precipitating a solid sulfide phase. All three treatment processes are inefficient and/or potentially expensive. The addition of sulfides also requires constant monitoring to avert a low pH that could cause the release of  $H_2S$  gas, and to reduce effluent concentrations of  $H_2S$ .

The In-Line Aeration and Neutralization System (ILS) incorporates the chemical treatment processes into a functionally closed system where the treatment reactions can be more closely monitored and accelerated in order to reduce the chemical reagent costs and reaction processing times [3]. Electro-precipitation processes accomplish similar results by the precipitation of metal hydroxides or by metal ion adsorption [9]. However, all of these processes, both chemical and physical, are severely limited in that they: (1) are unable to treat the sometimes excessive sulfate concentrations associated with most acid-mine drainage, (2) impart a high degree of hardness to the water, and (3) produce waste sludge which requires additional treatment and/or disposal [10]. These methods can also require large capital costs, operations, and maintenance.

An alternative method has been investigated for the removal of manganese from circum-neutral waters. Gordon [11] reported successful removal of manganese using a packed column reactor filled with 2–5 cm chert stones from the Duck River below Normandy Dam, Tennessee. A “black slime” developed on the stones, but the exact nature of the removal mechanisms is yet undetermined. Possibilities range from adsorption,

chemical oxidation and even bacterial mediation to achieve the near 100% removal efficiency. It was later discovered that the “black slime” could be successfully transferred to other medium, such as glass marbles. The efficiency of the system was solely a function of substrate surface area and hydraulic loading rates [12,13]. This system has only been tested on manganese concentrations of 2–15 mg/L, so it is uncertain if it would be equally efficient at concentrations in excess of 100 mg/L, such as those found at many sites such as in the Golf Tunnel discharges at Chalk Creek, CO.

#### 4. Passive mine drainage treatment systems

One innovative technique for the treatment of ARD has been the use of natural and artificial wetlands as a biological pollution abatement process. The focus of these passive mine drainage treatment systems (PMDTS) [14] is to apply bio-geochemical water treatment mechanisms at or near the source of the mine drainage to concentrate and immobilize metals and raise pH. Prototype PMDTS have been constructed in Colorado, Pennsylvania, and West Virginia, Canada, South Africa and Australia. The cost and maintenance of a PMDTS is a fraction of that of a conventional treatment system. Eger and Lapakko [15] estimated that PMDTS were less than 1/2 the capital costs and 1/20 the maintenance costs of conventional plants.

It has been observed for many years that natural wetland ecosystems have the capability to remove metals from mine drainage. The emphasis, for a decade or more, has been to strive to emulate the natural wetland ecosystem by constructing a peat-based system with emergent vegetation. Vegetation was retained because it was accepted that plants probably remove metals into their biomass, and that they supplied microaerobic zones for bacteria that may assist in the removal process. Past PMDTS technologies were based on constructing shallow ponds or cells that resembled natural wetlands [16]. These systems were filled with peat or other organic substrate. Cattails, sedges, and rushes were then transplanted from natural wetlands [16]. Approximately 400 constructed wetland treatment systems had been constructed in the U.S. by the mid-1990s (Fig. 2).

It is now known that mushroom compost, as well as other organic substrates, serve to supply sulfate-reducing bacteria, like *desulfovibrio* and *desulfotomaculum*, with an organic substrate [6]. They then can reduce sulfate to sulfides that react with metals to form metal sulfides and hydrogen sulfide. Other bacteria assist in raising the system pH to more neutral levels. In the upper few inches of substrate, manganese oxidizers and iron oxidizers precipitate metal oxides and co-precipitate other metals and metal hydroxides. The bulk

of the beneficiary activities, though, are due to the sulfate reducers [6].

The properties of biological treatment systems reduce (but do not eliminate) the need for continuous maintenance, offering an attractive, alternative abatement technology to the conventional systems. The biological systems also avoid the production of copious amounts of wet sludge associated with oxidative and hydrolytic processes. These passive biological systems can also operate at a fraction of the production and maintenance costs of the conventional chemical and physical treatment methods.

Optimally, the system should be designed to accept large flows to minimize the need for extensive area coverage of wetlands. Flows that are too large, however, exceed the system hydraulic conductivity capabilities and the water seeks the path of least resistance – across the surface. Major portions of the flow through the system will therefore not be treated. Thus, the hydraulic conductivity and design of the wetland system must be optimized to permit the highest possible flows through the system, while maintaining a long contact time with the substrate for metals removal.

If flow is too high, the sulfate reducers, preferring near-neutral pH, will be overwhelmed with low pH waters. Their nutritional needs must also be considered.

#### 5. PMDTS evolution and limitations

Klusman and Machermer [18] listed the possible major metals removal processes in a PMDTS system: (1) adsorption and complexation of metals by organic substrates; (2) microbial sulfate reduction, followed by precipitation of metals as sulfides; (3) precipitation of ferric and manganese oxides; (4) adsorption of metals by ferric hydroxides; (5) metal uptake by plants; (6) filtration of suspended and colloidal materials.

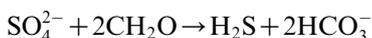
The following discussion will focus on the evidence for and against metal removal and pH increase in PMDTS by the above-stated mechanisms. The evidence will be used in combination with data from hydraulic studies to suggest design configurations and parameters.

Reynolds et al. [19] and Machermer [20] examined the chemical and biological processes in wetland treatment (PMDTS) systems receiving AMD and found that the rate of sulfate reduction to sulfide was the most crucial process involved. This conclusion has been corroborated by additional research on the utilization of natural and artificial wetlands for acid-mine drainage treatment [21,6,22–24]. The activity of the sulfate-reducing bacteria in these systems controls the efficiency of metal decontamination [21,25,18]. During respiratory processes, the sulfate-reducing bacteria oxidize simple organic compounds (represented by the chemical formula  $\text{CH}_2\text{O}$ ) resulting in the formation of hydrogen



Fig. 2. Wetland treatment system for discharge from the King Solomon mine in Creede, CO.

sulfide and bicarbonate ions at the circum-neutral pH's of "optimal" sulfate reduction [24,26]:



The electron donors for this reaction can include molecular hydrogen ( $\text{H}_2$ ) and organic compounds such as acetate and lactate. The formation of bicarbonate indicates the ability of the sulfate-reducing bacteria to control the pH of their particular microenvironment [6,22]. The form of the sulfide from the bacterially-mediated sulfate reduction is dependent upon the pH of the reaction and is represented by the equilibrium:

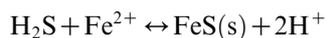


Both  $\text{HS}^-$ , which occurs at neutral pH, and  $\text{S}^{2-}$ , which occurs at high pH, are soluble in water, while  $\text{H}_2\text{S}$ , the predominant form at low pH, is not soluble and tends to evolve from solution, even at neutral pH where it is in equilibrium with  $\text{HS}^-$ .

The bicarbonate ion formed during sulfate reduction will equilibrate between  $\text{CO}_2$ ,  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ . However, the predominant form at the "optimal" pH range for dissimilatory sulfate reduction will be the bicarbonate ion. This rise in pH will facilitate the hydrolysis and precipitation of some contaminant metals from acidic waters as insoluble hydroxides and oxides [1]:



The hydrogen sulfide created during the reduction reaction also will react with many metal species, forming insoluble metal sulfide precipitates:



These processes all contribute to the removal of contaminant metals from waste waters through the action of sulfate-reducing bacteria.

Other processes that may contribute to metal removal within these systems include the precipitation of ferric hydroxides and manganese carbonates, the subsequent adsorption of metals by the ferric hydroxide, adsorption to the organic substrate, and the physical removal of colloidal particles through filtration by the substrate matrix. Such mechanisms were postulated and modeled by Walker and Hurl [27].

In order for the sulfate-reducing bacteria to thrive, they require a strict anaerobic environment (they are obligate anaerobes) with a pH in the range of 5–8 [25,28]. When pH and/or redox conditions are not optimum, the rate of microbial sulfate reduction declines. This, in turn, reduces metal removal capacity. The rapid influx of acidic, aerobic waters appears to drive the pH of the treatment system down and redox up, thus inhibiting bacterial sulfate reducing processes. The metal removal efficiency and loading capacity of the

treatment system then becomes a function of not only size and hydraulic conductivity, but also the acidity and oxygen content of the influent water [25].

## 6. Results, evidence, and implications

### 6.1. The role of vascular plants in PMDTS

Many studies in the late-1980s either focused on plants or assumed plant uptake of metals was an important process in metal removal [29–31]. Girts and Kleinmann [16] suggested that metal removal was mediated by plants and bacteria. Such was the emphasis on plant removal processes, that Guntenspergen et al. [30] evaluated 1000 plant species. Considerable emphasis was placed on cattail species, a plant found in most wetlands that is particularly resistant to poisoning by heavy metals and low pH [31–33]. At the Big Five Tunnel, Idaho Springs, CO, cattails not only dominated the PMDTS, but almost completely out-competed the other plants and formed very high biomass densities. There is, however, evidence that plants account for as little as 1–5% of metal accumulation. Evidence for uptake of metals into root, stem and leaves of plants shows that there is probably no net uptake of metals over the period of the experiments [32,34–36]. There is a possible, but not statistically supportable, increase in plant tissue metals for some metals, but that is probably within the range of analytical and sampling error. There is as much evidence for decreasing metal concentrations in the plants. In addition, one 3 by 3 by 1 m unit at the Idaho Springs, CO site was covered with hay and a black, opaque liner. It proved to be the most successful design for removing metals.

Cheng et al. [37] claim that plants removed metals to very low levels in a wetland treatment system. One-third of the copper and manganese was adsorbed by plant roots, but other metals and the rest of the Cu and Mn were removed in other parts of the system. The study was more of an input–output analysis that did not differentiate between metal removal by substrate sorption, microbes and plants. The authors assumed that plants were the primary metal removal mechanism.

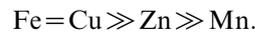
Mays and Edwards [38] reported that plants did take up metals, but the plants accounted for a small percentage of the removal that took place in both a constructed and a natural wetland.

Although plants may not accumulate metals, they may promote metal removal by generating microzones of oxidizing or reducing conditions in the organic substrate. Microzones around the root mass may be more aerobic due to oxygen excretion [32], inducing precipitation of manganese iron as Fe(III). The process of Mn and Fe oxide formation may be a removal mechanism for the metals, but it occurs in only 2% of

the volume of the system. The remaining 98% of the system is anaerobic. Thus, plants most likely are not the dominant removal mechanism for metals.

### 6.2. Adsorption and complexation processes in metal removal

Adsorption is a process in which a cation, like  $\text{Fe}^{+2}$ , is bound to the solid phase that contains a residual negative charge on its surface (usually in the form of a hydroxide ion). Complexation can be the result of humic materials terminating in phenolic and carboxylic groups that dissociate under particular pH conditions. Kerndorf and Schnitzer [39] found that the strength of sorption to humic materials varied with metal species as follows:



The adsorption process also varies with pH.

For natural and constructed wetland systems, metal removal due to adsorption takes place until sorption sites are saturated. Thus, the system will have a fixed life cycle, perhaps as short as a month [25]. When sulfate-reducing bacteria activity was terminated by poisons specific to their systems, Willow and Cohen [25] reported that all metals were removed by the organic substrate (approximately 20 days), then manganese, zinc, copper and iron started breaking through to the effluent (Fig. 3).

The interpretation of the data is that there is competition for the sorption sites, particularly as the sites become saturated. Fe and Cu, which bind to the particles more strongly than Zn and Mn, cause desorption of the Zn and Mn in an ion exchange process. Therefore, in 20–30 days, the sorption sites were becoming saturated with metals. It is apparent that

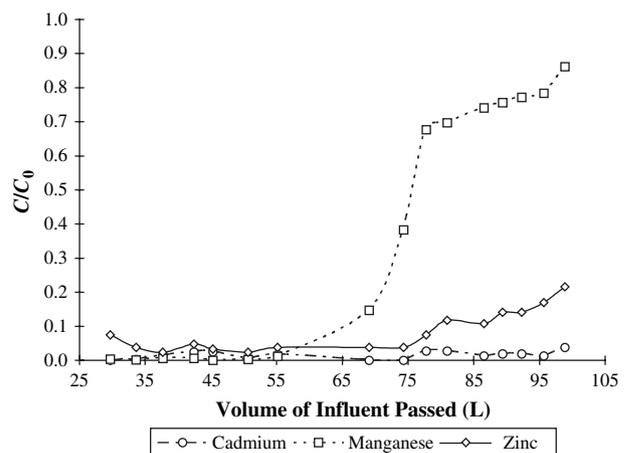


Fig. 3. Adsorption column effluent concentration vs. volume of influent passed. Manganese is the first constituent metal to appear in the effluent [25].

sorption processes are not the removal mechanism of choice if PMDTS are to have multiyear life cycles. Thus, a significant proportion of the short-term removal of metal ions was due to adsorption onto the organic manure substrate. Experimental results indicate that adsorption was limited by the sorptive capacity of the substrate. Different substrate materials will have different sorptive capacities. Combined with the fact that adsorption in these systems is also a function of pH and the particular metal species being removed, each system will be unique in character. A universal sorption capacity cannot be assigned to passive mine drainage systems as a whole, but the experimental results are similar to those of Machemer [20], that is, adsorptive capacity lasts for 30–60 days; reactor life-span is 4–6 years. Thus, most attention should be focused on optimizing conditions for sulfate reduction.

### 6.3. Sulfate-reducing bacteria (SRB)

Sulfate-reducing bacteria (SRB) are obligate anaerobes that decompose simple organic compounds using sulfate as the terminal electron acceptor [40]. The result is the production of sulfide that may be given off as H<sub>2</sub>S gas or react with metals to form metal sulfides. Much of the following comes from a review by Cohen and Staub [6] and work by Willow and Cohen [25]. As discussed in the introduction, SRB reduce sulfate to sulfide, followed by precipitation of metals as metal sulfides. FeS is one of a family of compounds called acid volatile sulfides (AVS). These compounds will generate H<sub>2</sub>S with the addition of acid. Reynolds et al. [19] reported that the dominant forms of sulfur resulting from SRB activity in PMDTS are H<sub>2</sub>S, dissolved S<sup>2-</sup>, and solid acid volatile sulfides (AVS). Reynolds et al. [19] also noted that H<sub>2</sub>S is present in small quantities when influent metal loadings are high, resulting in AVS being the major form (over 90%). Herlihy and Mills [41] also reported that most of the sulfide readily reacts with metal ions to precipitate them as metal sulfides.

Hedin et al. [42] have suggested that a good estimate for rates of sulfate reduction was 300 nmol S<sup>-</sup> produced

per cm<sup>3</sup> of substrate per day. Reynolds et al. [19] reported typical levels to be 600–1200 nmol/cm<sup>3</sup>/day using both radio-labeled tracers and measurements of rates of production of AVS (Table 1).

These numbers permit the calculation of theoretical treatment capacities of PMDTS if it is assumed that most removal of metals can be accounted for by sulfate reduction and the metals are removed on a one-to-one molar basis as AVS. Reynolds et al. [19] reported that sulfate reducing activity could account for all of the removal of metals in the Big Five Tunnel PMDTS. In addition, work at the late US Bureau of Mines and Colorado School of Mines demonstrated that as metals were removed, pH increased and alkalinity increased [43,8]. This is not unexpected because there is production of bicarbonate and loss of H<sub>2</sub>S gas represents a loss of hydrogen ions.

Updegraff [44] and Reynolds et al. [19] reported on experiments examining the effects of substrates and other variables of SRB activity. They used many different substrates, including spent mushroom compost, peat, corn wastes, rice waste, decomposed wood chips and composted cow manure. Results from the Big Five Tunnel PMDTS showed that peat-based systems were ineffective, even when limestone was added [45]. The wood chips and cow manure gave the highest activity rates, with other materials often yielding near-zero activity. Decomposed wood chips and composted cow manure also had the highest buffering capacity and pHs of the tested substrates. If pH-raising additives and alkalinity were added to the poor substrates, activities of SRB increased substantially. Reynolds et al. [19] also found that water extracts of hay could increase sulfate reduction between 2.5 and 7 times. SRB cannot utilize complex organics. They require simple, volatile, organic acids such as acetate and lactate. Indeed, additions of these nutrients enhanced SRB activity. There probably is a consortium of heterotrophic bacteria in the substrate that decomposes complex organics into forms available to the SRB.

Willow and Cohen [25] demonstrated that pH of the influent water is more critical to reactor efficiency than was the oxygen content of the influent water. The metal

Table 1  
Results of isotopic and acid volatile sulfide (AVS) analyses of the rate of sulfate reduction in samples taken from a pilot scale system [19]

Rates of sulfate reduction in nanomoles of S <sup>2-</sup> per cm <sup>3</sup> of substrate per day			
Location sample taken in reactor	Date	Rate	% Standard deviation (number of samples)
<i>Isotopic method <sup>35</sup>S</i>			
Surface	10/90	600	10.9 (4)
Bottom	11/90	440	10.4 (3)
Surface	11/90	750	8.6 (6)
Control	11/90	12.2	29 (3)
<i>AVS method</i>			
Surface	11/90	670	35 (6)

loading rate capacity of a wet-substrate bioreactor can be enhanced, and hydraulic detention times reduced, by modifying the pH of the influent to near neutral. The neutral pH permits enhanced activity of SRB, the production of sulfide, and the removal of metals as metal sulfide precipitates. Since the SRB are not only obligate anaerobes, but also require a narrow pH range near neutral for “optimal” sulfate reduction, it is reasonable to assume that pH is the predominant controlling factor. Optimization and enhancement of the reactors may be accomplished through the modification of mine drainage to near-neutral pH. Perhaps the use of anoxic limestone drains before the SRB bioreactor would raise the pH to levels more acceptable to the bacteria.

Lemke [46] found that biofilms clog the pores of substrates used in horizontal flow units, regardless of the size or number of amendments. Cohen and Staub [6] used composted cow manure and hay in a 4 to 1 by-volume ratio. The hay served as a bulking agent to help maintain hydraulic conductivity. The substrate used by Cohen and Staub was able to efficiently (98–100% metal removal) treat between 4 and 8 times the mine drainage flow rates of previous systems used in the Rocky Mountain region. The advantages of using the cow manure and hay can be seen in the section on sulfate reduction. The manure pH was 8–9 and it had high buffering capacity. The hay has been demonstrated to enhance SRB activity by 250–700%. The composted manure can neutralize the pH of the mine drainage. The organic matter in the manure decomposes to forms available to SRB and low redox potentials are generated, conditions ideal for SRB. Thus, the SRB can become established and can then modify their own micro-environments.

#### 6.4. Structural and hydraulic considerations

The Big Five Tunnel system in Idaho Springs, CO, was constructed in a horizontal flow pattern similar to natural wetlands and other constructed wetland-like systems. Studies showed that hydraulic conductivity of the substrate decreased two to three orders of magnitude in a few weeks [46]. As a result, very small quantities of wastewater passed through in contact with the substrate and most of the water flowed, untreated, over the surface of the system. The system was reconfigured to force the water through the substrate [45]. Lemke’s work [46] showed that upflow systems retained their hydraulic conductivity longer and guaranteed wastewater contact with substrate. A cell of the Big Five PMDTS was divided into an upflow cell and a downflow cell. The upflow cell substrate remained saturated throughout the experiments and treated mine water efficiently. The downflow cell worked well, but flows that were too low followed channels through the substrate and a large percentage of the

substrate remained dry and unutilized. The upflow configuration seems to fulfill the requirement of steady hydraulic conductivity and water substrate contact. Fig. 4 shows a schematic of an upflow reactor [45,46].

Five hundred gallon, upflow reactors were constructed in one area of the Eagle Mine, Minturn, CO, and filled with composted livestock manure. The influent distribution pipe, made of perforated irrigation tubing, was protected against clogging by the particulates in the substrate. In the 500 gallon pilot system, the influent pipe was covered with pea gravel and landscape fabric which, in turn, was covered with a water permeable geomembrane as in Fig. 4. The gravel disperses the inflow and the geomembrane separates the influent chamber from the substrate. Larger systems may require the construction of an influent plenum that is covered with geomembrane.

#### 6.5. Results of the pilot scale experiments

Research at CSM has demonstrated that SRB activity is approximately 600 nmol of sulfide produced per  $\text{cm}^3$  of substrate per day [19]. Calculations based on the metal loading rates at the Eagle Mine and the 37  $\text{ft}^3$  of substrate of the PMDT, that the system should theoretically reach its limit of metal removal at a flow of between 200 and 400 ml/min. Maximum removal rates of 97–100% have occurred for all metals except for manganese at the 200 ml/min and 400 ml/min flow rate (Fig. 5).

On both 27 August and 28 October, there seemingly is a dramatic decrease in metal removal efficiency for iron, zinc, lead, copper and cadmium. On or before 27 August, an inflow valve to the systems inadvertently was turned up to very high flows, flooding the system at levels significantly beyond their treatment design limits.

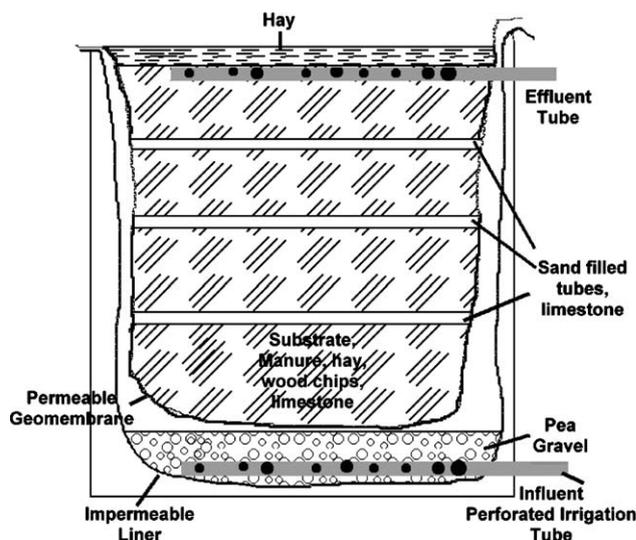


Fig. 4. Upflow PMDTS.

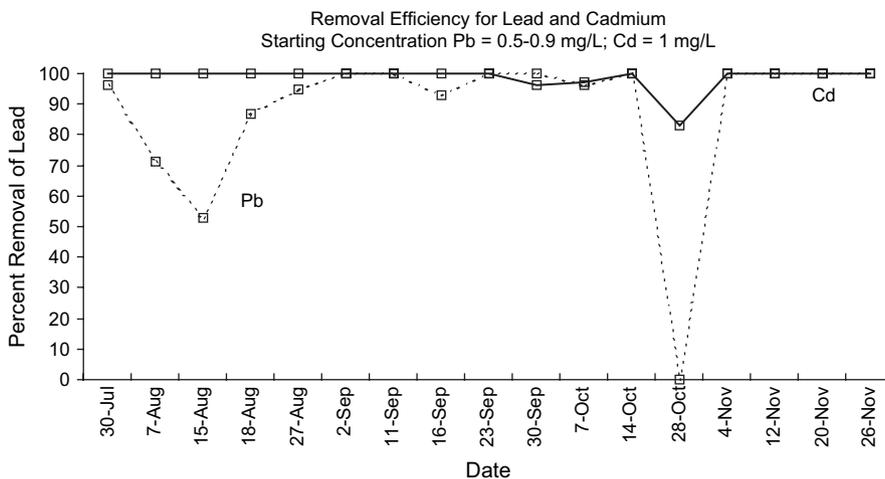


Fig. 5. Removal efficiency for cadmium and lead in a PMDTS [6].

Prior to 28 October, there was a backup of untreated water into the system. The drain to which overflowing, untreated water is discharged was clogged with metal hydroxides. This water backed up and mixed with the treated water near the sampling point. Evidence that it is not a failure of the system's treatment capability is demonstrated by the fact that, the following week, treatment efficiencies for all metals except manganese were again near 100%. Flows were doubled from 50 to 100 ml/min on 3 September and from 100 to 200 ml/min on 15 October. Near the end of the study, flows were doubled to 400 ml/min. The times of flow adjustment are shown as arrows in Fig. 4.

#### 6.6. Further improvements

Until PMDTS optimization research and development reduced hydraulic detention times required for 99% or better metal removal rates to 40 h or less [6], PMDTS were constrained in their volume/discharge handling capacity. Older PMDTS could treat metal waste streams effectively (99% or greater removal) for hydraulic detention times of 250–300 h, or remove 40–70% of metals at shorter hydraulic detention times.

In order for the sulfate-reducing bacteria to have high sulfate reducing activity, and therefore a system with high metal removal capacity, they require a strict anaerobic environment (they are obligate anaerobes) with a pH in the range of 5–8 [28,25]. When pH and/or redox conditions are not optimum, the rate of microbial sulfate reduction declines. The rapid influx of acidic, aerobic waters appears to reduce the pH of the treatment system and increase redox, thus inhibiting bacterial sulfate reduction. The metal removal efficiency and loading capacity of the treatment system then becomes a function not only of size and hydraulic conductivity, but also of the acidity and oxygen content

of the influent water. One of the limiting factors appears to be the pH of the influent water [25].

A second possible limiting factor in the PMDTS at higher inflow rates is the introduction of dissolved oxygen to the system by the AMD. An increased loading rate of oxygen would reduce the capacity of sulfate-reducing bacteria, which require anaerobic conditions, to produce hydrogen sulfide. With a greater influx of dissolved oxygen, it is reasonable to assume that redox would increase, sulfate reduction would decrease, and metals treatment capacity would decrease. If the dissolved oxygen content of the influent mine drainage could be reduced prior to entering the treatment system, the anaerobic conditions could be maintained for a greater volume of the treatment substrate, thus allowing for an increased influent treatment capacity.

Our experiments have shown that pH is more critical to reactor efficiency than dissolved oxygen. The metal loading rate capacity of a wet-substrate bioreactor can be enhanced, and hydraulic detention times reduced to as low as 16 h by modifying the pH of the influent to near neutral [25]. The neutral pH permits enhanced activity of SRBs, the production of sulfide, and the removal of metals as metal sulfide precipitates. The dissolved oxygen was completely removed in the first few centimeters upon entering the reactors, while the pH required a greater proportion of the substrate in order to reach suitable levels for SRB activity. As the pH of the influent increased, the rate of sulfate reduction increased, increasing the metal removal capacity of the system.

#### 6.7. Treatment of oxyanions – arsenate and chromate

Not all metals appear as positively charged cations. Some appear as negatively charged oxyanions with metals like arsenic, selenium and chromium bound to

oxygen to yield arsenate, selenate, selenite, chromate and chromite. Can an anaerobic bioreactor dominated by SRBs remove metal oxyanions? 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, MINTEQAQ [47], modified from MINTEQA2, was utilized for the inverse modeling of the bioreactor. Ninety to over 99% of the arsenic and 86–94% of the chromium were removed [26]. Cadmium, copper, iron, lead, and zinc were also removed from the feedwater. 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 MINTEQAQ confirmed that most (90–95%) 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  $[\text{Cr}(\text{OH})_{3(s)}]$ , and reduction of As(V) to As(III), followed by precipitation of arsenic sulfides ( $\text{As}_2\text{S}_3$  or  $\text{AsS}$ ). The use of a passive mine drainage treatment system was effective for wastewater and acid-mine drainage with elevated concentrations of arsenic and chromium (Fig. 6).

The results of Ozawa and Cohen [26] are in contrast to those of Walker and Hurl [27] who reported an increase in As and no change in Cr in a stormwater treatment wetland.

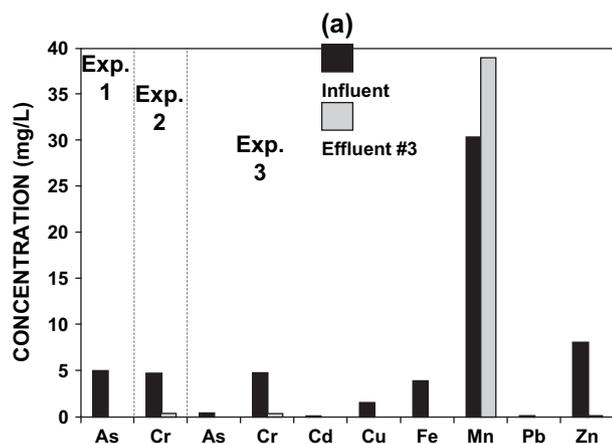


Fig. 6. Results of 3 experiments on removal of arsenic (experiment 1), chromium (experiment 2) and arsenic and chromium with other metals (experiment 3).

## 7. Summary, conclusions, and design and construction recommendations

The final design and construction decisions will be based on the flow rate to be treated, the loading rates of metals, and the space available for the bioreactor. The decision whether or not to use plants should be based solely on the aesthetic and erosion considerations, not on plants as a major contributor to metal removal or system longevity. Once operating, one can expect an effective life of 4–6 years from a single load of substrate, based on experience at the Big Five Tunnel, Idaho Springs, CO or longer, according to William Pulles [48]. At the end of the system life, the concentrated acid volatile sulfides must be disposed of as a RCRA waste or recovered from the organic substrate. Future work is required to determine if recovery can be efficient enough to justify recycling the metal values from the laden sludge.

### 7.1. Mass loading rates of metals

Metal concentrations must be converted from mass/volume (mg/L) to mol/L. Then:  $\text{mol/day} = Q \times c$ , where  $Q$  = discharge in volume/time (L/day) and  $c$  = concentration, mass/volume (mg/L).

For example, at the Eagle Mine, the mass loadings for the major metals were 1.49 mol metals/day for 100 ml/min, 2.97 mol metals/day for 200 ml/min. Once metal loading rates are known, the total volume of substrate required must be determined using estimated SRB activity. At the Eagle Mine, the substrate volume was  $2.46 \times 10^6 \text{ cm}^3$ . The estimated sulfide production rate based on our own study results and literature values range from 300 to 1200  $\text{Nm S}^-/\text{cm}^3/\text{day}$ . Therefore, moles of  $\text{S}^-$  produced/day =  $V$  (volume of substrate)  $\times$  SRB activity rate (mass/volume/day) and for this system the sulfide produced should be 0.74–2.95 mol  $\text{S}^-/\text{day}$ . Waters with lower metals concentrations than the Eagle Mine water, such as the effluent at the Big Five Tunnel (an order of magnitude lower concentrations) could either be treated with a smaller system or with higher flow rates.

Alternatively, and perhaps even better is to assume an empty bed hydraulic residence time of 20–40 h. Forty hours would be used if the feedwaters were below pH 5 and 20 h if the feedwaters were near-neutral pH.

### 7.2. Configuration to minimize hydraulic problems and maximize SRB activity

The upflow configuration has been shown to pose the least hydraulic problems of systems studied. Construction is more complicated than for horizontal flow systems, but suffers from fewer hydraulic conductivity or surface flow problems. The water is forced into

contact with the substrate. Downflow units are possible but require careful control of flow rates. If flow is too high, water pools on the surface. If flow is too low, water moves through channels, leaving most of the substrate dry and unused.

Distribution pipes in the upflow configuration require protection against contact with the substrate. There are two possible solutions: (1) distribution pipes can be buried in pea gravel (or similar material), then covered with a geomembrane or landscape fabric; (2) a plenum can be built to house the distribution system. The plenum is then covered with landscape fabric or geomembranes. The substrate then is placed upon the geomembrane.

If a system is to be passive, the driving force for the mine water is hydraulic head. Recent work suggests that 3 m of head will drive water through the system for the long term, although less head may suffice. Systems have been built with valve control of flow rates. Globe and ball valves rapidly clog with hydroxide precipitates and need frequent maintenance. Butterfly and gate valves seem to clog less frequently but need periodic (weekly) adjustment and cleaning. The higher the flow maintained through the system, the less frequent are the requirements to remedy clogging and flow rate adjustment. The Eagle Mine system uses a constant head tank to control flow to the reactors.

The reactor itself can be HDPE or similar plastic tanks, or of wood or concrete support structure covered by an HDPE liner. The Big Five Tunnel system uses a concrete base and a Hypalon liner. If tailings ponds or tailings dams are available, they can be lined and fitted as a PMDT. The Eagle Mine research also suggests that units can be run in series and will act as a single larger unit. There is also the possibility of constructing units to run in parallel. The result would be a group of smaller, easier to access units.

Maintenance is a consideration. I would advise that plumbing be configured to be as accessible as possible. Ports could be designed to access distribution pipes for cleaning and unclogging. Pipes should be oversized to reduce clogging by sediments of frozen water. All delivery and effluent systems should be buried or insulated to minimize frozen pipes.

### 7.3. Substrates

The previous sections suggest that composted cow manure, mixed with hay, is a good substrate, supplying acid neutralizing capacity to bring pH into the ideal range for SRB's and organic nutrients for growth of the complex microbiological consortium that develops in PMDT's. The hay can act as a bulking agent, helping to maintain hydraulic conductivity. We have found that porous ceramics used as a bulking agent for enhanced drainage on golf courses and athletic fields, when mixed in a ratio of 9 units of volume of substrate to 1 unit of

bulking agent (7.5 to 2.5 is even better for hydraulic conductivity), is considerably better than hay for long term permeability. In our studies, we used Turface™ and Profile™. This is not to say that it is the best or only substrate for this purpose. Decomposed wood chips act in a similar fashion. Additions of simple organic compounds like lactate, are known to enhance SRB activity. They can be added to the solid organic substrate by a feed mechanism. In fact, the future may bring active, batch units filled with liquid nutrient media to which mine drainage is added for the requisite contact period, and AVS precipitated in a clarifier. Other substrates, like peat and “mushroom compost” can be shown, both in the laboratory and field, to be inferior to composted cow manure. They lack the buffering capacity or nutrient composition to efficiently enhance SRB activity. Substrate amendments, like potting soil, dilute the nutrient and buffering capacity of the manure do not appreciably enhance hydraulic conductivity.

There are problems associated with any organic substrate, be it manure or wood chips. There will be a dissolved organic carbon (DOC) component leached from the system. There also might be nitrogenous materials like nitrate or ammonia in the effluent. A plant-based polishing treatment step might be required to bring the effluent to standards.

Ultimately, an efficient, long lasting, low sludge generation, inexpensive, low maintenance system can be constructed to treat metal laden and acidic (or neutral) wastewaters.

## References

- [1] Stumm W, Morgan RR. Aquatic chemistry. 2nd ed. John Wiley and Sons; 1981. p. 780.
- [2] Taylor BE, Wheeler MC, Nordstrom DK. Stable isotope geochemistry of acid mine drainage: experimental oxidation of pyrite. *Geochimica et Cosmochimica Acta* 1984;48:2669–78.
- [3] Kleinmann RLP. Acid mine drainage: U.S. Bureau of Mines, Research and Developments, control methods for both coal and metal mines. *Engineering and Mining Journal* 1989;190:16i–n.
- [4] Rahn PH. A method to mitigate acid-mine drainage in the Shamokin area, Pennsylvania, U.S.A. *Environmental Geology and Water Science* 1992;19:47–53.
- [5] Wildeman TR, et al. Passive bioremediation of metals from water using reactors or constructed wetlands in emerging technology for bioremediation of metals. In: Means Jeffrey L, Hincbee Robert E, editors. *Emerging technology for bioremediation of metals*. Ann Arbor: Lewis Publishers; 1994.
- [6] Cohen RRH, Staub SW. Technical manual for the design and operation of a passive mine drainage treatment system. U.S. Bureau of Land Management and U.S. Bureau of Reclamation; 1992. p. 69.
- [7] Kleinmann RLP, Hedin RS. Treat minewater using passive methods. *Pollution Engineering* 1993;25(13):20–2.
- [8] Bolis Judith, Wildeman Thomas, Cohen Ronald. The use of bench scale permeameters for preliminary analysis of metal removal from acid mine drainage by wetlands. Paper presented at the 1991 National Meeting of the American Society for Surface Mining and Reclamation, Durango, Colorado, May 14–17; 1991.

- [9] Jenke DR, Diebold FE. Electroprecipitation treatment of acid mine wastewaters. *Water Research* 1984;18(7):855–9.
- [10] Grimm EC, Hill RD. Environmental protection in surface mining of coal. Cincinnati, OH: EPA 093 National Environmental Research Center, U.S. Environmental Protection Agency; 1974.
- [11] Gordon JA. Manganese oxidation in the releases from dams. Proceedings of the 1985 Triangle Conference on Environmental Technology, North Carolina State University, Raleigh, North Carolina; 1985.
- [12] Gordon JA, Burr JL. Treatment of manganese from mining seep using packed column. *Journal of Environmental Engineering* 1989;15(2):386–94.
- [13] O'Neill Jr EB. Manganese removal from natural waters by stone-filled columns. Master's thesis. Cookeville, TN: Tennessee Technological University; 1986.
- [14] Holme JD, Elmore T. Passive mine drainage treatment using artificial and natural wetlands. Proceedings of the High Altitude Revegetation Workshop, No. 7, 41–48 meeting, St. Louis, MO; 1986.
- [15] Eger P, Lapakko K. Nickel and copper removal from mine drainage by a natural wetland. In: US Bureau of Mines Circular 9183. 1988. p. 301–9.
- [16] Girts MA, Kleinmann RLP. Constructed wetlands for treatment of mine water. AIME fall meeting, St. Louis, MO.
- [18] Klusman RW, Machemer SD. Natural processes of acidity reduction and metal removal from acid mine drainage. In: Peters DC, editor. *Geology in the coal resource utilization*. Fairfax, VA: Tech Books; 1991. p. 513–40.
- [19] Reynolds Julie, Machemer Steven, Wildeman Thomas, Updegraff Dave, Cohen Ronald. Determination of the rate of sulfide production in a constructed wetland receiving acid mine drainage. Paper presented at the 1991 National Meeting of the American Society for Surface Mining and Reclamation, Durango, Colorado, May 14–17; 1991.
- [20] Machemer SD. Measurements and modelling of the chemical processes in a constructed wetland built to treat acid mine drainage. CSM thesis T-4074; 1992.
- [21] Willow MA. pH and dissolved oxygen as factors controlling treatment efficiencies in wet substrate bioreactors dominated by sulfate reducing bacteria. Colorado School of Mines thesis #T-4747; 1995.
- [22] Staub MW. Passive mine drainage treatment in a bioreactor: the significance of flow, area, and residence time. Colorado School of Mines thesis T-4090; 1992.
- [23] Tuttle JH, Dugan PR, Randalls CI. Microbial sulfate reduction and its potential utility as an acid mine water pollution abatement procedure. *Applied Microbiology* 1969;17(2):297–302.
- [24] Dvorak HD, Hedin RS, Harry ME. Treatment of metal-contaminated water using bacterial sulfate reduction: results from pilot-scale reactors. *Biotechnology and Bioengineering* 1992; 40:609.
- [25] Willow Mark A, Cohen Ronald RH. pH, dissolved oxygen, and adsorption effects on metal removal in anaerobic bioreactors. *Journal of Environmental Quality* 2004;2003(32):1212–21.
- [26] Ozawa T, Cohen RR, Klusman RW. Biogeochemistry and behavior of arsenic and chromium in an anaerobic bioreactor. June 5, 1995 in the symposium volume of the American Society for Surface Mining and Reclamation, Gillette, Wyoming; 1995.
- [27] Walker David J, Hurl Sigrid. The reduction of heavy metals in a stormwater wetland. *Ecological Engineering* 2001;18:407–14.
- [28] Brown DE, Groves GR, Miller JDA. pH and Eh control of cultures of sulfate reducing bacteria. *Journal of Applied Chemical and Biotechnology* 1993;23:141–9.
- [29] Brodie GA, Hammer DA, Tomjanovich DA. Treatment of acid drainage with a constructed wetland at the Tennessee valley authority coal mine. In: *Constructed wetlands for wastewater treatment*. Chelsea, MI: Lewis Publishers; 1989. p. 201–9.
- [30] Guntenspergen GR, Stearns F, Kadlec JA. Wetland vegetation. In: *Constructed wetlands for wastewater treatment*. Chelsea, MI: Lewis Publishers; 1989. p. 73–88.
- [31] Samuel E, Sencindiver JC, Rauch HW. Water and soil parameters affecting growth of cattails; pilot studies in W. Virginia mines. Annual Meeting, ASSMR, Pittsburgh, PA; 1988.
- [32] Sencindiver JC, Bhumbra DK. Effects of cattails on metal removal from mine drainage. In: US Bureau of Mines Circular 9183. Kent State University Press; 1988. p. 359–66.
- [33] Wenerick WR, Stevens SE, et al. Tolerance of three wetland plant species to acid mine drainage: a greenhouse study. *Constructed wetlands for wastewater treatment*. Chelsea, MI: Lewis Publishers; 1989.
- [34] Emerick JC, Cohen RRH, Wildeman TR, Klusman RW. Results of an experiment using a pilot scale constructed wetland for remediation of acid mine discharge at Idaho Springs, CO. Thorne Wildlife Symposium, Snowmass, CO; 1990.
- [35] Heil MT, Kerins FJ. Tracy wetlands: a case study of two passive mine drainage treatment systems in Montana. Annual Meeting, ASSMR, Pittsburgh, PA; 1988.
- [36] Emerick JC, Cohen RRH. Results of an experiment using a pilot scale constructed wetland for remediation of acid mine drainage at Idaho Springs, CO. Guidebook for surface water contamination and its remediation near Idaho Springs, CO. USGS Circular; 1991.
- [37] Cheng S, Grosse W, Karrenbrock F, Thoennessen M. Efficiency of constructed wetlands in decontamination of water polluted by heavy metals. *Ecological Engineering* 2002;18:317–25.
- [38] Mays PA, Edwards GS. Comparison of heavy metal accumulation in a natural wetland and constructed wetlands receiving acid mine drainage. *Ecological Engineering* 2001;16:487–500.
- [39] Kerndorf H, Schnitzer M. Sorption of metals onto humic acid. *Geochimica et Cosmochimica Acta* 1980;44:1701–8.
- [40] Hamilton WA. Microbiology and microbial ecology of sulphate reducing bacteria. TMR the sulphur cycle. *Environmental Science and Technology*, April 19–24, 1998, Wageningen, The Netherlands; 1998.
- [41] Herlihy Alan T, Mills Aaron L. Sulfate reduction in freshwater sediments receiving acid mine drainage. *Applied and Environmental Microbiology* January 1985;49(1):179–86.
- [42] Hedin RS, Hammack R, Hyman D. Potential importance of sulfate reduction processes in wetlands constructed to treat mine drainage. *Constructed wetlands for wastewater treatment*. Chelsea, MI: Lewis Publishers; 1989. p. 508–14.
- [43] McIntire PE, Edenborn HM, Hammack RW. Incorporation of bacterial sulfate reduction into constructed wetlands for the treatment of acid and metal mine drainage. National Symposium on mining: a new beginning. Knoxville, TN. Report no. 284. Lexington, KY: University of Kentucky, OES Publications; 1990.
- [44] Updegraff David. Personal communication; 1998.
- [45] Cohen Ronald, Lemke Peter, Batal Wafa, Machemer Steven, Updegraff David. October 1988 through May 1989 Year End Report for the big five tunnel constructed wetland treatment system. Contract No. CR 815325, Site Emergency Technology Project, Colorado School of Mines; 1989.
- [46] Lemke Peter R. Analysis and optimization of physical and hydraulic properties of constructed wetlands substrates for passive treatment of acid mine drainage. Master's thesis No. 3823. Golden, CO: Colorado School of Mines; 1989.
- [47] Klusman RW, Dvorak DH, Borek SL. Modeling of wetlands and reactor systems used for mine drainage treatment. In: Proceedings. vol. II. The challenge of integrating diverse perspectives in reclamation, 10th National Meeting, Spokane, Washington, May 16–19; 1993. p. 685–704.
- [48] Pulles William. Personal communications, South Africa treatment system at Hlobane; 2000.