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Review

Role of Microorganisms in Mining: Generation of Acid Rock Drainage and its Mitigation and Treatment

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ABSTRACT

Natural oxidation of sulphide mineral wastes during their disposal and storage at the mining sites may result in generation of acid rock drainage (ARD) that may contain high acidity and high concentrations of dissolved metals and sulphates. Due to its chemical composition, ARD poses potential harm to the environment and significant challenges to many mining industries requiring development and application of effective ARD management plans to the mining sites during the operational and post-closure periods. If the generation of ARD cannot be prevented or controlled, it must be collected and treated to eliminate acidity and reduce the concentration of heavy metals and suspended solids before its release to the environment.
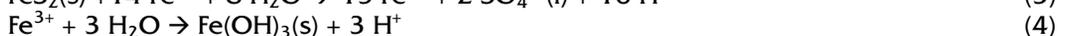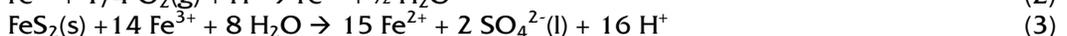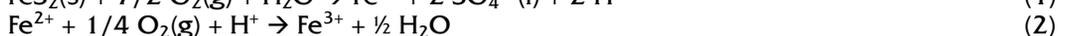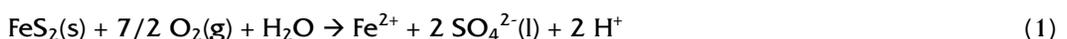
As a group of microorganisms may contribute to the generation of ARD, different types of microorganisms may play a vital role in the development of microbiological prevention, control and treatment technologies. The role of microorganisms in the generation of ARD and their role in the methods used for prevention, control and treatment are discussed in this paper. Benefits, limitations and design criteria for passive biological processes are presented using examples of passive and biological treatment processes that have been developed recently and implemented to mine sites. © 2002 SDU. All rights reserved.

Keywords:

1. INTRODUCTION

1.1. Acid Rock Drainage (ARD)

The management of waste materials such as tailings and waste rocks from mining of sulphidic metal and uranium ores and coal mines poses an environmental challenge to mining companies. Acid generation occurs when sulphide minerals (predominantly pyrite, FeS_2 and pyrrhotite, FeS) contained in the waste material are exposed to oxygen and water. The primary step is the oxidation of the sulphide minerals and generation of acid and subsequently, leaching of oxidized products occurs as rainwater and snowmelt enters the waste pile or dump. If sufficient alkaline or buffering minerals (for example, calcite) are not present to neutralize the acid, the resulting leach water becomes acidic. This water is generally known as acid rock drainage "ARD" (or as acid mine drainage "AMD"). ARD is characterized by high acidity (e.g., low pH) and high concentrations of sulphate and metals such as iron (Fe), manganese (Mn), aluminium (Al), zinc (Zn), copper (Cu), nickel (Ni), lead (Pb), cadmium (Cd), arsenic (As), etc. The steps involved in the ARD generation process, using pyrite as the example, can be represented by the following reactions:

$$FeS_2(s) + 7/2\ O_2(g) + H_2O \rightarrow Fe^{2+} + 2\ SO_4^{2-}(l) + 2\ H^+ \tag{1}$$
$$Fe^{2+} + 1/4\ O_2(g) + H^+ \rightarrow Fe^{3+} + \tfrac{1}{2}\ H_2O \tag{2}$$
$$FeS_2(s) + 14\ Fe^{3+} + 8\ H_2O \rightarrow 15\ Fe^{2+} + 2\ SO_4^{2-}(l) + 16\ H^+ \tag{3}$$
$$Fe^{3+} + 3\ H_2O \rightarrow Fe(OH)_3(s) + 3\ H^+ \tag{4}$$

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The overall sulphide to sulphate oxidation is summarized as follows:

$$FeS_2(s) + 15/4\ O_2(g) + 7/2\ H_2O \rightarrow Fe(OH)_3(s) + 2\ SO_4^{2-}(aq.) + 4\ H^+ \qquad (5)$$
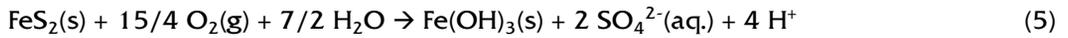
The principle components required for the formation of ARD are: (i) wastes containing reactive sulphides, including sulphide minerals (S^{2-} or S_2^{2-}), elemental sulphur (S^o) and various sulphur intermediates (e.g., thiosalts, $S_2O_3^{2-}$); (ii) molecular oxygen; and (iii) water as shown in Equations 1 and 2. Other factors influencing the rate of acid generation include bacterial activity, temperature, pH, the presence of alternate oxidants (e.g., ferric iron, Fe^{3+} and manganese, Mn^{3+} or Mn^{4+}) as illustrated by Eq. 3, and the presence of buffer or alkaline minerals (e.g., calcite and silicates). The hydrolysis of ferric iron and precipitation of ferric hydroxide also produce acid as shown in Eq. 4. Chemical oxidation of sulphides by ferric iron or manganese and hydrolysis reactions can take place under anoxic conditions. Equations 1 and 2 could occur as a result of either an abiotic or microbially catalyzed chemical reaction. Microbial oxidation reactions require the presence of oxygen. The role of microorganisms in the acid generation process is briefly discussed below.

In addition to waste rock and tailings, the sources of ARD from mining operations may include underground mines, open pit mines, spoil piles, stock piles and spent heap-leach piles. The development of ARD is time dependent and, at some sites, may evolve over a period of years after mine closure or occur during the initial stages of mining operations. If the formation of ARD cannot be prevented, it should be collected and treated. Otherwise, it has the potential to contaminate groundwater and local water courses, damaging the health of plants, fish and possibly humans.

1.2. Chemical Composition of ARD

The chemical quality of ARD can vary widely because it is dictated by the physical, chemical, mineralogical and microbiological properties of each site. Physical site characteristics include the size, density and source (e.g., tailings, waste rock, etc.) of the waste material; as well as the hydrological properties of the site. The type of mining operation, coal versus metal or uranium mining, may also have a large influence on ARD composition. However, recently, MEND, an industry government consortium on Mine Environment Neutral Drainage, in Canada conducted a nation-wide survey and compiled the results in a report (MEND, 1994). Studies conducted by Paine (1987) and Bhole (1994) also reported typical characteristics and ranges for ARD. Reported characteristics and ranges of ARD for coal mining operations are summarized in Table 1. Usually, metal mining ARD contains a greater variety of metals, such as copper, zinc, cadmium, arsenic, lead, nickel etc., in elevated concentrations.

Table 1
Reported characteristics of ARD from coal mine drainage (Paine, 1987)

Parameter	Unit	Range
Flow Rate	l/s	57-157
pH	-	1.4-6.2
Total Acidity as $CaCO_3$	mg/l	0-45,000
Fe	mg/l	1-10,000
Al	mg/l	1-2,000
Mn	mg/l	1-50
SO_4	mg/l	1-20,000
Total Suspended Solids (TSS)	mg/l	5-3,000

Dissolved salts other than sulphates (calcium, magnesium, etc.) may also be present in ARD. The expected concentration range for total dissolved salts (TDS) is generally in the order of 100mg/l to 30 000mg/l. These levels can lead to a reduction in the quality of potable surface and ground water supplies and therefore these compounds require removal/reduction from the ARD before it reaches the natural environment or water resources.

1.3. Factors Affecting ARD Generation

The type of sulphur minerals, the presence of oxygen (air) and the quality and quantity of alkaline minerals found in the waste are the primary factors that affect ARD generation. In addition, temperature, pH, distribution of sulphide and alkaline materials in the waste pile and the surface area of sulphide minerals are important variables in controlling the rate of oxidation and, hence ARD generation. Since bacteria play a catalytic role, temperature and pH appear to be the determining factors for microbial growth in mine waste environments.

Differences in conditions found in tailings and waste rocks such as particle size, surface area of sulphide minerals and the homogeneous distribution of sulphide and alkaline mineral content affects the rate of potential oxidation and neutralization process and, hence the quality of ARD. As the mean particle size diameter in the waste rock environment is typically greater than 20cm, tailing material is much finer, generally less than 0.2mm (Nicholson, 1994; Broughton and Robertson, 1992). The finer particle size has a larger surface area for sulphide minerals and enhances the rate of oxidation. On the other hand, the finer and more homogeneous material that facilitates the neutralization process within the tailing impoundment, allows the alkaline materials to be in close proximity to the acid generating sulphide minerals.

The oxidation of sulphide minerals is an exothermic process. Thus, a significant quantity of heat is released and an interior temperature encountered in mine waste piles can reach 80°C. Microorganisms, mainly bacteria as mentioned above, are indigenous to the ARD environment. These microorganisms play a role in the direct and indirect oxidation of sulphur minerals (e.g., pyrite, pyrrhotite). Normally, sulphur oxidizing bacteria are incorporated for extracting metals from ores in metallurgical processes. Bioleaching processes will not be discussed and is beyond the scope of this report. The concepts of ARD generation and bacterial oxidation of pyrite via direct and indirect pathways are illustrated in Figure 1.
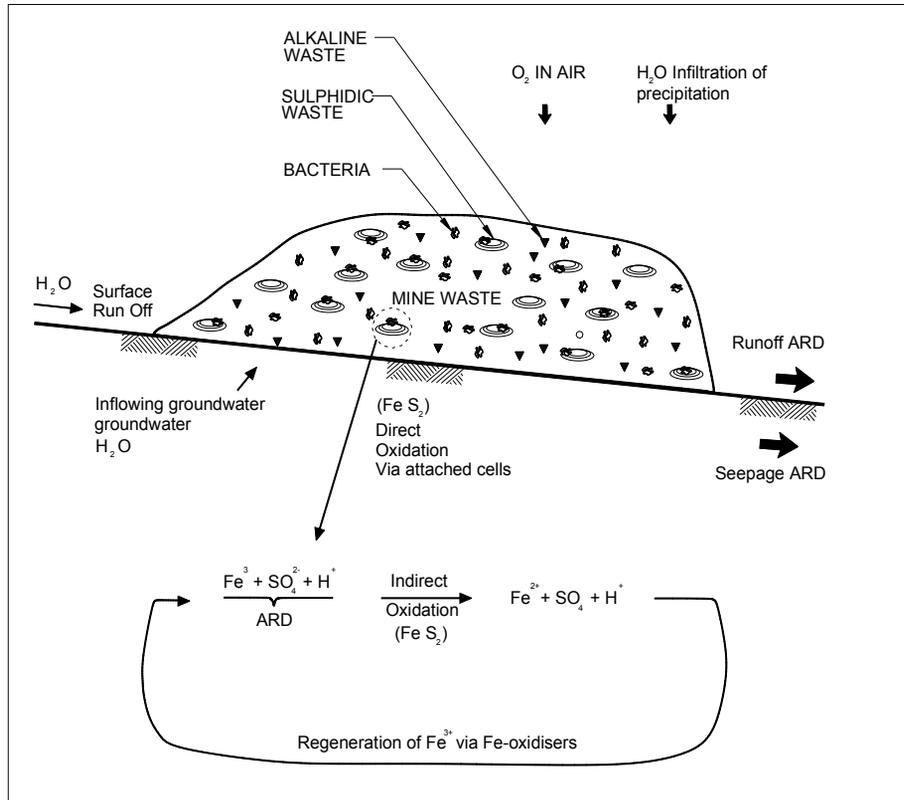

Figure 1. The concepts of ARD generation and bacterial oxidation of pyrite via direct and indirect pathways

2. ROLE OF MICOORGANISMS IN MINING

2.1. Role of Microorganisms in ARD Production

It has been reported that a number of acidofilic or acid-tolerant bacteria namely *Thiobacillus*, *Leptospirillum*, *Sulfolobus*, *Sulfobacillus* and *Metallogenium* are associated with the mediation of acid generation from sulphide minerals at pH levels below 4. Most of these microorganisms are gram-negative, autotrophic mesophile and chemolithotrophs who exhibit high tolerance to various metal ions and also to some anions such as arsenate.

The *Thiobacillus* species, except *Thiobacillus ferroxidans*, are unable to oxidize iron but can oxidize reduced sulphur compounds to sulphuric acid (H_2SO_4). *T. ferrooxidans* has been the most documented microorganism having a catalytic role in the production of ARD. *Thiobacillus* species are typically mesophiles with optimum growth observed between 25°C and 35°C. *T. thiooxidans* is the most acid-tolerant of the thiobacilli with growth occurring over a pH range of 0.5 to 4. *T. denitrificans* can use nitrate as a terminal electron acceptor for the oxidation of reduced sulphur compounds at pH levels of 6.0 to 8.0 and can reduce nitrate (NO_3^-) or nitrite (NO_2^-) to nitrogen gas (N_2). *T. novellus* is a chemolithoheterotroph capable of growing on inorganic and organic substrates.

Similarly to *Thiobacillus* bacteria, *Leptospirillum* bacteria are also gram-negative, mesopihilic, acidophilic and obligate autotroph. Although *Leptospirillum ferrooxidans* cannot oxidize sulphur, like it *Thiobacillus* counterparts, it is capable of oxidizing ferrous iron in acidic environments at temperature above 20°C. *Sulfolobus* sp. and *Sulfobacillus* sp. are thermophilic acidophiles capable of oxidizing both sulphur and iron. These bacteria can grow at pH levels 1 to 3 over a temperature range of 50°C to 90°C.

2.2. Microorganisms in ARD Prediction, Control/Prevention and Treatment

Several research programs have been undertaken to understand the role of bacteria in the generation of ARD. The results obtained from these investigations have helped to develop strategies to control ARD and methods to predict, prevent and treat ARD. For instance, a method where activities of *T. ferrooxidans* are enhanced, was developed to predict the potential for ARD generation from a given mine waste. The use of anionic surfactants has been found to be useful to inhibit the growth of *T. ferrooxidans* (US EPA, 1995).

It has been revealed that creating anoxic conditions would not only inhibit the activities of sulphur oxidizing bacteria, but also would enhance activities of sulphide reducing bacteria (SRB) which could successfully be used in preventing and/or treating ARD. Several methods to control, prevent and treat ARD have been developed based on SRB. This paper discusses the main biological processes that have been developed or are under development for ARD prevention, control and treatment.

3. ARD CONTROL/PREVENTION AND TREATMENT METHODS

3.1. Principle of ARD Prevention/Control and Available Methods

Mining industries face the challenge of preventing ARD occurrences in a cost-effective manner. The most feasible method would be to control and prevent ARD at the source (Kuyucak, 2001a). The best way to achieve this is to remove one or more principle ingredients of the ARD generation process such as limiting the penetration of oxygen and water in to sulphide minerals and/or increasing the portion of neutralizing minerals in the mine waste. Several methods including the use of soil (or dry) and water (or sub-aqueous) covers have been investigated to curtail oxidation of sulphidic wastes (MEND, 1993; 1994). It has been demonstrated that soil covers, particularly multi-layer methods, are effective, but in many cases, are not economically feasible (Yanful and Nicholson, 1991). Water covers have been shown to be an economical alternative to dry covers. Because of the low oxygen diffusion rate in water

with respect to air, the oxidation of reactive wastes can be minimized and water covers can be an effective long-term control method for acid generation. However, the application of water covers is limited to site conditions. Site conditions with respect to hydrology, topography and the presence of a water source in the vicinity should be suitable for the water cover application (Davé, 1992; St-Germain and Kuyucak, 1998). In addition, although water covers can significantly reduce acid generation, a slow-release of some metals may still occur to the water column resulting in an increase in some metal concentrations which may exceed regulated water standards, Thus, the water will require treatment before its discharge to the environment. The use of biological materials and growth of aquatic plants in situ ("Biologically Supported Water Cover") have been recommended to further improve the effectiveness of water covers. The concept and the process will be discussed in detail below.

3.2. Prevention of ARD by Water Covers and Biological Methods

Since the effectiveness of a water cover is based on the solubility and diffusion rate of oxygen in water, the rate of oxygen transport through water can be sufficiently slow so that acid generation is insignificant. However, tests carried out at the Noranda Technology Centre in Canada revealed that although water covering unoxidized waste rock could reduce the acid generation rate more than 99.7 percent, concentrations of some metals such as lead and zinc increased to 4 and 6mg/l, respectively (Aubé *et al.*, 1995). In order to further improve the effectiveness of water cover, feasibility of a layer of organic material on top of tailings in addition to the water cover was investigated (St-Germain and Kuyucak, 1998; Beckett *et al.*, 1998; St-Germain *et al.*, 1997). The organic layer could be built up by growing aquatic plants in situ in the water cover. Aquatic plants could consist of emergent, floating and submerged species. The investigations revealed that the benefit of the organic layer at the tailings and water interface was two- fold:

1. Oxygen would be consumed in the organic material through bacterial activity and thus oxygen diffusion from the surface water to the tailings pore water would be prevented,

2. Upward metal fluxes from the tailings to the water cover would be prevented due to formation and precipitation of metal sulphide complexes through the activity of sulphate reducing bacteria (SRB). The organic material degrades to lower molecular organic compounds during the consumption of oxygen by aerobic microorganisms and anaerobic conditions at the interface are also created. Presence of lower molecular organic compounds and the anaerobic conditions enhances the growth of SRB as explained by equations below (Section 5.2, Eq. 4 and 5). Additionally, metals form complexes with organic compounds and, biosorption and bioaccumulation phenomena occur in the system. These processes further help to retain metals at the tailings-water interface.

Further more, the presence of plants in the tailings impoundment, especially emergent plants growing at the edges, improve the physical stability of the tailings through their roots. The concept of biologically supported water cover is illustrated in Figure 2.
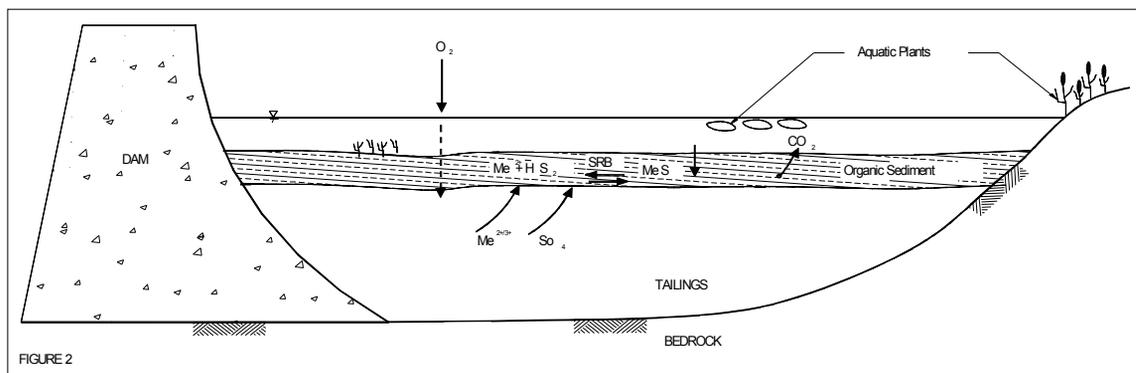

Figure 2. The concept of biologically supported water cover

4. CONVENTIONAL AND BIOLOGICAL METHODS TO MITIGATE ARD

If the occurrence of ARD cannot be prevented, the ARD must be treated by means of a chemical and/or biological process to eliminate or minimize its impact on the environment. Acidity is neutralized and metals are removed or reduced to acceptable levels to comply with regulated water standards.

Heavy metals such as iron, zinc, copper, lead, cadmium, aluminium, manganese found in acid mine waters become insoluble and precipitate in solution at a certain pH level when they react with a chemical reagent to form a metal complex (Hedin *et al.*, 1994; Kuyucak, 1995). Type of chemical reagent, pH of the water, oxidation/reduction and hydrolysis reactions, the presence of biotic and abiotic catalysts and the retention time of the water are important parameters which govern metal-removal processes.

4.1. Conventional and Chemical Methods

4.1.1. Neutralization/Precipitation Methods

Neutralization of waters and precipitation of metals with a neutralizing reagent (e.g., calcium or sodium hydroxide and calcium or sodium carbonate) in a treatment plant is a common method due to its simplicity and low cost. Neutralization/precipitation processes are particularly feasible for treating large volumes of highly contaminated waters. Precipitation of metals such as copper, zinc, cadmium, manganese, lead and ferrous iron (Fe^{2+}) require a pH greater than 9 which provides a low solubility for each of the given metal ions. Ferric iron (Fe^{3+}) and aluminium are hydrolyzed and precipitate out at pH levels less than 5. In some cases, neutralization processes may be inefficient to reduce metals to low levels as desired.

Lime neutralization/precipitation, referred to as "Chemical Process", is the most common method used for treating ARD within the mining industry. The use of lime as CaO or $Ca(OH)_2$, is often preferred over other alkaline reagents, particularly for treating ARD in large quantities, due to its high reactivity and abundance (Kuyucak, 2001b). In the lime neutralization process, metals and sulphate (SO_4) are precipitated as metal hydroxide complexes and gypsum ($CaSO_4$), respectively. The precipitate called "Sludge" is separated from the neutralized water in ponds or through a mechanical solid/liquid separation device (e.g., clarifier/thickener) to obtain clean water and is stored in a controlled area. The mode of process application may vary from a simple to sophisticated fashion depending on the level of control and equipment used in the process. The lime neutralization/precipitation process may pose some drawbacks. It produces a large volume of sludge and may result in inefficient treatment, specifically for removal of some metal ions to low levels.

Removal of metals to low levels can be achieved with the help of sulphide reagents (e.g., Na_2S, H_2S, CaS, etc.) by forming sulphide compounds at pH levels less than 6. Metal concentrations in the treated water are usually lower than those obtained with hydroxide precipitates. Metal sulphide complexes offer some advantages over hydroxide precipitates because they are less voluminous and are chemically more stable, being less susceptible to changes in pH as long as they are stored under anaerobic conditions. However, due to colloidal nature of metal sulphide precipitates, the treated effluent may require filtration to obtain low suspended solids in the treated effluent. In addition, sulphide precipitation process is more expensive than that of lime neutralization. Therefore, its application is limited to site specific conditions. Biologically generated sulphide precipitation process has been investigated as an alternative treatment method.

4.2. Role of Microorganisms in ARD Mitigation

Microorganisms can be involved in ARD abatement primarily through the reduction of metals and sulphates as well as other alkalinity generating processes. The extent to which each process may contribute to the neutralization of ARD depends upon the chemical composition of

ARD, availability of necessary electron donors and acceptors, as well as temperature and pH conditions within the mine waste environment.

Acidophilic heterotrophic bacteria present in the ARD environment may be involved in ARD mitigation, as well as iron and manganese recycling. Some of these bacteria such as *Acidiphilicum* sp. play a passive role by metabolizing organic materials that are potentially toxic to iron-oxidizing bacteria, thereby inhibiting biologically mediated iron oxidation reactions (Johnson, 1995). Other species demonstrated the ability to reduce iron, present either as soluble or as solid phase compounds, to ferrous iron.

Manganese and iron reduction may also contribute to the neutralization process. Microorganisms including heterotrophic bacteria *Pseudomonas, Clostridium* and *Desulfovibrio* can directly reduce manganese and iron by using them as final electron acceptors under anaerobic conditions. The ability to reduce ferric iron is widespread amongst acidophilic heterotrophic bacteria such as *Thiobacillus ferrooxidans* growing on elemental sulphur. When ferric iron is reduced to ferrous iron, removal of iron from ARD becomes easier because ferrous iron reacts with sulphide produced by sulphate reduction, and ultimately results in the removal of iron and promotes alkalinity generation. Sulphate reduction leads to permenant alkalinity production when hydrogen sulphide gas (H_2S), is released from the mine waste environment.

Other biologically mediated processes which can contribute to ARD neutralization by ultimately consuming H^+ ions include: ammonification by various microorganisms and enzymes; denitrification, where a number of bacterial species such as *Pseudomonas, Paracoccus, Flavobacterium, Alcaligenes, and Bacillus* sp. convert ammonia to nitrates under anaerobic conditions; and methane generation by methanogenic bacteria. The processes involved in sulphate reduction will be discussed in the following section.

5. PASSIVE - BIOLOGICAL ARD TREATMENT METHODS

These processes in some circumstances, have been proven to be feasible alternatives to conventional lime neutralization/precipitation and sulphide precipitation methods (Hedin *et al.,* 1991; Blowes *et al.,* 1995). Properly functioning passive treatment systems can produce compliance level effluents with no additional costs other than the initial construction and limited periodical maintenance. Even if supplementary chemical treatment is required to meet effluent limits at some sites, it still can be cost effective. They are particularly ideal for decommissioned sites and treatment of seepage where the temperature, flow rate and chemical composition do not fluctuate and remain optimal all year round. Alkalinity required for removing acidity and metals can be generated by two naturally occurring processes: (1) the dissolution of limestone or other carbonate rocks and (2) bacterial sulphate reduction which also generates sulphide to precipitate/remove metals.

The most common passive treatment systems are anoxic limestone drains, constructed anaerobic and aerobic wetlands and biosorption. The performance of individual systems is a function of both quality and quantity of the raw mine drainage. Experience has demonstrated that the influent flow rate, contaminant concentrations, pH and alkalinity (or acidity) are all extremely important to system performance. And, in addition to temperature, the capacity of the biological treatment system is significantly affected by the changes in pH.

5.1. Anoxic Limestone Drain (ALD)

Anoxic limestone drains (ALD) treat ARD having a net acidity by adding alkalinity to the water. The system consists of a bed of crushed limestone ($CaCO_3$) which is installed below the ground surface and covered with a fabric filter and clay (or other materials) to promote anoxic conditions (Brodie *et al.,* 1992). ARD flow through the ALD is gravity driven, allowing the acid component of the water to react with the limestone and release carbonic acid (Brant *et al.,* 1995; Brodie *et al.,* 1991) as presented in Eq. 6. The carbonic acid then reacts with the limestone to produce bicarbonate (HCO_3^-) alkalinity and, as a consequence, the pH level in the water is increased (Eq. 7).

$$CaCO_3 + 2\ H^+ \rightarrow Ca^{2+} + H_2CO_3 \qquad\qquad (6)$$
$$CaCO_3 + H_2CO_3 \rightarrow Ca^{2+} + 2\ HCO_3^{\ -} \qquad\qquad (7)$$

When the pH in the water increases above 6.4, bicarbonate becomes the dominant dissolved carbonate species. At this stage, the acidity reacts with limestone generating alkalinity directly as shown in Eq. 8.

$$CaCO_3 + H^+ \rightarrow Ca^{2+} + HCO_3^{\ -} \qquad\qquad (8)$$

The degree of dissolution is enhanced in anaerobic environments because of a decrease in the formation of ferric iron precipitate that armours carbonate surfaces and because of an increase in the partial pressure of dissolved CO_2 that directly affects the solubility of carbonate compounds (Hedin and Nairn, 1992). Development of high CO_2 partial pressures increases the alkalinity beyond that possible under normal atmospheric conditions (Kilborn, 1996). The size of the limestone particles comprimises the quantity of free flow and the presence of sufficient surface area for dissolution. Faulkner and Skousen (1994) suggested that the larger particle sizes (8cm-25cm) along with the finer particles (2cm-4cm) should be used in an ALD to increase the hydraulic conductivity and reduce the potential for plugging. Mine water having low dissolved oxygen (O_2), ferric iron (Fe^{3+}) and aluminum is ideally suited for the treatment with ALD, based on the results reported by United States Bureau of Mines (USBM) for the treatment of ARD at coal mines in the United States.

The waters with sulphate concentrations greater than 2000mg/l should also be treated with care to prevent $CaSO_4$ (gypsum) formation and its potential precipitation in the system. For instance, partial neutralization of ARD and/or operation of the system with high velocity lowers the risk of plugging and system failure.

5.2. Microbiological Sulphate Reduction Principles of SRB Processes

A group of bacteria called sulphate reducers "SRB" such as *Desulphovibrio* sp., can convert sulphate contained in ARD to sulphide and can generate bicarbonate (HCO_3^{-}) in the presence of organic carbon (nutrient) sources using it as an electron donor under anoxic and reducing conditions. Sulphate reduction first produces hydrogen sulphide (HS^-) which attracts free hydrogen ions (H^+) and produces hydrogen sulphide (H_2S). The hydrogen sulphide generated forms insoluble metal complexes and results in the removal of metals. The bicarbonate released results in an increase in alkalinity (i.e., pH). The reactions are explained with Eq. 4 and 5:

$$2\ CH_2O + SO_4^{\ 2-} \rightarrow HS^- + 2\ HCO_3^{\ -} + H^+ \qquad\qquad (9)$$
$$Me + H_2S \rightarrow MeS \qquad\qquad (10)$$

Where CH_2O and Me represent the organic matter and heavy metal, respectively. SRB are known to be natural soil bacteria and can be found in soils, sewage sludge and manure. They require low-molecular weight organic carbon compounds (e.g., simple organic acids), suitable concentrations of sulphate (>200mg/L), a pH level greater than 4.5 and low Eh levels (<-150mV). SRB can function in the absence of oxidizing agents such as O_2 and Fe^{3+}. Low-molecular weight carbon compounds (e.g., lactic acid, acetate) used by SRB are common products of natural degradation (i.e., microbial fermentation) processes that occur in anoxic environments (Kuyucak and St-Germain, 1994 a, b; Béchard *et al.*, 1994; Eger *et al.*, 1997; Wetland, 1992). A wide variety of materials, depending on their cost and availability, can be used as nutrients. They may include several industrial wastes such as molasses, sewage sludge, compost, manure, wood chips, wood or paper pulp, brewery waste and hay. Natural organic materials provide slow release, long-term nutrient sources to SRB. The materials can be supplemented with materials containing nitrogen and/or phosphorus to obtain the optimal nutrient (i.e., substrate) composition required. Bacteria found in natural organic substrates other than SRB utilize oxygen to degrade the material and, in turn, create anoxic conditions required for the SRB.

The pH requirements are obtained by the alkalinity generated by microbial activity and carbonate dissolution. Temperature significantly affects the rate of microbiological processes. At low temperature levels ($<10^{\circ}C$), the reaction rate decreases more than 50% of that which could be obtained at $20^{\circ}C$. The average sulphate reduction (or H_2S generation) rate has been found to be 0.3 mol per m^3 of the nutrient per day at temperature levels higher than $10^{\circ}C$ in the tests conducted where agricultural wastes were used as nutrients (Kuyucak and St-Germain, 1994). However, since the average temperature found in seepage and in the groundwater is known to be above $10^{\circ}C$ year-round and does not fluctuate seasonally, temperature requirements do not pose serious limitations for treatment of these water bodies.

The overall process results in improvement in the water quality due to precipitation of metals as sulphides with the H_2S generated in organic substrate and neutralization of the acidity due to the bicarbonate released during sulphate reduction. This process is particularly effective for removing heavy metals such as cadmium, copper, lead, mercury, zinc and iron to low concentrations.

6. BIOLOGICAL AND SRB-BASED TREATMENT PROCESSES

The SRB can be used as a cost-effective alternative to the conventional lime neutralization process for treating ARD. The function of SRB can be used in either the wetland systems, in the bioreactive walls of which are placed at the leading edge of an underground acid plume or in bioreactors operated under controlled conditions. The SRB-based passive processes are able to treat seepage, small streams and acid water accumulated in open pits or lagoons at mine sites.

6.1. Constructed Wetlands

Wetlands are composite systems where a variety of physical, chemical, microbiological and plant-mediated processes occur that can cause significant changes in the water chemistry. Investigations on natural wetlands indicated that wetlands have a significant capacity for removing trace metals and improving low pH values (MEND, 1990; Pett *et al.*, 1990; Wheeler *et al.*, 1991). Alkalinity generation and improvements in metal and acidity removal enhanced by the addition of organic amendments and nutrients to the natural systems and directing the flow path through the system was observed.

The general removal mechanisms which have been identified in both natural and constructed wetlands include oxidation and precipitation of metal oxides, adsorption and complexation of metals by organic substrates, sedimentation, filtration of suspended and colloidal particles, active plant uptake and microbial sulphate reduction followed by precipitation of metal sulphides (Cohen, 1996). Plant growth and decay in the wetland provide a constant source of organic substrate. The organic matter provides ion-exchange and adsorption sites while stimulating a consortium of bacterial activity, which promote a number of biotic and abiotic processes. Wetland vegetation also provides attachment sites for bacterial growth and flow channels, thereby increasing contact with the microorganisms and nutrients and promoting good flow distribution within the system.

In most wetlands where a free water surface is present, aerobic conditions exist throughout the water column and anaerobic conditions develop mainly below the sediment surface. Metals are removed in the aerobic zones by oxidation, precipitation, adsorption and complexation reactions, while neutralization is primarily achieved in the anaerobic zones by SRB activity and by the increase in alkalinity associated with other chemical and microbial reactions, such as the dissolution of limestone beds.

In recent years, wetlands have been constructed for the treatment of ARD. Constructed wetlands can be classified as either aerobic, anaerobic or combined wetlands. Sequential (or staged) treatment using a combination of aerobic and anaerobic wetland cells has been found to be effective in generating alkalinity and attenuating ARD (Kepler and McCleary, 1994). An example of staged aerobic and anaerobic constructed wetland cells is illustrated in Figure 3.
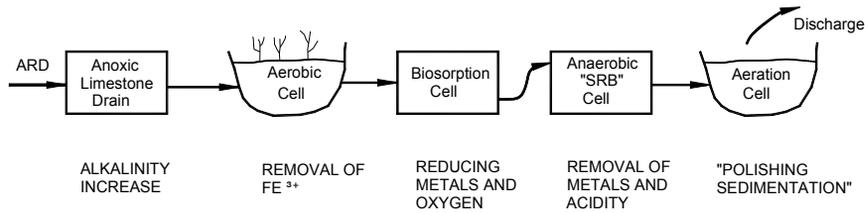
Figure 3. An example of combined passive treatment systems

6.1.1. Anaerobic Wetlands

Anaerobic wetlands are generally employed to treat drainage waters which contain elevated concentrations of iron and aluminium and has a total acidity greater than 300 mg/l as $CaCO_3$ or a pH of less than 4 (Hedin *et al.,* 1994). Anaerobic wetlands may also be referred to as compost wetlands or subsurface wetlands. Subsurface flow through a 30cm to 45cm deep organic substrate under anaerobic conditions promotes chemical and microbial processes, which generate alkalinity. The substrate usually consists of a low-cost, high organic content material, such as mushroom compost, sawdust, manure, leaf mulch, hay or peat which acts as a nutrient source for reducing bacteria. A neutralizing source such as limestone is also generally included below the substrate layer (Kleinmann and Hedin, 1993).

6.1.2. Aerobic Wetlands (Marshes)

Aerobic wetland cells are typically 15cm to 45cm deep water columns designed to maximize oxidation reactions which cause the precipitation of metals, primarily iron and/or manganese as oxides and hydroxides. The precipitates are then incorporated into the wetland sediment. This type of wetland is most effective for drainage waters which are alkaline in nature (Hedin, 1996). As dissolved metals are oxidized, a net increase in acidity is generally observed in these systems due to the release of H^+ ions and/or consumption of alkalinity associated with oxidation reactions. Thus, the use of an aerobic wetland followed by an ALD system has been highly recommended if the quality of ARD is suitable for the use of an ALD system.

Most aerobic wetlands (or marshes) contain plants growing in a clay or soil substrate. The dense stands of reeds growing in these systems serve as a support for bacteria and algae, and act as a hydraulic barrier to reduce the flow of the water enhance its residence time. In aerobic wetlands, several oxidation reactions take place. As a result of these reactions, metals (e.g., Fe, Mn) precipitate as oxides and hydroxides, which provide adsorption, sites for metal ions, and organic material decomposes (Hedin *et al.,* 1994). Metals can form complexes with organic materials becoming immobilized and as a result are retained in the system. The wetland also acts as a filter and enhances the settling of suspended solids.

An aerobic wetland system was recently adapted to a ditch treatment method at mine sites located in the United States and in Quebec, Canada (Eger *et al.,* 1997; Kuyucak, 2001b). An open ditch was constructed around waste rock piles and the seepage originating from the waste rock dumps was collected into it. The ditch was furnished with a layer of limestone and peat obtained from a nearby bog area. Stone berms were also installed in the ditch to control the flow of the ARD. The ARD remained in contact with atmospheric air and the iron species found in the ARD was oxidized to ferric iron, and subsequently, was precipitated in the ditch. A small ALD system was installed at the outlet of the ditch to neutralize the acidity of the water, which consisted predominantly of protons (H^+) that resulted from the oxidation, hydrolysis and precipitation of iron. No plant transplantation or growth was involved in these systems. The system installed in Quebec, Canada in 1997 has been performing well and producing good effluent quality which can meet the regulated standards, even in under Canadian winter conditions (Kuyucak, 2001b). The aerobic ditch treatment system was illustrated in Figure 4.
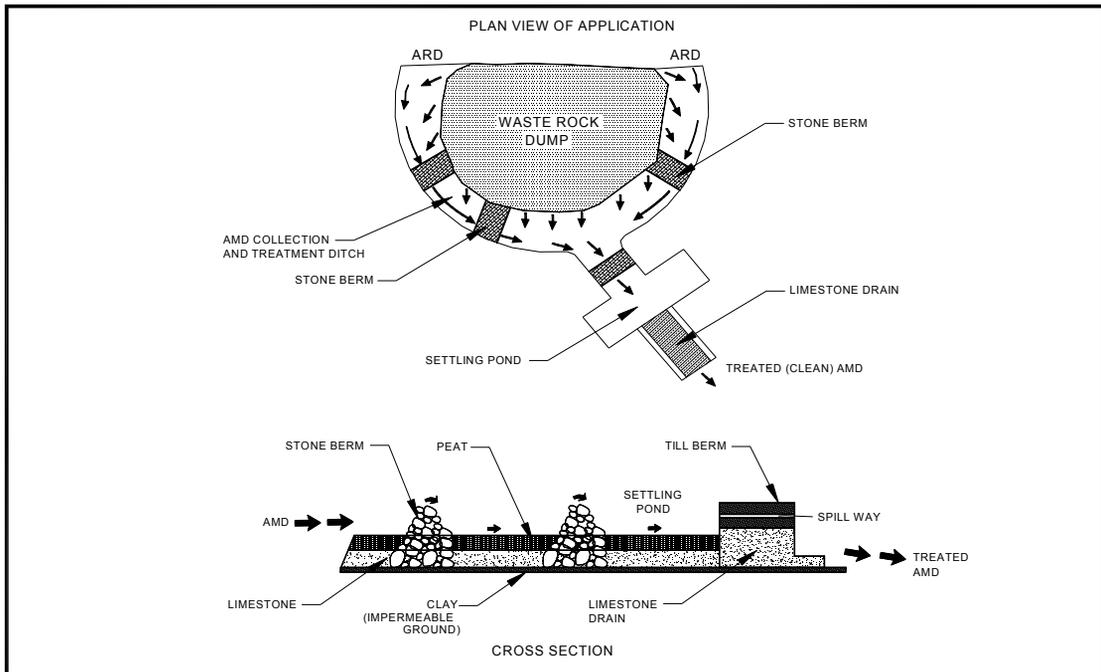
Figure 4. Aerobic- Non Vegetated AMD Treatment System

6.2. Sulphate Reduction and Bioreactor Systems

Bioreactor treatment systems are designed to optimize wetland treatment systems without presence of wetland plants. They are strictly dependent on bacterial activities. In biochemical reactor applications where the process parameters such as pH, temperature, anaerobic, nutrients and SRB are controlled, a short-chain organic acid or fatty acid is used as substrate. For instance, Budelco Mining and Smelter in the Netherlands had evaluated the effectiveness of several processes (e.g., SRB, ion exchange and membrane) with laboratory and pilot scale tests and found that the microbial sulphate reduction was the most cost-effective method for the treatment of ground water containing trace metals (e.g., Zn, Cd, Cu) and sulphate. At the site, reduction in sulphate concentrations from 1500mg/l to less than 500mg/l was required to comply with the regulated standards. This could be achieved with the microbial sulphate reduction process. In the full-scale process operation, ethanol is used as the nutrient for the bacteria and a flow of $250m^3/hr$ to $400m^3/hr$ is treated in up-flow anaerobic blanket reactors. All the process parameters are controlled and adjusted to optimal levels. Excess H_2S, which is left after the precipitation and separation of trace metals from the system, is converted to elemental sulphur with the help of photosynthetic sulphur oxidizing bacteria.

In passive processes, biodegradable substrates are used as nutrients and the efficiency of these processes is dependent on a consortium of bacteria rather than that of SRB alone. Biodegradable substrates are first metabolized and organic acids are produced which become available for the growth of SRB. As in wetland systems, the microbial activity within the bioreactors can be supplemented with inorganic chemical reactions, such as pH neutralization via limestone dissolution or other neutralizing reagents (Eger *et al.*, 1997). The bioreactors rely on several microbial reactions, which require different levels of oxygen to treat ARD. A cellulose-based material must be supplied to the system on a periodic basis or when it has been consumed. The substrate is degraded by cellulolytic bacteria, which generate free sugars and other metabolites. Aerobic and facultative heterotrophs can then further metabolize these products to provide substrates for the growth of fermentative anaerobes. Under anaerobic conditions, the free sugars are fermented to short -chain organic acids or short-chain fatty acids

which are suitable substrates to support the growth of the SRB. The SRBs then reduce sulphate to hydrogen sulphide which react with dissolved metal ions forming low solubility metal sulphide precipitates. The SRBs concurrently consume hydrogen ions and produce carbon dioxide that generates the alkalinity, thereby increasing pH levels. This is due to the reduced concentration of hydrogen ions and the buffering effect of bicarbonates. Other biologically mediated reactions such as ammonification, metal reduction and methane production can also contribute to the alkalinity generation depending upon the chemical composition of ARD, as mentioned above.

Bioreactor applications that have been investigated for ARD mitigation have included open pit, underground mines, biotrench and a series of cell configurations (e.g., acid reduction using microbiology ARUM process, Fyson et al, 1995). These processes involve the addition of SRB inoculum along with the necessary substrate and nutrients to the acidic drainage. Due to activities of a consortium of bacteria, anaerobic and reducing conditions are generated in situ in the open pit, in trenches or in cells. These, then, promote the growth of SRB resulting in alkalinity generation, acidity reduction, metal precipitation and, hence, ARD mitigation (Kuyucak and St-Germain, 1994 a, b; Béchard *et al.*, 1995; Fyson *et al.*, 1995). In the biotrench and cell applications, the cells are physically separated by a clay wall and the drainage is allowed to flow slowly through the system. Various organic substrates are used in these systems such as wood shavings, straw, manure, alfalfa, etc. A bag of organic substrate was prepared and suspended to an abandoned mine shaft with cables for the mitigation of acid mine water in place (Canty, 1998). Except during the time of spring run-off, the process is capable of producing a high quality effluent.

6.3. Bio-reactive Walls

The use of sulphate reducing reactive walls has been proposed for those waters affected by sulphide mineral oxidation which migrate downward to aquifers underlying the mine waste environment. These can be transported through the groundwater system eventually discharging into a surface receiving stream (Blowes *et al.*, 1995; Benner *et al.*, 1998). A portion of the existing aquifer is excavated and the original material is replaced with an organic substrate. The porosity of the wall is an important factor; the wall should be sufficiently permeable (e.g., 10^{-3}cm/s) to allow water flow through (Waybrant *et al.*, 1995).

A field test was conducted in 1995, where mine drainage flowing within an aquifer is intercepted and treated using a 15m wide, 4m thick and 3.6m deep wall. The design rate of flux through the wall was 288m^3/yr (Blowes *et al.*, 1997). The results to date revealed that the downstream sulphate concentrations had been reduced by fifty percent as iron concentrations were reduced by ninety-five percent (Benner, *et al.*, 1998). The pH was raised from 5.8 to 7.0 coinciding with an increase in alkalinity from 0mg/l to 50mg/l as $CaCO_3$.

6.4. Biosorption and Bio-Accumulation Systems

Materials of biological origin that have the ability to remove metal ions from aquatic solutions are called "Biosorbent". Sorption processes that play the role in removing metals are referred to as "Biosorption". Usually, biosorption refers to as the removal of metals by non-living biomass as bio-accumulation is attributed to the removal of metals by living cells. The main sorption mechanisms involved include surface adsorption, ion exchange, complex formation and/or precipitation with either living or non-living biomass. These processes exhibit potential as ARD abatement methods (Kuyucak, 1987).

6.4.1. Metals Removal via Living Cells ("Bio-Accumulation")

Wetlands for ARD mitigation were initially designed to imitate natural *Sphagnum* peat moss wetlands which exhibit relatively high adsorption capacities for heavy metals. Investigations conducted on a white cedar bog in Minnesota revealed that the natural peat land removed eighty percent of the nickel and nearly one hundred percent of the copper from the tailings

drainage (Eger and Lapakko, 1988; Eger *et al.*, 1980). Removal by the peat accounted for more than ninety percent of the total metal reduction. In Canada, Kalin and her colleagues (1990) reported that acidic drainage from a coal mine in Nova Scotia, containing elevated heavy metal concentrations and low pH levels, caused considerable damage to a peat bog's natural vegetation, with the exception of *Typha* and *Sphagnum* species. It has been found that *Sphagnum* moss generally accumulates metals to toxic levels after several months of exposure to mine drainage, but it is not readily available and is difficult to transplant (Hedin and Nairn, 1993). A laboratory study indicated that the formation of organically bound iron was the principle phenomenon for the removal of dissolved iron from a coal mine drainage (Tarleton *et al.*, 1984). The high metal sorption capacity of peat (living or non-living) has been attributed to presence of humic substances containing carboxyl, amino, quinone, hydroxyl and other functional groups. These functional groups are responsible for surface adsorption, ion exchange, chelation and complex formation with metals in solutions.

The underwater meadow of macrophytic algae chara, *Nitella flexilis*, was evaluated for its capability to treat a mine drainage from a uranium mine (Smith and Kalin, 1989). The algae act as a filter, which effectively transfers the contaminant radium (Ra^{226}) and to a lesser extent uranium (U) from the water to the sediment, during their continuous growth and decay.

The use of microbial mats immobilized on glass wool or wire mesh has been examined in constructed ponds to reduce concentrations of dissolved metals such as zinc, manganese and iron from mine drainage (Phillips *et al.*, 1995; Vatcharapijarn *et al.*, 1994). Microbial mats are composed of heterotrophic and autotrophic microorganisms mainly blue-green algae *Oscillatoria* sp., green filamentous algae and *Chromatium* sp. It has been reported that the microorganisms are held together by slimy secretions and extracellular metal deposits could be responsible for minimizing toxic effects in the biological system. Since these mats are both nitrogen fixing and photosynthetic, they are self-sufficient solar-driven with few growth requirements. As well, microorganisms have been genetically engineered to sequester metals such as gold, silver, platinum, cadmium, cobalt, copper, uranium and mercury mainly for the purpose of metal extraction and recovery (Ledin and Pedersen, 1996; Smith *et al.*, 1994). However, in spite of several pilot studies conducted under low flow rates of 2 liter/min to 5 liter/min, these processes have not yet been tested on a large scale.

6.4.2. Metals Removal via Non-Living Cells ("Biosorption")

The drawbacks associated with the use of living cells, such as metal toxicity, adverse climatic conditions and costs of nutrient supply and culture maintenance, can be avoided with the use of non-living biomass as biosorbents (Kuyucak, 1987; Kuyucak and Volesky, 1989; Kilborn, 1996).

Dried non-living biomass such as marine algae (*Ulva* sp.), blue-green algae (*Spirulina* sp.), yeast (*Saccharomyces cerevisiae*), common duck weed (*Lemna* sp.), and finely ground peat (*Sphagnum* peat moss) have been immobilized by blending them into a high-density polysulfone, dissolved in an organic solvent. BIO-FIX beads are fabricated from the immobilized biomass and are commercially used to reduce dissolved metal concentrations including arsenic, cadmium, copper, silver, lead, manganese and zinc from the mine waste environment (Jeffers, *et al.*, 1989; Bennet, *et al.*, 1991; Kilborn, 1996). BIO-FIX beads are generally enclosed in meshed polypropylene bags and placed either in a trough, directly into an ARD stream or in conventional equipment (e.g., a tank). The beads are particularly effective in adsorbing metals from dilute ARD streams.

Biosorbents such as marine and fresh algae are also employed for the recovery of metals including silver, copper, cobalt, mercury or cadmium from aqueous solutions (Kuyucak, 1987; Kuyucak and Volesky, 1989). An immobilized algae, mainly *Chlorella vulgaris*, which commercially is called AlgaSORB, has been found to be effective for treating groundwater (Darnall *et al.*, 1989). The levels of metal ions can be reduced in concentration from low ppm levels to low ppb levels.

Another commercial biosorbent (Mediaflex$_{MC}$) consisting of *Sphagnum* peat moss and a carbon material has been reported to be effective in treating various types of wastewaters including ARD (Belanger *et al.*, 1995). Mediaflex$_{MC}$ was able to treat drainage from an abandoned copper mine in a basin with a capacity of 6000m^3, using flow rates in the order of 250m^3/d. Mediaflex$_{MC}$ acts as a biofilter having adsorptive and precipitation properties and can remove Fe, Zn, Cu, Ni, Cr and Cd.

Chitosan (a natural biopolymer from chitin) and calcium-alginate have been investigated to remove cadmium, barium, uranium and zinc (Kilborn, 1996). Forest products and celluloytic materials such as bark, sawdust and litters also show metal sorption capacity (Kuyucak and St-Germain, 1993 and 1994).

When the biosorbent in use becomes saturated, it is replaced with fresh material. Metals can be stripped from the metal-laden biosorbent and can be recovered. The biosorbent can be used several times or it can be disposed of into a safe environment.

7. POTENTIAL BENEFITS AND SUITABILITY OF PASSIVE TREATMENT

With the placement of the cover over the tailings, the quantity and quality of the seepage originating from the tailings pond will become manageable for passive in-situ treatment processes. Foreseen potential benefits are as follows:

- Passive systems are a sustainable alternative to the expensive chemical methods.
- They form a potentially low maintenance option with low requirements for energy and material input compared with chemical treatment plants.
- Passive systems can protect ground water resources and remediate contaminated plumes. They can keep constituents immobilized in the system.
- They incorporate a range of physical, chemical and biological processes which can reduce metal concentrations to very low levels similar to the chemical sulphide precipitation process.
- The passive systems eliminate sludge collection and disposal concerns.
- In passive systems natural materials are used rather than chemical reagents and they are aesthetically attractive with consequent "green" appeal. Wetland can eventually become a valuable wildlife refuge.

7.1. Examples to Passive Treatment Field Applications

The technology is relatively new and has been undergoing continuous development. Although, there are several field-scale applications, information related to their long-term performance is limited. The field-scale process applications including pilot tests are summarized below.

7.1.1. ALD Systems

Over the last decade, it has been reported that more than 30 ALD systems have been installed in the USA (Brodie *et al.*, 1992; Hedin and Nairn, 1992). Usually, ALD systems have been combined with an aerated wetland and have been used for the removal of acidity and metals (mainly Fe and Mn).

7.1.2. SRB Based Treatment Systems

The SRB based processes are still under development. There are some examples of pilot-scale applications. The technology has mainly been incorporated with mine closure plans to eliminate maintenance requirements and the potential for future environmental liability and/or remedial action. The examples include:

- Suspended organic nutrients in an abandoned mineshaft in Montana, USA treats ARD in place (Kuyucak, 1995; Canty, 1998).
- A reactive Wall (or Barrier) has been implemented in the field for the treatment of seepage originating from a nickel mine in Sudbury, Canada (Blowes *et al.,* 1998)
- An anaerobic wetland has been constructed to treat seepage from heap leach ponds in Nevada, USA (Homestake Mining).
- Treatment of seepage from waste rock stockpile in Minnesota, USA (LTV Steel Mining Company). This is a combination of limestone-peat substrate and an aerated wetland. For five years, it has been successfully treating a flow of 19 liter/sec to 38 liter/sec, containing Cu, Ni and Fe, with a pH of 5 (Eger *et al.,* 1997).

7.1.3. Aerated Wetlands

Most of the full-scale wetlands constructed for the treatment of mine waters have consisted of aerated wetlands and lagoon components, located in existing stream channels. Several have been successfully used in Australia, South Africa and USA. The performance of these systems is closely associated with the near-neutral pH and low concentrations of metals in waters. Inclusion of riffle zones and algae in addition to other aquatic plants have been found to be effective in removing metals particularly Mn. It has been reported that waters with flow rates up to 3000m^3/day (i.e., 35 liter/sec) can be treated. Some examples include:

- Removal of acidity, Zn, Fe, Mn and thallium: Hilton Mine, Australia.
- Removal of uranium: Ranger Mine, Australia.
- Removal of arsenic, Zn, Cu, Cd, Pb, Fe, Mn: Tom's Gully Gold Mine, Australia.
- Removal of Cd, Pb, Cu and Zn: Woodcutters Mine, Australia.
- Removal of Mn, Pb, Fe and SO$_4$ from the tailings dam: Hellyer Mine, Australia.

8. LIMITATIONS OF BIOLOGICAL PASSIVE TREATMENT SYSTEMS

Although passive treatment systems offer several benefits, their application is limited to site conditions. Except for the ALD systems, their performance is highly dependent on temperature. At temperatures lower than 10°C, they do not function well (Kuyucak and St-Germain, 1994a,b). Their performance is limited and dependent on the flow rate and the chemical composition of water being treated. These systems cannot handle high loading situations and fluctuations in both flow rate and chemical composition. It has been reported that during spring run-off, an SRB system suspended in a mine shaft could not produce the same water quality as it could before the spring run off took place. The system was capable of recovering to normal condition after a few months (Canty, 1998). Even flow distribution and the contact of acid water with bacteria in the passive system are important factors affecting the efficiency. Channelling in the system often occurs and causes inefficient use of the system and, consequently inefficient treatment of the mine water. The presence of alkalinity, sulphate and nutrients in sufficient quantities and anaerobic and reducing conditions in the system are especially necessary for the SRB based processes. The removal of Mn cannot be achieved with an anaerobic process alone.

The performance of ALD depends mainly on the chemical composition of the mine water. For instance, the presence of heavy metals such as copper (eg. Cu, Al), ferric iron and sulphate in high concentrations, and dissolved oxygen in the acid water deteriorates the performance of ALD because these compounds cause precipitation and clogging in the system.

The cost of passive systems is highly dependent on the site conditions, chemical composition of the acid water and treatment requirements. In some cases, the application of passive systems may be more expensive or as expensive as a chemical treatment option (Hedin *et al.,* 1994; Kuyucak and St-Germain, 1994). The size of the system, simplicity of construction, and quantity and availability of the nutrients required dictate the cost of the process. Therefore, the use of locally available materials as nutrient or organic substrate in these systems has been recommended.

In most cases, one method alone is not sufficient to achieve the treatment requirements at the site. A combined passive system such as ALD with an aerobic and/or anaerobic wetland could provide a continuous means of treatment to the site. Due to its constant flow rate, chemical composition and temperature, passive treatment systems (e.g., reactive walls) have been suggested as ideal for the treatment of seepage of waste water.

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