THEORETICAL VERSUS OBSERVED HYDROCHEMICAL PERFORMANCE OF SULFATE-REDUCING BIOREACTORS IN SOUTH-WESTERN INDIANA[1]

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

Abstract: Sulfate-reducing bioreactor performance depends on a number of biological, chemical, and hydrological factors. Bioreactors are supposed to neutralize active acidity, generate alkalinity, and reduce sulfate concentrations. The performance of three bioreactors constructed to treat acidic seeps discharging from abandoned coal mine sites in south-central Indiana was evaluated through a number of means including the collection of chemical data over time that was used to calculate a dimensionless sulfate-reducing bioreactor index (SRBI). Internal monitoring of SRBI demonstrates that sulfur reduction is not uniformly efficient. Pockets of maximum sulfate reduction correspond to maximum sulfur isotopic fractionation, typically 40 to 60 per mil greater than the inflow $\delta^{34}S$ ratios (SRBI values 3 to >5). Such high fractionation is interpreted to indicate near-stagnant flow. Preferred flow paths are inferred in zones where inflow sulfate concentration is reduced by only 5 to 20 percent, and $\delta^{34}S$ ratios differ from inflows by no more than 5 to 6 per mil (SRBI values -0.5 to 0.5). The results of this study indicate that the current generation of field-scale sulfur-reducing bioreactors is not performing optimally, and premature failures are possible. More attention to all aspects of bioreactor design including size, morphology, plumbing, and changes in the physico-chemical composition of the matrix, must be considered to insure maximum degree and duration of performance.

Additional Key Words: passive treatment, acid mine drainage mitigation, abandoned mine land

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Introduction

Over the past decade, sulfate-reducing bioreactors (SRBs) were determined to provide a viable option for passive treatment of acid-mine drainage (AMD) (Gusek, 2002). SRBs are constructed in a wide variety of mining environments, from coal fields of the Eastern United States to metal mines in the west, and in other countries around the world. Although some research has been published related to design criteria such as substrate compositions, cell configuration, plumbing design, seasonal and climate impacts, contaminant loadings, and flow rates (Neculita et al., 2007; Doshi, 2006), much of the work was based on limited field data. In most cases hydrochemical evaluation was based solely on inflow and outflow chemistry. However, as shown in this study, we can gain much insight by monitoring the spatial distribution of hydrochemical conditions within the bioreactors over time.

Sulfate-reducing bioreactors were conceived from observations on the development of anoxic zones and microbial sulfate reduction in natural wetlands (Ehrlich, 1981). Constructed bioreactors have a limited life span, because they consist of a finite volume of microbial growth-enhancing substrate for treating AMD, which does not replenish itself. The general recipe for SRB substrate consists of a framework material such as wood chips, cellulolytic material in the form of straw or hay, organic-rich material containing readily biodegradable carbon-chain molecules such as occurs in compost, and a fine-grained carbonate material, all homogenized into a uniform blend. In theory, an ideal sulfate-reducing bioreactor will treat low-pH water consisting of a wide range of total acidity/sulfate/metals composition by first neutralizing the active acidity (increasing pH) followed by removing dissolved oxygen from solution. These conditions allow sulfate reduction to occur, producing hydrogen sulfide that will degas or react to form metal sulfides, while simultaneously increasing alkalinity within the treated waters. Reactions describing these changes can be illustrated from simple chemical equations and begin with the neutralization of active acidity when hydrogen ion reacts with calcium carbonate within the substrate:

$$H^{+} + CaCO_3 = Ca^{+2} + HCO_3^{-} \quad (1)$$

This initial abiotic reaction causes the pH to rise and the solubility of ferric iron and aluminum to decrease rapidly. Complex sulfate mineral deposits of aluminum can form as

observed by Thomas and Romanek (2002) but may be limited in distribution to the neutralization interface if the acid conditions behind this advancing front are sufficiently aggressive to re-dissolve neutralization-generated mineral phases. Beyond the neutralization front, higher pH conditions are more conducive to robust microbial growth (Waybrant et al., 1998). For anaerobic conditions to develop, oxygen must be depleted from the neutralized mine waters. A minor amount can be removed through precipitation of ferric iron oxide minerals that potentially form at the neutralization front. Because the neutralization front should advance through the cell as calcium carbonate is depleted, permanent oxygen removal by these reactions will be minimal as the mineral phases continue to re-dissolve and precipitate at the advancing neutralization boundary. The main process to remove oxygen is the activity of aerobic bacteria, which create the anoxic conditions just beyond the neutralization front within the organic-rich substrate. A simple variation of this microbially catalyzed reaction might be expressed as:

$$CH_2O + O_2 \rightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \quad (2)$$

where CH_2O represents degradable organic compounds. Important to this oxidation reaction is the formation of carbonic acid, which can dissociate to bicarbonate (reaction 2), or decompose to carbon dioxide gas and water, depending on the pH and partial pressure of carbon dioxide within the system.

Once the mine water is depleted of dissolved oxygen, anaerobic bacteria grow as long as there are appropriate nutrients and a chemical source of oxygen to be extracted for their metabolic processes. The most abundant chemical source of oxygen in mine waters is sulfate. Sulfate-reducing bacteria such as *Desulfovibrio* use sulfate oxygen in the metabolic decomposition of simple organic molecules to generate both hydrogen sulfide and alkalinity as shown in reaction 3:

$$SO_4^{-2} + 2CH_2O \rightarrow H_2S + 2HCO_3^- \quad (3)$$

Some hydrogen sulfide gas may escape the system, while some will dissociate to sulfide and react with metals to produce insoluble metal sulfides, as shown for iron monosulfide in reaction 4. The generated hydrogen ion is then available for reacting with available calcium carbonate in the substrate mix to generate even more alkalinity (reaction 1) that, in turn, buffers the pH.

$$H_2S + Fe^{+2} \rightarrow FeS + 2H^{+} \quad (4)$$

Precipitates that form in the anaerobic regions of an SRB cell are susceptible to oxidation and remobilization as the redox and acid neutralization fronts advance through the cell; however they may re-precipitate again further within the cell as long as an anoxic zone is sustained within the cell, which depends on sufficient substrate to maintain bacterial communities. Ultimately, a sulfate-reducing bioreactor will fail. Failure of a bioreactor is evident from a downward trend in alkalinity and the eventual return of acidic conditions accompanied by increased sulfate and metals in discharge effluent.

Several factors influence how well a sulfate-reducing bioreactor functions (Gusek, 2004). The substrate must be sufficiently homogenized to eliminate variable permeability that leads to preferred flow paths and eventual short-circuiting, allowing untreated AMD to pass through the cell. Complete homogenization of the substrate also minimizes chemical reaction variability that can influence the rate that reaction fronts migrate through the cell. The capacity of the SRB to completely neutralize acid and allow microbe communities to develop sufficiently to be effective can be compromised by excessive AMD loading. The plumbing design has to allow maximum distribution of influent AMD into the cell and collection of treated water in an effective manner that prevents flow channeling and overloading of a small percentage of the substrate. The design of a bioreactor should provide a sufficient residence time to allow all microbial-mediated reactions to equilibrate or at least proceed to a cost-effective extent. A complex system of microbes is required to sustain the bioactivity within a SRB (Seyler et al., 2003; Unten et al., 1998) and a short residence time is detrimental to the development of complex microbial communities and preservation of necessary anaerobic conditions. This paper focuses on how the chemical data collected from three bioreactors in south-central Indiana provide a means to evaluate bioreactor performance. A companion paper by Waddle and Olyphant (2012) in this volume provides a hydrologic evaluation of the three study bioreactors.

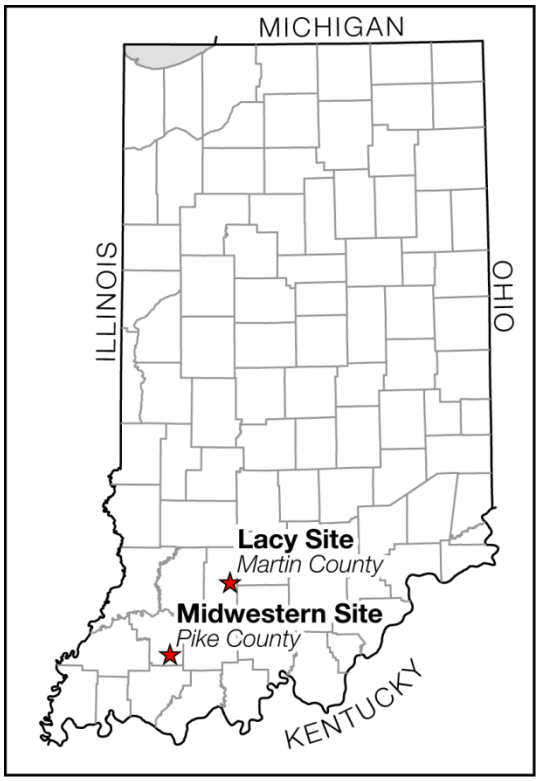

Figure 1 Map of Indiana showing the location of study sites for three sulfate-reducing bioreactors, two at the Lacy site, and one at the Midwestern site.

Study Sites

The three bioreactors we studied are located in the coal mining region of southwestern Indiana (Fig. 1). All three are composed of essentially the same blend of reactive substrate: 50% wood chips-by volume, 30% straw, 10% limestone, and 10% compost. Materials were blended using backhoes and front-end loaders, either onsite or at a nearby mixing station.

Midwestern Bioreactor

A bioreactor was constructed at the Midwestern Abandoned Mine Land Site to treat the perennial low-flow (50 L/min) discharge of AMD from a flooded underground mine. The bioreactor contains ~ 3,645 m^3 of reactive substrate. The bioreactor cell is 2 m deep at the inflow end and thins to approximately 0.5 m deep at the outflow end. A network of perforated pipes collects water from the base of the reactive substrate and funnels the water to an outflow pipe at the shallow end of the cell. Details of the bioreactor design and its planned performance are provided

in a companion paper (Waddle and Olyphant, this volume). Monitoring of the bioreactor commenced upon its completion in late 2008. The cell remains saturated most of the time but the water level drops during extended dry periods. During the driest times of the year, levels drop to the point that no discharge from the cell outflow pipe occurs.

Lacy Bioreactors

Two bioreactor cells were constructed on national forest land near the junction of Indiana State Road 550 and U.S. Highway 150 (Fig. 2). The Lacy North bioreactor is located on the east side of U.S. 150 near the base of a ridge where multiple adits were present from small, abandoned mines. The seepage is low flow (typically <4 L/min), but AMD concentrations are greater than those at the Midwestern Site (Table 1). The total volume of reactive substrate is only about 131 m^3, with the cell being built on top of two AMD seeps so that water has to flow upward to an outflow pipe that fixes the water level in the bioreactor and discharges the treated AMD into an adjacent settling pond.

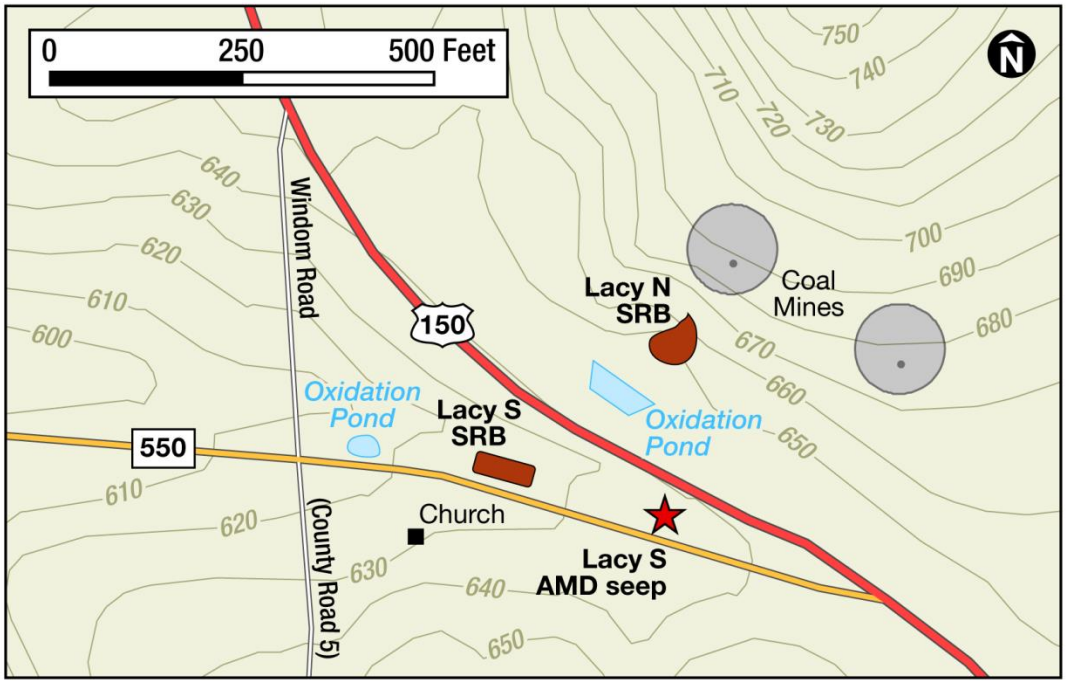

Figure 2. Map showing the location of Lacy North and South bioreactors.

Table 1. Physical and chemical data on AMD seeps treated by Midwestern and Lacy SRB cells

	Midwestern Spring (n=23)	*Lacy-North (n=12)*	*Lacy-South (n=23)*
pH	2.8	2.5	2.5
Acidity (mg/L)[1]	500	1400	1300
Alkalinity (mg/L)[1]	0	0	0
SO_4 (mg/L)	2400	2100	1600
Fe(II) (mg/L)	53	490	280
Al (mg/L)	10	67	75
Flow (L/min)	50	4.4	3.4

[1] as $CaCO_3$

The Lacy South bioreactor was constructed on the south side of a triangle formed by the intersection of S.R. 550, U.S. 150, and C.R. 5 (Fig. 2). An AMD seep emerges on the side of S.R. 550 after passing under U.S. 150, where it is believed to originate from mine adits along the

same ridge but to the south of the seeps that feed Lacy North. Acid-mine drainage inflow to the Lacy South bioreactor averaged about 3.5 L/min prior to its failure and decommissioning in late summer of 2011. Water quality is similar to that of Lacy North. The cell contained ~101 m^3 of reactive substrate and, unlike the other two study bioreactors, was covered by approximately 1.5 m of soil. Acid-mine drainage entered the bioreactor from a distribution pipe network at the bottom of the cell, and was collected in a pipe network at the top of the cell, approximately 2 m above the base where it discharges through a p-trap pipe into the roadside ditch and an oxidation pond (see Waddle and Olyphant, this volume).

Methods

Ongoing research by personnel of Indiana University and the Indiana Geological Survey concentrated on the hydrology of sulfur-reducing bioreactors and chemical reactions that occur within the systems through time. In addition to monitoring their inflows of raw AMD and outflows of treated water, we installed internal monitoring ports to allow sampling of waters in immediate contact with the reactive substrate of each of the three systems (Reeder et al., 2010). The sampling ports were constructed of two slotted PVC pipes approximately 15 cm long, capped at one end and connected by a t-joint with a tube port at the other end (Fig. 3a). Additional tube ports were connected to the internal pipe network of the Midwestern cell, and in both distribution and collection pipe networks of the Lacy South bioreactor (Fig. 3b). A length of tubing was attached to the sampler ports and buried in the substrate with the ends being

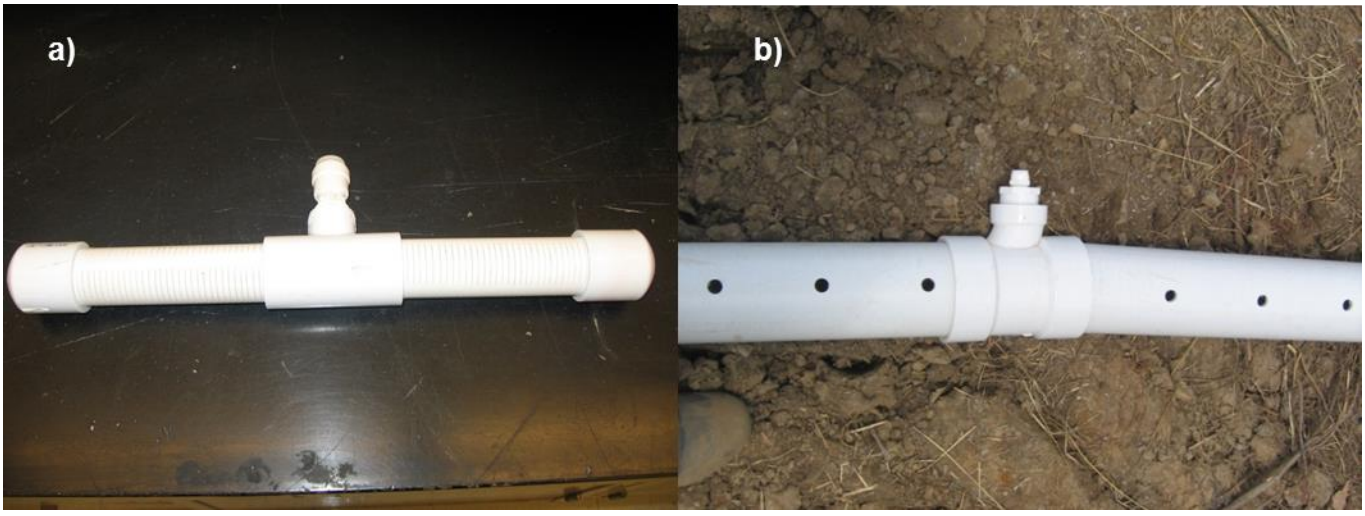

Figure 3 Photographs of water sampling devices placed inside bioreactor cells.

collected together and placed in lock boxes. The tubing ends were fitted with screw-cap adapters to prevent air from entering when water is not being siphoned through the tubes. Field measurements of pH, conductivity, dissolved oxygen, oxidation-reduction potential and temperature were obtained by connecting the tubing adapter to a fitting attached to c-flex tubing threaded through a peristaltic pump, with the other end connected to a flow-through cell. The water was pumped through a flow-through cell that housed a YSI multi-parameter sonde containing the appropriate probes for measuring field parameters. The sonde was connected to a YSI 650 data logger that provided continuous readings of the sonde probe measurements. The time required to obtain consistent readings was a function of both the pumping rate and sample port tubing length. Efforts to minimize over pumping and prevent excessive drawdown at the sampler were made by not exceeding a pumping rate of 1 liter/minute. By employing this procedure, the time required to obtain consistent field data readings varied from a minimum of 10 minutes for short tube lengths up to 20 minutes for the longest tube lengths. Upon achieving stable field measurements, the tubing from the peristaltic pump was disconnected from the flow-through cell and attached to a stainless steel tripod filtration unit containing a 0.45-micron cellulose nitrate filter for processing sample aliquots; these were either directly stored at 4°C, or treated with preservative and then stored at 4°C. The samples were returned to a laboratory where they were analyzed for sulfide, total alkalinity, total potential acidity, ferrous iron, COD, major anions, major cations, and trace metals using standard methods.

Our ongoing studies indicate that the most important chemical components associated with sulfate-reducing bioreactors mentioned above are sulfate, alkalinity, and ferrous iron. These components are associated with biological activity within the reactive substrate of bioreactor cells (reactions 2-4 above). One way to combine these variables into a single measure of bioreactor performance is to formulate a dimensionless sulfate-reducing bioreactor index (SRBI):

$$SRBI = \log_{10}[(Alk/Acy)/(SO_4^{=}{}_{port}/SO_4^{=}{}_{in})/(Fe^{+2}{}_{port}/Fe^{+2}{}_{in})] \quad (5)$$

where Alk/Acy is the total alkalinity to total potential acidity ratio of a sample (components in mg/L of calcium carbonate equivalent), $SO_4^{=}{}_{port}/SO_4^{=}{}_{in}$ is the ratio of sulfate concentration (mg/L) of water discharging from, or residing within, a bioreactor to that of the inflow, and $Fe^{+2}{}_{port}/Fe^{+2}{}_{in}$ is the same ratio of port to AMD inflow concentrations for ferrous iron. Antilog values of SRBI (raw SRB activity values) vary by as much as six orders of magnitude, so

logarithmic transformation simplifies plotting, tabulation and statistical analysis. In some of our samples, reducing conditions resulted sulfate and iron concentrations that were below method detection limits. In those cases, a value of half the detection limit was used in subsequent analyses. This typically resulted in an SRBI of > 5.

In a previous paper (Reeder et al., 2010), we introduced the value of measuring sulfur isotopic ratios as a tool for evaluating bioreactor performance. 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 reaction 3 described above, an identifiable isotopic signature is created when sulfate containing "lighter" sulfur ($^{32}SO_4^{-2}$) is converted to sulfide more readily, compared to sulfate composed of the "heavier" sulfur ($^{34}SO_4^{-2}$). This selective usage is a result of microbial preference based on 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 net result is that 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.

Sample collection, preparation, and method of analysis were described in Reeder et al. (2010). All isotope analyses were conducted in the laboratories of Indiana University. 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 (‰):

$$\delta^{34}S_{sample} = \left\{ \frac{\frac{^{34}S}{^{32}S}sample}{\frac{^{34}S}{^{32}S}VCDT} - 1 \right\} \times 1000‰ \quad (6)$$

The notation used to indicate the difference between the $\delta^{34}S$ for SO_4 collected from a sampling port and the inflowing AMD SO_4 on a specific date is a capital delta (Δ):

$$\Delta^{34}S_{SO4} = \delta^{34}S_{port} - \delta^{34}S_{AMD} \quad (7)$$

Results

The statistical relationship between the SRB index and sulfur isotopic fractionation of mine waters subjected to passive treatment by sulfate-reducing bioreactors is of interest because both variables are presumably influenced by microbial activity. Most of the water samples we subjected to isotopic analysis to date were collected from the Lacy South bioreactor and so the following preliminary analysis is based on those data (Fig. 4). The most notable result in the data is that most of the variability is associated with the sampling ports placed in the middle portion of the reactive substrate (triangles in Figure 4). The best-fit line is the trend line generated using only the data from the outlet and the upper (outlet pipe) collection ports. The coefficient of determination for that trend is $r^2 = 0.76$ (N=100), but when all the data are included in the statistical analysis, the coefficient of determination declines to $r^2 = 0.53$. The correlation is statistically significant in either case (99% confidence level), but the discrepancy in shared variance warrants discussion. One possible source of noise in the data is the fact that waters in the vicinity of floating sampler ports 6-8 were episodically experiencing very long residence times, which resulted in extreme sulfur reduction. The resulting low concentrations of sulfate in those samples made quantitative extraction difficult and may have carried over into the analyses of sulfur isotopes. Alternately, the low sulfate concentrations may have been approaching detection limits or experienced interference from unidentified chemical components in these excessive anoxic pockets, resulting in anomalous calculations of the SRBI values. Regardless of the ultimate sources of discrepancy, further work on the nature of the SRBI - $\Delta^{34}S$ relationship is warranted.

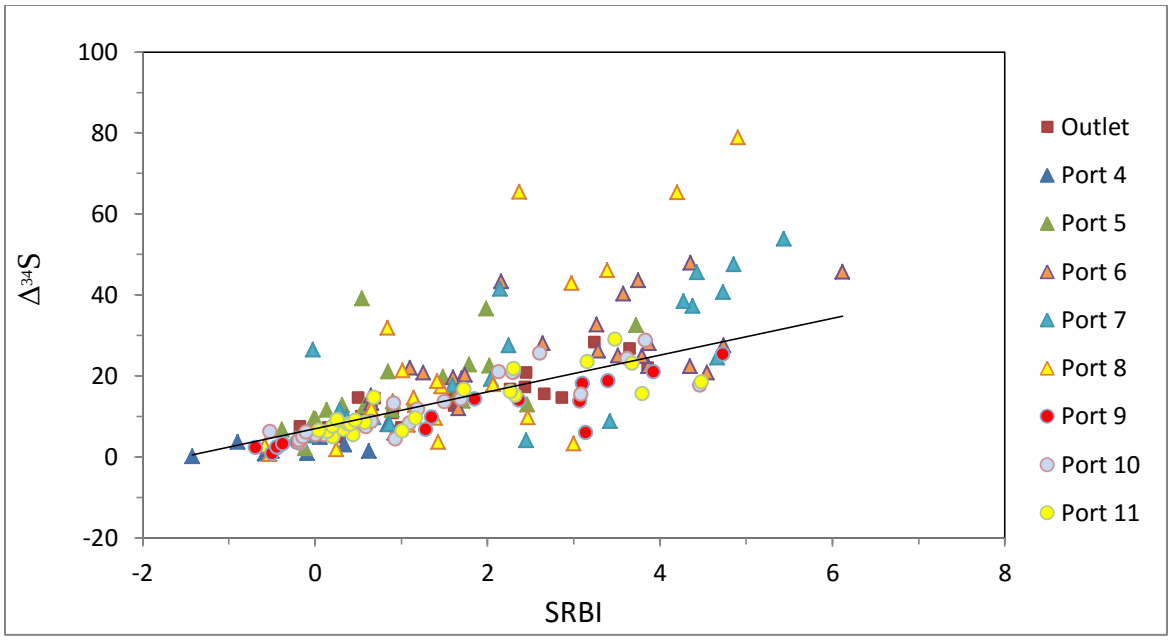

Figure 4. Scatter-plot showing relationship between computed values of SRBI and laboratory determinations of $\Delta^{34}S$ for all water samples collected at the Lacy South Bioreactor (N=191). Note strong trend between the measures of microbial reduction in the upper ports (along the outflow pipe) and tendency for positive anomalies to be associated with samples collected from the middle of the bioreactor.

Statistical correlations among the hydrochemical attributes show strongest correlations between SRBI and the attributes of its calculation, especially Fe^{II} and SO_4, but there are also statistically significant correlations between the attributes themselves (Table 2). In particular, there are negative correlations of sulfate and iron concentration with alkalinity, and an equally strong, but positive, correlation between Fe^{II} and acidity. This is likely due to the reactant/product relationships (reactions 3 and 4) in which alkalinity is generated from the conversion of sulfate to hydrogen sulfide that reacts to sequester Fe^{II}. The positive correlation between Fe^{II} and acidity is expected because Fe^{II} is a form of mineral acidity and a major contributor to potential acidity under near-neutral pH conditions.

Table 2. Correlation matrix between selected hydrochemical attributes of the three study bioreactors

	Tw	Acid	Alk	SO_4	Fe^{II}	Sulfide	SRBI
Tw	1.00	-0.03	0.09	-0.21	-0.14	0.25	0.29
Acid		1.00	0.09	0.08	0.47	-0.18	-0.38
Alk			1.00	-0.46	-0.51	0.05	0.61

SO4				1.00	0.47	0.02	-0.77
Fe^{II}					1.00	-0.22	-0.84
Sulfide						1.00	0.09
SRBI							1.00

Notes: Correlations based on a sample size of N=461. A correlation coefficient of 0.15 or greater is statistically significant at the 99% confidence level.

In an effort to further elucidate the primary controls on the SRBI, we undertook a multiple regression analysis. The model we evaluated was as follows:

$$SRBI = b_0 + b_1 \log(Tw) + b_2 \log(Acid) + b_3 \log(Alk) + b_4 \log(SO_4) + b_5 \log(Fe^{II}) + b_6 \log(Sulfide) + e \quad (8)$$

where the parameters $b_0 \ldots b_6$ are regression coefficients representing the relative contributions of each chemical attribute to the observed variance in the SRB index, and *e* is a random disturbance term assumed to have a mean of zero. The analysis used all the data collected from the three study bioreactors (Table 3).

Table 3. Best-fit parameter estimated for a multiple regression model relating measured chemical attributes to the computed SRB index. As noted in the table, all of the parameters estimated are statistically different from zero, indicating that each of the selected chemical attributes is contributing significantly to the SRB index of water samples collected from the three study bioreactors.

Parameter	Parameter Estimate	Standard Error	t-ratio
b0	34.28	0.328	10.45
b1	0.625	0.067	9.27
b2	-0.272	0.032	-8.50
b3	0.319	0.031	10.28
b4	-0.447	0.017	-26.11
b5	-0.398	0.016	-24.95
b6	-0.089	0.017	-5.38

Note: N=461; R^2=0.93; all parameters estimated are statistically different from zero at the 99% confidence level.

Based on the magnitude of the computed t-ratios, sulfate concentration has the strongest partial effect on SRBI variation, followed closely by Fe^{II} and then, to a lesser but still strong degree, alkalinity. The combined effects of the chemical attributes account for 93 percent of the observed variability in the SRB index. The degree of correlation between chemical attributes and SRBI is

essentially independent of the bioreactor or monitoring port from which the water samples are collected (Fig. 5). Taken together, these findings indicate that the SRBI is, indeed, a generally applicable indicator of the efficacy of water treatment by sulfur-reducing bioreactors.

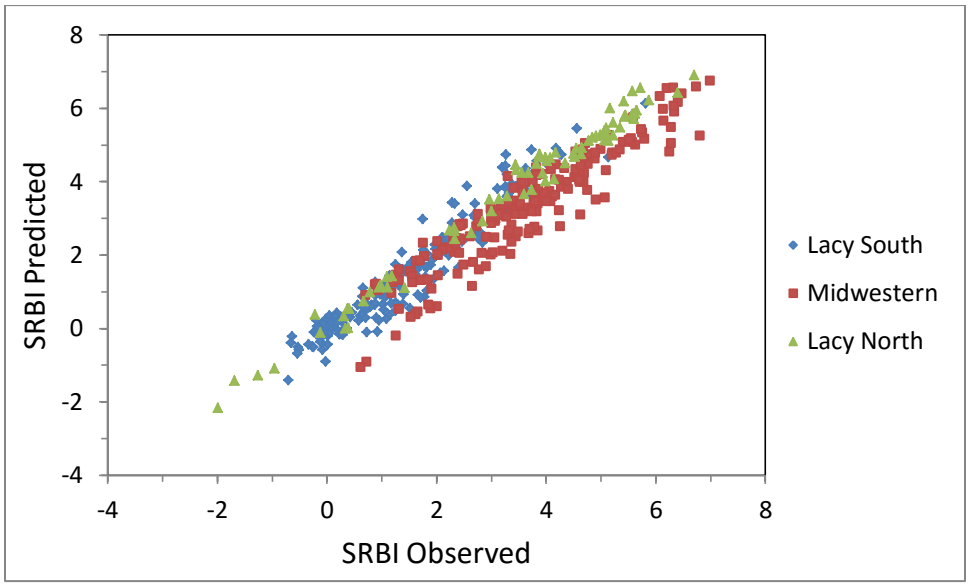

Figure 5. Scatter plot showing correlation between measured SRBI from equation 5 (Observed SRBI measurements) and SRBI computed from equation 8 (Predicted). Most of the data straddle the line of 1:1 correspondence with little difference in fit between data sets

During the two-year monitoring period at Lacy South, approximately 25 samples were collected from each monitoring port. Representative data used to calculate the SRB activity and SRBI are presented in Appendix A as an illustration of how the various components affect the index number. Because the sulfate and ferrous iron ratios of water from the sampling port compared to the AMD source flowing into a functioning bioreactor are likely never to exceed a value of 1, the alkalinity to acidity ratio is critical for producing a negative SRBI. Acidity exceeding alkalinity is an obvious flag, producing a negative SRBI which implies that microbial activity is not occurring at the sample location. Reversal of SRBI from positive to negative can result from depletion of substrate, development of preferential flow paths, excessive contaminant loading, or temperature fluctuations.

Our intensive monitoring of the Lacy South bioreactor revealed that the SRBI was effective at delineating where AMD treatment (by microbial communities) was most active within the cell. Patterns changed significantly within just one year of deployment, and there is evidence of short-circuiting in the bioreactor cell along the path of low SRBI values (Fig. 6). The changes in SRBI are supported by the measured values of $\Delta^{34}S$, which changed from 18.8 to 3.2 at the upper right sample port and from 5.0 to 0 in the substrate immediately below.

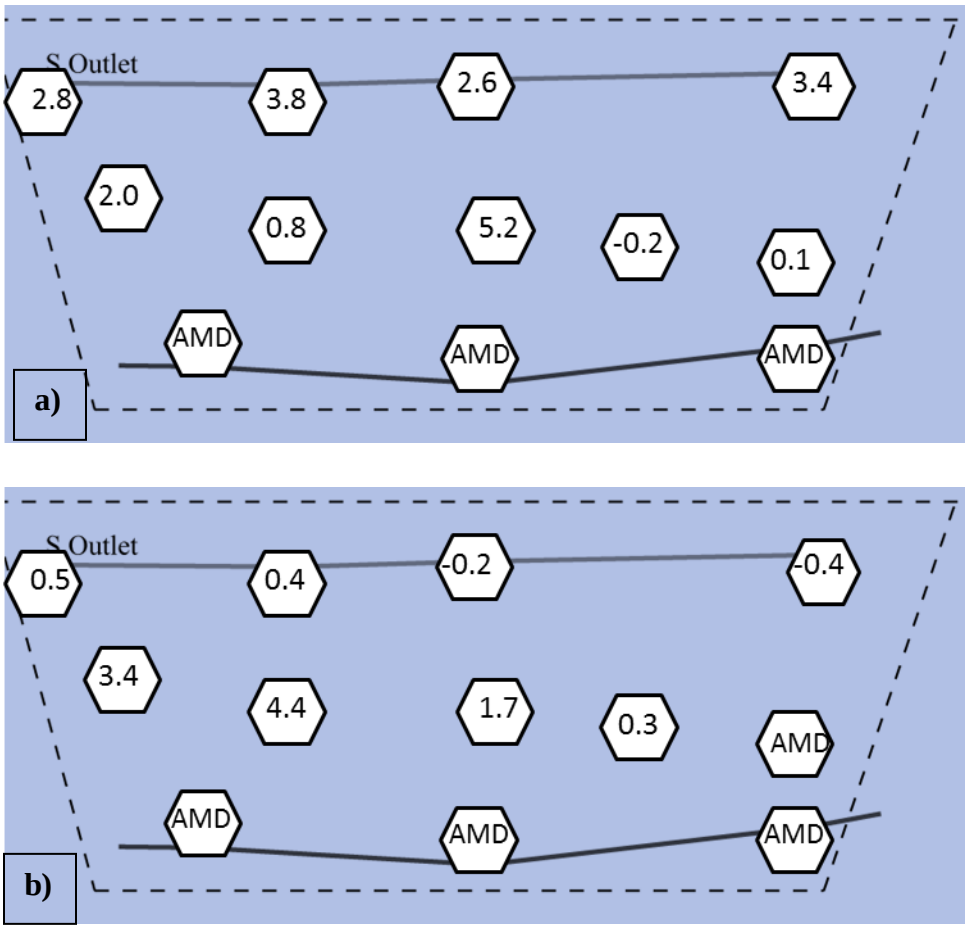

Figure 6 Schematic diagrams illustrating a cross sectional view of Lacy South SRB cell showing internal sampling port locations and SRBI numbers for a) September 1, 2009, and b) September 1, 2010. (Cell became operational June, 2009)

Discussion

The relatively inexpensive development of an accurate index number for characterizing sulfate-reducing bioreactor performance was a major goal of this research. A mathematical

expression using critical reactants and products of microbe-controlled reactions into a single dimensionless ratio (SRBI) seems to be a good start. When acidity equals or exceeds alkalinity, the SRBI value is less than 1, and in some cases even negative (Appendix). At the other extreme, highly anoxic reducing conditions lower sulfate and ferrous iron concentrations in water samples to detection limits, causing the SRBI to increase by orders of magnitude. Our findings to date indicate that it is possible to devise a rating scale for the level of reducing activity being generated within a bioreactor cell (Table 4).

Table 4. Scale for using SRBI number to evaluate bioreactor cell performance correlated to impacted parameters and estimation of microbial activity from chemical data.

SRBI range	Alkalinity level	Sulfate reduction	Degree of ferrous iron removal	Microbial activity indication
<1	Insufficient to slight excess	Minimal	Minimal to significant	Little to none
1 – 2.9	Net minor to high	Minor to significant	Significant to high	minor
3 – 4.9	High	Significant to very high	High to extreme	significant
≥5	High to very high	Very high to extreme	Extreme to complete	major

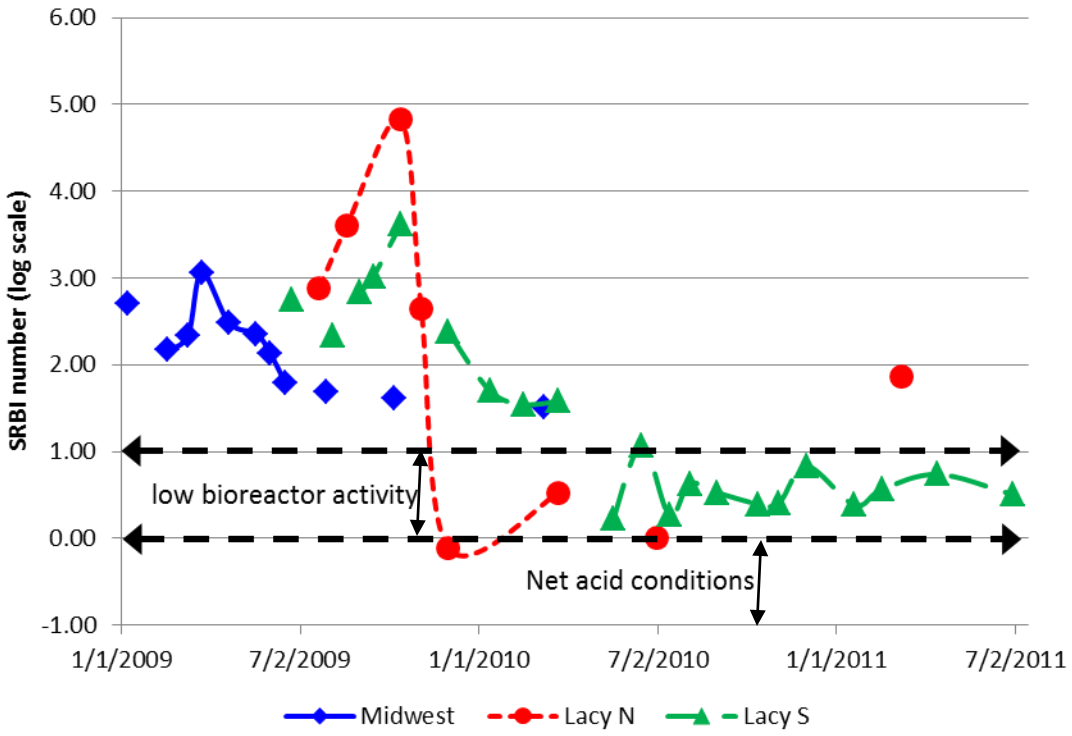

Figure 7. Graph of time-dependent performances of Indiana bioreactors based on calculated SRBI numbers.

After only about one year of deployment, low SRBI numbers for Lacy South outfall indicates no resurgence in microbial activity; the cell was ultimately decommissioned (Fig. 7). The decreases in SRBI number at Lacy North were a temporary condition (rather than short-circuiting or complete failure) and that cell continues to operate to date. Reasons for reduced productivity at the outflow are not discernible, but internal monitoring ports can provide a clearer picture of the reasons for cell failure. A changing internal pattern of SRBI at the Lacy South cell (Fig. 6) was already providing early warning of failure by short-circuiting within one year of its deployment.

In the larger bioreactor deployed at the Midwestern site, averaged data from 16 months of monitoring has helped to identify the development of a preferred flow pattern, and to recognize pockets of sustained bioactivity (Fig. 8). The SRBI values for the deeper part of the cell indicate a sustained, relatively high level of microbial activity. In contrast, the pattern generated from water collected from the shallow samplers is more mixed, with SRBI values near the outflow in the moderate range of performance (Table 4). This pattern of lower SRBI towards the outlet may

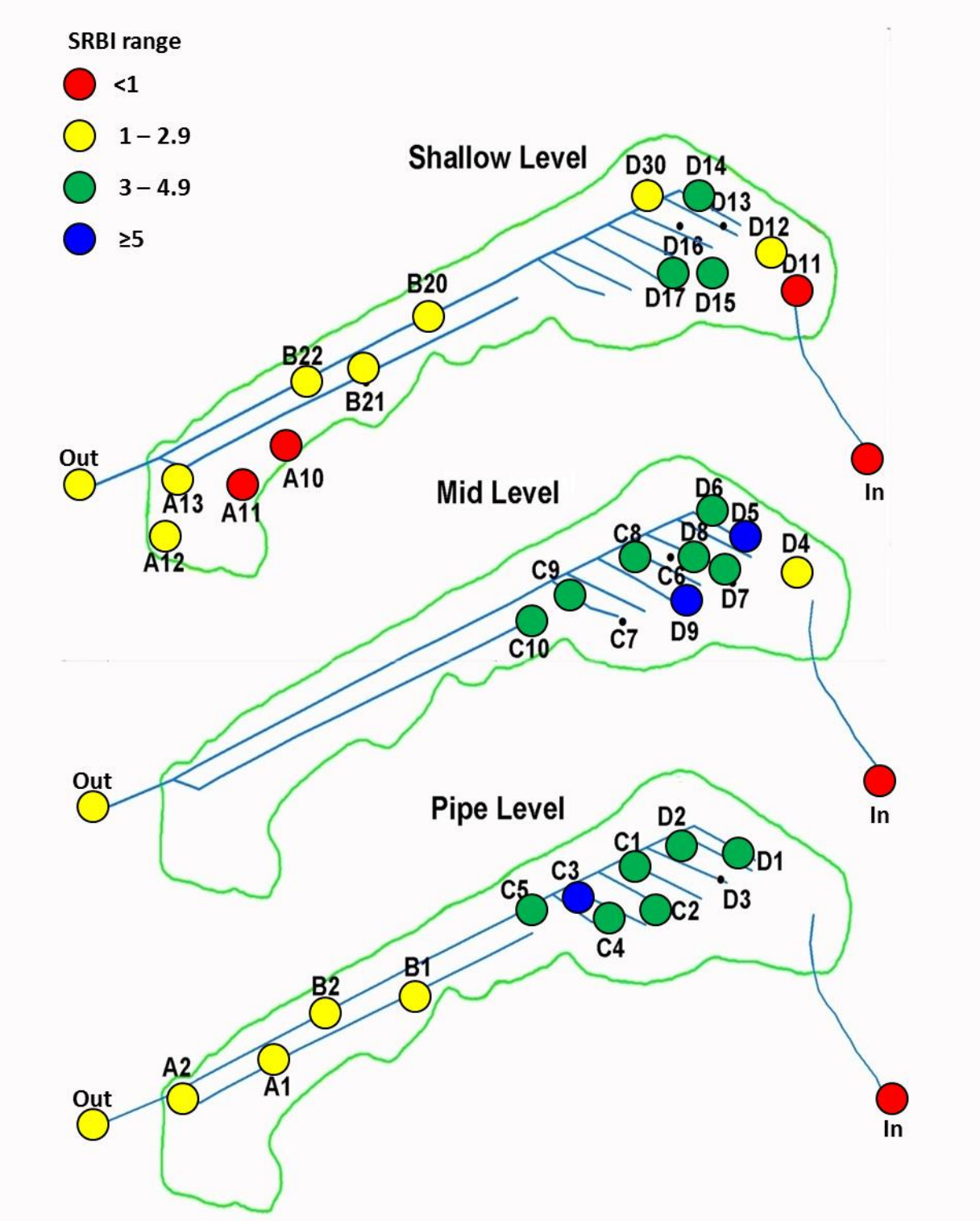

Figure 8 Schematic diagram showing the distribution of internal samplers, inflow, and outflow locations at Midwestern bioreactor cell with color coded SRBI values. The SRBI numbers derived from averaged parameter ratios were used to determine number.

be a consequence of ponding on the surface of the bioreactor which promotes flow of AMD above the substrate. These findings suggest that current designs are not optimal and that more considerations should be given to promoting long residence times in field-scale constructed bioreactors.

Conclusions

The ability to internally monitor bioreactors enhances the information obtained regarding their spatial and temporal performance. Isotopic analyses of sulfur provide an accurate assessment of how active sulfate-reducing bacteria are within an SRB. In the absence of sulfur isotope data, chemical relationships between parameters affected by bacterial-mediated reactions may provide an alternative to evaluating SRB performance. The combination of internal monitoring with this proposed SRB bioactivity index number is effective in showing where cells constructed in southern Indiana have experienced failures and provides indications of the causes.

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Appendix

Table A1. Subset of chemical data used to calculate sulfate-reducing bioreactor activity and index values. Data shown collected from floating sampler S7 at Lacy South bioreactor and acid mine drainage flow into the bioreactor.

Date	Alkalinity[1] mg/L $CaCO_3$	Acidity[2] mg/L $CaCO_3$	SO_4 mg/L	$SO_{4\ AMD}$ mg/L	Fe^{+2} mg/L	Fe^{+2}_{AMD} mg/L
6/24/2009	737	104	1438	2024	9	263
8/5/2009	393	164	1106	1255	115	202
8/19/2009	444	151	1369	2299	93	298
9/1/2009	410	257	1133	2203	170	367
9/15/2009	388	469	1577	2760	260	403
10/13/2009	496	111	103	1184	52	152
11/3/2009	653	76	56	1172	20	142
12/1/2009	273	198	569	1458	24	182
1/12/2010	419	229	115	702	6.6	151
2/16/2010	699	44	3	1216	2.6	111
2/23/2010	723	48	4	414	1.3	38
3/23/2010	246	142	330	468	76	55
5/18/2010	524	134	1044	1416	47	250
7/14/2010	1083	37	39	1856	1.6	321
8/4/2010	1135	61	187	2108	1.0	353
9/1/2010	1193	160	669	2258	0.5	469
10/13/2010	1135	80	817	2841	0.1	540
11/3/2010	1148	110	885	2568	0.6	542
12/1/2010	977	45	857	1620	0.7	342
1/19/2011	456	31	1362	1885	2.9	375
2/16/2011	328	782	1351	1795	222	376
4/14/2011	309	240	411	668	110	121
6/30/2011	458	454	1120	1093	143	183

[1]Total alkalinity determined to pH inflection point in lab analyses, and to color change endpoint in field analyses.

[2]Total potential acidity to pH endpoint 8.3 determined from alkalinity-titrated samples. Only acid added to lower pH beyond alkalinity neutralization point was subtracted from acidity determinations, resulting in no negative (net) acidity values.

Table A2. Ratios of key components related to microbial activity, calculated SRB activity and SRBI, and sulfate isotope signature of water samples collected from floating sampler S7 at Lacy South bioreactor.

Date	Alk/ Acy	SO_4/ $SO_{4\ AMD}$	Fe^{+2}/ $Fe^{+2}{}_{AMD}$	SRB activity	SRB index	$\delta^{34}S_{sulfate}$
6/24/2009	3.55	0.71	0.04	141	2.15	4.2
8/5/2009	2.4	0.88	0.57	5	0.68	10.0
8/19/2009	1.28	0.6	0.31	7	0.84	11.4
9/1/2009	1.59	0.51	0.46	7	0.83	9.3
9/15/2009	0.83	0.57	0.64	2	0.35	6.2
10/13/2009	4.45	0.09	0.34	150	2.18	41.7
11/3/2009	8.55	0.06	0.13	965	2.98	
12/1/2009	1.38	0.53	0.14	19	1.28	19.5
1/12/2010	1.83	0.15	0.04	282	2.45	27.4
2/16/2010	13.81	0.004	0.02	162614	5.21	
2/23/2010	14.51	0.01	0.03	44517	4.65	24.4
3/23/2010	1.74	0.71	1.4	2	0.25	11.4
5/18/2010	3.92	0.74	0.19	28	1.45	17.9
7/14/2010	29.34	0.02	0.01	276146	5.44	54.5
8/4/2010	18.52	0.09	0.003	71604	4.85	48.2
9/1/2010	7.46	0.3	0.001	24107	4.38	38.3
10/13/2010	14.12	0.29	0.0002	264927	5.42	42.1
11/3/2010	10.48	0.34	0.001	26898	4.43	47.4
12/1/2010	21.74	0.75	0.002	13205	4.12	40.7
1/19/2011	14.91	0.72	0.01	2643	3.42	10.6
2/16/2011	0.42	0.75	0.59	1	-0.03	27.7
4/14/2011	1.29	0.61	0.91	2	0.36	10.3
6/30/2011	1.01	1.02	0.78	1	0.1	12.8