Alkalinity Generation in a Novel Multi-stage High-strength Acid Mine Drainage and Municipal Wastewater Passive Co-treatment System

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Alkalinity Generation in a Novel Multi-stage High-strength Acid Mine Drainage and Municipal Wastewater Passive Co-treatment System

W. H. Strosnider · B. K. Winfrey · R. W. Nairn

Abstract Passive co-treatment of high-strength acid mine drainage (AMD) and municipal wastewater (MWW) was examined in a laboratory-scale, four-stage continuous flow reactor system with a total residence time of 6.6 d. Synthetic AMD of pH 2.60 and an acidity of 1,870 mg/L (as $CaCO_3$) was mixed at a 1:2 ratio with raw MWW (pH 7.67, 288 mg/L alkalinity (as $CaCO_3$), and 265 mg/L BOD_5) from the City of Norman, Oklahoma and introduced into the system. Alkalinity generated by limestone dissolution and bacterial SO_4^{2-} reduction (BSR) processes was sufficient to support various metal removal processes and produce an effluent with circumneutral pH (6.98) and a net alkalinity of 10.4 mg/L (as $CaCO_3$). Alkalinity generation from limestone dissolution was comparable with conventional AMD passive treatment systems. BSR proceeded at a relatively high rate (0.56 mol/m^3 day) despite inhibitory pH and metals concentrations. Results indicate that the diverse electron donors in the MWW may be as suitable for BSR and their supporting microbial communities as commonly used substrates, presenting an opportunity to use a common waste as a resource for passive treatment.

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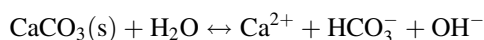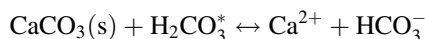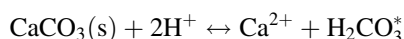e-mail: bill.strosnider@gmail.com

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Introduction

Generally, acid mine drainage (AMD) contains elevated concentrations of hydrogen ion, trace metals, SO_4^{2-}, and acidity (Watzlaf et al. 2004). Because AMD is often net acidic, passive treatment usually necessitates alkalinity generation and pH augmentation, which is often realized via limestone dissolution and/or bacterial SO_4^{2-} reduction (BSR). The dissolution of calcite, the principal component of limestone, occurs via a combination of reactions (Stumm and Morgan 1996):

$$CaCO_3(s) + 2H^+ \leftrightarrow Ca^{2+} + H_2CO_3^*$$

$$CaCO_3(s) + H_2CO_3^* \leftrightarrow Ca^{2+} + HCO_3^-$$

$$CaCO_3(s) + H_2O \leftrightarrow Ca^{2+} + HCO_3^- + OH^-$$

where $[H_2CO_3^*] = [CO_{2(aq)}] + [H_2CO_3]$. The three reactions affect pH and alkalinity via changes in HCO_3^- and OH^-. The Ca concentrations increase stoichiometrically with each mole of calcite dissolved, creating a signature that can be used to track dissolution.

Passive treatment of AMD often requires suitable organic substrate electron donors for dissolved oxygen (DO) stripping, BSR, and the bacterially-mediated removal of metals, such as Fe. Using SO_4^{2-} reducing bacteria (SRB) to generate alkalinity via bicarbonate production and remove metals from solution via sulfide precipitation was first suggested by Tuttle et al. (1969). The reactions can be generally expressed as follows (Neculita et al. 2007):

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$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S$$

$$H_2S + M^{2+} \rightarrow MS + 2H^+$$
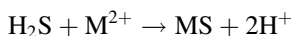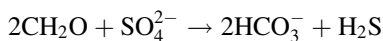
where CH_2O is a simple sugar and M^{2+} is a metal ion such as Pb^{2+} or Zn^{2+}.

Numerous carbon sources, including horse and cow manure, chicken litter, ethanol, methanol, municipal sewage sludge, and compost, have been successfully applied to encourage BSR (Benner et al. 2002; Cocos et al. 2002; Kaksonen and Puhakka 2007; Waybrant et al. 1998; Younger et al. 2002). Generally, BSR is coupled with limestone dissolution in passive treatment cells such as reducing and alkalinity producing systems (RAPS) or vertical flow bioreactors (VFB) to maximize alkalinity generation and metals retention. However, BSR alone can be effective for alkalinity generation, as Benner et al. (2002), Cocos et al. (2002) have demonstrated.

Sewage sludge has been used as a BSR carbon source to treat AMD. Waybrant et al. (1998) observed that sewage sludge encouraged higher levels of SO_4^{2-} reduction than seven other commonly used organic carbon sources. However, studies have noted that mixtures of MWW sludge with multiple organic carbon sources generally promote higher SO_4^{2-} reduction rates than single sources (McCullough et al. 2008; Waybrant et al. 1998). MWW contains a wide variety of organic compounds ranging from simple sugars to more recalcitrant compounds, such as cellulose (Metcalf and Eddy, Inc 2002). SRB and their associated supporting communities also require sufficient nutrients for optimum operation (Neculita et al. 2007). BSR substrates have been supplemented with nitrogen and/or phosphorus to encourage greater BSR rates (Kaksonen and Puhakka 2007). MWW contains a mix of labile and recalcitrant organic substrates as well as other nutrients (Metcalf and Eddy, Inc 2002), which indicates that it could support a rich and diverse community of SRB and supporting microbes.

Metals and therefore acidity can also be removed from solution by sorption to the organic substrates in AMD passive treatment system unit processes (Neculita et al. 2007). Organic substrates such as peat (Champagne et al. 2008), algae (Van Hille et al. 1999), biosolids (Norton et al. 2004) and many of the aforementioned SRB carbon sources have demonstrated abiotic AMD treatment via metals sorption. Relative to co-treatment, elevated concentrations of bacteria in raw MWW are present within activated sludge. Utgikar et al. (2000) demonstrated that non-viable activated sludge from conventional MWW treatment could be an effective abiotic biosorbent for metals in low pH AMD.

The passive co-treatment of municipal wastewater (MWW) and high-strength acid mine drainage (AMD) blends aspects of passive AMD treatment and conventional active MWW treatment. Conventional active MWW treatment can require electron acceptors for bacterial oxidation of carbon substrate, chemicals or ultraviolet light for pathogen removal, and physical/chemical filtration or flocculation for solids removal. Passive co-treatment potentially provides electron acceptors and does not require nonrenewable energy or refined material inputs.

Despite its promise, little research has been undertaken to explore the use of MWW as a substrate for AMD treatment. In laboratory microcosm studies, Van Hille et al. (1999) used MWW to create algae-rich high pH water, which was mixed with AMD. McCullough et al. (2008) documented water quality improvement due to BSR in an evaporation pond in which high-strength AMD was accidentally introduced to secondary MWW. Strosnider and Nairn (2010) found that incubating AMD with MWW, with and without limestone, could decrease acidity and create alkalinity. Strosnider et al. (in review-a,b) documented highly efficient metals, biochemical oxygen demand, and phosphate removal within the same system as described in this manuscript. Winfrey et al. (in press) noted that the system reduced total coliforms, fecal coliforms, E. coli, and fecal streptococci by 100%.

The object of this study was to investigate the extent to which alkalinity could be biotically and abiotically generated in a high-strength AMD and raw MWW passive co-treatment system. Considerable energy and material savings could result if MWW were used as a substrate in passive treatment systems. This would be especially suitable for those settings where other organic substrates are scarce, such as in Potosí, Bolivia. Untreated high-strength AMD and MWW pollute the headwaters of the Rio Pilcomayo there (Strosnider and Nairn 2010), which prompted this research.

Methods

Experimental Design

The experimental setup involved four serial unit processes in quadruplicate (Fig. 1). The first unit process step was to mix the AMD and MWW, which would raise the AMD pH to a pH that less inhibitory to SRB. This step was also intended to provide primary clarification for the MWW, reduce Fe(III) in the AMD to Fe(II), complex dissolved metals with organic ligands, flocculate Fe and Al with phosphate from the MWW, strip DO, and settle solids.

The second and third unit processes together emulated VFB. The upper column section was filled with inert Kaldnes K3 biofilm media. These polyethylene high surface area (500 m^2/m^3) components are typically used in moving bed biofilm wastewater and drinking water treatment

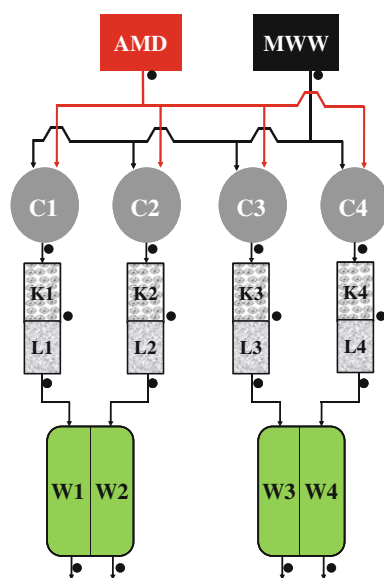**Fig. 1** Conceptual experimental layout. *C1-4* represent the clarifiers, *K1-4* the Kaldnes, *L1-4* the limestone, and *W1-4* the wetlands; *black dots* indicate sampling points

(Saliling et al. 2007), and were intended to encourage further DO stripping, Fe(III) reduction, and BSR. The bottom of the column was filled with limestone for abiotic alkalinity generation via calcite dissolution and additional BSR. The final unit process step involved aerobic wetland mesocosms for sequential Fe, then Mn, oxidation and precipitation (which consumes alkalinity) as well as the removal of remaining As, Cd, Pb and Zn via sorption to Fe oxyhydroxides. Each unit process was connected to the next via clear vinyl tubing and sampled at its outflow.

Transverse 4 cm deep baffles and 2.5 cm radius semi-circular weirs served as the physical structures in the polyethylene basins that comprised the clarifier. Clarifier residence time was 32 h.

The VFB emulation columns were 91.5 cm in height and 12.5 cm in diameter. The bottom 38 cm of the columns were filled with high quality (>90% $CaCO_3$ per manufacturer certification by Marble City, OK) limestone washed of all fines and separated by sieve analysis adapted from ASTM D422 with the fraction passing a 2.54 cm sieve yet retained by a 1.27 cm sieve. The remaining top 53.5 cm of the columns were packed with the Kaldnes biofilm media. Following Pruden et al.'s (2007) findings of the importance of inoculation to SO_4^{2-} reducing bioreactor performance, the Kaldnes stage was inoculated with 100 mL of VFB substrate from two mature passive coal mine AMD treatment systems in Pittsburg and Latimer Counties, OK. Each column was wrapped in aluminum foil to emulate the lightless conditions in VFB substrate. Residence time was 42 and 18 h for the Kaldnes and limestone stages, respectively.

The aerobic constructed treatment wetland mesocosms were two shallow polyethylene storage containers. Each

wetland container was bisected longitudinally with plastic to create the necessary four treatment trains. Wetland soil was collected from an existing constructed mitigation wetland that received tertiary wastewater at the Midwest City, Oklahoma MWW Treatment Plant. The surface flow mesocosms were planted with *Hydrocotyle verticillata* and *Nasturtium officinale*. The wetlands were placed under timed grow-lights on a 12 h/d cycle. Wetland residence time was 67 h. Further design details are presented in Winfrey et al. (in press).

Raw MWW was collected after grit screening at the City of Norman, Oklahoma Water Pollution Control Facility. Synthetic AMD, approximating that found at the base/ precious metal mining district of Cerro Rico de Potosí, Bolivia, was introduced to the system at a 2:1 ratio (MWW:AMD). All four replications continuously treated the mixed influent for 135 day.

The AMD and MWW were introduced with peristaltic pumps at a combined flow rate (3.8 L/d) to produce an 18 h residence time in the limestone stage, which is greater than the recommended minimum 15 h design residence time suggested for anoxic limestone drains and the limestone drain components of VFB (Younger et al. 2002). The system was gravity flow from the first (clarifier) to the last (wetland) unit processes. MWW was collected from the treatment plant weekly, homogenized during pumping, and refrigerated at 4°C before introduction to the system. AMD was prepared weekly and stored at room temperature (20°C) until use. All unit processes were maintained at room temperature throughout the experiment.

Data Collection

Alkalinity titrations were conducted in accordance with standard methods (APHA 1998) and Hach Method 8203 (Hach 2002). A properly calibrated Orion 1230 multimeter was used to determine pH. Quantification of SO_4^{2-} concentrations followed EPA method 300: samples were immediately filtered through Dionex OnGuard® II H cartridges and 0.2 µm nylon filters, then analyzed on a MetrOhm® 761 compact ion chromatograph unit. Alkalinity titrations, pH readings, and SO_4^{2-} samples were taken for a total of ten sampling events spaced throughout the duration of the experiment.

Biological activity reaction tests (BART™) by Droycon Bioconcepts Inc. (DBI) were applied to estimate SRB populations. BART™ tests have been successfully used in the past to estimate SRB populations in various harsh environments (Hrapovic and Rowe 2002; Pfiffner et al. 1997; Sahrani et al. 2008). BART™ tubes were incubated at 22°C and read every 12 or 24 h. DBI QuickPop software was used to estimate populations. BART™ tests were initiated on day 85, 112, 123, and 130.

Data Analysis

Because direct sampling of the clarifier influent mix was impossible due to the experimental design, the theoretical mixed influent constituent concentrations (theomix) were calculated using the ratio of AMD to MWW. Net acidity was calculated following Younger et al. (2002). For acidity calculations, all Fe was assumed to be Fe(II) when pH > 3 and Fe(III) when pH < 3, because Fe(III) predominates for most solutions with pH < 3 (Kirby and Cravotta 2005; Watzlaf et al. 2004). Metals data for acidity calculations were taken from Strosnider et al. (in review-a). Prior to median testing, data were tested for normality with the Anderson–Darling test and similarity of variance. Due to the prevalence of unequal variances and non-normality, the nonparametric Kruskal–Wallis Multiple Comparisons test was used to determine statistical differences between the medians of each unit process. All statistical calculations were completed with Minitab® version 15 using $\alpha = 0.05$.

Results and Discussion

Influent Characteristics

Influent characteristics were relatively uniform throughout the 135 day experiment (Tables 1 and 2). As intended, the

Table 1 Mean influent AMD and MWW physiochemical properties and SO_4^{2-} concentration

	pH	mg/L, as $CaCO_3$ equivalent		SO_4^{2-}
		Alkalinity	Net acidity	mg/L
MWW	7.67	288	−287	70
s.d.	0.12	20	20	16
AMD	2.60	0	1,865	1,920
s.d.	0.04	0	91	140

$n = 10$

Table 2 Mean influent, theoretical mixed influent constituent concentrations (theomix), and wetland effluent dissolved metal concentrations, in mg/L (Strosnider et al. in review-a)

	MWW	AMD	Theomix	Effluent
Al	0.108	45.8	15.1	0.034
As	<0.022	0.25	0.090	<0.022
Ca	39.6	82.8	53.4	209.0
Cd	0.0010	2.02	0.665	0.015
Fe	0.315	292	96.4	0.183
Mn	0.056	54.6	18.0	15.5
Pb	0.015	1.21	0.404	0.049
Zn	0.045	391	129	34.3

high strength synthetic AMD proved to be similar in composition to that generated at Cerro Rico de Potosí (Strosnider and Nairn 2010). The mean alkalinity, BOD_5 (265 ± 94 mg/L), Cl^- (69 ± 3.8 mg/L), and SO_4^{2-} concentrations place the MWW used between the "medium" and "strong" designations of MWW created by Metcalf and Eddy, Inc (2002) (Strosnider et al. in review-b).

Limestone Dissolution

Alkalinity was generated via abiotic and biotic processes from the clarifier to the limestone stages (Fig. 2). Dissolved Ca remained statistically unchanged until the limestone stage where it increased from a mean 57.4–183 mg/L which was calculated to correspond to generation of 314 mg/L of alkalinity as $CaCO_3$ (Strosnider et al. in review-a). This corresponded relatively well with the mean 285 mg/L drop in net acidity in the limestone stage. The alkalinity produced by the limestone stage was representative of real-world ALD performance (Watzlaf et al. 2000, 2004). This is further evidence that mixing AMD with MWW may not considerably inhibit alkalinity generation via limestone dissolution despite the negative effects that increased pH and decreased ionic strength from mixing can have on calcite dissolution (Strosnider and Nairn 2010).

Sulfate Reduction

Sulfate concentrations decreased from a mean 680–610 mg/L from the theomix to the clarifier effluent and then averaged 607, 612 and 650 mg/L in the Kaldnes, limestone and wetland effluent, respectively. Although SO_4^{2-} concentrations appear to have risen in the wetlands, the increase was not statistically significant. The wetlands were an aerobic unit process where other constituents, such as Na and Cl, increased by a similar percentage due to evapoconcentration (Strosnider et al. in review-a). BSR in the clarifiers was
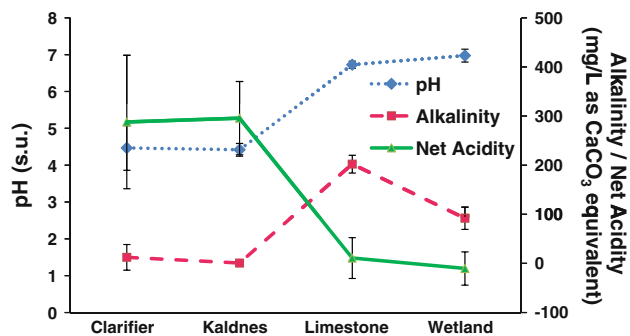

Fig. 2 Mean of all sampling events ($n = 40$) for pH, alkalinity and net acidity throughout the system. *Error bars* represent one standard deviation above and below the mean and are not visible when *bars* are smaller than the *symbol*

identified as the primary SO_4^{2-} removal mechanism. Other possible SO_4^{2-} removal mechanisms include the formation of schwertmannite, alunite, and gypsum. Schwertmannite ($Fe_8O_8(OH)_{8-2x}(SO_4)_x$), where $1 \leq x \leq 1.75$, was likely formed in the clarifiers to some extent and will remove SO_4^{2-} from solution upon formation (Bigham et al. 1996; Strosnider et al. in review-a). However, if one assumes that all dissolved Fe was removed from solution by schwertmannite formation, which is highly unlikely because of the other aforementioned plausible removal mechanisms, it could only account for a maximum SO_4^{2-} removal of 15.1 mg/L (21.4% of the total clarifier removal). Alunite formation was likely not a substantial removal mechanism (Strosnider et al. in review-a). SO_4^{2-} removal was not noted in the limestone stages, which is where gypsum formation could have been favored due to the elevated Ca concentrations. Therefore, all SO_4^{2-} removal was assumed to be via BSR despite the marginal error likely inherent in this assumption.

Following this assumption, BSR in the clarifiers was calculated, before metal sulfide formation, to generate 147 mg/L alkalinity, as $CaCO_3$ equivalent. Aside from generating alkalinity, BSR likely facilitated the removal of As, Cd, Cu, Fe, Ni, Pb, and Zn as sulfides, as these contaminants were removed in the clarifiers (Strosnider et al. in review-a), though co-precipitation of some of these contaminants with iron hydroxide was also likely taking place. Overall, the alkalinity provided by the MWW and generated in the system was sufficient to produce a net-alkaline effluent and buffer pH in the wetland, where H^+ liberating reactions such as Fe, Mn, and NH_4^+ oxidation occurred (Strosnider et al. in review-a,b). In addition, BSR contributed to the system's highly efficient processing of BOD_5 and DOC, as noted in Strosnider et al. (in review-b).

BART[TM] tests indicated the presence of SRB in the influent MWW (10^{6-7} cfu/mL), clarifiers (10^{0-2} cfu/mL), clarifier sludge (10^{0-4} cfu/mL), Kaldnes stage (10^{0-3} cfu/mL), limestone stage (10^{1-3} cfu/mL), and wetlands (10^{0-2} cfu/mL). The wide range of values may be explained by the limitations of the BART[TM] method, which does not directly measure bacterial concentrations, but the black metal sulfide product of SRB activity. In addition, SRB populations are generally associated with biofilms or bio-colloids, of which water column samples may not exhibit representative bacterial concentrations (DBI 2002). The supernatant from clarifier sludge samples was used in the BART[TM] tests, while the bulk of SRB were likely bound to the sludge solids. Although the MWW contained high concentrations of organic particulates upon which SRB likely congregated, the very high concentrations of SRB in the MWW and low concentrations in the clarifier water columns suggest die-off of substantial proportions of SRB upon exposure to the AMD. The clarifiers may have acted as a species filter or sieve, building a more resistant SRB community throughout the system as non-AMD-resistant SRB perished. Overall, the BART test results suggest that SRB were present throughout the system, which is evidence of SRB tolerance to low pH (clarifiers, Kaldnes stage), aerobic (wetlands) waters, and highly elevated Zn and other metals (entire system). Substantial concentrations of simple to complex carbohydrates present in the MWW likely directly and/or indirectly supported SRB populations (Metcalf and Eddy, Inc 2002). SRB require simple sugars or alcohols as substrate, which could have been gleaned from the MWW directly and/or created by other microbial consortia, such as cellulolytic and fermentative bacteria, breaking down more complex carbohydrates (Neculita et al. 2007).

The mean clarifier SO_4^{2-} removal rate was 0.56 mol/m^3 day, which is greater than the approximate 0.3 mol/m^3 day found under optimal BSR field conditions (Neculita et al. 2007), yet less than the ≈ 3 mol/m^3 day that Tsukamoto et al. (2004), Kolmert and Johnson (2001) found under laboratory conditions at similar pH. However, Tsukamoto et al. (2004) constructed their reactors with sand and horse manure substrate, and fed those reactors with high quality refined electron donors (methanol and ethanol) and synthetic AMD that only contained Fe (≈ 100 mg/L) and SO_4^{2-} (900 mg/L) with no Zn added. Kolmert and Johnson (2001) fed ethanol, lactic acid, and glycerol to bioreactors treating nutrient-augmented AMD with acidity and metals concentrations orders of magnitude less than the AMD used in this study. The co-treatment clarifier reduced SO_4^{2-} more rapidly than the various mixes of bark, post peel, and compost tested by McCauley et al. (2009) (0.28–0.38 mol/m^3 day) and the pilot-scale bioreactors of spent mushroom compost of Dvorak et al. (1992) (0.214 and 0.333 mol/m^3 day); though both of those studies used AMD with lower concentrations of acidity and most metals of interest.

The BSR rate was unexpectedly high, given the elevated Zn and other metal concentrations and suboptimal pH. The concentration of Zn present in the clarifiers (78.6 mg/L), Kaldnes (80.9 mg/L), and limestone (64.7 mg/L) unit processes likely reduced BSR activity somewhat. Zn concentrations ranging from 13 to 65 mg/L have been found to be toxic and severely inhibitive to SRB (Kaksonen and Puhakka 2007; Neculita et al. 2007). In addition, studies have suggested additive individual metal toxicity (Neculita et al. 2007), indicating that the other toxic metals within the system could have had an additive inhibitory effect on SRB. BSR rates also begin to decline at pH < 5 (Neculita et al. 2007), which likely slowed BSR in the clarifier and Kaldnes stages where effluent pH was 4.47 ± 0.61 and 4.42 ± 0.17, respectively. It is assumed that BSR could have been greater if the AMD Zn concentrations were less and/or the ratio of MWW to AMD was increased to raise

pH. Because much of the dissolved organic carbon and organic solids provided by the MWW were removed from solution within the clarifier, BSR in the Kaldnes and limestone stages was possibly also limited by the lack of suitable electron donors (Strosnider et al. in review-b).

Nevertheless, BSR rates were relatively high, suggesting that a rich and diverse SRB community was supported by the electron donors supplied by the MWW. It is likely that microenvironments with elevated pH in the organic and metal-rich sludge at the bottom of the clarifiers supported increased BSR rates (e.g. Fortin et al. 1996; Stockdale et al. 2009). The capacity of particle enclosures, biofilms, and associated metal sulfide precipitates to allow formation of SRB-suitable microenvironment is not quantifiable; however, inferences can be made from the literature. Studies have documented the protective capacity of sheltered attachment surfaces and biofilms, demonstrating that biofilms bind aqueous heavy metals, accumulate metal sulfides, and retard diffusion of hydrogen ions and other toxic species (Beyenal and Lewandowski 2001; Curtin and Cormican 2003; Ito et al. 2002; Kaksonen and Puhakka 2007; Labrenz et al. 2000; Stone et al. 2006; Teitzel and Parsek 2003; Vroom et al. 1999; White and Gadd 2000). Vroom et al. (1999) documented pH gradients of >3 units in fewer than 10 μm of heterotrophic biofilm. Teitzel and Parsek (2003) noted that bacteria embedded in biofilms were up to 600 times more resistant to heavy metal stress than planktonic cells. Stone et al. (2006) attributed increased Zn tolerance of the SRB *Shewanella putrefaciens* to the protective capacity of biofilms and related attachment surfaces. Researchers have solid evidence of metal sulfide precipitation within and overlying SRB biofilms (Labrenz et al. 2000; White and Gadd 2000). The activity of acid and metal tolerant SRB, many species of which have been recently isolated (Kolmert and Johnson 2001) and less metal tolerant or neutrophilic SRB embedded in suitable biofilm microenvironments, is also likely responsible for the relatively high BSR rates observed.

Conclusions

Passive abiotic (limestone dissolution) and biotic (BSR) alkalinity generation processes produced an effluent with circumneutral pH and positive net alkalinity. SRB were able to maintain a relatively high level of SO_4^{2-} reduction despite inhibitory pH and metals concentrations. Results indicate that the diverse electron donors in MWW may be as suitable for BSR as commonly used substrates. Future studies may investigate mixing ratios to determine optimum BSR conditions and supplementing existing VFBs with MWW to encourage greater sustainability and efficiency.

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