ASSESSMENT OF TWO FIELD-SCALE SULFATE REDUCING BIOREACTORS USING SULFUR ISOTOPES[1]

M.D. Reeder[2], T.D. Branam, and G.A. Olyphant

Abstract: Sulfate-reducing bioreactors (SRBRs) have shown promise as a cost-effective option in the passive remediation of acid mine drainage (AMD). While these systems do provide the necessary conditions for increased bacterial activity, little is known about the internal dynamics and the functional lifespan of the systems in field settings. To help address these issues, two field-scale bioreactors are being monitored using an array of sampling ports distributed at varying depths throughout the treatment cells. These internal monitoring ports are located in such a way as to observe 3-D trends in activity occurring within the system.

Water samples collected from the ports, as well as samples from the AMD inflow and outflow, have been analyzed for $\delta^{34}S$ of sulfate as well as standard chemical parameters. Preliminary results indicate that in both systems, bacterial sulfate reduction is occurring yet the degree of reduction is not uniform throughout the cells. Within each system, areas where only a limited amount of bacterial sulfate reduction has occurred are characterized by high concentrations of sulfate coupled with $\delta^{34}S$ values only slightly different than the influent AMD. In contrast, low sulfate concentrations together with large $\delta^{34}S$ fractionations are found in areas where extensive bacterial sulfate reduction has taken place. The observed range in fractionation values likely reflects the development of preferential flow paths and points of stagnation within the systems. This implies that not all of the reactive substrate put into a cell will contribute to AMD treatment. The results of this study provide information not typically attainable in smaller laboratory-scale studies and point to the need for further engineering of SRBRs to optimize field-scale applications.

Additional Key Words: sulfate-reducing bioreactor, stable sulfur isotopes

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[2] Matthew D. Reeder, Research Associate, Department of Geological Sciences, Indiana University, Bloomington, IN 47405 *mdreeder@indiana.edu*. Tracy D. Branam, Research Scientist, Indiana Geological Survey, Bloomington, IN 47405. Greg A. Olyphant, Associate Professor, Department of Geological Sciences, Indiana University, Bloomington, IN 47405.

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Introduction

Sulfate-Reducing Bacteria

Since microbially-mediated sulfate reduction was first proposed as a means to treat streams affected by AMD (Tuttle, 1969), several studies have incorporated sulfate-reducing bacteria (SRB) into AMD treatment designs (Benner *et al.*, 1997; Costa *et al.*, 2008). Bacteria capable of sulfate reduction use dissolved sulfate as an electron acceptor in a dissimilatory reaction (energy is generated but the sulfur is not incorporated into the cell). In this reaction, SRB couple the oxidation of an available carbon source (represented here by generic CH_2O) to bicarbonate (HCO_3^-) with the reduction of sulfate (SO_4^{2-}) to sulfide (H_2S):

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \quad (1)$$

The bicarbonate that is generated in this reaction decreases the overall toxicity of the water by increasing the pH and net alkalinity of the system. Another positive effect is that the resulting H_2S readily complexes with dissolved metals and forms insoluble sulfide minerals:

$$H_2S + Metal^{2+} \rightarrow MS_{(s)} + 2H^+ \quad (2)$$

In this reaction, divalent cations such as iron (Fe^{2+}) will be precipitated as stable metal-sulfides (MS). Trace metals, such as cadmium (Cd^{2+}), copper (Cu^{2+}), lead (Pb^{2+}), nickel (Ni^{2+}), and zinc (Zn^{2+}) can also be removed from the water either directly as metal-sulfide minerals or by co-precipitation and sorption.

In order for the activity of sulfate-reducing bacteria to serve as an effective AMD treatment strategy, certain environmental conditions must be established. The system must be anaerobic and contain dissolved sulfate, available organic carbon (such as acetate, ethanol, formate, lactate), and have a near-neutral pH (Neculita *et al.*, 2007).

Sulfate-Reducing Bioreactors

Sulfate reducing bioreactors (SRBRs) contain a blend of materials intended to promote bacterial sulfate reduction by providing the necessary conditions described above. Typical SRBRs are comprised of a mixture of the following main components: a source of bacteria (inoculum), a readily consumable carbon source, pH neutralization capacity, and a matrix

material. These passive remediation systems are intended to function for an extended period of time with minimal maintenance interaction.

A diverse microbial population is likely to be present (albeit at low numbers) in all of the materials used in the bioreactor. In order to accelerate the bacterial activity of the cell, materials such as compost (McCauley *et* al., 2009) or creek sediment (Zagury *et al.*, 2006) are added to the system to increase the population of bacteria capable of sulfate reduction.

Sulfate reducing bacteria are not capable of directly using complex organic matter such as cellulose (Chang *et al.*, 2000). In order to thrive, the SRB depend on a community of other bacteria in the system to break down complicated organic materials to simpler carbon molecules that are accessible and easily degradable. Small organic molecules such as lactate, acetate, and ethanol have been shown to be effective as carbon sources in bench-scale bioreactor experiments (Tsukamoto *et al.*, 2004).

Reactive materials such as limestone are incorporated into the system in order to neutralize the AMD to a point where microbially-mediated sulfate reduction is possible (pH 5-8) (Waybrant *et al.*, 1998).

The matrix material (typically straw, hay, wood chips, and/or saw dust) comprises the bulk of the system and provides a structure for the bioreactor. This framework maintains the physical conditions of the system by preventing over-compaction and the development of preferential flow paths as well as aiding in sequestering precipitates without clogging the system.

The type and relative proportion of the materials used in the bioreactor depends on local availability of the materials and site-specific characteristics such as available space and contaminant loading rates.

Sulfur Isotopes Related to AMD Remediation

The two main stable sulfur isotopes found in nature are ^{32}S (95%) and ^{34}S (4%). During AMD remediation, these isotopes partition between the un-reacted sulfate reservoir and the produced sulfur species in a manner reflective of bacterial sulfate reduction. For the reaction described above (eqn 1), an identifiable isotopic signature is created when sulfate containing "lighter" sulfur ($^{32}SO_4^{2-}$) is converted to sulfide more readily compared to sulfate comprised of the "heavier" sulfur ($^{34}SO_4^{2-}$). This selective usage is a result of thermodynamic considerations

whereby ^{32}S-oxygen bonds require less energy to break compared to ^{34}S-oxygen bonds (the net energy gain by the bacteria is maximized). The result of this preferential use is that the residual sulfate (*i.e.* the sulfate not yet consumed in the reaction) will be enriched in ^{34}S and the sulfide that is generated will be depleted in ^{34}S relative to the isotopic composition of the original sulfate.

Sulfur isotope values are reported in delta (δ) notation relative to the Vienna Canyon Diablo Troilite (VCDT) international standard and have units of parts per thousand or permil (‰):

$$\delta^{34}S_{sample} = \left\{ \frac{\frac{^{34}S}{^{32}S}sample}{\frac{^{34}S}{^{32}S}VCDT} - 1 \right\} \times 1000‰ \quad (3)$$

Study Sites

Midwestern SRBR

The Midwestern SRBR, located in Pike County, IN (Fig. 1), treats a perennial low-flow (<30 L/min) AMD spring and an ephemeral stream that drains a small adjacent watershed (Table 1). The bioreactor, built in August 2008, contains ~1800m^3 of reactive substrate (50% wood chips-by volume, 30% straw, 10% limestone, and 10% compost). During the construction of the system, isolated sampling ports were distributed within the reactive substrate at a variety of depths in order to observe any three-dimensional trends in activity (Fig. 2). AMD enters the system at the surface of the east end and is discharged at the west end through a pipe connected to a collection network at the base of the reactive substrate layer. A column of water (0.1 to 0.75m) persists above the reactive substrate throughout the year.

Lacy – South SRBR

The Lacy – South SRBR, located in Martin County, IN (Fig. 1) was constructed in June 2009 and also treats a low-flow (<15 L/min) AMD seep (Table 1). This bioreactor contains ~120m^3 of reactive substrate with a similar composition to that used in the Midwestern SRBR described above. Due to the size of this SRBR, fewer sampling ports were installed in order to create a vertical profile through the system (Fig. 3). In contrast to the Midwestern SRBR, this system is buried by approximately 1.5m of fill. AMD enters the bioreactor from a pipe at the bottom and exits by means of a pipe connected to a collection network at the top of the reactive substrate (substrate – fill interface).

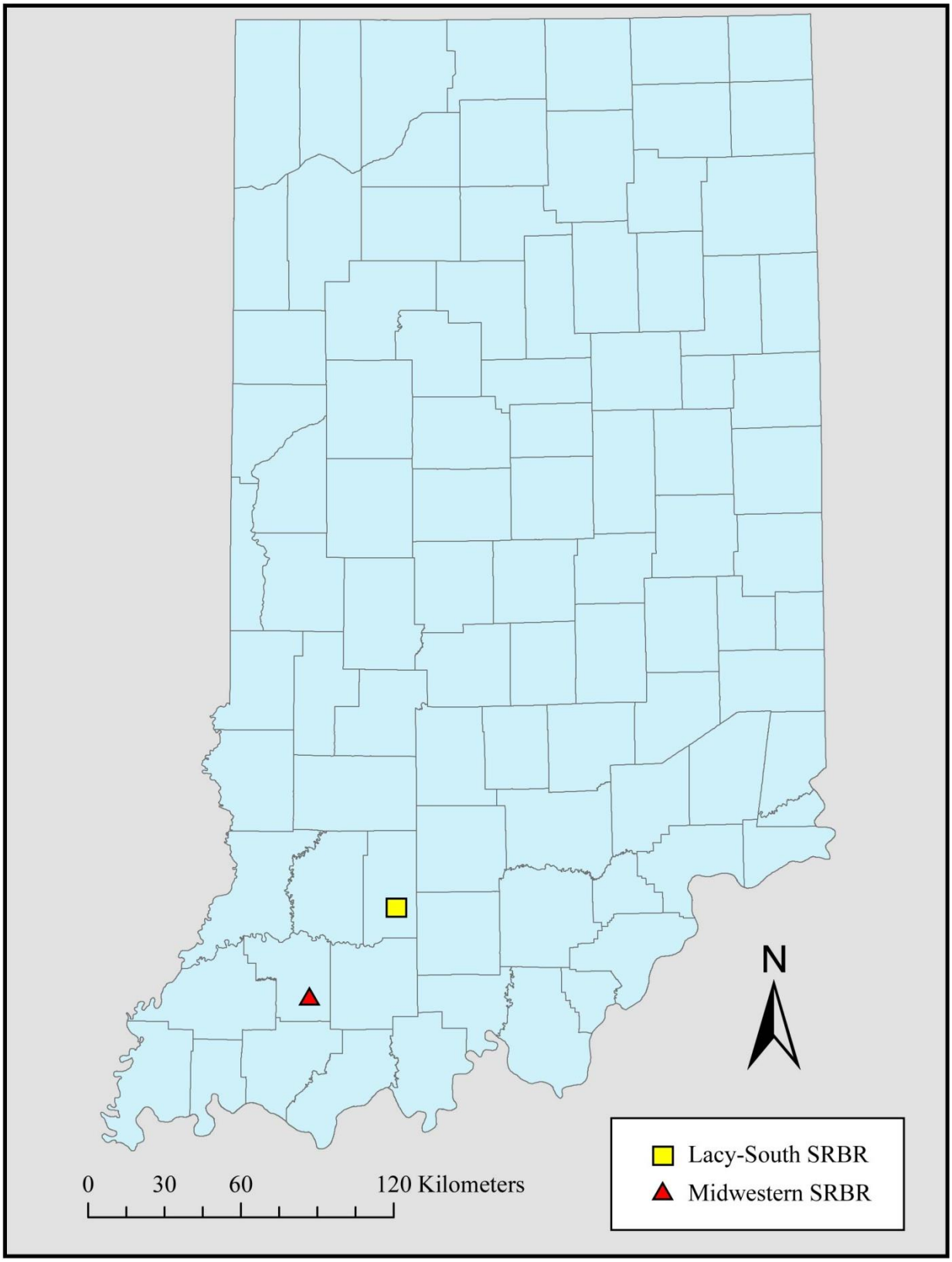

Figure 1. Location of Midwestern SRBR in Pike County, Indiana (red triangle) and Lacy-South SRBR in Martin County, Indiana (yellow square).

Table 1. AMD seeps treated by Midwestern and Lacy - South SRBRs

	Midwestern Spring (n=7)	*Midwestern Watershed (n=5)*	*Lacy-South (n=6)*
Conductivity (µS/cm)	3539	1798	3624
pH	2.8	6.0	2.4
Eh (mV)	683	219	644
Acidity (mg/L)[1]	605	70	1780
Alkalinity (mg/L)[1]	0	101	0
Cl (mg/L)	6	9	23
SO_4 (mg/L)	2696	1153	2220
Ca (mg/L)	499	350	61
Mg (mg/L)	162	53	66
$Fe_{(total)}$ (mg/L)	177	34	373
Fe(II) (mg/L)	57	34	264
Mn (mg/L)	12	9	4.1
Al (mg/L)	9	<0.5	115

[1] as $CaCO_3$

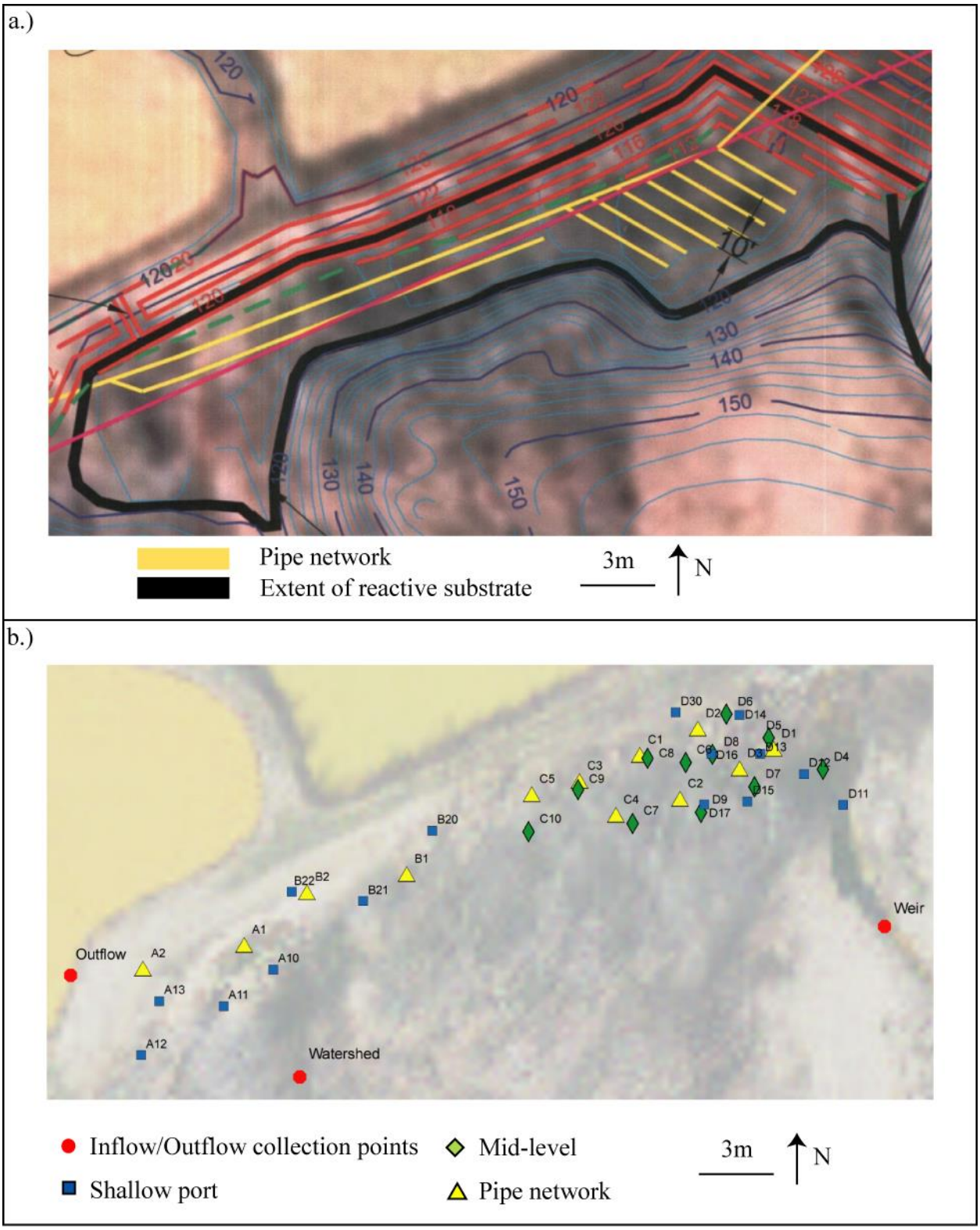

Figure 2. Midwestern SRBR layout: a.) map view; b.) sampler locations

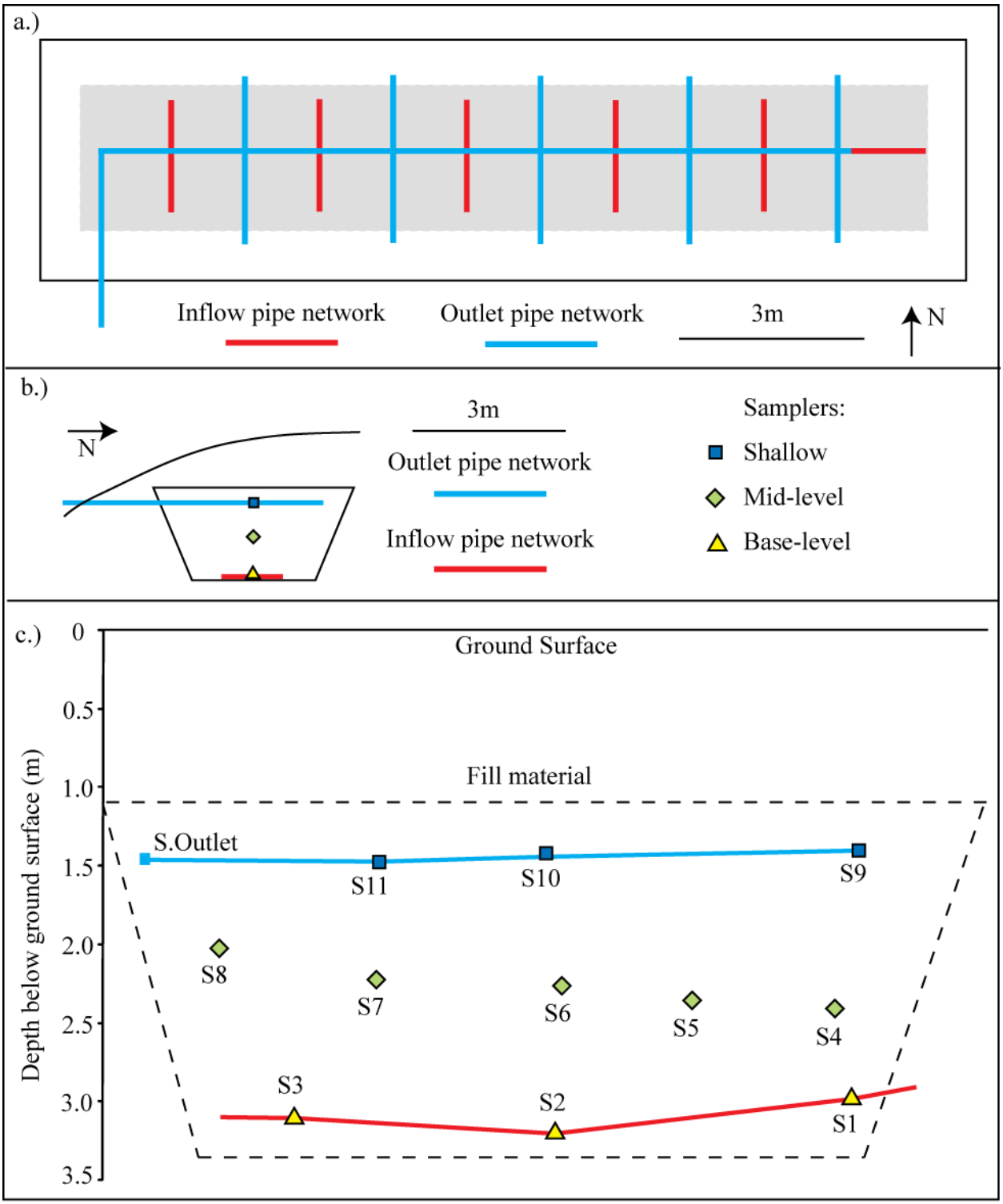

Figure 3. Lacy-South SRBR layout: a.) map view; b.) cross-section; c.) sampler locations.

Methods

Water Chemistry Sampling

Sampling was initiated at the Midwestern SRBR only after the system filled to capacity and produced a measurable outflow (January 2009, approximately five months after construction was completed). In contrast, samples were collected from the Lacy – South SRBR three weeks after construction was complete (June 2009).

Samples collected for untreated AMD and at the cell outflow pipes were obtained using the grab sample method. Field data collected from these two locations (pH, temperature, oxidation/reduction potential, dissolved oxygen, and specific conductivity) were obtained by submerging a YSI Multi-parameter sonde into the stream of water and recording data on a YSI 650 MDS display/logging unit.

Water samples and field data collected from the sampling ports located within the cell were obtained by using a peristaltic pump connected to a flow-thru cell in which the sonde was placed and field parameters monitored. Samples were collected in 1L bottles and placed in a portable refrigerator for transport back to the lab where they were filtered, separated into aliquots for various analyses and preserved per standard protocol. Commencing in May, 2009 unfiltered sample aliquots for iron and sulfide analyses were collected in the field, preserved with HCl and NaOH respectively, and placed in refrigeration for transportation.

Sulfur Isotope Sampling

Samples for sulfur isotopic analysis were collected in a separate 250 ml bottle pretreated with $CdCl_2$ (to preserve dissolved sulfide) according to the procedure described by Clark and Fritz (1997). The samples were prepared for analysis as $BaSO_4$ following the method outlined by Carmody *et al.* (1998). Sulfur isotopes were measured on a Finnigan MAT 252 mass spectrometer equipped with an elemental analyzer.

Results and Discussion

Midwestern SRBR

As noted, this bioreactor receives water from two distinct sources: the perennial low-flow AMD spring and an ephemeral stream that discharges storm run-off from a small watershed. The AMD entering the bioreactor has an average sulfate concentration of 2,570 mg/L (n=10)

with a consistent $\delta^{34}S$ value of -6.3‰ (Fig. 4). The discharge of the ephemeral stream has an average sulfate concentration of 1,140 mg/L (n=5) and a consistent, yet more depleted, $\delta^{34}S$ value of -9.8 ‰.

The outflow from the SRBR has lower sulfate concentrations than either of the inflows (910 mg/L; n=9) with a trend towards more enriched $\delta^{34}S$ values over time (Fig. 4). The initial $\delta^{34}S$ value of -8.0‰ measured at the outflow, which was more depleted than the AMD input, is more like the water coming from the ephemeral stream that enters the SRBR closer to the outlet pipe. That inflow only experiences a minimal amount of bacterial sulfate reduction due to a short residence time within the reactive substrate. Over a period of eight months, $\delta^{34}S$ values at the SRBR outflow were progressively more enriched, reaching a maximum of 6.1‰ in August of 2009. However, since most of the samples collected from the outlet have $\delta^{34}S$ values less than -2.0‰, it appears that the outflow from the SRBR is being dominated by quickflow from the ephemeral stream that experiences a low residence time in the reactive substrate. This interpretation is supported by the fact that the SRBR outflow with the most enriched $\delta^{34}S$ value (6.1‰) was collected when the ephemeral stream was dry.

Within the bioreactor itself, sulfate concentrations range from a maximum of 1,950 mg/L down to <50 mg/L while $\delta^{34}S$ values range from -6.1‰ up to 51.8‰. Samples collected closest to where the AMD enters the cell (D4, D11, D12) have $\delta^{34}S$ values similar to, but slightly more enriched than those of the AMD spring while the sulfate concentrations of those same samples are approximately 600 mg/L less than those of the spring. These results indicate that while bacterial sulfate reduction is actually occurring in this area, sulfate is constantly being replenished as reflected by the small enrichment in $\delta^{34}S$ values.

Water samples collected from the mid-level ports (D5-D9, C6-C10) have significantly lower sulfate concentrations (720 mg/L, n=7) and are more enriched ($\delta^{34}S$ = 22.1‰; n=7) relative to the AMD spring. Based on the limited data collected so far, this mid-level portion of the SRBR is experiencing the greatest amount of bacterial sulfate reduction. Samples collected from within the pipe network at the base of the bioreactor (D1-D3, C1-C5) have regularly yielded sulfate concentrations that are so low (<10mg/L) that they have not been analyzed for $\delta^{34}S$. The AMD entering the SRBR is apparently unable to penetrate to the deepest part of the cell. Future work

involving the analysis of $\delta^{34}S$ of dissolved sulfide will allow this issue (the development of preferential flow and stagnation) to be addressed further.

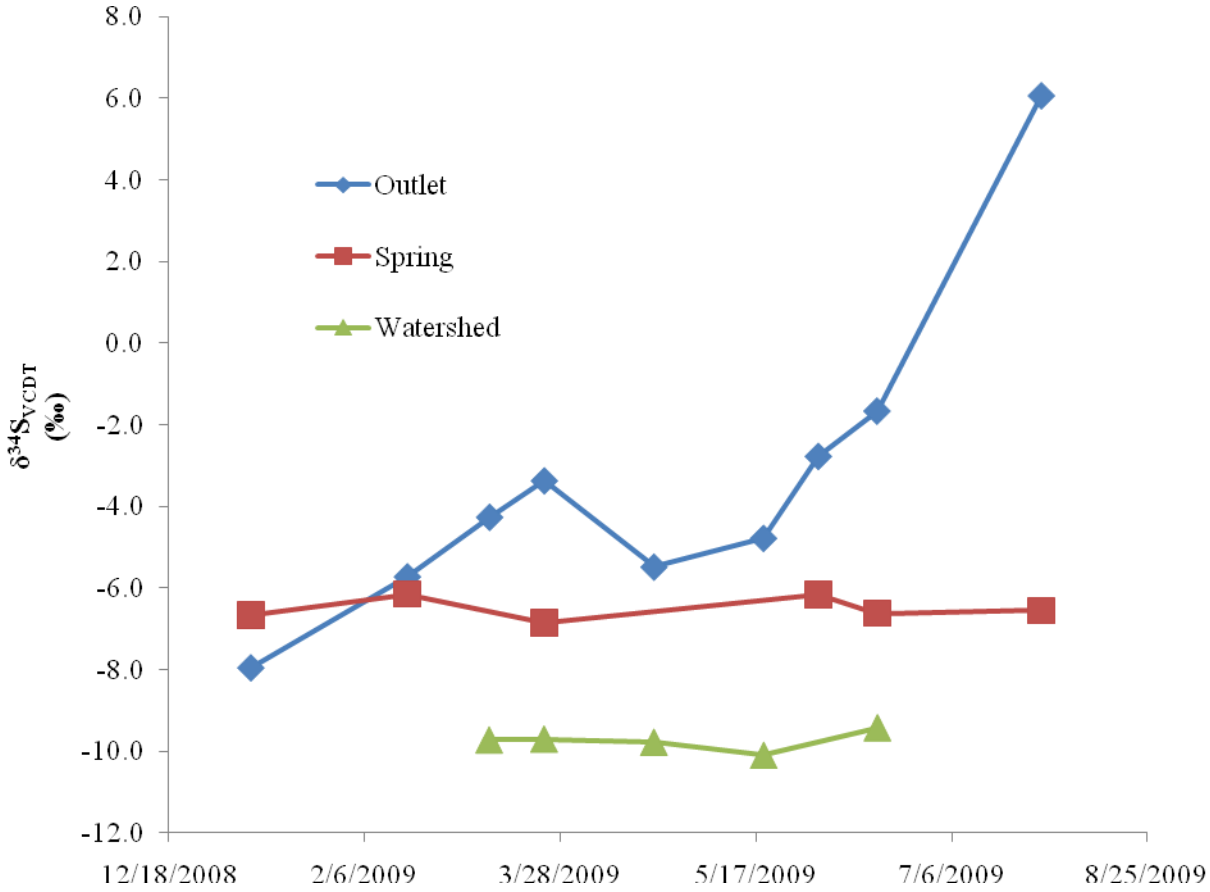

Figure 4 – $\delta^{34}S$ of sulfate at the Midwestern SRBR.

Lacy – South SRBR

Samples collected from the AMD seep, as well as the base of the bioreactor (S1, S2, S3) yielded consistent $\delta^{34}S$ values of 0.5‰ (n=10) during the first two months of operation (Fig. 5). These $\delta^{34}S$ values, together with consistently high sulfate concentrations (1,280 mg/L; n=14) indicate that the AMD is being distributed uniformly across the base of the SRBR.

Dissolved sulfate in the water discharging from the SRBR (860 mg/L; n=4) has significantly enriched $\delta^{34}S$ values (18.6‰; n=4) relative to the AMD entering the cell. The decrease in the concentration of sulfate coupled with the enriched $\delta^{34}S$ values indicate that bacterial sulfate reduction is effectively occurring within the bioreactor.

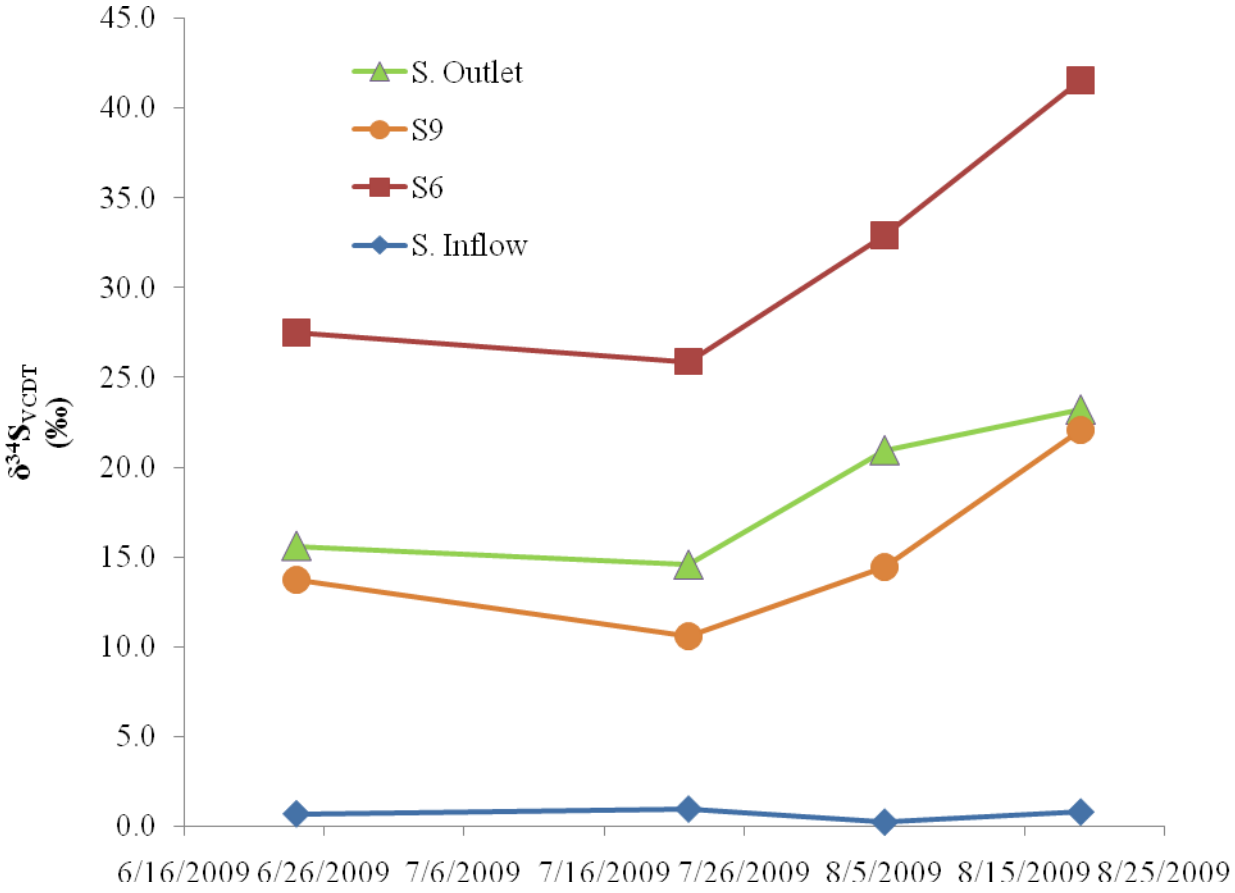

Figure 5 – Temporal trend of $\delta^{34}S$ of sulfate at the Lacy – South SRBR.

Waters collected from the monitoring ports within the SRBR during the first sampling event (6/24/09) showed that the SRBR was functioning as designed from its initiation. In this bioreactor, substantial decreases in sulfate and iron concentrations are accompanied by increases in pH, alkalinity, and an enriched $\delta^{34}S$ value (Table 2). Interestingly, the most enriched $\delta^{34}S$ value (27.5‰) was observed in a sample collected from the middle of the cell (port S6). This sample also has the lowest sulfate and iron concentrations and the highest amount of alkalinity in the sample set. The overall spatial trends in these initial samples indicate that the middle portion of the bioreactor is providing the greatest degree of bacterial sulfate reduction with diminishing reduction occurring towards the boundaries of the cell.

Table 2. Lacy-South SRBR 6/24/2009 sampling event.

	Inflow (*S1,S2,S3*)	Mid-level (*S4,S5,S7,S8*)	Outflow (*S9,S10,S11*)	*S6*
pH	2.5	6.2	6.4	6.4
Alkalinity (mg/L)[1]	0	873	2070	2501
SO_4^{-2} (mg/L)	2167	1349	704	269
$\delta^{34}S$ (‰)	0.6	7.5	16.3	27.5
Iron (mg/L)	213	49	15	0

[1] as $CaCO_3$

The temporal trends of $\delta^{34}S$ values for various sampling locations within the SRBR are also shown in Fig. 5. These trends indicate that during the first two months of operation, AMD entering the system has maintained a consistent $\delta^{34}S$ value whereas the $\delta^{34}S$ values in the reactive portions of the cell have become progressively more enriched. This observed enrichment could be due to a decrease in the activity of the bacteria resulting in a lower rate of sulfate reduction or the development of preferential flow-paths resulting in points of stagnation within the bioreactor. In order to further address these issues, future work, including the analysis of $\delta^{34}S$ of dissolved sulfide, will focus on evaluating and tracking the rate of sulfate reduction in various portions of the cell.

Conclusions

Our limited data collected to date indicate that the two SRBRs installed to treat AMD in southwestern Indiana are functioning as designed when considering the observed changes in typical AMD indicators (*e.g.* sulfate and iron concentrations are decreased and pH and alkalinity are increased). However, the sulfur isotope analysis of water collected at various locations within the SRBRs indicates that the rate and extent of bacterial sulfate reduction is highly variable within the bioreactors. Consequently, the functional lifespan of such field-scale systems (primarily related to the rate of substrate utilization by the bacteria) would be difficult to evaluate by monitoring standard AMD parameters in the inflows and outflows alone.

In an effort to quantify the degree of variability within the Indiana bioreactors, more emphasis will be placed on the analysis of $\delta^{34}S$ of dissolved sulfide and its relationship to the variability in $\delta^{34}S$ of sulfate that has already been observed. It is hoped that this additional work

will lead to a better understanding of the life-cycle of these passive remediation systems and aid in creating better designs for optimal performance in subsequent field-scale applications of sulfate-reduction bioreactors.

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