ARD passive treatment using waste mussel shells- Part II: System autopsy and biogeochemical investigations

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ABSTRACT

Acid rock drainage (ARD) impacted waters are a worldwide concern for the mining industry; both active and passive technologies are employed for the treatment of such waters. System autopsy and biogeochemical investigations are presented here for a novel, fully operational, mussel shell bioreactor used to treat low pH effluents elevated in Al, Fe, Ni, and Zn. This bioreactor is within the Whirlwind catchment at the Stockton Coal Mine, located on the West Coast of New Zealand. The Whirlwind bioreactor utilizes a mussel shell matrix, which is similar in concept to a vertical flow successive alkalinity producing system (SAP), for the passive treatment of an ARD seep derived from acid-forming overburden. The bioreactor has been in operation since September 2012; since construction it has effectively treated ≈99% of metals and continues to neutralize the influent acidity resulting in a circum-neutral effluent.

To understand the performance and functionality of the bioreactor a systematic approach has been undertaken to investigate the bio-physico-chemical dynamics of the system. An autopsy was performed in May 2013 (after 8 months operation) and in June 2014 to better understand the contributing biogeochemical mechanisms occurring. Within the bioreactor exists a complex redox gradient controlled by first order reaction kinetics, which are defined by both its physico-chemical environment (adsorption & precipitation) and microbiology (*i.e.* Complex Fe and S cycling). A horizontal and vertical grid pattern was used for sampling across, and within, the bioreactor. Bio-physico-chemical investigations to date indicate homogenous treatment across the bioreactor with no restrictions to vertical flow even with continued sediment loads to the system.

The work describes a comprehensive investigation of the chemistry, microbiology, and functionality of this novel passive treatment approach and sheds light on the functionality and performance for global technology transfer.

Keywords: geomicrobiology, mussel shell bioreactor, passive treatment, Acid rock drainage, metals.

INTRODUCTION

Acid Rock Drainage (ARD) within the mining industry is associated with the oxidation of sulfide minerals within exposed overburden and other waste rock sources including tailings. Unfortunately it remains an ongoing legacy for some mining operations worldwide. In the United States approximately 200,000 ARD sites exist; in Europe there are over 5000 km of watersheds impacted by direct ARD effluents some predating 1000 years (Lieβmann, 1992; Hochella *et al.,* 1999; Bakers and Banfield 2003; Schippers et al. 2010). The exposure of sulfide minerals to water and oxygen in the presence of bacteria (*e.g. Thiobacillus ferrooxidans*) will result in solutions with increased loads of dissolved metals, sulfate, and net acidity. The Brunner Coal Measures (BCM) associated with the Stockton opencast coal mine in New Zealand has a legacy of ARD which is well documented (*e.g.,* McCauley *et al*., 2010) (Figure 1).

Figure 1 The Stockton Coal Mine located on the West Coast of the South Island of New Zealand

The BCM were formed as part of a marginal marine setting consisting of carbonaceous mudstones, sandstones and coal containing elevated pyritic sulfide sequences (Flores & Sykes 1996; Black et al. 2005; Pope *et al.,* 2006). The sequence can result in significant acid generation and Fe, Al, and S release during the oxidation of pyritic overburden (Weisener & Weber 2010). Studies by McCauley *et al.,* (2010) have provide valuable baseline chemical data on the seep effluent chemistry associated with the Stockton Coal Mine, which has resulted in several viable remediation solutions for passive treatment of ARD impacted waterways. ARD control using passive treatment systems, some referred to as vertical flow wetlands (VFW), or biochemical reactors (BCR), have proven to be effective options to treat isolated geographically confined ARD seeps. These systems contain a porous media, which can range from organic mulch blended with crushed limestone, or systems unique to this particular study that utilise weathered mussel shells. The latter provides exceptional permeability and reactive surface area with extensive neutralization capacity and ability to remove 99% metals (McCauley *et al.,* 2010).

To gain a better understanding of the performance and functionality of the bioreactors using mussel shells, a systematic approach was used to investigate the bio-physico-chemical dynamics of the system. Physical sampling of an existing full-scale treatment cell was performed in May 2013 (after 8 months operation) and again in June 2014 to better understand the contributing biogeochemical mechanisms occurring within the system. Within the bioreactor exists a complex redox gradient

controlled by first order reaction kinetics, which are defined by both its physico-chemical environment (adsorption & precipitation) and a succession of microbial communities (*i.e.,* complex Fe and S cycling). Investigations to date indicate that homogenous treatment across the bioreactor with no restrictions to vertical flow even after continued sediment load to the system.

METHODOLOGY

Sample Collection

Samples for both bacterial and geochemical analysis were collected from the Whirlwind bioreactor (Figure 2) at 8 and 18 months after the system commenced treatment. Acid neutralization capacity (ANC) and metal distributions were performed on material collected at 8 months, while bacterial analysis was performed on 18 month samples. The samples were collected both horizontally and vertically from several pits within a square grid. Subsequent samples were collected at a 18 month time period in areas undisturbed by the previous 6 month sampling. Four vertical layers where targeted; the allochtonous overlying sediment, the iron precipitate layer, the aluminum precipitate layer, and the underlying unreacted shell layers. In addition to porewater and geochemical solid phase characterization, sub samples were also collected for molecular studies (*e.g.,* total DNA and RNA functional analyses). These samples were flash frozen using liquid nitrogen at site to preserve DNA and RNA for shipment to the laboratory.

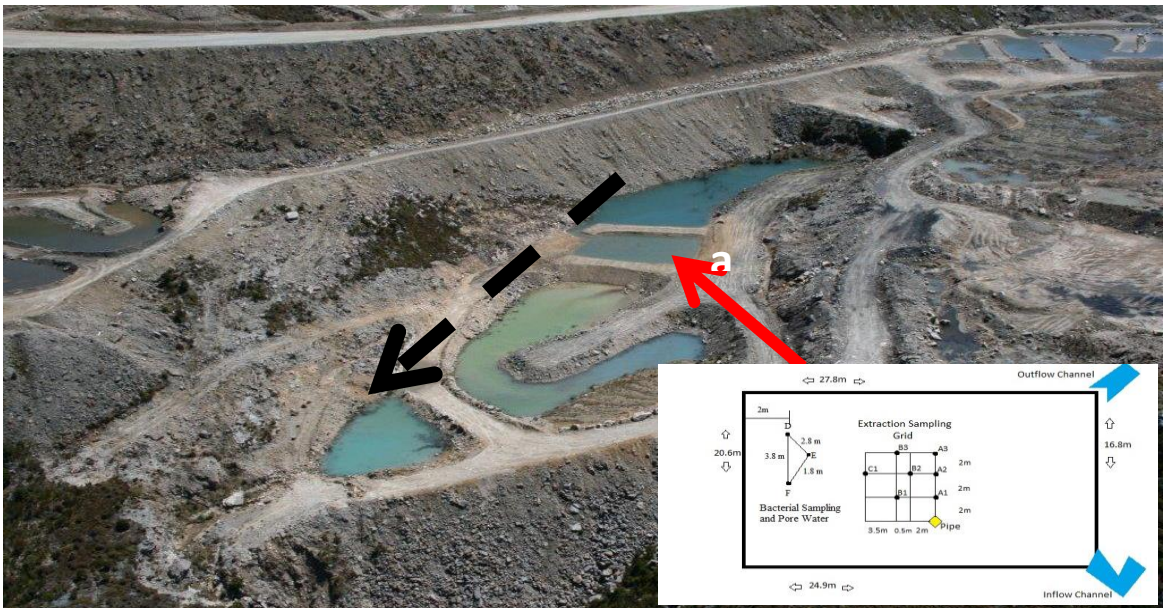

Figure 2 Emplacement of mussel shell bioreactor (location a); dashed line denotes direction of flow from the upper sediment pond, through the bioreactor and then two sediment ponds before discharge

Geochemical Characterization

The ANC test was used to evaluate the acid neutralization capacity of the bioreactor material from the different vertical sampling zones. This test was modified after Sobek *et al.* (1978), and the AMIRA ARD Test Handbook (AMIRA, 2002). Values from ANC testing are presented in kg of H_2SO_4 equivalent and as a percentage of $CaCO_3$ present. All Fe (total), pH and Eh values were measured from pore water collected from the bioreactor based on horizontal and vertical location and are shown averaged including statistical error. Approximately 100 ml of pore water were extracted from different depths within the bioreactor using rhizon pore water samples (Rhizosphere Research Products). Both pH and Eh determined from collected pore water was measured using Orion 8102BN probe by Thermo Scientific for pH and an Orion 01301MD (Duraprobe 4) by Thermo Scientific for Eh. Total iron concentrations were determined using ICPOES.

Bacterial Characterization

Three growth media were used to enrich the acidophilic iron oxidizers, neutrophillic iron oxidizers, and sulfate reducing bacteria (SRB). The media and method used for the enrichment of iron oxidizing bacteria was done using a modified 9K media described in Silverman & Lundgren (1958). Neutrophillic iron oxidizing bacteria were enriched using the Wolfe's media and methodology described in Emerson & Moyer (2002). The SRB were enriched using the Postgate media C and the methodology described in Postgate (1979). A Hucker's gram stain method (Gephardt *et al.*, 1981) was performed on samples extracted from bacterial enrichments. The bacteria were then examined using an Olympus BX61 petrographic microscope coupled with a Lumenera Infinity 1 digital video camera to determine relative abundances of gram (+) and gram (-) bacteria as well as examine their morphologies. Further characterization was performed using a FEI Quanta 200 FEG (Field Emission Gun) Variable Pressure Scanning Electron Microscope (SEM) with an EDAX® SiLi detector for bacterial morphology characterization. DNA extractions were performed using MoBio PowerSoil DNA isolation kit, following the manufacturer's instructions, and three sets of primers, which were used for amplicon targeting (PCR_1) within the 16S rRNA region for each sample. The thermocycling profile for PCR_1 conformed to the following parameters: initial denaturation for 5 min at 95°C followed by 34 cycles of 15 sec at 94°C, 15 sec at 48/55°C (bacteria/archaea), and 30 sec at 72°C, and a final extension of 1 min at 72°C. A second PCR was then performed for barcoding each of the samples (PCR_2), using a unique barcode for each sample as the forward primer and a universal reverse primer known as UniB-P1 (Table 1). The samples were diluted to a final concentration of 25 ng/μL and combined together in preparation for next generation sequencing using Ion Torrent platform (Life Technologies).

RESULTS AND DISCUSSION

Pore water and Solid Phase Characterization

A cross-section autopsy of the bioreactor shows a transitional environment dominated by geochemically distinct reactive layers of mineralization (Figure 3).

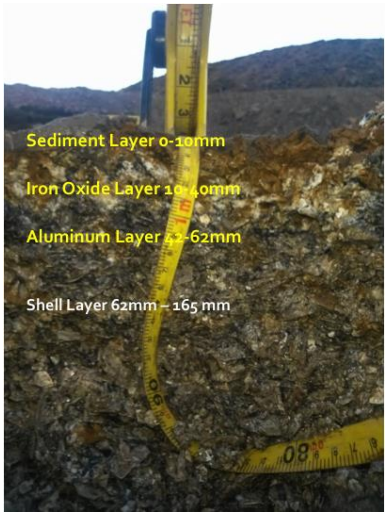

Figure 3 Geochemical gradient based on downward vertical flow within the mussel shell bioreactor

A summary of the average ANC profiles, Fe(III) distribution, pH and Eh from the bioreactor is provided in Figure 4. The ANC determinations, in terms of kg of H_2SO_4 (Figure 4a), as well as the distribution of Fe (III) within the vertical transect is shown in (Figure 4b). Both pH (Figure 4c) and Eh (Figure 4d) measurements show dramatic changes along the vertical depth suggesting a defined redox gradient. This assumption is supported by the physical appearance of distinct geochemical zones of precipitation observed in Figure 3. The top layer (0 – 10 mm thick) has a Eh of +199 mV and a measured pH of 3.6. This location correlates to ANC values of <5 kg H_2SO_4/t (<0.5 wt% $CaCO_3$) for ANC potential within the top sediment horizon. The capping allochthonous sediments is both oxidized and acidic with little, if any, capacity to neutralize incoming AMD effluent. The subsequent underlying iron oxide layer is dominated by reactive iron oxyhydroxides that extended from 11 to 40 mm depth and shows a rapid change in both porewater Eh and pH. Eh descreases to +26 mV and pH increases to 5.3 from 3.6. The ANC capacity within this layer increases to ~100 kg H_2SO_4/t (~10 wt% $CaCO_3$). The hydrolytic reactions involving iron observed within this layer are very characteristic of iron hydrolysis reactions that lead to its insolubility as pH increases above ~3.5. Below this reactive iron layer exists a thin zone dominated by white precipitates, which have been identified as amorphous aluminum hydroxide (unpublished data). The layer extends from a vertical depth of 40 to 62 mm with the reactor. Both Eh and pH continue to change with Eh from +26 mV to more reducing conditions of -33 mV and a subsequent pH increase from 5.2 to 6.5. The measured ANC values for the porewater collected within this layer is ~700 kg H_2SO_4/t (70 wt%

$CaCO_3$). The aluminum layer is characterized as a moderately reducing, circumneutral environment with high acid neutralization capacity. The bottom layer which extends from ~62 mm to 1655 mm (the base of the bioreactor) represents the unreacted mussel shell matrix. Porewater collected from within this layer shows low Eh values of -55 to -60 mV with an average pH of 7.2. The measured ANC values are slightly higher at 800 kg H_2SO_4/t (70 – 85 wt% $CaCO_3$). The unreacted shell layer represents a reduced environment with circum-neutral pH and a significant capacity to neutralize incoming acidic effluent. The redox reactions, which dominate the bioreactor, are controlled by a series of abiotic chemical and biotic catalyzed reactions, which give rise to favoured mineralogical phases, as well as direct influence to dominant microbial species present. Soluble Fe(III) (Figure 4b) correlates well with the observed iron oxyhydroxide interface noted in the profile.

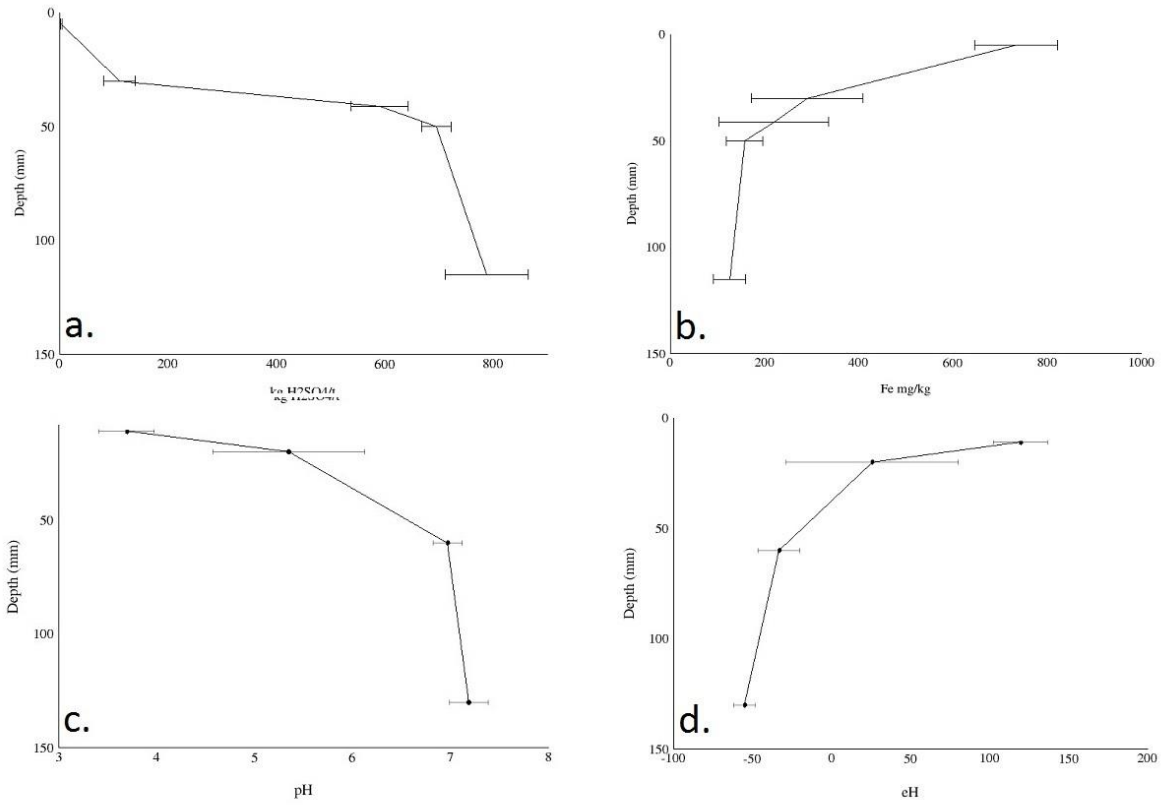

Figure 4 A summary of the of the neutralization capacity, iron distribution pH and Eh as a function of depth for the bioreactor (a) Acid Neutralization Capacity (ANC) at different depths (mm) within the bioreactor is based on kg H_2SO_4/T of material (b) Distribution of total Fe(III) within the vertical depth of the bioreactor (c) and (d) show pH and Eh profiles as a function of depth in the bioreactor

Bacterial Enrichment and Geochemical Conditions

The iron and sulfur dominant bacterial species were assessed using iron and sulfur enrichment cultures and molecular genomics (e.g. next generation sequencing- Ion torrent platform) to assess

the geochemical layers (Figure 5 & 6). This approach was used to differentiate prospective bacteria as a function of geochemical environments. A modified 9K media (Silverman & Lundgren, 1958) was used to target both iron and sulfur bacteria within the allochtonous sediment layer. This approach was successful allowing the enrichment of acidophilic iron oxidizers. The most common species at sites with similar effluent and geochemical conditions include *Thiobacillus ferrooxidans, Ferrobacillus ferrooxidans, Acidothiobacillus ferrooxidans, Aciothiobacillus thiooxidans, Leptospirillum ferrooxidans,* and *Ferrimicrobium spp.* (Silverman & Lundgren, 1958; Johnson 1998; Hallberg & Johnson, 2003; Johnson & Hallberg, 2003; Baker & Banfield, 2003; Hallberg, 2010; Schippers *et al.*, 2010). Based on the molecular characterization the dominant acid tolerant species identified was *Acidovorax sp*. This species is capable of metabolizing iron by coupling iron oxidation in the presence of nitrate and acetate. The dominant iron metabolizing species within the iron oxyhydroxide layer consisted of *Sideroxydans lithotrophicus* along with an increase in abundance of *Desulfotomacculum acetooxidans* a strict sulfate reducing anaerobe. The presence of *Sideroxydans lithotrophicus* within the iron oxide layer is interesting as this species is an neutrophillic aerobic oxidizer of Fe (II), and is capable of growing on other reduced mineral precipitates (e.g. siderite and pyrrhotite) at oxic–anoxic interfaces (Liu et al. 2012, Hedrich et al. 2011). Although suitable substrates within the reactor are possible other vectors should be determined considering the available HCO_3^- and Fe(II) in the transition zone making it possible for other enzymatic electron transport systems (EETS) to operate freely. No iron metabolizing bacteria were detected within the deeper profiles of the reactor including the aluminum oxide layer and the reduced unreacted shell matrix. A combination of both SRB enrichments and molecular investigation confirm the presence of *Desulfotomacculum acetooxidans* which is a spore forming SRB bacteria, and is more resistant to extreme environmental change (*e.g.,* periods of desiccation and oxic conditions) (Castro *et el.,* 2000). Postgate media C (Postgate, 1979) was used to enrich all SRBs present within the layers sampled from the bioreactor. In all layers positive growth was observed. Material collected below the sediment cap showed the fastest growth within the first 24 h compared to 2 - 3 week lag period for the cap sediment. This is likely due to oxygen stress at the top of the profile. Of note is the increased abundance of Archaea (e.g. methanogens) detected within the unreacted zone in Figure 5.

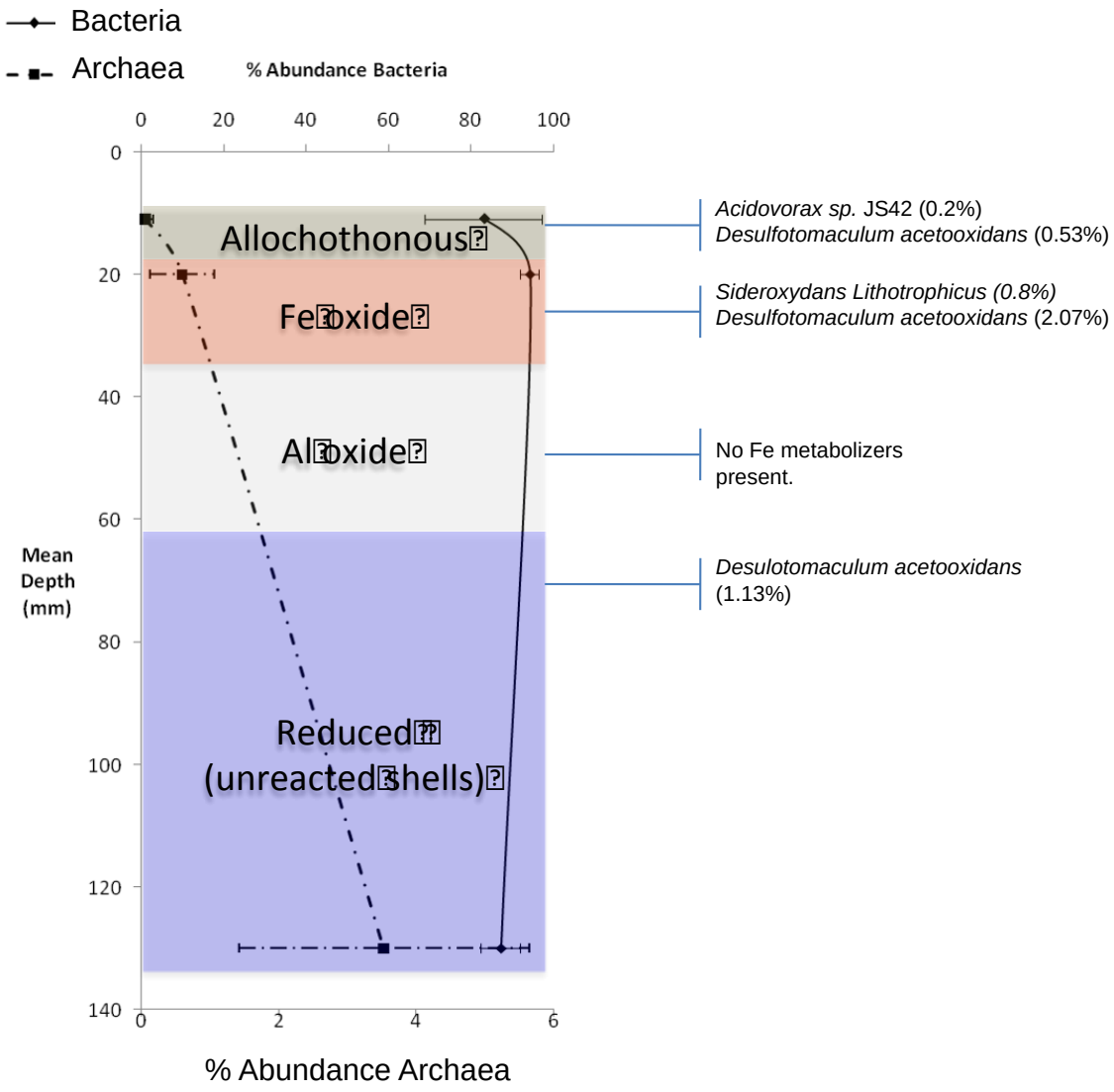

Figure 5 Correlation of iron and sulfur metabolizing bacteria and archaea within the bioreactor profile as a function of depth.

CONCLUSION

The formation of the geochemical profile within the mussel shell bioreactor and its influence on the development of microbial species is in part controlled by a series of abiotic chemical reactions that form during *in-situ* treatment of the ARD effluent. The rate of Fe(II) oxidation at pH values above 5 can be described by the following equation:

$$d[Fe^{2+}]/dt = k_1[Fe^{2+}][OH]^2 P_{O2} \quad (1)$$

where $k_1 = 8.0 \times 10^{13}$ min $^{-1}$ atm^{-1} mol^{-2}

It is noted that a 100-fold increase in the reaction rate occurs for every unit increase in pH (Gazea *et al.,* 1996). Given this situation the most important role of the *in-situ* reaction process would be adequate retention time for the dissolved constituents (Fe, Al) to oxidise and precipitate within the system. It should be noted that under these conditions the oxidation is slow, thus the contribution from iron oxidizing bacteria becomes increasingly important. The presence of acidophilic *Acidovorax sp.* and neutrophilic species such as *Sideroxydans lithotrophicus* and other chemoautotrophic species such as *Desulfotmacullum acetooxidans* (*e.g.*, SRB) which tolerate pH values 2-3 and 5-7 respectively will serve as key electron facilitators thus increasing the rate of iron oxidation or sulfur reduction by several orders of magnitude. Bacterial sulfate reduction in some respects is limited to very specific environmental conditions, which function best at pH >4 in the absence of other oxidising agents such as O_2 and Fe^{3+}(Postgate, 1984). The increase in SRB activity noted in the subsequent layers below the precipitated iron oxide layer confirms this fact. Additionally the abundance of amorphous zinc sulfide proximal to organic matter (data not shown) within this reduced zone (unreacted shells) is further testimony and results directly after iron is removed via oxidation and precipitation from waters flowing down through the reactor. These rapid precipitation fronts characterised by Fe, Al, and reduced metals contribute to the change in SRB activity and pH gradients. Preliminary investigations of the sulfur reducing consortia suggest a range of species able to capitalize on this, characterized by facultative acid tolerant species (gram + and -) near the surface of the reactor with a succession of species dominated by strict anaerobic chemotrophic neutrophilic SRBs within the reduced zones of the bioreactor. The SRB process involved in this case can be considered the reverse of pyritic sulfide oxidation with the end result contributing to the net consumption of acidity by the sulfate reduction process. In addition, considering that metal oxidation and hydrolysis reactions are not effective for removal of metals such as Zn, Tl, and Mn at pH values below 8 compared to bacterial produced hydrogen sulfide which readily reacts with metals above pH 3 forming insoluble metal sulfides in a reduced setting.

ACKNOWLEDGEMENTS

The authors wish to thank Solid Energy (SENZ) Ltd for permission to publish this work and their original and ongoing support of the research programme.

NOMENCLATURE

F(x)	cumulative probability density function
e	natural logarithm
α	scale parameter
β	shape parameter
x	random variable

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