A Strategy to Simulate Indigenous Bacterial Communities to Effectively Remediate Mine Drainages

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Abstract

Drainages from mining operations frequently contain elevated levels of contaminants of concern (CoC) . Indigenous adapted bacterial communities are characterized and their ability to reduce many CoC are showcased. Each contaminated site consists of a distinct prokaryotic community that in turn requires a specific C:N:P balanced environments to contribute to site remediation. This balanced bioremedial strategy is managed both for in situ or fix-filmed bioreactors, using electron donor selection and ratios, redox potential, and hydraulic retention times. These communities can effectively treat elevated levels of hexavalent chromium (10 mg/L), nitrate (110 mg/L), and sulfate (1 250 mg/L) in a one-pot balanced system.

Keywords: Bacterial Diversity, C:N:P Stoichiometric Balance, In Situ Treatment, Fixed-Film Bioreactors, Redox Ladder

Introduction

Mining operations in South Africa demand a high volume of water usage during operations and tailing deposits. These water volumes often leach or drain causing the mobilisation of elevated contaminants of concern (CoC), including dissolved metals, metalloids, nitrate, and sulfate compounds. In most mine drainages, these CoC are primarily in reducing chemical forms and act as essential oxidants for organic carbon and other reducing chemicals (Fowler *et al.* 2013), where oxidation states are more toxic to the environment and human end receptors.

In recent years in South Africa, concerns have developed around poor managing practices in disposing of CoC. A mirid of treatment options is deployed that include filtration, absorption, and chemical reduction. Each treatment has specific benefits, but often capital and operating costs are high, and many could produce additional, often still hazardous, by-products. Biological reduction of CoC using indigenous bacteria can be an environmentally sound alternative technology that lowers operational costs with less or no hazardous by-products. Bacteria metabolic reactions often use CoC as a terminal electron acceptor, ultimately precipitating compounds as insoluble hydroxides or metal-sulfides (Cason *et al.* 2017; Peng and Zhu 2006).

A better understanding of the biochemical cycles of contaminates, including carbon utilisation, can identify cooperative metabolic functioning within bacterial communities. This metabolic functioning and bacterial community compositions can hold the key to developing more sustainable and advanced biological treatment systems. Using the knowledge and characterization of strategic management practices, each specific environments' indigenous microbes can become powerful bioremediation tools (Lau *et al.* 2016).

This paper deals with the management and stimulation of high metabolic rates of biomes by creating C:N:P stoichiometrically balanced environments, while dominance within species can naturally attenuate in these circumstances. This is demonstrated both, in situ and in fixed-film bioreactors, to treat contaminated mine drainages on-sites. These remedial strategies can remove several contaminants, including hexavalent chromium (Cr_{6+}), nitrate, and sulfate while improving the overall water quality. Using targeted metagenomics, the microbial community and unique biogeochemical cycles are characterised to balance the C, N and P cycles to environmental geochemical conditions.

Methods

Water quality analysis and site selection

Two sites with different concentrations of the selected CoC were sampled. Analyses were conducted at accredited laboratories, using anion analysers, ICP-MS/OES, and auto-titration methods, including benchtop physicochemical analysis. The study source sites are in the KwaZulu Natal (Site A) and North West (Site B) provinces of South Africa. Site A is a chromium process facility that mainly operates leather tanning, while Site B is a chromium smelter that actively mines and processes chrome ore. Activities on both sites resulted in ground and surface water contamination with Cr_{6+} and nitrate.

Microbial diversity and metabolic capabilities

Extracted and purified DNA (Lau *et al*. 2014) was analysed using 16S targeted metagenomic sequencing on an Illumina MiSeq system. Sequence reads were analysed using QIIME 2 (https://qiime2.org) (Bolyen *et al*. 2018). Taxonomy was assigned to amplicon sequence variants (ASVs) against the SILVA 132 99% OTUs reference sequences (SILVA) (Quast *et al*. 2013; Yilmaz *et al*. 2014). The functional potential was predicted using FAPROTAX.

Microcosm studies

Using the data from section 2.1 the N (Potassium Nitrate) and P (Di-Potassium Hydro-orthophosphate) supplementation were balanced. The optimal assimilation and utilisation of electron donors, simple - and complex carbon sources, were assessed for the indigenous bacterial communities. These rates and geochemical data sets are used to calculate the specific C:N:P ratio, while balancing metabolic function to sustain a chosen redox condition for treatment by calculated molar ratios (Appendix – Table 1 & 2). For excess and balanced ratios, the stoichiometrically balanced mixtures were inoculated into site water samples and incubated in 250 mL Scott bottles, at 25 °C, for five days and 20 days. The depletion in dissolved oxygen (DO) concentrations were measured for each incubation period. Refinement of concentration was done with continuous flow measuring reduction of CoC while optimising donor versus acceptor rations.

In situ design

The treatment system for site A was implemented directly at the site of the contaminant (*in situ*) (Appendix – Figure 1). Injection – and extraction boreholes, which is 100 meters apart, were used as the treatment site. Initially, crucial information of the heterogeneous matrix between the selected boreholes was studied for the treatment implementation design and selection. An EC profile of the injection borehole was created to determine the primary fracture depth. At this depth, a stoichiometrically balanced electron donor (Emulsified vegetable Oil – EVO) mixed with groundwater, was injected. This amended groundwater made a final solution containing 2% by volume. A subsurface groundwater pull was created, by withdrawing groundwater from the bottom extraction boreholes, to control the treatment hydraulic retention time (HRT).

iWater treatability plant

A pump-and-treat semi-active strategy was implemented at site B (Appendix – Figure 2). iWaters' proprietary treatability plant design consists of modular fix-filmed bioreactors (5 000 L, each) and treats 80 000 L per day. Inlet site water was continuously dosed with a balanced electron donor (sodium acetate) mixture. All operations, in terms of electron donor dosing concentrations,

redox conditions, and HRT were controlled remotely with a Program Logic Controller (PLC). The PLC also allowed for individual parameters (e.g., EC, flow rate and quantity, pH, and ORP) monitoring and recording. Once reducing conditions were established within the bioreactors, the electron donor was incrementally lowered until the minimum donor requirement was determined empirically. The final treatment stage involved removing any residual organic carbon through various filtration methods (activated carbon) and sterilisation techniques (chlorination).

Results and Discussions

Site selections

It is crucial to understand each contaminated site geochemistry data to design an effective tailored bioremedial strategy (Williams *et al.* 2014). The treatment focuses on creating an optimal environment for the indigenous bacteria to select and stimulate selected community members to reduce site pollutants. Due to the repeated processes of soaking raw hides and wringing them out during the leather tanning process, large amounts of wastewater is created. If this wastewater is not properly managed is often leads to surface and groundwater contamination. Site A contained fluctuating contamination levels of Cr_{6+} and nitrate of around 10 mg/L, and 35 mg/L, respectively. Site B contained similar concentrations of Cr_{6+} (8 mg/L) levels, but with additionally elevated concentrations of nitrate (110 mg/L) and sulfate (1 250 mg/L). The solid products obtained from the smelting of ferrochrome are metal, slag, and dust. This slag by-product is often disposed of as tailings storage facilities (TSF). Slag is regarded as harmless, as the chromium is predominantly present as trivalent chromium (Cr_{3+}). However, there exists a great deal of controversy regarding the possibility of oxidation of Cr_{3+} to Cr_{6+}. Thus, if the TSF is not properly manage or lined it often result in Cr_{6+} leachate into the surface and underground water. In addition, a great environmental risk also lies in the sludge from the gas cleaning system. This is particularly the case for open-top furnaces where chromium is readily oxidised to Cr_{6+} in the off-gas dust. The remaining parameters, of both sites, does not raise any concerns (Appendix – Table 5 & 6).

Microcosm studies

It is essential to understand the indigenous bacterial community's optimum electron donor type and concentration demand using a microcosm study (Lau *et al.* 2016). To determine this balance one can ensure that enough free energy is available for the desirable reductive metabolisms to functions. Site A's indigenous bacterial community is more selective towards a complex long-chain carbon source, with minimum phosphate supplementation required, while site B's community prefers a simple carbon source, also with minimum phosphate supplementation (Appendix – Table 3 & 4). Both indigenous bacterial consortia require similar electron donor concentrations to reduce Cr6+ and nitrate. Site B's consortia interestingly can also reach sulfate reduction if the donor concentration is increased, as it can improve this site's water treatment.

On-site treatment and bacterial composition

Treatment strategies of both sites were implemented as described in sections 2.4 and 2.5. Before and during the treatments, the

Table 1 *Geochemical dataset of inorganic parameters during in situ treatment on site A.*

Elemental concentration of heavy metals (μgL^{-1})					
Determinants	Units	Initial	Injection borehole	Extraction borehole	Extraction borehole
Total alkalinity as $CaCO_3$	mg/L	254	472	198	452
pH		7.04	7.66	8.29	7.05
ORP	mV	+148	-10	-68	-15
Chromium as Cr_{6+}	mg/L	9.87	< 0.02	1.23	< 0.02
Nitrate as N	mg/L	35	< 0.35	< 0.35	< 0.35
Sulfate as SO_4^{2-}	mg/L	120	129	133	38.7

change in bacterial diversity was analyzed and their metabolic functionality could be correlated to the water chemistry changes. At site A, the optimum redox conditions for nitrate, and consequently Cr_{6+}, reduction was reached after 30 days of treatment implementation. Due to the created stoichiometrically balanced environment, the indigenous bacteria were able to reduce both Cr_{6+} and nitrate by 99.9% even after one year of treatment (table 1). By using the groundwater pull-push method, the electron donor was effectively migrated through the entire treatment site. In the initial phase of treatment, the extraction borehole showed delayed Cr_{6+} reduction due to the slower migration of electron donor, illustrate the importance of an engendered balance environment. A major advantage of bacterial metabolic activity is the creation of water alkalinity, in the form of calcium carbonate ($CaCO_3$), that improves the overall buffering capability of a water source.

Before and after one year, the bacterial composition, up to the genus level, was evaluated between the different boreholes. All boreholes were initially dominated by Acinetobacter (39%), Rhodobacter (11%), Prosthecobacter (8%), Acidovorax (8%) and other minor representative groups (MRC) (fig. 1). Figure 1 shows shifts in bacterial communities throughout the treatment site over the course of a one-year treatment. The treatment site is now mainly dominated by (i) Legionella (25% – Pathogenic), Pseudarthrobacter (16%) and (ii) Novosphingobium (56% – aromatic compound degradation) between the injection, and extraction boreholes, respectively. What is of note is that both the injection and extraction boreholes had a dramatic increase in minor representative groups (54% and 30%, respectively). The data also shows that none of the genera co-exists between the different boreholes, suggesting no flow-through of the dominant bacteria through the site, however, Cr_{6+} and nitrate reduction is still evident in all the boreholes.

Interestingly, even though the communities of the separate boreholes are different after treatment, the functional annotation is more closely related, compared to the initial functionality of the communities. Nevertheless, after one year of treatment, the nitrate reduction metabolism functionality seems to be present in all the boreholes (>5%).

At site B, optimum operations and CoC reduction were reached at an HRT of 8 h at 80 000 L per day treated. Using sodium acetate continued dosing, effective Cr_{6+} and nitrate reduction were achieved, with a 99%

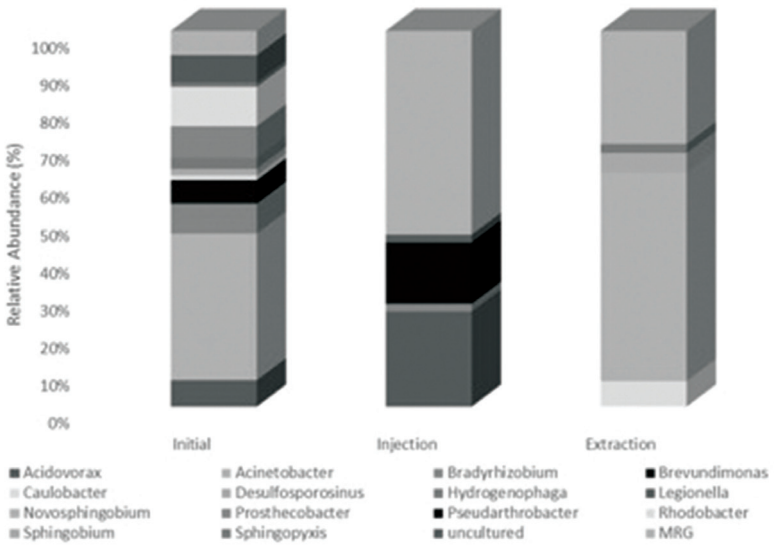

Figure 1 *Taxonomic information (genus level) based on 16S rRNA gene sequences of the indigenous bacteria during Site A's treatment.*

Table 2 Geochemical dataset of inorganic parameters during a pump-and-treat plant treatment on site B. The data represents the final parameters after one year of treatment.

			Nitrate reduction strategy	Sulfate reduction strategy
Determinants	Units	Plant inlet	Final sample	Final sample
Total alkalinity as $CaCO_3$	mg/L	229	692	1425
pH		7.85	7.95	7.36
ORP	mV	+87	-73	-325
Chromium as Cr_{6+}	mg/L	8.19	0.42	< 0.02
Nitrate as N	mg/L	110	0.61	< 0.35
Sulfate as SO_4^{2-}	mg/L	1244	1013	66

reduction of both parameters. The water quality dataset (table 2) represents the final analysis perform one year after treatment implementation. Note that only specific redox metabolism is active at this balanced electron donor mixture, for example, nitrate respiration and denitrification.

In contrast, electron acceptors like sulfate are not metabolised. As an additional, to showcase the treatment strategy capability, the HRT tempo was lowered, and the electron donor ratio changed to achieve a 95% sulfate reduction from 1 244 mg/L (table 2). During this treatment strategy, the overall balance of the water chemistry was improved where alkalinity was increased, and parameters such as total hardness, iron, potassium, and zinc were decreased.

Bacterial compositions of the treatment plant inlet water (In) and within the reactors (Out) was studied at a genus level. The inlet dominance of Pseudomonas (53%), Exiguobacteria (15%), Rhizobium (8%), Stenotrophomona (6%) change to Pseudomonas (65%), Stenotrophomona (3%), Exiguobacteria (2%), and interesting minor representative groups (13%), within the bioreactors (Fig. 2). It is interesting to note that even though Site A and B's indigenous communities are similar, at the phylum level, it demands different electron donor composition. This indicates the importance of the microcosm study to define treatment strategies for each site.

The functional annotation analysis shows that the inlet water's bacterial community

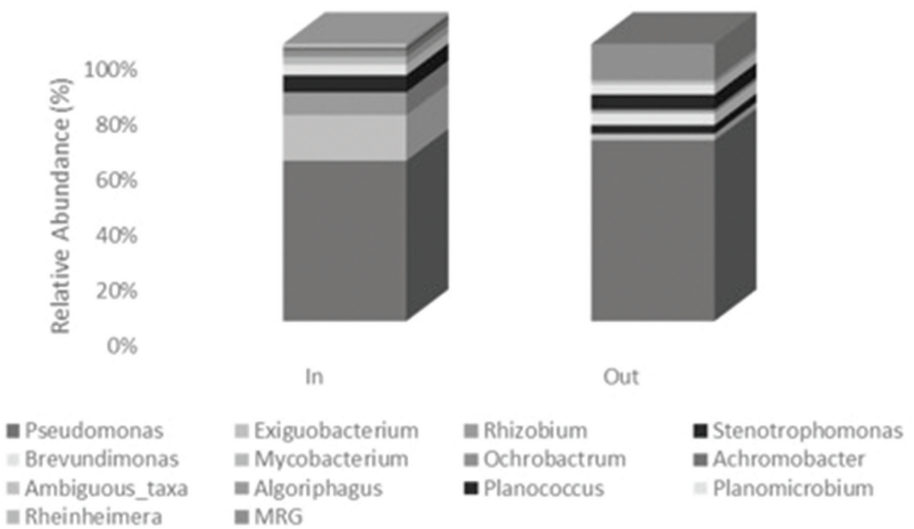

Figure 2 Taxonomic information (genus level) based on 16S rRNA gene sequences of the indigenous bacteria during Site B's treatment.

and those within the bioreactor are specialised for nitrate metabolic potentials (Appendix – Figure 3). The fact that this functional annotation is accelerated within the bioreactors illustrate the importance of a balanced energy flux and that natural attenuation will struggle without supplementation.

At Site A, there appears to be a flow-through of the minor representative groups, causing a specialised functionality towards nitrate, and consequently Cr_{6+}, reduction towards boreholes further away from the injection site. Whereas for site B, the data shows that minor representative groups are only present in the bioreactors. It is essential to mention that several studies confirmed that less dominant bacterial groups support the major communities (Hemme *et al*. 2010; Hugenholtz *et al*. 1998; Tanner *et al*. 1998; Wang *et al*. 2013) and that they are essential for successful reduction of CoC. This means the minor representative groups, present at both sites A and B, should not be eliminated from the indigenous community composition and should be carefully managed.

Conclusions

Anthropogenic activities on both sites have led to groundwater contamination with COC, mainly Cr_{6+}, nitrate, and sulfate. The data sets generated in this paper shows that even if bacterial community compositions are similar, indigenous bacteria from separates environments have different metabolic capabilities and electron donor requirements. This illustrates the importance of understanding the geochemical data and correlating it to the C:N:P ratios of the environment. Through creating a stoichiometrically balanced environment, that is correlated to the free energy flux requirements of the indigenous bacterial communities, dominant bacterial species was allowed to naturally attenuate. Interesting minor representative groups showed an important functionality to unsure that these dominant communities can successfully reduce CoC. This paper illustrates that adapted bacteria acclimate to every change in chemical composition. Thus, it is essential to identify all the informational gaps of each site to tailor the remediation strategy since no aspect from geochemistry, bacterial composition, and their metabolic capacities stand alone. All the generated knowledge will optimise bioremediation strategies to extend to treatment implementation beyond the CoC levels illustrated in this paper.

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Appendixes

Table 1 *Calculated molar ratios of stoichiometric electron donor demand for redox reactions of anaerobic metabolic electron acceptors, using acetate.*

Electron acceptor	Balanced reaction	Molar ratio
Oxygen	$CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O$	½
Nitrate	$5CH_3COOH + 8NO_3^- + 8H^+ \rightarrow 10CO_2 + 4N_2 + 14H_2O$	5/8
Chromate	$3CH_3COOH + 8CrO_4^{2-} + 40H^+ \rightarrow 6CO_2 + 8Cr^{3+} + 26H_2O$	3/8
Sulfate	$CH_3COOH + SO_4^{2-} + 2H^+ \rightarrow 2CO_2 + H_2S + 2H_2O$	1/1

Table 2 *Calculated molar ratios of stoichiometric electron donor demand for redox reactions of anaerobic metabolic electron acceptors, using emulsified vegetable oil – EVO.*

Electron acceptor	Balanced reaction	Molar ratio
Oxygen	$C_{57}H_{98}O_{12} + 75.5O_2 \rightarrow 57CO_2 + 49H_2O$	1/77
Nitrate	$C_{57}H_{98}O_{12} + 50.34NO_3^- \rightarrow 57CO_2 + 25.17N_2 + 49H_2O$	1/50
Chromate	$3C_{57}H_{98}O_{12} + 25.75SO42- \rightarrow 57CO_2 + 25.75H_2S + H_2O + 44.5H$	3/77
Sulfate	$C_{57}H_{98}O_{12} + 37.75CrO42- \rightarrow 57CO_2 + 37.75Cr^{3+}\ 49H_2O$	2/77

Table 3 *Microcosm study results of site A's indigenous bacteria oxygen demand from different electron donors.*

Electron donor	DO_0 (mg/L)	DO_5 (mg/L)	DO_{20} (mg/L)	Oxygen demand value
Control	3.40	3.34	-	0
Sodium acetate	3.37	0.99	-	0.48
Sodium acetate + Phosphate	3.48	3.23	-	0.19
EVO	3.48	-	0.08	0.75
EVO + Phosphate	3.45	-	0.23	0.68

Table 4 *Microcosm study results of site B's indigenous bacteria oxygen demand from different electron donors.*

Electron donor	DO_0 (mg/L)	DO_5 (mg/L)	Oxygen demand value
Control	5.12	5.08	0
Sodium acetate	5.22	3.28	0.39
Sodium acetate + Phosphate	5.19	3.35	0.29
Glucose	5.17	4.14	0.17
Glycerol	5.17	4.68	0.08

Table 5 Geochemical dataset of inorganic parameters during in situ treatment on site A.

			30 days after injection		1 year after injection
Determinants	Units	Initial	Injection borehole	Extraction borehole	Extraction borehole
Total alkalinity as $CaCO_3$	mg/L	254	472	198	452
Total dissolved solids	mg/L	595	624	366	600
Total hardness	mg $CaCO_3$/L	171	286	114	314
pH		7.04	7.66	8.29	7.05
ORP	mV	+148	-10	-68	-15
Chromium as Cr^{6+}	mg/L	9.87	< 0.02	1.23	< 0.02
Nitrate as N	mg/L	35	< 0.35	< 0.35	< 0.35
Otrhophosphate	mg/L	4.0	< 0.03	< 0.03	< 0.03
Sulfate as SO_4^{2-}	mg/L	120	129	133	38.7

***Table 6** Hydrochemical dataset of inorganic parameters during a pump-and-treat plant treatment on site B. The data represents the final parameters after one year of treatment.*

			Nitrate reduction strategy	Sulfate reduction strategy
Determinants	Units	Plant inlet	Final sample	Final sample
Total alkalinity as $CaCO_3$	mg/L	229	692	1425
pH		7.85	7.95	7.36
Total dissolved solids	mg/L	3406	2798	3486
Total hardness	mg/L	1690	1480	257
ORP	mV	+87	-73	-325
Chromium as Cr^{6+}	mg/L	8.19	0.42	< 0.02
Nitrate as N	mg/L	110	0.61	< 0.35
Otrhophosphate	mg/L	0.61	5.92	2.26
Sulfate as SO_4^{2-}	mg/L	1244	1013	66
Iron – total	mg/L	2.40	< 0.01	< 0.01
Potassium	mg/L	23	3.91	4.3
Zinc	mg/L	4.12	0.2	< 0.01

Figure 1 *In situ treatment implemented at site A, located in the KwaZulu Natal province of South Africa.*

Figure 2 *Semi-active pump and treat plant implemented at site B, located in the North West province of South Africa.*

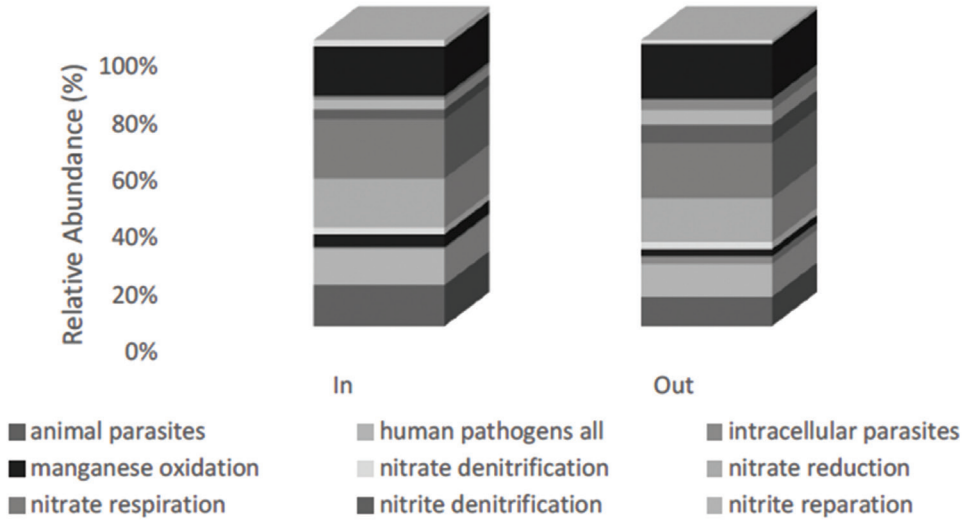

Figure 3 *Functionality assignments of the indigenous bacteria during Site B's treatment.*