SUDBURY 2003
BIOREMEDIATION OF ROCK DRAINAGE USING SULPHATE-REDUCING BACTERIA

James P. Higgins, Barbara C. Hard,
Jacques Whitford Environment Limited,
Oakville, Ontario
And

Al Mattes
Nature Works Remediation Corporation
Trail. BC

ABSTRACT

Dealing with high concentrations of sulphates and metals in mine drainage is one of the major problems associated with many base metal and coal mines. Large volumes of acidic water can be generated from mine workings, waste rock piles and tailings. Usually, this acid rock drainage (ARD) cannot be disposed off until it has been treated in some way as it poses a direct threat to drinking water, agriculture, vegetation, wildlife and waterways. Traditional active treatment processes such as reverse osmosis or the addition of chemicals employed are often not very efficient and can be quite costly. In some cases they are simply not feasible. Therefore, alternative methods have to be considered.

Anaerobic bioremediation, a process in which sulphate-reducing bacteria (SRB) are used to decontaminate mine drainage in a stand alone bioreactor or one forming part of a constructed wetland (CW) system is such an alternative: Sulphate-reducing bacteria are able to remove metals such as iron, zinc, copper, and others, raise the pH of the water and lower sulphate concentrations. With anaerobic bioremediation, SRB reduce metal sulphates to insoluble sulphides as part of their metabolic activity. These metal sulfides precipitate, removing them from the water. Indeed, some species of SRB even are able to reduce otherwise hard-to-handle metals, for example converting soluble uranium (VI) to insoluble uranium (IV). Additionally, in anaerobic bioreactors, metals such as aluminum that cannot be removed by precipitation can be removed by biosorption, either through accumulation of the ions in bacterial cells or by adsorption on their celluar surfaces. At the same time, the reduction of sulphate anions by the SRB uses up protons, and this in turn raises the pH. Conditions in CWs can serve two purposes with this form of bioremediation: they provide carbon and energy sources for autochthonous bacteria in form of organic matter from the plants and/or added waste products, and can also provide a level of phytoremediation (bioremediation involving plants) through adsorption of dissolved metals on the surfaces of their rhizomes (sub-surface root nodules).

With engineered wetlands (EWs), a more advanced, semi-passive form of constructed wetlands, these processes can be enhanced and/or wetland plants even can be selected that have further phytoremediating properties which allow them to take up and metabolize organics and heavy metals from the mine drainage passing through their root systems. The SRB-mediated anaerobic bioremediation process is not limited to acidic mine waters, it can be applied to various industrial waste waters of different pH which are high in sulphates and metals and/or organic contaminants. The process can be designed in different ways to accommodate different locations and natures of the contaminated waters.

INTRODUCTION

Acid mine drainage is formed when pyrite and other mineral sulphides are exposed to air (oxygen) and water. The bacterially and/or chemically-catalyzed oxidation of the pyrite releases ferrous iron, sulphate and hydrogen ions (H^+). The ferrous iron can then further oxidize to ferric iron, which then may hydrolyze resulting in the precipitation of ferric oxyhydroxide, producing more hydrogen ions. Ferric iron also can oxidize more pyrite, resulting in the formation of still more hydrogen ions.

The main sources of ARD are the contact of oxygen-containing water with the workings of base metal, precious metal and coal mines; the passage of water through pyrite-containing mine tailings piles; and contact of water with pyrite-containing, removed cover material, base rocks, coal beds and/or mine waste rock. Depending on the pH and the nature of the rock involved, ARD may mobilize a wide variety of other metal ions into solution. Generally, iron, aluminum, copper and zinc are found in the highest proportions.

ARD may be managed by prevention (stop it from forming in the first place), control (limit or control the migration of water and/or materials/contaminants that might cause ARD), treatment (deal with it after it has been formed), or enhancement (cause it to be formed faster than normal to "burn" acid-causing materials out of waste accumulations over relatively short times).

ARD treatment may be carried out by active, semi-passive or passive methods, and there are a variety of biotic and abiotic processes available for each. Often, active treatment processes are used at operating facilities, while passive and semi-passive systems are preferred for facilities that are no longer in operation, yet still continue to generate mine drainage (Skoussen, 1998, Brown et al., 2002). Passive systems are generally more economic to build and operate than semi-passive systems, which in turn are cheaper than active ones. Combinations of active, passive and semi-passive treatment units are possible.

ARD may be neutralized by contact with limestone or similar alkaline materials, but the resulting neutral mine drainage (NMD, with pH values from 6 to 9) may still contain elevated levels of dissolved metals and sulphates. The treatment of ARD usually requires creating both aerobic conditions (minewater oxidation) and anaerobic conditions (sulfate reduction) and aims to raise pH, remove suspended solids, allow metals that easily form (oxy) hydroxides to precipitate, and to remove the sulphates.

SULPHATE-REDUCING BACTERIA

Typically, mine drainage contains >500 mg/l of sulphates and these can be removed from it by reduction to sulphides, by biological uptake, and/or by the formation of organic esters on plant decomposition in a vegetated wetland cell (biosorption). Sulphate reduction is carried out by anaerobic, sulphate-reducing bacteria. SRB need sources of organic carbon (for biomass) and suphates as electron acceptors for their metabolism. There are a large number of species of sulphate-reducing bacteria and their distribution is ubiquitous. Generally, SRB do not grow well at pH values below 5.5 and prefer higher levels of alkalinity, with 6.6 being optimal (Govind et al., 1999). Therefore, a treatment system, such as a CW, should include a process step in which the pH of the mine drainage is first raised (e.g., passing it through an upstream oxic limestone drain).

The basic equations for SRB-mediated sulphate reduction can be represented by:

$$2CH_2O + SO_4^{=} + H^+ \rightarrow H_2S + 2\,HCO_3^- \qquad (1)$$

$$Me^{2+} + H_2S \rightarrow MeS\downarrow + 2\,H^+ \quad (2)$$

where CH_2O represents a carbon source and Me is typically a dissolved divalent metal cation. The hydrogen sulphide quickly reacts with any dissolved cationic metals in the water (e.g., Zn, Cd, Ni) and this results in the precipitation of relatively insoluble metal sulphides. It is noted that two moles of alkalinity and one mole of acidity are the net result of reactions 1 and 2, so the action of the SRB is to raise alkalinity and buffer the solution.

Anaerobic bioreactors using SRB represent a rapidly developing new method for removing contaminants such as sulphates and dissolved metals from NMD. They work by passing it through a suitable packed bed under oxygen-free conditions. The bed can be an inactive substrate such as gravel (in which case a liquid organic material is added as the carbon source), or it can be made of an organic substrate which itself serves as the carbon source. Anaerobic bioreactors can be part of active treatment facilities or semi-passive ones. For the latter, mine drainage can be fed into them using distributors buried in or below one of the substrate layers or, in warmer climes for downflow operation, by surface distributors or sprayers. In colder climates, buried inlet and outlet distributors can be used to direct flow through the reactors. In active modes, the precipitated metal sulphides can be separated out, while in semi-passive modes they accumulate in the packed bed and/or in downstream filters (e.g., CW basins, more commonly referred to as "cells"). Where the mine drainage being treated contains a dissolved metal with an economic value (e.g., zinc), the packed beds and their filters can later be "harvested" to recover the metal for re-use.

The carbon source can be any type of carbon material (e.g., sawdust, manure), the decaying roots/detritus of the wetland plants, an added soluble carbon-based liquid material (e.g., methanol) (Hard et al., 1997), or a layer of carbonaceous substrate material such as municipal compost or biosolids. The organic layers in an anaerobic biofilter not only serve as carbon sources, they also physically retain metal sulphides.

Sulphate-reducing bacteria are sensitive to temperature, and reaction rates are lower where colder water is involved. However, unlike the eukaryotic algae used for the biosorption of dissolved metals in some treatment processes, SRB are prokaryotes and are less affected by cold water. Bacterial communities in ecosystems usually include a number different species of SRB, some of which are cold-adapted and can thrive down to temperatures as low as 4 ºC, with increased numbers of bacteria compensating for lower reaction rates at these temperatures (Fortin et al., 2000).

CONSTRUCTED WETLANDS FOR TREATING MINE DRAINAGE

CWs are passive systems that can be used to neutralize acidity in mine drainage and precipitate metals. There are different types of cells that can be used in constructed wetlands. Pond wetlands are simple open water basins. Free Water Surface (FWS) wetland are artificial marsh ecosystems and water in them flows on the surface in a basin through emergent wetland vegetation. In them, the submerged portions of wetland plants, as well as the soil, underlying materials, and detritus act as substrates for micro-organisms, and these and physical filtration are responsible for much of the pollution removal. CWs involving series of mostly FWS basins (cells) are the most common type. With a Sub-Surface Flow (SSF) constructed wetland, water flows just under the surface in porous materials. Pollutant removal is via microbial biofilms in the interstices of these substrates and via vegetation root systems growing in them. Although wetland vegetation can be present in a SSF wetland, its surface is largely dry. Generally, a SSF wetland cell consists of one or more vegetated beds of rock, gravel, aggregate, or soil.

In some CWs, both aerobic and anaerobic conditions can occur simultaneously in the same cells, and the metals of concern in mine drainage can be precipitated as (oxy)hydroxides and/or sulphides. The most important metal removal processes involved are redox reactions, and these are complemented by others such as precipitation; by the sorption of metals by algae, bacteria, plant debris, organic substrates, and/or oxides/hydroxides; and by plant uptake. Any metals in mine drainage that do not get fixed to organic or mineral matter probably do not exist as free ions but rather as ions associated with suspended colloids. Accordingly, if conditions in constructed wetlands can be optimized, various heterogeneous chemical reactions (accompanied by filtration, coagulation, and/or flocculation processes) can be exploited to remove the metals from any mine drainage passing through them.

Studies in the late 1970s and early 1980s in the US determined that ARD passing through natural *Spaghnum* bogs were not adversely affecting them (Huntsman et al., 1978, Girts and Kleinmann, 1986). This led to abortive attempts at the pilot and full scale level to use artificial *Sphagnum* bogs for the treatment of acidic coal mine drainage (Kleinmann and Girts, 1987, Brown et al., 2002.), but the moss proved too difficult to manage in CWs.

Beginning in the 1980s, R&D on CWs for treating ARD then focused on the potential of using open water, cattail-vegetated wetlands (i.e., FWS cells) to reduce ferrous iron and raise pH (Kadlec and Knight, 1986). These are called aerobic wetlands and are designed with very shallow water depths (often only a few cm). They are underlain with layers of soil and/or organic matter from 30 - 90 cm thick. Their concept is to provide residence time and aeration for metal oxidation and hydrolysis so that the metal hydroxides of iron, aluminum and manganese can be precipitated out and retained (Skousen, 1998). Often, they are preceded by cascades and other methods of oxygenating the ARD prior to the aerobic wetlands.

Aerobic wetlands were found to be most appropriate where the ARD being treated contained net alkalinity to neutralize metal acidity (Skousen, 1998). *et al*., 1994). The aerobic wetland concept was quickly followed by that of using anaerobic or compost wetlands. These are shallow (2 - 8 cm deep) cattail-planted or un-vegetated open water wetlands containing an organic substrate (e.g., compost) 30 - 70 cm thick below the water. Flow through the substrate is encouraged (i.e., operation in a semi-SSF mode). Anaerobic wetlands are recommended for use where the mine drainage has net acidity. They are suggested for the treatment of small flows of poorer quality ARD with higher Fe and dissolved oxygen contents than can be treated in aerobic wetlands. Much of their success results from the action of SRB in their substrates.

By 1989, there were at least 300 CWs in operation in the US at mining facilities (Kadlec and Knight, 1986) but few of these were designed using ecological engineering principles. Cell geometries, water depths, residence times, hydraulics, and other aspects for them were decidedly sub-standard in relation to modern wetlands engineering methods. Because the performances of many such badly designed aerobic and anaerobic wetlands were poor, erroneous negative perceptions of the use of CWs to treat mine drainage were generated.

For too may constructed wetlands used earlier by the mining industry, little attention was given to optimal cell geometries, water depth, hydraulics, temperature effects, or winter operabilities. In addition to the poor design problems endemic with aerobic wetlands, anaerobic wetlands had to contend with those of substrate blinding and plugging due lack of understanding by many of their designers of basic chemical engineering methods for the flow of fluids in porous media. More attention has recently been paid to mine drainage geochemistry, however designs based on modern wetlands engineering principles are still rare. In mining applications. Indeed, even the basic concept that CWs need to be designed as low aspect ratio, multi cell, multi-train systems has been slow to be appreciated (Higgins & Mattes, 2003).

Nevertheless, throughout the 1990s, constructed wetlands technology for the treatment of mine drainage continued to develop and systems combining CWs with other types of mine drainage treatment became more common. Before the end of the twentieth century, over 1000 constructed wetlands had been built to treat mine drainage (Skoussen, 1998).

ENGINEERED WETLANDS FOR TREATING MINE DRAINAGE

Engineered wetlands (EWs) are advanced forms of CWs. They also involve more active manipulation of process conditions than is usual for ordinary, constructed wetlands, which are largely passive systems. For example, EW systems may involve aspects such as cell aeration, the addition of chemicals and/or energy, active phytoremediation, and/or use of specialty substrates that chemically interact with certain wastewater pollutants. EWs are usually semi-passive systems in that some of their cells may contain materials (e.g., limestone, biosolids) that are used up during operation and need to be periodically replaced. Indeed, other kinds of semi-passive mine drainage treatment processes such as oxic limestone drains or anaerobic bioreactors can be "expressed" as some of the cells in engineered wetlands (Higgins et al., 1999, Higgins, 2000, Higgins & Mattes, 2003).

Engineered wetlands show excellent potential to be one of the major choices in the next generation new technologies to be used for the treatment of mine drainage. Their multiple cell designs allow the various reactions needed to treat ARD or NMD to be carried out in highly efficient manners in the successive cells of their treatment trains. This avoids the use of wetland cells containing "messy" mixtures of different substrates, for example, the use of compost and limestone layers in the same cell, a configuration that is hard to operate and difficult to clean out (such wetland cells are called Successive Alkalinity Producing Systems or SAPS, Watzlaf et al., 2000, Brown et al., 2002).

The engineered wetland concept has already been proven at pilot and demonstration scale test facilities. Full scale, SSF wetlands treating relatively high volumes of influent (> 15 L/s) are already operating treating stormwater (Higgins & MacLean, 2002), and ones treating even larger volumes of water are planned. Contaminant removal kinetic rate constants for them can be determined using treatability tests, so the highly limited loading method used in past to design constructed wetlands for mining applications need no longer be used in wetland sizing (Higgins & Mattes, 2003).

For example, one engineered wetland concept that is now being pilot tested to treat high aluminum ARD is to use aerated SSF wetland cells followed by oxic limestone drains in engineered wetland treatment trains to enhance ferrous iron and other metal oxidation, then to run the water through FWS cells to filter out precipitating hydroxides. During the late 1990s, the first pilot and demonstration scale, semi-passive anaerobic bioreactors to remove dissolved metals from NMD was built and tested, and in the last few years, anaerobic bioreactors of this sort have been tested as part of engineered wetland systems.

A demonstration scale, six cell engineered wetland system for treating an NMD incorporating anaerobic bioreactor cells has already been built and has been operating since 1997 at an operating lead-zinc smelter in Western Canada. It treats cadmium- and zinc-contaminated landfill leachate and seepage from the site of an old arsenic scrubbing pond. The resultant NMD contains elevated levels of arsenic, cadmium and zinc. The engineered wetland system that treats this wastewater involves two upflow, anaerobic bioreactor vertical SSF (VSSF) wetland cells (700 m^3/18 m by 30 m, 600 m^3/18 m by 25 m) with biosolids-based substrates (60% pulp mill biosolids, 35% sand and 5% cow manure underlain by a layer of dolomitic limestone), followed by three gravel horizontal SSF (HSSF) cells (5 m by 10 m, 5 m by 10 m and 10 m by 30 m, all 0.7 m thick) vegetated with cattails and other plants, and a final pond wetland cell (20 m by 25 m by 0.8 m deep) (Mattes, 2002, Mattes et al., 2002, Mattes et al., 2003).

Table 1 presents average metal removal results for this engineered wetlands system from April to the end of 2001 during which 1.86 MM L of NMD was treated at rates varying from 8,000 to 15,000 L/d.

Table 1
METALS REMOVAL IN ANAEROBIC/AEROBIC ENGINEERED WETLAND SYSTEM IN 2001

	Average Influent Concentration (mg/l, N=61)	Effluent Concentration (mg/l)	Removal (%)
Arsenic	98	0.4	99.6
Cadmium	5	0.03	99.3
Zinc	321	5.2	98.4

Metal removal rates are high, both in summer and winter. This demonstration unit has shown that anaerobic bioreactors can be used as part of engineered wetland systems, and it is providing valuable information needed to work out the practical aspects for the designs needed to progress to larger, full scale systems (e.g., pH control, hydraulics, winterability, compaction, precipitates, best distributor types). For example, several years of operations seem to indicate that precipitated sulphides will not plug up substrate beds as they are very fine and colloidal in nature.

The bioremediation of dissolved metals in mine drainage using SRB in anaerobic bioreactors is rapidly becoming a "best available technology". The combination of this anaerobic bioreactor technology with advanced, semi-passive engineered wetland technology presents a powerful new way to treat mine drainage.

REFERENCES

Brown, M., Barley, B. and Wood, W. 2002. Minewater treatment, technology, application and policy. IWA Publishing, London, UK.

Fortin, D., Goulet, R, and Roy M. 2000. Seasonal cycling of Fe and S in a constructed wetland: the role of sulfate-reducing bacteria. Geomicrobiol. J., 17: 221-235.

Girts, M. and Kleinmann, R. 1986. Constructed wetlands for treatment of acid mine drainage, preliminary review. *In* Proceedings of National Symposium on Mining, Hydrology, Sedimentology and Reclamation, U of Kentucky. pp. 165-171.

Govind, R., Yong, W. and Tabak H. 1999. Studies on biorecovery of metals from acid mine drainage. *In* Bioremediation of metals and inorganic compounds, A. Leeson and B. Allemans (eds), Battelle Press, Columbus, OH. p. 37.

Hard, B., Friednich, S., and Babel, W. 1997. Bioremediation of acid mine water using facultative methylotrophic metal-tolerant sulfate-reducing bacteria. Microbiol. Res., 152: 65–73.

Higgins, J., Hurd, S. and Weil, C. 1999. The use of engineered wetlands to treat recalcitrant wastewaters. 4th Internat. Conf. on Ecol. Eng., Oslo, Norway, June, 1999.

Higgins, J. 2000. The use of engineered wetlands to treat recalcitrant wastewaters, advances in ecological sciences. J. Environ. Sci. Health, A35: 1309-1334.

Higgins, J. & MacLean M. 2002, The use of a very large constructed sub-surface flow wetland to treat glycol-contaminated stormwater from aircraft de-icing operations, 31st Central Canadian Symposium on Water Pollution Research, Burlington, ON, Feb. 5, 2002.

Higgins, J. & Mattes, A. 2003, The use of engineered wetlands to treat mine drainage, German-North American Environmental Conference. The Rehabilitation of Industrial Wasteland and Post Mining Landscapes, Gorliz, Germany, April 12, 2003.

Huntsman, B., Solch, J. and Potter, D. 1978. Utilization of *Spaghnum* species dominated bogs for coal mine drainage abatement. Geologic. Soc. America, 91st Annual Meeting, Ottawa, ON.

Kadlec, R, and Knight, R. 1996. Treatment wetlands. Lewis Publishers, Boca Raton, FL.

Kleinman, R. and Girts, M. 1987. Acid mine drainage treatment in wetlands: an overview of an Emergent Technology. *In* Aquatic plants for water treatment and resource recovery, K. Reddy and W. Smith (eds), Magnolia Publishing. p. 255.

Mattes, A. 2002. Anaerobic/aerobic treatment system for removal of heavy metals constructed in Trail BC for Cominco Limited. Nature Works Remediation Corporation, Progress Report for Environment Canada.

Mattes, A., Gould D. & Higgins, J. 2003, Biological metal removal in an engineered wetland system, US EPA International Applied Phytotechnologies Conference, Chicago, Illinois, USA, March 3 - 5, 2003.

Mattes A., Gould W. and Duncan B. 2002. Multi-stage treatment system for removal of heavy metal contaminants. ESSA conference, Banff, Alberta, October, 2002.

Skousen, J. 1998. Overview of passive systems for treating acid mine drainage. *In* Acid mine drainage control and treatment. American Society for Agronomy, and the American Society for Surface Mining and Reclamation http://www.wvu.edu/-agexten/landrec/passtrt/passtrt.htm

Watzlaf, G., Schroeder, K., and Kairies, C. 2000. Long term performance of alkalinity-producing passive systems for the treatment of mine drainage, at A New Era of Land Reclamation, Proceedings 17 Annual Meeting, ASSMR, Tampa, FL, June, 2000.