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## Research article

## Novel cost effective full scale mussel shell bioreactors for metal removal and acid neutralization

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## ABSTRACT

Acid mine drainage (AMD) impacted waters are a worldwide concern for the mining industry and countries dealing with this issue; both active and passive technologies are employed for the treatment of such waters. Mussel shell bioreactors (MSB) represent a passive technology that utilizes waste from the shellfish industry as a novel substrate. The aim of this study is to provide insight into the biogeochemical dynamics of a novel full scale MSB for AMD treatment. A combination of water quality data, targeted geochemical extractions, and metagenomic analyses were used to evaluate MSB performance. The MSB raised the effluent pH from 3.4 to 8.3 while removing up to ~99% of the dissolved Al, and Fe and >90% Ni, Ti, and Zn. A geochemical gradient was observed progressing from oxidized to reduced conditions with depth. The redox conditions helped define the microbial consortium that consists of a specialized niche of organisms that influence elemental cycling (i.e. complex Fe and S cycling). MSB technology represents an economic and effective means of full scale, passive AMD treatment that is an attractive alternative for developing economies due to its low cost and ease of implementation.

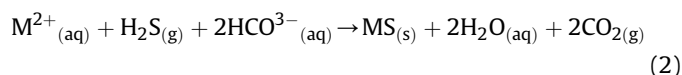
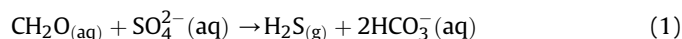
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## 1. Introduction

Acid Mine Drainage (AMD) caused by the oxidation of sulfide minerals within overburden and mine tailings is a persistent issue and of concern for the international mining community. In the United States alone approximately 200,000 AMD sites exist, and within Europe there are over 5000 km of AMD impacted watersheds some predating 1000 years (Liebmann, 1992; Hochella et al., 1999; Blowes et al., 2013). The weathering of sulfide minerals in the presence of bacteria (e.g. *Thiobacillus ferrooxidans*) will often accelerate the rates of reaction resulting in increased concentrations of dissolved metals, sulfate, and net acidity in watersheds (Baker and Banfield, 2003; Blowes et al., 2013). To address AMD a variety of treatment methods have been developed and can be broadly grouped into passive and active treatments. The implementation of these strategies varies across sites based on the nature of the AMD effluent treated (i.e. net acidity/alkalinity, DO, [Fe<sup>3+</sup>],

[Al<sup>3+</sup>], and flow rate) (Hedin et al., 1994; Skousen, 1997), as well as access to infrastructure. Since many AMD seeps are isolated geographically, control using passive treatment systems, such as vertical flow wetlands (VFW), or biochemical reactors (BCR), are proving to be a more effective treatment options in the 21st century (Neculita et al., 2007).

The aim of most AMD treating BCR, or bioreactors, is the promotion of bacterial sulfate reduction pathways under chemically reducing conditions (reactions (1) and (2) for heterotrophic sulfate reduction) (Stumm and Morgan, 1996).



The purpose of promoting these pathways ((1) and (2)) is to facilitate subsequent alkalinity generating reactions and provide conditions favourable for the cycling of S (SO<sub>4</sub> ↔ H<sub>2</sub>S) coupled with the complexation of reduced metals such as Fe(II), Zn(II), Mn(II), or As(III). Many of these systems use a porous base media,

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which can range from organic mulch blended with crushed limestone to other biological treatments (eg. Lindsay et al., 2011; ; Zipper and Skousen, 2014). Since their development, sulfur reducing bioreactors have operated with a variety of organic carbon sources (Liamlean and Annachatre, 2007; Papirio et al., 2013). For example manures (cow, pig, goat, and buffalo), sawdust, rice straw, woodchips, sugarcane waste, mushroom compost and chitinous material have all been used with variable levels of success (eg. Zagury et al., 2006; Robinson-Lora and Brennan, 2009; Zhang and Wang, 2014). Bioreactor substrates containing composites of labile (e.g. manures) and recalcitrant carbon sources (e.g. chitin, cellulose) have been shown to achieve greater sulfate reduction rates than those with only a single carbon source (e.g. pure lactate or ethanol based) (Waybrant et al., 2002; Zagury et al., 2006; Neculita et al., 2007). The applications of certain forms of chitin (i.e. crab) in these systems have been shown to be an efficient alternative product for treating AMD and metals, but they are still economically prohibitive compared to other substrates [Table 1]. A drawback to mixed systems is often related to their reliance on obtaining biological inoculants (e.g. stimulus) and logistical issues pertaining to acquisition of components, as well as complexity in design.

The application of mussel shell materials addresses these above-mentioned concerns and is cost effective. Mussel shells contain up to 5–12 wt% organic content (Kawaguchi and Watabe, 1993; Crombie et al., 2011) and have a structure that consists of amorphous CaCO<sub>3</sub> with interlamellar sheets of chitin in a “brick and mortar” arrangement, which provides effective surface area (Jacob et al., 2008). Due to these characteristics mussel shells can be considered a favourable substrate, providing both labile and recalcitrant carbon, and alkalinity. Mussel-shell based bioreactors (MSBs) are an emerging technology to passively treat AMD. Initial MSB performance has been investigated at bench and pilot scale conditions (McCauley et al., 2008, 2009a, 2009b, 2010; Uster et al., 2014; DiLoreto et al., 2016). However, this is the first study to compare the performance of a full scale MSB treatment system under active mine conditions as well as evaluate the microbial aspects. The research described here provides mechanistic insight into its long-term efficiency, cost evaluation and limitations as a technology. Here the performance of this single source system in contrast to mixed source treatment systems is discussed. Details on the geochemical performance along with correlations to the existing microbiology after 2 years of operation will be discussed. The potential low cost associated with MSB systems make it an attractive alternative approach to passive treatment, especially to countries with developing economies and coastal settings where mussel shell waste may be in sufficient supply and underutilized.

## 2. Materials and methods

### 2.1. Site description

The Brunner Coal Measures (BCM) within the Stockton opencast coal mine in New Zealand has a legacy of AMD (McCauley et al., 2010). The BCM were part of a marginal marine basin, which consisted of carbonaceous mudstones, sandstones and coal within elevated pyritic sulfide sequences (Flores and Sykes, 1996; Black et al., 2005; Pope et al., 2006). These coal measures release AMD due to the high sulfide content in their waste rock and overburden coupled with high rain fall ( $\approx 7000 \text{ mm y}^{-1}$ ) and an annual average temperature of 8 °C. The BCM commonly contains up to 1 wt% sulfur and the overlying marine mudstones contain up to 5 wt% pyrite (FeS<sub>2</sub>), as well as a large fraction of aluminosilicate minerals (Al<sub>2</sub>SiO<sub>5</sub>) (Weber et al., 2004; Pope et al., 2010a; Weisener and Weber, 2010). Oxidation and dissolution of these materials result in the formation of acidic AMD effluents which are elevated in Fe, Al, Zn, Ni, Mn ± As, Cd, Cu, Pb, & Tl (Pope et al., 2010b; Pope and Trumm, 2015).

### 2.2. Bioreactor design

The MSB system consists of 3 cells; a sediment retention pond, the bioreactor, and an outflow channel [Fig. 1]. With a trapezoidal design, the bioreactor measures 32 m × 20m at the top tapering down 1.2 m vertically to 24 m × 12m at the bottom and is saturated with 200 mm of water cover. The MSB was filled with 362 T (~1t m<sup>-3</sup> density) of mussel shell waste product with a pore volume of 192 m<sup>3</sup>. The drainage network contains 6 lengths of megaflo drainage pipe wrapped in filter cloth with PVC capped ends to prevent clogging. These pipes were arranged in a rib like pattern and are connected to a central PVC pipe drain which flows out a riser into a final settling cell before discharge. The MSB was drained and sampled in May 2013 (8 months operational) and again in June 2014 (20 months operational). Samples were collected for geochemical and biological analyses. The samples were collected using a 4 × 4m spatial grid pattern [Fig. 1]. At each location, samples were collected as a function of depth into the MSB system and in response to layering in the system [Fig. 1].

### 2.3. Water chemistry & selective extractions

Influent and effluent water samples were collected on a bimonthly basis from 2012 to 2014 and analyzed for pH, total metals, sulfate, nitrogen, and phosphorous (Hill Laboratories, New Zealand) with data collection ongoing. While the MSB drained pore-water was collected using Rhizon samplers (Rhizosphere Research Products) and frozen on dry ice. Pore-water pH and Eh was measured using Orion 8102BN and 01301MD probes (Thermo

**Table 1**  
Cost analysis of various passive AMD treatment options modified from Grembi et al. (2015). MSB technology represents a cheaper alternative to other passive systems and higher efficiency than conventional passive systems.

Substrate or treatment type	Capital cost	Organic substrate cost	Annual operations and maintenance cost	20 year total cost
100% Waste Mussel Shell Bioreactor	\$50,000 (NZD)	\$0 <sup>b</sup>	\$9000 over 10	\$68,000
70% Crabshell + 30% Spent Mushroom Compost <sup>a</sup>	\$149,000	\$688,000	\$500	\$847,000
<sup>c</sup> Anoxic Limestone Drain	N/A	N/A	N/A	\$36,744*
<sup>c</sup> Open Limestone Channel	N/A	N/A	N/A	\$27,409*
<sup>c</sup> Limestone Leach Bed	N/A	N/A	N/A	\$68,997*
<sup>c</sup> Vertical Flow Wetland	N/A	N/A	N/A	\$51,151*
<sup>c</sup> Anaerobic Wetland	N/A	N/A	N/A	\$126,110*

\*Not including replacement costs.

<sup>a</sup> Grembi et al. (2015).

<sup>b</sup> Substrate obtained freely, shipping costs included in capital.

<sup>c</sup> Estimates from Ziemkiewicz et al., 2003.

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