OPPORTUNITIES OF MICROBIAL GEOCHEMISTRY IN THE BIOREMEDIATION OF MINE-IMPACTED WATER

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Methods for conventional treatment of mine effluent are often costly and, in some cases, ineffective at meeting environmental water quality objectives. Microbially-mediated reduction and biotransformation of inorganic contaminants in mine-impacted water (MIW) can provide an innovative and cost-effective solution. This presentation will discuss two geochemical systems: anaerobic treatment of selenium and nitrate; and anaerobic treatment of sulphate and metals. One type of technology in which these processes can be implemented is a semi-passive biotreatment reactor called a Gravel Bed Reactor (GBR™). A GBR is an engineered biochemical reactor that has been proven to effectively decrease concentrations of metals, metalloids, other inorganics such as nitrate or sulphate, and organic chemicals through microbial and geochemical processes. The chemistry of MIW can vary widely including highly acidic or alkaline pH and elevated and variable concentrations of metals and inorganics. The ability to understand and control the target microbial and geochemical processes, and to adapt to influent chemistry are important considerations in designing and achieving long-term effective performance using biological treatment systems. This presentation will discuss the considerations and opportunities to applying microbial remediation as an innovative and cost-effective treatment technology at Canadian mine sites.

Key Words: biotreatment, mine-impacted water, sulphate reducing bacteria, anaerobic, microbial geochemistry

INTRODUCTION

Innovative biotreatment technologies such as in-situ passive and semi-passive treatment offer significant opportunities for treatment of MIW (USEPA, 2014). Here we discuss semi-passive anaerobic biotreatment processes applied to stimulate nitrate, selenium and sulphate reducing bacteria for biotreatment of MIW.

Mine-Impacted Water

Inorganic constituents that may be present in MIW include metal cations (e.g. cadmium, lead, zinc), transition metals (iron, manganese, copper, chromium, mercury), non-metals (sulfur, nitrogen, selenium), metalloids (arsenic, antimony), and actinides (uranium). These constituents can be present in the geological formation of the target resource and become mobilized through disturbance of material and exposure to the atmosphere and/or aerated waters. The leaching of inorganic constituents can occur in neutral or acidic pH water; with acidic pH typically associated with accelerated rates of metal leaching through acid rock drainage (ARD) that can develop through activities related to ore processing and tailings management (INAP, 2009). Specifically, MIW typically consists of elevated concentrations of sulphate in 1000s of milligrams per liter (mg/L) as a product of iron sulphide oxidation when reduced iron minerals are exposed to oxygen and water at surface. MIW can also consist of nitrogen compounds that are the degradation products from cyanide (a product of gold cyanidation processing), or due to the dissolution of residual waste from nitrate- and ammonia-based explosives. Further, coal mining operations exhibit elevated selenium in MIW from the weathering of seleniferous soils and rocks, often associated with coal-bearing geological formations.

The behavior of elements that are redox-sensitive, meaning their biogeochemical cycles, including partitioning between aqueous and solid phases, are driven by the transfer of electrons, i.e. reduction and oxidation ("redox") reactions. This group comprises major elements (carbon, nitrogen, sulfur, iron, manganese) and trace elements (arsenic, chromium, selenium, uranium). The aqueous fate and transport of non-redox sensitive elements, such as metal cations (e.g., copper, zinc, cadmium, cobalt), may be indirectly impacted by redox processes that control the redox-sensitive elements, including coprecipitation with sulphide minerals or adsorption onto organic matter and iron and manganese (hydr)oxide minerals, and iron sulphides.

Biotreatment of MIW

Passive and semi-passive anaerobic bioreactors range widely in design specifications. Examples include GBRs, in-situ mine pit treatment, engineered wetlands, flow-through bioreactors, and permeable reactive barriers (PRBs) (Higgins et al., 2017; ITRC, 2013; USEPA, 2014). These treatment technologies have common goals to passively or semi-passively stimulate biological and geochemical processes to provide effective water treatment at a reduced capital and operating cost. Semi-passive bioreactors, in comparison to passive bioreactors (e.g. engineered wetlands, PRBs), perform the same role of reducing and immobilizing the constituents of concern in MIW while typically providing a higher control of treatment of water and smaller footprint. Soluble electron donor (e.g. ethanol or methanol)-dosed reactors such as a

GBR, in contrast to compost or organo-rich substrate reactors, provide several advantages including: higher porosity (rock matrix vs compost), controlled reduction rate, and higher rates of reduction and microbial activity (ITRC, 2013).

Active bioreactors are dependent on significant infrastructure, and power-consumptive operation and maintenance controls (INAP, 2009). Examples of active biotreatment for MIW include moving bed bioreactor (MBBR), packed bed bioreactor (PBR), fluidized bed bioreactor (FBR), upflow anaerobic sludge bed (UASB) reactors, and biofilters (ABMet) (ITRC, 2013). The advantages of passive and semi-passive anaerobic bioreactors compared to active bioreactors includes less infrastructure and operation and maintenance costs (USEPA, 2014).

Biotreatment is gaining acceptance at mine sites. Pilot-scale tests have proven that this approach is reliable in the treatment of metals, metalloids, sulphate, and nitrate and the technology is being used at full-scale operations at several locations (USEPA, 2014). As an example, a passive bioreactor in Pennsylvania functioned to treat ARD for eight years during which some maintenance (mixing and addition of reactants) was required to restore the system's permeability (Skousen et al., 2017). At another mine site, in Utah, an active biotreatment system was implemented to reduce and immobilize dissolved selenium (USEPA, 2014). The system was noted to have the ability to treat flow rates from 28 m^3/d to 7,600 m^3/d while meeting effluent discharge levels of 5 µg/L of selenium. Some other examples of bioreactors that employed sulphate reduction for treatment of MIW include (Ness et al., 2014):

- a pilot-scale treatment cell that effectively treated metals (antimony, manganese, and zinc from 80 to 99% removal efficiency) in United Keno Hill Mine in the Yukon (2-year trial) (Ness et al., 2014);
- a full-scale hybrid system including anaerobic bioreactors and wetlands that effectively treated metals (arsenic, cadmium and zinc from 91 to 100% removal efficiency) at Teck Metals Smelter in British Columbia (>5 years) (Duncan et al., 2004);
- a full-scale bioreactor that effectively treated metals (aluminum, copper, iron, nickel and zinc from 86 to 99% removal efficiency) at Leviathan Mine in California (>5 years) (USEPA, 2005); and,
- a full-scale vertical bioreactor and limestone drain that effectively treated aluminum (up to 90% removal efficiency) in Indiana County, Pennsylvania (7 years).

KEY GEOCHEMICAL CONSIDERATIONS FOR BIOTREATMENT

From pilot and laboratory investigations on biotreatment, circumneutral pH (e.g. 6 to 8 standard units) has been found to be the target condition for nitrate, selenium and sulphate reduction. Although many species that are active in biotreatment systems may exhibit the ability to tolerate extreme pH, these conditions are generally found to create stresses and sub optimal treatment performance, likely attributed to interference with the enzymatic function of the microbes (Garcia et al, 2001). Anions such as nitrate, nitrite, phosphate, chlorate, perchlorate and sulphate can be biologically reduced (broken down into their mineral elements) under anaerobic conditions in the presence of natural or added electron donors (carbon substrates). Redox-sensitive metals can be reduced under appropriate redox conditions from soluble forms to insoluble forms that precipitate from solution. Heavy metals present in aqueous forms as divalent cations (e.g., zinc, copper) can be immobilized by adsorption by shifting the pH to the alkaline range. Selected metals and divalent cations (e.g., arsenic, zinc) can be precipitated as metal sulfides through inducing microbial sulphate reduction to sulfide through addition of electron donor when sulphate is present or added.

Variability in major ion concentrations in MIW is likely. An engineered bioreactor should be monitored to balance the source of electron donor with electron acceptors (inorganic constituents including dissolved oxygen, nitrate and selenate), to achieve the ORP that is required to support target bacterial populations.

Electron donor dosing concentration can be calculated based on stoichiometric electron (e^-) demand, expected removal efficiency rate, and safety factor (SF). An example dosing calculation is provided based on methanol as the electron donor and reduction of dissolved oxygen and nitrate, using the following expression:

(Eq. 1)

$$MethanolDose\left(\frac{mg}{L}\right) = \left(\frac{C_{DO}}{1.5} + \frac{C_{NO3-N}}{0.525}\right) \times SF$$

where: C_{DO} – dissolved oxygen concentration (mg/L)

$C_{NO3\text{-}N}$ – dissolved nitrate concertation (mg/L as N)

SF – safety factor

This calculation assumes an e^- demand requirement to consume DO and reduce nitrate to nitrogen gas (N_2). The reaction for complete denitrification to N_2 is expressed as:

$$2NO_3^- + 10H+ + 10e^- = N_2 + 2OH^- + 4H_2O \qquad \text{(Eq. 2)}$$

And the methanol oxidation reaction is expressed as:

$$CH_4O + H_2O = CO_2 + 6H^+ + 6e^- \qquad \text{(Eq. 3)}$$

The net electron demand is then calculated. The safety factor is used to account for heterogeneities in electron donor distribution and variability in microbial zones expected in a field system. An ORP that is outside of the targeted range may occur through under- or over-dosing of electron donor.

APPLICATIONS OF SEMI-PASSIVE ANAEROBIC BIOTREATMENT

Application 1: Nitrate and Selenium Reduction

Nitrate/nitrite and selenium (i.e., selenate and selenite) are compounds that can be treated under sub-oxic conditions usually associated with nitrate and iron reduction. Nitrate reducing bacteria are responsible for the reduction of nitrate to nitrite and nitrite to nitrogen gas and are commonly used to remove excess nitrate in agricultural effluent and waste water (Christianson, 2011). Similarly, several otherwise aerobic species, examples including some *Pseudomonas* and *Thiobacillus denitrificans*, can convert nitrate and nitrite to elemental nitrogen gas (Vaccari et al., 2006). Dissimilatory selenium reduction, which is the reduction of selenate/selenite to elemental selenium is also carried under nitrate and iron reducing conditions by selenium reducing bacteria (Nancharaiah & Lens, 2015).

This has led to the application of denitrifying reactors at various mine sites (Herbert et al, 2014). Similar to nitrate reducers, selenium reducers need an electron donor (e.g. carbon source) to reduce selenate/selenite to elemental selenium which means the same reactor fed with a single electron donor can remove both selenium and nitrate (Geosyntec 2010).

A conceptual representation of the microbially mediated processes responsible for the remediation of nitrate and selenium are presented in Figure 1.

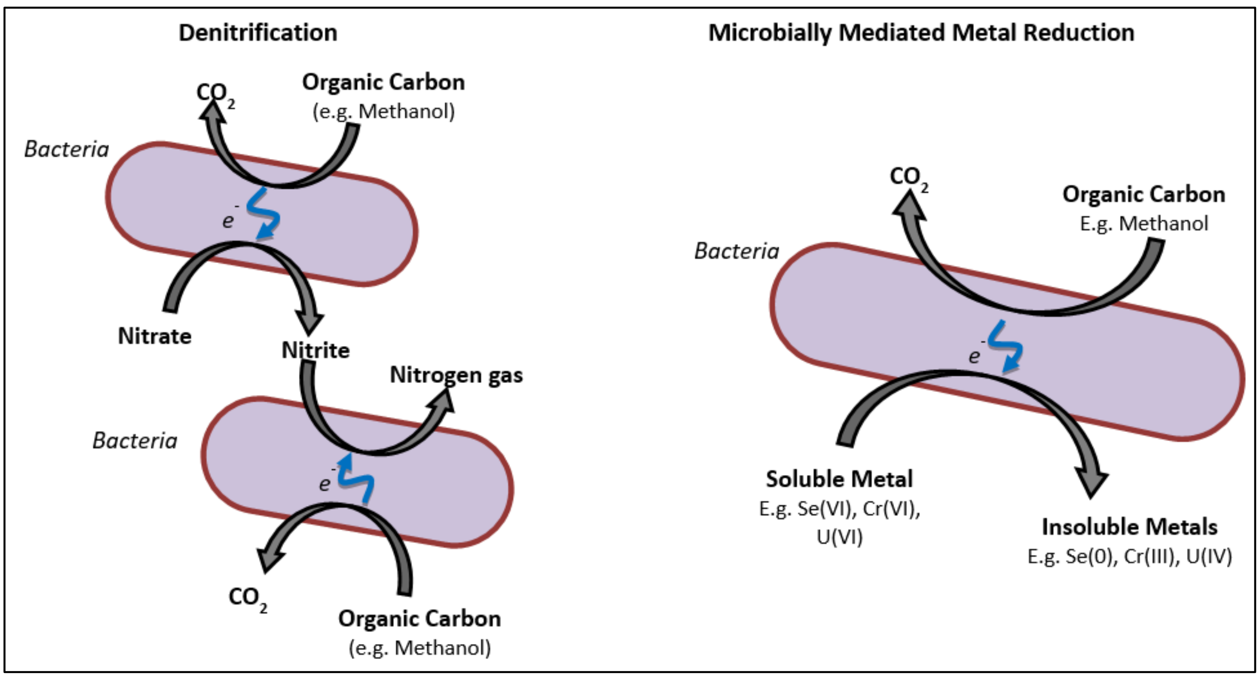

Figure 1: Conceptual Representation of Microbially Mediated Processes for Denitrification and Reduction of Metals

To remediate nitrate, electron donors are added to the influent water to promote the biological reduction of nitrate via nitrite to dinitrogen gas, a process referred to as nitrate reduction or denitrification. To remediate selenium, electron donors are added to the influent water to promote the development of appropriate geochemical conditions that result in the biological reduction of selenate (SeVI) and selenite (SeIV), the predominant oxidized forms present in surface waters, to elemental selenium (Se0), which is generally immobile. Elemental selenium precipitates and can therefore be retained within biomass as solid surface precipitates within the media bed of a bioreactor. These biological reactions are well documented in literature (Sobolewski, 2005; Knotek-Smith, 2006; Esawayah et al., 2016; and Deen et al., 2018). The nitrate- and selenium-reducing bacteria that mediate the biological reactions are naturally found in geographically diverse, pristine and contaminated waters (Nancharaiah and Lens, 2015).

Case Study: Biotreatment of Nitrate and Selenium in Coal Mine Runoff

A GBR was implemented in 2012 to treat MIW from coal mine runoff (Mancini, 2019) to concentrations below target treatment levels (e.g. USEPA water quality guidelines for the protection of freshwater aquatic life; 5 µg/L total selenium and 10 mg/L for nitrate) using anaerobic biological reduction. A GBR is an in-situ anaerobic semi-passive biochemical reactor that has been operated for pilot and full-scale industrial and mining applications. GBRs can treat a variety of water quality issues, including acidity, metals and metalloids, inorganics, and organic chemicals (Geosyntec 2003, 2005a, and 2010). The GBR design consists of an engineered bed of gravel or crushed rock media within which, when soluble, biologically available electron donors and trace nutrients are dosed along with influent water, a biofilm of native microbes grows and adheres to the rock surfaces. Nitrate- and selenium-reducing bacteria grow within the targeted conditions of the bioreactor. These bacteria can be sourced from the environment surrounding the impacted water, often having naturally acclimated to the selenium- and nitrate-enriched waters (Nancharaiah and Lens, 2015). The aim of GBR treatment of dissolved total selenium is to provide reduction of selenate and selenite to elemental Se (Se0), which is generally immobile and retained in the reactor bed within the biomass and solid surface precipitates. The reactor is monitored to balance the source of electron donor with electron acceptors (inorganic constituents such as nitrate and selenate), to achieve the ORP that is required to support the growth of the target bacterial populations.

The GBR was installed beneath a parking lot at the facility to accommodate limited available space. The field application was designed to be capable of treating flows up to 550 m^3/day, although during operations it averaged a flow rate of approximately 270 m^3/day. The field application ran from March to May 2012 and was intended to continue operating, however the coal company went into bankruptcy and GBR operations, although successful, were suspended. The seep source water had an aerobic ORP averaging 55 mV, DO averaging 4.8 mg/L, neutral pH (7.0), a temperature ranging from 13 to 18 ºC, selenium ranging from 15 to 25 µg/L, and nitrate averaging 6 mg/L as N.

The geochemical controls for this GBR included:

- Electron donor dosing system to add citric acid or acetic acid to the influent of the GBR.
- ORP probe at the outlet of the GBR to monitor effluent ORP to provide feedback to guide the electron donor dosing rate (adjustments to the dosing rate were conducted manually).
- Associated controls and monitoring equipment for the GBR.

Application 2: Sulphate Reduction and Metals Precipitation

In the context of ARD, which is a common occurrence at mine sites that occurs due to the stockpiling of sulfidic rock waste on surface, acidophilic bacteria catalyze acid-formation by the oxidation of iron sulphides contained in the stored geologic material. However, the activity of bacteria can also be harnessed in reductive pathways, providing the potential to reverse the acid-generating process of sulphide oxidation to sulphate reduction. SRB catalyze the reduction of sulphate to sulphide, producing carbonate alkalinity and soluble sulphide, according to the following reaction:

$$2\ CH_2O + SO_4^{2-} \Rightarrow 2HCO_3^{-} + H_2S \quad \text{(Eq. 4)}$$

Soluble sulphide reacts with metals to form metal sulphides, thus removing soluble metals from the mine influent according to the following:

$$H_2S + M^{2+} \Rightarrow MeS\downarrow + 2H^{+} \quad \text{(Eq. 5)}$$

Where Me can be a metal cation (e.g. cadmium, lead, zinc) or transition metals (e.g. iron, manganese, copper, chromium, mercury).

SRB populations are ubiquitous in the natural environment, and therefore are easily introduced to bioreactors, (Keller et al., 2011; Neculita et al, 2007; and Stottmeister et al., 2003). SRB activity within a bioreactor requires sufficient electron donor (carbon, added as an organic carbon solution) to facilitate the biologically mediated electron transfer to other electron acceptors such as dissolved oxygen, nitrate and ferric iron to create sulphate reducing conditions. For this reason, long-term treatment with a bioreactor may require the electron donor to be replenished.

Opportunity: Sulphate Mediated Metal Reduction in Semi-Passive Bioreactor

The process of sulphate reduction, production of H_2S, and subsequent precipitation of metals as metal sulphides has been implemented in several biotreatment applications such as engineered wetlands or biochemical reactors using organic substrate as the reactor media bed (Lenz et al., 2008; Baldwin et al., 2015; Mirjafari et al., 2015; Mirjafari et al., 2016; Skousen 2017; Rezadehbashi and Baldwin, 2018; and Nielsen et al., 2018).

The use of SRB and metal sulphide reduction in a GBR has not yet been investigated. However, as demonstrated in the case study presented here in use of a GBR for treatment of nitrate and selenium from coal mine runoff (Mancini, 2019), we propose that a semi-passive bioreactor with a soluble electron donor and rock matrix, like a GBR, may have the potential to provide the geochemical controls required to maintain optimal SRB growth, flow, and treatment, while providing large porosity with lower risk of clogging from mineral precipitates and biomass.

Geosyntec is currently conducting research using a SRB microbial culture that has shown promise for biogeochemical transformation of metals. This in-situ sulphate mediated metal remediation (i-SMMR™) process is being developed for a broad range of applications including treatment of mine and industrial process water.

Proof of concept bench-scale treatability testing and optimization of the i-SMMR process are in progress and will include both batch and column studies. The batch studies, which are currently in progress, are assessing the application of an enriched SRB culture under both high and low pH conditions. The SRB culture was enriched from a mixed consortium of bacteria (KB-1®) that originally included dechlorinating bacteria, SRBs, fermentative bacteria and methanogens that have been widely used for bioremediation of chlorinated solvents in groundwater (Major et al., 2002). The base culture has been enriched for SRB through addition of sulphate as an electron acceptor rather than chlorinated solvents.

Planned studies will entail bench-scale rock matrix columns (of approximately 50-centimeter (cm) length by 10-cm width) that will be filled with crushed rock to approximately 4-cm diameter. The columns will receive variable sources of MIW, be inoculated with the i-SMMR culture, and dosed with electron donor. A primary objective of column testing will include the evaluation of different MIW influents to investigate under what conditions effective constituent removal can be maintained. Additional questions to be addressed include:

- What are the upper and lower pH thresholds?
- What range of metal concentration is tolerated and treated?
- What are the optimal electron donor dose rates and hydraulic retention times?
- Are the biomass and metal precipitates retained on the crushed rock surface?

Future research objectives may include further stress testing to understand the effect on the i-SMMR performance due to fouling from metal precipitates and biomass, and low temperature. The SRB population in the i-SMMR culture will be quantified using SRB quantitative polymerase chain reaction (qPCR) prior to and during column testing.

CONCLUSION

Here we have discussed two biological treatment processes that provide promise for effective treatment of nitrate, selenium, sulphate and metals. The chemistry of MIW can vary including highly acidic or alkaline pH and elevated and variable concentrations of metals and inorganics. Continued laboratory and field-scale investigations are underway to optimize selenium, nitrate, sulphate and metals treatment through anaerobic semi-passive reactors. This will help to further refine our understanding of site-specific constraints and opportunities for these types of systems in the Canadian mining sector. The ability to understand and control the target microbial and geochemical processes, and to adapt to influent chemistry are important considerations in achieving long-term effective performance using biological treatment systems.

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