EVALUATION OF THE SUITABILITY TO USE SULFIDE-REDUCTION BACTERIA IN WETLANDS AND BIORREACTORS TO BIOREMEDIATE ACID DRAINAGE FROM COPPER MINING IN ECUADOR

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DIANA KARINA AYALA MUÑOZ
549592

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Supervisor: Dr. Graham Moore
Supervisor’s Department: Engineering and Infrastructure
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Diana Karina Ayala Muñoz
Abstract

Large scale-mining of copper industry is going to be developed in Ecuador as one of the major goals to diversify the country’s economy. The first large scale-mining project is in the field “Mirador” located in Zamora Chinchipe province, Ecuador. This field is an open pit copper mine from where it will be extracted 60000 tonnes of copper ore per day. Two rock dumps located in each of the sides of the pit will contain 44% of the extracted ore in the form of sulphide ore (mostly chalcopyrite), which if it is exposed to water and oxygen will form Acid Mine Drainage (AMD). AMD is characterized to have low pH, high concentration of sulphates and heavy metals. Due to high rainfall rates and geochemical characteristics of waste rocks in the Mirador Project, AMD produced will be in high flows and highly concentrated. It will threaten water quality and fisheries of the watersheds surrounding the mine site, thus causing environmental, social and economical impacts. As AMD is a complex problem in biological, chemical and ecological terms, its remediation is also complex. Aspects like climate and terrain of the Mirador Project, scale of its mining operation, and pollution prevention measures for AMD are addressed in this research to evaluate how they can influence the application of AMD remediation techniques. This research focuses on the bioremediation of AMD by using Sulphate-Reducing Bacteria (SRB) in anaerobic wetlands and bioreactors as a possible sustainable alternative. SRB are capable to treat AMD by increasing pH, sulphate reduction and metal remotion. To evaluate if SRB are suitable to be used in bioreactors and wetlands to treat AMD in the Mirador Project, this research analyses the most important factors that influence the growth and long-activity of SRB: substrate, pH, temperature, toxicity of metals and heterogeneous microenvironments. In addition, an analysis of the wastes produced by bioremediation of AMD with SRB and their management mechanisms is conducted to show that this bioremediation system may be sustainable in terms of energy efficiency, economic viability and little generation of waste. Finally, the evaluation of suitability of SRB in anaerobic wetlands and bioreactors is developed by considering AMD characteristics, topography and climate conditions of the site. Since the Mirador Project is still in his exploration phase, planning of AMD sustainable management is still on time to be addressed. This research recommends developing a phase scale study to decide what the best treatment option for AMD produced in the waste rock dumps in the Mirador Project could be. The outcomes of the
evaluation conducted in this research show that application of SRB may be suitable to remediate AMD in an anaerobic bioreactor. This literature review could be used to establish a framework of action to manage AMD mitigation and remediation since its results could be useful for other mining operations with similar conditions in Ecuador.
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INTRODUCTION

Mining activity is of great economic importance around the world (Andrade 2010). In Ecuador, large-scale mining projects are part of the strategy to stimulate the country’s economy (Eguiuguren & Jiménez 2011). The first large scale-mining in Ecuador is a mining project in the field “Mirador”, which plans to exploit ore in the chemical form of sulfide cooper (Sacher 2011). From the amount of exploited ore per day, 44 percent is waste rock stored in dumps (Terrambiente 2007). Poor environmental management of this kind of solid wastes produces pollution by acid mine drainage (AMD) considered as one of the most detrimental environmental problems that large-scale mining in Mirador project will cause (Sacher 2011). In an aim to search for sustainable solutions for AMD, this research seeks to generate information useful for mining companies, Ecuadorian government, communities, and policy makers to help decision-making regarding to reduction and remediation of acid mine drainage generated in waste rock dumps in Mirador project, Ecuador. This literature review mainly focuses on bioremediation alternatives of AMD, specifically passive treatments using Sulphate-Reducing Bacteria (SRB). The main purpose is to evaluate the suitability of SRB application in anaerobic wetlands and bioreactors as treatment options for AMD. To accomplish this goal, an understanding of the context of copper mining in Ecuador, specifically Mirador Project, the generation of AMD and its impacts, possible preventive measures, and bioremediation of AMD using SRB, has been addressed.

MINING IN ECUADOR

The huge demand of metals by countries like China and India, and the world depletion of petroleum reserves, have made of mining an important economic activity for countries like Ecuador (Sacher 2011). To diversify Ecuador’s economy and reduce its dependence on oil exports, large scale-mining of copper (Valencia 2012), will situate Ecuador as one of the sixth largest exporter of copper worldwide (Fernández 2012). The
new Ecuadorian mining policy supports the activation of overseas inversion in large-scale mining projects, especially in the southern region of Ecuador, in Zamora Chinchipe province (Eguiguren & Jiménez 2011). Although, large-scale mining is a priority for the Ecuadorian government, it is unclear how this activity can sustainably protect Ecuadorian environmental services and human rights of affected communities while supporting to the economic growth of Ecuador.

**Mirador Project: the context**

The first and most developed large scale-mining of copper is in the field “Mirador” located in Zamora Chinchipe province (Kuhn 2011). It is one of the five mining strategic projects for Ecuadorian Government since it is a copper reserve of 11.000 million pounds, valued in 220.000 million dollar (Kuhn 2011). Currently, this project is in an advanced phase of exploration (Sacher 2011), and it is expected to start an exploitation phase by 2014-2015 (Pillajo 2010). Therefore, there is still time to plan of strategies to mitigate Mirador project operational impacts.

Mirador Project is located in the Copper Belt zone of the Cóndor Mountain Range in the southeast of Ecuador in an altitude from 800 to 1400 metres above sea level (Kuhn 2011). This site constitutes a second-growth tropical forest with numerous clearings at lower elevations (Sivertz 2006). It is a deposit of copper, gold, and silver with an extension of 1600km² (Kuhn 2011) located in the Zamora River Basin (Sacher 2011), 10km east of the Zamora River (Sivertz 2006). The mine site consists on alluvial deposits in a relatively flat topography with humid climate, annual rainfall of 2700mm, and an average temperature of 22,4°C (Terrambiente 2007). It is an area with space-time variability in rainfall (Sacher 2011) due to variations in the local terrain (Sivertz 2006). Finally, in the site there is abundance of ground water (Kuhn 2011). These factors need to be considered to establish strategies for mitigation of impacts.
Ecologically, Mirador project is located in a zone of great interest for biological conservation (Eguiguren & Jiménez 2011). Regionally, this area is considered as crucial in the hydrological cycle that joins los Andes mountain range with the Amazon forest (Eguiguren & Jiménez 2011). It is known internationally for having a high biological diversity (Kuhn 2011) with 16 different ecosystems (CEDHU & FIDH 2010). This zone is of great environmental importance, and so, principles of control, cleaner production and remediation must be applied, to develop a sustainable large-scale mining.

Scale of mining operation

Mirador project is an open pit mine (Fernández 2012) planned to operate for 17 years (Sacher 2011). In the operation, it will be extracted 54,000 tons of copper ore per day (Sacher 2011). From this extraction, 24,000 tons are waste rocks stored in two rock dumps located in each of the sides of the pit, and 30,000 tons are useful rocks that have 2% of concentrated copper (Sacher 2011). The useful rock is ground and milled to be exposed to a flotation treatment to concentrate copper (Terrambiente 2007). All the material that is not floated and concentrated is transported directly to a tailings dam (Terrambiente 2007). Only 572 tons of copper per day is concentrated and ready to be exported (Pillajo 2010). In total, 190,000 tons of concentrated copper per year is expected to be exported (Pillajo 2010), but more than 19 million tons of waste rock per year will be generated and disposed in rock dumps and tailings dams.

ACID MINE DRAINAGE (AMD), THE MOST POLLUTING IMPACT IN MIRADOR PROJECT

The ore that is planned to be exploited in Mirador project is in the chemical form of sulphide cooper; mainly chalcopyrite (CuFeS$_2$) and other sulphide ores in less concentration like pyrite (FeS$_2$) (Sivertz et al. 2006). Mines with one to five percent of sulphur content in the ore have a higher likelihood of generating Acid Mine Drainage.
(AMD) (Tiwary 2001). As Mirador Project will produce high amounts of waste rock characterized by being sulphide rich and carbonates poor (Shannon 2004), it is unlikely to produce alkaline or neutral mine water (Ziemkiewicz et al. 2003), thus increasing the risk of AMD impacts. Currently, AMD cannot be stopped once it occurs on a large scale in open pit mines (Jennings et al. 2008), constituting one of the most polluting impacts of mining (Sacher 2011). In this context, there is a high necessity to study, develop and apply environmental strategies that can remediate the generation of AMD.

**Understanding the generation of Acid Mine Drainage (AMD)**

Acid mine drainage (AMD) occurs when rainfall and air get in contact with extracted rocks that contain sulphide ore (Andrade 2010) and generate water with low pH, high concentration of metals, sulphate and precipitates, see equation 1.

\[
\text{Sulphide ore} + \text{air} + \text{rain water} \rightarrow \text{low pH} + \text{dissolved metals and sulphates} + \text{precipitates} \quad (1)
\]

Considering chalcopyrite as the principal sulphide ore in Mirador project, the overall reaction that explains the generation of acid mine drainage in this mine site will be:

\[
\text{Chalcopyrite} + \text{oxygen} + \text{water} \rightarrow \text{low pH} + \text{cupric ion} + \text{ferric iron} + \text{sulphate ion} + \text{ferric oxide} \quad (2)
\]

Chemically, this reaction is represented as follows (Thurston et al. 2010):

\[
8 \text{CuFeS}_2 + 34 \text{O}_2(\text{aq}) + 2 \text{H}_2\text{O} \rightleftharpoons 4 \text{H}^{+} + 8 \text{Cu}^{2+} + 4 \text{Fe}^{3+} + 16 \text{SO}_4^{2-} + 2 \text{Fe}_2\text{O}_3 \quad (3)
\]

Three important steps explain the reaction (3) (Pope et al. 2010):

- Oxidation of sulphide ore (CuFeS$_2$) to sulphate ion (SO$_4^{2-}$).
- Oxidation of ferrous iron to ferric iron (Fe$^{3+}$).
• Hydrolysis of ferric iron (Fe\(^{3+}\)) that precipitates as ferric oxide (Fe\(_2\)O\(_3\)) at pH 2.3 – 3.5.

From equation 3, H\(^+\) (hydron) and SO\(_4^{2-}\) (sulphate ion) increase acidity and presence of dissolved solids in the waste water (Gray 1997). Dissolved metals like Cu\(^{2+}\) (cupric ion) and Fe\(^{3+}\) (ferric iron) can be transported downstream and precipitated (Kimball et al. 2009), affecting ecosystems because of their toxicity (Mayes et al. 2009). Precipitates like Fe\(_2\)O\(_3\) (ferric oxide) in huge amounts can choke out aquatic life and kill fish (Gray 1998). Ferric iron (Fe\(^{3+}\)) that does not precipitate can oxidize sulphide ore (equations 4 and 5) (Sheoran et al. 2010) generating more acidity and additional sulphate, ferrous iron, and cupric ion that contribute to the perpetual generation of AMD (Andrade 2010). Moreover, as the sulphide ore of a copper mine typically contains metals like copper (Cu), zinc (Zn), lead (Pb), and arsenic (As), a broad range of dissolved metals can be released to the environment (Kant et al. 2007).

With chalcocite (CuFeS\(_2\)):

\[
\text{CuFeS}_2 + 16\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow \text{Cu}^{2+} + 2\text{SO}_4^{2-} + 17\text{Fe}^{2+} + 16\text{H}^+ \quad (4)
\]

With pyrite (FeS\(_2\)):

\[
\text{FeS}_2 + 14\text{Fe}^{3+} + \text{H}_2\text{O} \rightarrow 12\text{Fe}^{2+} + 2\text{SO}_4^{2+} + 16\text{H}^+ \quad (5)
\]

Finally, bacteria that colonize tailings and rock dumps in conditions of low pH accelerate the Generation of AMD (Escobar et al. 2010). The most common bacteria involved in AMD production are *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* (Escobar et al. 2010) that at pH of 2-4 can increase the oxidation rate by 5 to 6 orders of magnitude (De la Torre et al. 2011).

As can be seen, AMD has low pH and is rich in sulphate and heavy metals, especially iron and copper for the specific case of Mirador Project (Johnson & Halberg...
However, a specific characterization of AMD produced in Mirador project is not yet possible since mine operations have not started yet. According to Liew and Sheppard (1997), AMD is typically characterized to have pH less than 3 and a concentration of sulphate greater than 3000 mg/L. Neculita et al. (2007) established that generation of AMD varies from site to site and mine to mine since it will depend on factors like geochemistry of waste rock, waste rock dumps permeability, surface area of exposed sulphide ore and mine site climate. This is why prediction, mitigation of AMD is challenging and costly (Sheoran et al. 2010).

**Possible Sources of AMD in Mirador Project**

In the Mirador Project, during the open pit mine operation, AMD can be produced in the pit where the surface rock will be exposed to atmospheric agents that can wash rocks and cause acidic and metalliferous water to flow to surface watersheds downstream the mine (Terrambiente 2007). Further, liquid and solid wastes from the flotation process to concentrate copper are stored in the tailings dam of 56,6 Ha (Escobar et al. 2010), and those wastes that can easily react with air and water to produce AMD. Finally, waste rocks, stored in the two rock dumps of 75 Ha and 47,9 Ha and located at 1300 metres above sea level (Terrambiente 2007), will allow generation of AMD by interaction between rainfall, oxygen and sulphide ore. The rock dumps of the Mirador Project will contain approximately 140 million tonnes of waste rocks. Consequently, since AMD generated in these rock dumps can be more concentrated and in higher volumes than the one generated in the tailings dam, there is likely to be a high pollution risk of downstream water and groundwater (Sacher 2011).

**AMD Impacts in Ecuador**

In this context, AMD is inevitable, hard to contain and with long-term impacts (Kuhn 2011). There are three important impacts of AMD: acidity, heavy metals and
sedimentation (Gray 1997). Acidity destroys the bicarbonate buffering capacity of a lotic system by reducing pH. Heavy metals released affects species because of their toxicity. Sedimentation affects lotic systems by the accumulation of sediments and turbidity. Therefore, AMD can affect the quality of surface and ground water (Kuhn 2011) causing elimination of sensitive species and reduction of biodiversity (Gray 1997). For example, in the South region of Ecuador where mining is the principal economical activity, the monitoring of rivers by Prodemica (1999) shows that metals like arsenic, copper, zinc and lead are in toxic concentrations for aquatic life (Andrade 2010). In the intersection of rivers Calera and Amarillo, fauna is absent, and downstream, accumulation of heavy metals in biota has been found (Guerra & Zaldumbide 2010). All of these impacts have been caused by small and medium scale mining. Therefore, now that large scale-mining in Ecuador will start, mitigation and remediation techniques have to be applied in order to reduce the degradation of Ecuadorian ecological services.

The AMD risks are greater in the Mirador Project due to the abundance of ground water and the high rate of precipitation (Kuhn 2011). Further, the alkalinity concentration of surface water samples from different points of watershed surrounding the zone are low (less than 5mg/L to 22 mg/L) making this water more vulnerable to AMD impacts due to a lack of natural neutralization capacity of the receiving watershed (Sacher 2011). AMD generated in the Mirador Project will affect the water quality of rivers like Wawayme, Paquintza, and so, economic activities and health of communities around the project will be extremely affected (Sacher 2011).

Despite increased community participation and consultation, greater community infrastructure inversion in affected areas, and more transparency of the budget deals of extractivist companies and government (Acosta 2012), still activists and local people complain that none of these actions will compensate for the damage that this kind of human activity will have in the environment (Becker 2012). Kuhn (2011) argues that the Ecuadorian Government is promoting large-scale mining without enough power to protect
the social rights and environmental services against the interests of huge mining companies. Therefore, policy that controls the generation of AMD in a mine site and encourages an accurate treatment of AMD prior to its release to the environment is a pressing necessity before operation of large-scale mines starts in Ecuador.

REDUCTION OF AMD

To avoid the generation of AMD, the sulphide ore must remain in its reduced state. Therefore, avoiding the contact of sulphide ore with oxygen and water is an alternative to prevent the oxidation of sulphide ore and stop the perpetual generation of AMD (Gray 1997). In this context, oxygen diffusion in rock dumps and prevention of water flow into dumps by using soil and water covers is one preventive measure to reduce AMD (Jordanov et al. 2007). In the Mirador project, waste rock dumps will be covered by a low permeability material like a geomembrane and gradually revegetated (Terrambiente 2007). A layer of rough waste tailings, impermeable clay soil, and organic soil will be the covered layer of the tailings dam (Terrambiente 2007). In addition, the Mirador project could consider elimination of bacteria that accelerate the sulphide oxidation by applying chemicals like sodium benzoate (Aguirre 2007). However, these preventive measures need to be better planned as the stability and sustainability of cover layers over the long term, are unclear. Also, application of biocide chemicals has a variable effectiveness and requires repeated applications of chemicals, which may not be environmentally sustainable (Johnson & Halberg 2005). As can be seen, further studies will need to be developed in order to define what best pollution prevention measures are to be applied in the Mirador Project.

Currently, there is no scientific evidence of avoidance of generation of AMD by large-scale mining in high rainfall areas (Kuhn 2011) like the Mirador Project in Ecuador, which means that AMD will be generated despite preventive measures. The Mirador project has focused on technologies to collect AMD on site. For example, in waste rock
dumps, a sub-drainage system to allow water flow under the structures of the tip will prevent infiltrations to ground water, and water collected from the drainage system will be directed to possible remediation systems (Terrambiente 2007). The application of these techniques conforms to the preventive measures described by Younger and Wolkersdorfer (2004) who recommend engineered mine drainage pathways to prevent AMD leakage to sensitive streams. However, in the Mirador Project, preventive measures for groundwater diversions are not considered. Younger and Wolkersdorfer (2004) recommend physical barriers like roadways and grout curtains that can be built to intercept water and divert it to treatment systems. Considering these facts, this research assesses treatment options for AMD collected in drainage system of waste rock dumps in the Mirador Project.

**REMEDIATION OF AMD**

It is crucial to evaluate strategies to deal with AMD. To plan and implement treatment options for AMD is necessary to characterize mine discharges and evaluate the current situation of receiving watersheds surrounding the mine site (Sheoran et al. 2010). This evaluation must be conducted over a long period of time and follow an impact assessment methodology (Sheoran et al. 2010) that includes hydrogeological and hydrochemical methods during wet and dry periods of time (Pope et al. 2010). In addition, synchronous measurements of flow and water quality must be conducted to characterize mine discharges and understand the temporal changes in the quality of mine water (Sheoran et al. 2010). A detailed description of the possible methods to apply for mine water characterization and monitoring is presented by Younger and Wolkersdorfer (2004) (pp. S45). Characterization and monitoring of mine water generated in Mirador Project will define if further pollution management measures are required to comply with the Ecuadorian environmental legislation (TULAS, Appendix 1, Book VI, Table 12, pp. 330). If mine water collected in drainage system needs more treatment, then bioremediation may be a sustainable treatment option.
Bioremediation of AMD by using Sulphate-Reducing Bacteria (SRB) in passive treatments

A sustainable solution would be energy efficient and economically viable, would generate little or no waste, and would be not a source itself of pollution (Kalin et al. 2006). Several treatments like lime neutralization have been broadly used as an option to treat AMD (Younger 2000). However, the addition of lime will not by itself produce a complete precipitation of iron and other metals (Kalin et al. 2006), is expensive, and generates residual unstable sludge in large quantities (Foucher et al. 2001). An alternative remediation process is using Sulphate-Reducing Bacteria (SRB) in a well-engineered passive treatment (Foucher et al. 2001). This bioremediation option is considered a sustainable solution since it is an ecological and self-renewing system (Kalin et al. 2006) that uses naturally available energy sources like microbial metabolic energy and photosynthesis, requires low maintenance and costs (Doshi 2006), and is capable of being integrated with natural ecosystems (Younger 2000). The aim of this literature review is to evaluate how feasible is the application of SRB to treat AMD generated in waste rock dumps in the Mirador Project in Ecuador.

SRB mechanisms to remediate AMD

Sulphate-reducing bacteria (SRB) are strict anaerobic soil microorganisms like Desulfovibrio and Desulfotomaculum (Liew & Sheppard 1997) that thrive in a range of different environmental conditions like in AMD at pH 2 (Muyzer & Stams 2008). SRB are used in AMD bioremediation since they achieve alkalization of low pH mine water and reduce sulphate and soluble toxic metal concentrations (Tebo & Obraztsova 1998) which are the principal detrimental characteristics of AMD.

In the absence of oxygen, SRB catalyses a dissimilatory sulphate reduction (Muyzer & Stams 2008) by using sulphate (SO\textsubscript{4}) as electron acceptor to oxidize a simple organic
carbon source (CH$_2$O), and produce carbonate (HCO$_3^-$) and hydrogen sulphide (H$_2$S) (Andrade 2010), see equation 7 (Doshi 2006).

$$2\text{CH}_2\text{O} + \text{SO}_4^{2-} \rightarrow 2\text{HCO}_3^- + \text{H}_2\text{S} \quad (7)$$

The generation of carbonate contributes to acid neutralization (Lindsay et al. 2011), and the produced hydrogen sulphide (H$_2$S) reacts with dissolved metals (M$^{2+}$) to form highly insoluble sulphides (MS) (Jhonson & Halberg 2005), see equation 8 (Doshi 2006).

$$\text{H}_2\text{S} + \text{M}^{2+} \rightarrow \text{MS} + 2\text{H}^+ \quad (8)$$

Hydrogen sulphide promotes the removal of metals like (iron) Fe$^{2+}$. This metal sulphide precipitates and, in this way, the dissolved metal concentration of AMD decreases. Other dissolved metals like zinc, copper, arsenic and lead are precipitated in the same way (Lindsay et al. 2011).

**Factors that influence the effectiveness of SRB activity**

To evaluate the suitability of SRB application in a passive treatment for AMD bioremediation, it is necessary to evaluate what factors affect their growth and long-activity. The most important factors are adequate substrate, pH and temperature of influent, toxicity of metals, and generation of microenvironments.

**Substrate**

Availability of carbon from an organic source is the most limiting factor for effective SRB performance. SRB growth and activity require the presence of an organic carbon as substrate (see equation 7) that has to be of small molecular weight (lactate, methanol, glucose) to be degraded by SRB (Muyzer & Stams 2008). In field application, as
AMD generally has low concentrations of organic carbon, under 10mg/L (Neculita et al. 2007), a mix of biodegradable materials like manure and more recalcitrant materials like sawdust are recommended to be added as substrate (Johnson & Hallberg 2005). The latter helps to keep the long term provision of organic sources required by SRB (Johnson & Hallberg 2005). To degrade this kind of materials to small molecular weight substrate, SRB are dependent on other microorganisms (Muyzer & Stams 2008). Depletion of organic source will affect the SRB effectiveness to treat AMD. Therefore, the selection of a suitable organic source is crucial to assure the long-term performance of SRB (Zagury et al. 2006). For bioremediation of AMD from waste rock dumps in the Mirador project, the best substrate option will have to be assessed in terms of its biodegradability, availability and costs (Neculita et al. 2007).

**Influent pH**

Kilborn (1996) established that SRB do not grow below a pH of 5.5 since they are sensitive to even mild acidity (Johnson and Hallberg 2005b), and Andrade (2010) defined that pH 5 to 8 is required for an optimal performance of SRB. On the other hand, Tsukamoto and Miller (2004) demonstrated the efficiency of SRB to reduce sulphate at low pH (2.5 to 3), and McCullough et al. (2008) demonstrated that pH under 5 did not inhibit sulphate reduction activity promoted by SRB. However, as SRB depend on other heterotrophic microorganisms to degrade complex substrate, a neutral pH is required for these microorganisms to thrive (Kilborn 1996). In this context, a pH greater than 5.5 is necessary to sustain SRB growth and activity (Kilborn 1996). As it is expected that AMD generated by waste rock dumps in the Mirador Project is highly acidic, passive systems must produce sufficient buffering capacity in a long term to raise the pH of AMD (Kilborn 1996). Doshi (2006) established that addition of sodium hydroxide in a pretreatment pond is recommendable to adjust pH because of its solubility and small dosage. Gusek (2002) recommends the addition of limestone. These options will have to be assessed in the designing process of a passive treatment using SRB.
Temperature

Tsukamoto and Miller (2004) demonstrated that temperature does not influence performance of SRB, once they are acclimated and adapted. SRB are tolerant to temperature ranges from -5°C to 75°C (Neculita et al. 2007). However, a high rate of sulphate reduction by SRB is achieved at mean temperatures (McCullough et al. 2006). For example, temperatures between 20-28°C in Collinsville, Australia, enhance biochemical rates of sulphate reduction by SRB (McCullough et al. 2006). In the Mirador project, temperature is not a fact that will affect SRB activity since the mean temperature is 22.4°C. Even in lower temperatures, its impact can be mitigated by designing anaerobic bioreactors with sufficient depth to keep a mean temperature (Doshi 2006).

Toxicity of metals

The effect of heavy metals on the efficiency of SRB varies depending on their concentrations in AMD (Utgicar et al. 2002). For example, 20 mg/L of cadmium and nickel, 25 mg/L of zinc, 60 mg/L of chromium and 65 mg/L of lead were toxic to a mixed culture of SRB, inhibiting sulphate reduction in an anaerobic bioreactor (Doshi 2006). To assure successful operation of a passive system using SRB, AMD has to have metal concentrations below inhibitory/toxic levels for SRB (Neculita et al. 2007). Therefore, before applying a passive treatment using SRB, it will be necessary to conduct metal toxicity assessments with the AMD generated by waste rock dumps in the Mirador project. In addition, in passive treatments using SRB, hydraulic problems like clogging have been produced by precipitation of metal sulphides. As this fact interferes with SRB activity (Kalin et al. 2006), it is necessary to design mechanisms to remove metal sulphides from the passive treatment before they become toxic or inhibitory for SRB (Utgicar et al. 2002).
Heterogeneous microenvironments

In nature, SRB are found congregated in soils and sediments where they interact with other microorganisms (Liew & Sheppard 1997). Other heterotrophic bacteria (acidogens, acetogens and methanogens) help SRB to obtain degradation and fermentation products necessary for their growth and sulphidogenic activity (Zagury et al. 2006). In field application, a physical support like compost beds with gravel for SRB and other microorganisms is necessary since they tend to aggregate in areas which offer some physical protection (Liew & Sheppard 1997). In these areas, SRB are able to condition the immediate environment forming a microenvironment conducive to their survival (Liew & Sheppard 1997). The development of heterogeneous microenvironments enhances the tolerance of SRB to acidic water, metal toxicity and presence of oxygen (Doshi 2006). Finally, Neculita et al. (2007, pp. 7) established that “the most effective design is typically when the substrate is sandwiched between pipes set an inert gravel in the top and bottom of the basin”, consideration that must be addressed in the application of passive treatments in the Mirador project.

Wastes of AMD bioremediation with SRB

Processes of sulphate reduction and metal removal catalyzed by SRB generate hydrogen sulphide (H$_2$S) and metal sulphides (MS), see equation 7 and 8. Hydrogen sulphide that does not react with dissolved metals could be toxic for SRB in concentrations of 477 to 617 mg/L of H$_2$S (Neculita et al. 2007). This extra hydrogen sulphide can be converted to elemental sulphur by action of sulphide-oxidizing bacteria in another reactor like in the THIOPAQ System referenced by Muyzer and Stams (2008, pp. 9).

When metal sulphides precipitate then form sludge that constitutes unstable waste (Johnson & Halberg 2005) that is usually disposed to landfill (Younger & Wolkerdorfer 2004). In high AMD flows like the ones expected in the Mirador Project,
metal precipitate accumulation rates will be high, and consequently frequent sludge removal will be required (Doshi 2006). For example, the Leviathan mine bioreactor removes sludge every year but has an extra pond for storage of sludge that reduces frequency of flushing (Doshi 2006). Ziemkiewicz et al. (2003) recommend placing a settling pond to collect sludge before and after treatment. From this collected sludge, metals can be recovered and reused (Muyzer & Stams 2008). To recover metal sulphides from sludge, the flotation technique planned to concentrate copper in the Mirador Project could be applied. However, this process will contribute to the oxidation of metal sulphides to sulphate, and to the formation of dissolved metals in water, regenerating AMD (Peng et al. 2009). There are other methods that can be more effective and economical. For example, ultrasonic-assisted extraction that shows a 92 to 100 percent of metal separation efficiency (Hanna et al. 2004). Even though, the management of this waste usually accounts for 25-50% of the total treatment cost (Younger & Wolkerdorfer 2004), the possibility to recover commercial grade metals from sludge makes passive systems more economical valuable than conventional treatment plants (Kalin et al. 2006).

In addition, a minimization of soluble and particulate iron entering the passive system is very important for treatment efficiency since release of ferrous iron in the treated effluent to the environment regenerates acidity (Johnson & Hallberg 2005b) as shown in equation 9. To solve this problem, after an anaerobic system, water could be aerated and filtered to remove iron (Younger 2002). Prior to the reaeration of water, sulphide metal sediments must be completely retired from the effluent to avoid a reacidification of water by oxidation of sulphides (Younger 2000). Roughing filters like filter rocks could be used to retain the oxidized ferrous iron Fe(OH)$_3$ that could be dried, stored and reused by local industry (Younger 2000). Finally, as passive treatments final effluent may have a high concentration of biological oxygen demand (BOD), fecal coliform bacteria and manganese, addition of aeration ponds and filtration could contribute to the treatment of this effluent (Doshi 2006).
Regarding the ecotoxicity of the treated effluent, Neculita et al. (2007) established that treatment systems that use SRB decrease the toxicity of mine water. For example, Song et al. (2001) observed 100% of survival of *Daphnia magna* and fathead minnows to the treated water from passive systems using SRB. This fact shows that the treated effluent has low levels of toxicity and can be released to the environment.

**Application of SRB in passive treatments**

Considering the factors that influence the SRB performance, anaerobic wetlands and bioreactors are two of the passive systems that can be evaluated by Mirador Project environmental managers to remediate AMD generated, especially in waste rock dumps. Anaerobic wetlands and bioreactors work to treat net acid water, pH lower than 4.5 (Skousen 1997), more than 1mg/L of Dissolved Oxygen and Ferric (Fe³⁺) (Ziemkiewicz et al. 2003) and high concentrations of dissolved metals (Andrade 2010). Both systems have an organic-rich anaerobic zone called compost that contains a mix of biodegradable materials like manure with recalcitrant materials like sawdust (Johnson & Hallberg 2002). In addition, the income water enters the anaerobic zone thanks to a subsurface flow of AMD into the compost in both systems (Johnson & Hallberg 2002). The principal difference between anaerobic wetlands and anaerobic bioreactors is that anaerobic wetlands are isolated from the atmosphere by standing water or overlying material where sorption or uptake of metals can be achieved by vegetation sorption. On the other hand, bioreactors are collected water drains in anoxic chambers with compost, but not vegetation (Doshi 2006). Moreover, bioreactors are designed as wastewater treatment facilities that may not provide ecosystem services like wetlands provide (Doshi 2006).

In general, passive treatments like anaerobic wetlands and bioreactors have low operational costs, high metal removal rate at low pH (3-6), low maintenance
requirements and minimum energy consumption (Andrade 2010). Further, both systems encourage community involvement by transplanting wetlands vegetation or collecting organic materials as carbon source for SRB (Gusek 2002). Both systems must have a proper drainage system that allow its maintenance, prevent clogging, and reduce the risk of percolation of treated or non-treated water to ground water (Doshi 2006). However, their effectiveness will depend on the land available to construct them, AMD flow and water quality.

**Anaerobic wetlands**

Anaerobic wetlands allow the sulphate and carbonate precipitation catalyzed by SRB, metal uptake and adsorption of wetland plants, and filtration of suspended solids (Gusek 2002). This system consists of wetland vegetation planted in deep (>30 cm), organic rich substrates with presence of SRB (compost), and limestone for acid neutralization (Skousen 1997). In general terms, anaerobic wetlands treat net acidic water with low flow and a design factor of 3.5 g acidity/m²/day, long residential times and large areas to treat large volumes of acidic AMD (Ziemkiewicz et al. 2003). In the USA, anaerobic wetlands have demonstrated to reduce up to 76% of acidity and 80% of Fe concentrations in AMD with a flow of 98L/min and 2400mg/L acidity (Ziemkiewicz et al. 2003). Another anaerobic wetland built near Cochocton, USA, is a long term successful system that has worked since 1985, but its success has depended on the presence of moderate water quality (neutral pH and Fe<100mg/L), periodic maintenance and high vegetative cover (Skousen 1997). According to Kilborn (1996), the estimate cost to build and maintain an anaerobic wetland that treats 60L/min of AMD is 45,350 dollars including excluding design costs. Finally, anaerobic wetlands are subject to seasonal and other variations since they cannot control hydraulic burden on the system or low temperatures (Johnson & Hallberg 2002). For example, a constructed wetland in Scotland decreased its metal removal and acid neutralization rates in winter (Johnson & Hallberg 2005). In mountainous areas like the Mirador Project zone, anaerobic wetlands could be successful in treating AMD with
small flows and moderate water quality (Ziemkiewicz et al. 2003). However, if proper design and application of preventive measures are not well-conducted in the Mirador Project, small flows and moderate water quality of AMD is not likely to happen because of high rainfall rate and topography of the mine site.

**Anaerobic bioreactors**

Sulphate-reducing bioreactors also achieve increase of water pH, less sulphate concentrations in the effluent and metal removal by action of SRB (Jhonson & Hallberg 2005b). These systems are totally enclosed, below ground level, with layers of organic matter (compost), and do not have wetland plants (Jhonson & Hallberg 2005). These systems have successfully treated flows from 4 to 4,800 L/min and moderate to high acidity water with 24 to 36 hours of residence time (Ziemkiewicz et al. 2003). The space required to build a sulphate-reducing bioreactor will depend on the mineral acidity of AMD. Typically, the higher the acidity, the more surface area is required. For treatment of an AMD with a flow of 120L/min and an acidity of 2000 mg/L, it is required 1,2 Ha of surface area (Gusek 2002). According to Kilborn (1996), the estimate cost to build and maintain an anaerobic bioreactor that treats 100L/min of AMD is 56000$ excluding design costs. Bioreactors are also resilient to metal-loading and climate variations, if properly designed. In Canada, at the Ferris Haggarty Copper mine, even though the water temperature could be less than 1°C, the bioreactor functions with a sulphate removal rate of 0,24m/d/m³ (Gusek 2002). In USA, the Leviathan mine bioreactor achieved 95% metal removal, and raising of pH from 3 to 7 despite high flow rates (Doshi 2006). Moreover, anaerobic bioreactors minimize evaporative water losses and control hydraulic burden as they are buried systems (Johnson & Hallberg 2002). Therefore, appropriate engineered and tested bioreactor designs lead their performance despite seasonal variations. In summary, given the topographical and climate conditions for the Mirador Project, the application of SRB in anaerobic bioreactors will be a more effective system than wetlands
for remediation of AMD produced by large scale copper mining. Bioreactors can treat higher flows in more controlled conditions.

FINAL CONSIDERATIONS AND RECOMMENDATIONS

Considering anaerobic bioreactors as a potential option to treat AMD from waste rock dumps in the Mirador Project, it is necessary to consider some operational recommendations to enhance its effectiveness. For example, anaerobic bioreactors must mature to develop adapted and acclimated SRB before throughput of AMD starts. The recommended bacteria source is from sediments in long established AMD streams (Johnson & Hallberg 2005b). To reduce bioreactor flushing requirements and stress on SRB, and enhance precipitation of metals in the settling pond, it is recommended to have “a recirculation mode that combines bioreactor-generated sulfides with base and AMD” (Doshi 2006, pp. 33). Finally, Johnson and Hallberg (2002) considered that technologies like anaerobic bioreactors must be applied in tandem with other remediation alternatives. For example, the technology known as ARUM, which is a composite system of aerobic and anaerobic bioreactors, has shown being an effective treatment option for AMD in subtropical and high altitude locations like the Mirador Project in Ecuador (Johnson and Hallberg 2002). In composite systems, anaerobic bioreactors should be placed before aerobic bioreactors to avoid oxygenation and acidification of AMD and the unnecessary bioprocessing of rain water (Johnson & Hallberg 2005b).

A phased design program is extremely important to apply Sulphate-reducing bioreactors in an industrial scale (Doshi 2006). Laboratory, bench and pilot scale phase increase the performance success of any bioremediation technology (Gusek 2002). Gusek (2001) accurately describes laboratory, bench and pilot tests to design passive treatment systems for AMD. Currently, there are also models like AMD Treat (for coal mine drainage), PHREEQC (geochemical modeling software) and BEST (for bioreactor design) that have been developed, studied and tested to design and evaluate AMD treatment
efficiency (Johnson & Hallberg 2002). As can be seen, it is possible for environmental managers of the Mirador project to develop a phased design program to consider the best treatment options for AMD in order to build an effective large-scale treatment technology.

CONCLUSION

The application of Sulphate-reducing bacteria (SRB) can be feasible for remediation of Acid Mine Drainage generated in waste rock dumps in the copper mine of the Mirador Project, especially if applied in an anaerobic bioreactor. However, the efficiency of this method is mainly controlled by SRB activity, and consequently, composition of AMD, adequate substrate and physical conditions that assure SRB to thrive will have to be considered in the designing process of a treatment system prior its construction and operation in an industrial scale. Bioremediation of AMD with SRB is also feasible since the evaluation of generated wastes and the wastes management mechanisms shows that bioremediation of AMD using SRB may be a sustainable technology for the Mirador project. Finally, it is relevant that a sustainable solution for AMD in Ecuador requires the application of preventive measures to reduce generation of AMD. This research describes some of the options that can be considered when operations start. However, further study needs to be conducted to establish the best pollution prevention measures for the Mirador Project.
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