RFP NO. OSM PA (AMD-04)

INNOVATIVE MINE DRAINAGE IN-SITU TREATMENT

IN SITU TREATMENT OF ABANDONED MINE DRAINAGE UTILIZING INDIGENOUS BACTERIA IN A REDUCED ENVIRONMENT

Initial Bench Study Incubation Phase 1 – Final Report

Submitted To

Pennsylvania Department of Environmental Protection

Division of Acid Mine Drainage Abatement

Bureau of Abandoned Mine Reclamation

Harrisburg, Pennsylvania

Prepared By:

Winner Global Energy & Environmental Services

32 West State Street

Sharon, Pennsylvania

Submitted By:

Western Pennsylvania Coalition for Abandoned Mine Reclamation

226 Donohoe Road

Suite 110

Greensburg, Pennsylvania 15601

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Introduction

Current technologies for remediation of acid mine drainage (AMD) primarily focus on the neutralization of effluents. While limestone ditches, anaerobic wetlands, anoxic drains and settling ponds decrease the pH of the effluent, these technologies do not guarantee the removal of sulfate and metals. In addition to the high costs associated with maintenance and monitoring, lime addition techniques face a common problem: encrustation of lime on surfaces.

Alternative treatment approaches for the remediation of mine effluents are being explored. In contrast to lime addition, biological treatments including the use of permeable barriers and reactive mixtures (Zagury et al., 2006; Gibert et al., 2004; Coetser et al., 2006) have been shown to offer more effective metal removal (Zagury et al., online source), to lower operation and maintenance cost, to reduce the use of chemicals, and to increase sulfate removal (Christensen et al., 1996). However, long term efficiency, *in situ* performance, and the source of organic carbon are still subjects that need to be evaluated.

The present study – phase 1 evaluated the potential of three industrial solid waste materials as organic donors for sulfate-reducing bacteria (SRB) in the treatment of acid mine drainage.

Materials and Methods

Water source. Mine effluents from Indianola TP Boring, Diamondville Upper and Indianola Medrad were collected anaerobically with a submersible pump and transported to the laboratory. The 25 gal containers were kept at 4 ºC until analysis.

Solid waste materials. Three organic wastes [S (sliced potatoes), L (sludge waste from potato processing), and P (potato peels)] were used as organic donors to stimulate sulfate reducing bacterial activity within each treatment vessel. These substrates were selected based on results from the chemical characterization study conducted prior to the bench study. The three substrates were assessed for waste characteristics (TCLP metals, volatiles, semi-volatiles, herbicides and pesticides) to ensure that the materials were not hazardous.

Incubation vessels. Glass jars (940 ml) were inoculated with a reactive mixture. The mixture composition was determined based on literature review and similar published studies. The mixture included a carbon source (selected substrates), a bacterial source (mine water), nutrients, inert porous support and lime. The detailed mixture composition can be found in Table 1. Incubation vessels were prepared anaerobically and kept at room temperature. A total of thirty incubation vessels per mine were constructed (including three non-substrate control vessels per mine). Twenty seven additional vessels were constructed for sacrificial sampling for metal analysis per mine. Table 2 displays the distribution of incubation vessels per mine. The vessels were incubated in the dark at room temperature in anaerobic glove bags with a flow of nitrogen.

Lime addition. Three different amounts of lime (as calcium carbonate) were also assayed during the bench study: X, Y, and O. See Table 1, below.

Table 1. Detailed mixture composition per mine and per treatment

Mixture composition X									
Mine	1			2			3		
Substrate	P	S	L	P	S	L	P	S	L
Carbon source (%)	1.05	1.03	1	1.02	0.95	0.93	0.91	1.14	0.99
Nutrients (%)	0.05	0.05	0.04	0.05	0.05	0.04	0.05	0.05	0.04
Lime (%)	0.07	0.07	0.06	0.07	0.07	0.06	0.07	0.07	0.06
Sand (g)	30	30	30	30	30	30	30	30	30
Total volume (ml)	755	710	840	755	710	840	755	710	840

Mixture composition Y									
Mine	1			2			3		
Substrate	P	S	L	P	S	L	P	S	L
Carbon source (%)	1.05	1.03	1	1.02	0.95	0.93	0.91	1.14	0.99
Nutrients (%)	0.05	0.05	0.04	0.05	0.05	0.04	0.05	0.05	0.04
Lime (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sand (g)	30	30	30	30	30	30	30	30	30
Total volume (ml)	755	710	840	755	710	840	755	710	840

Mixture composition O									
Mine	1			2			3		
Substrate	P	S	L	P	S	L	P	S	L
Carbon source (%)	1.05	1.03	1	1.02	0.95	0.93	0.91	1.14	0.99
Nutrients (%)	0.05	0.05	0.04	0.05	0.05	0.04	0.05	0.05	0.04
Lime (%)	0	0	0	0	0	0	0	0	0
Sand (g)	30	30	30	30	30	30	30	30	30
Total volume (ml)	755	710	840	755	710	840	755	710	840

Substrate identification:
P: Potato peels
S: Sliced potatoes
L: Sludge waste from potato processing

Mixture composition:
X: 0.52 g of $CaCO_3$ added per incubation vessel
Y: 0.05 g of $CaCO_3$ added per incubation vessel
O: 0 g of $CaCO_3$ added per incubation vessel

Nutrients:
0.35 g of urea were added to each incubation vessel

Table 2. Distribution of incubation vessels, sacrificial vessels and treatments.

Mine	Treatment	No of vessels		Substrate	Lime addition
		For metals	For incubation		
Indianola TP	1	3	3	P	X
Indianola TP	2	3	3	P	Y
Indianola TP	3	3	3	P	O
Indianola TP	4	3	3	S	X
Indianola TP	5	3	3	S	Y
Indianola TP	6	3	3	S	O
Indianola TP	7	3	3	L	X
Indianola TP	8	3	3	L	Y
Indianola TP	9	3	3	L	O
Diamonville Upper	10	3	3	P	X
Diamonville Upper	11	3	3	P	Y
Diamonville Upper	12	3	3	P	O
Diamonville Upper	13	3	3	S	X
Diamonville Upper	14	3	3	S	Y
Diamonville Upper	15	3	3	S	O
Diamonville Upper	16	3	3	L	X
Diamonville Upper	17	3	3	L	Y
Diamonville Upper	18	3	3	L	O
Indianola Medrad	19	3	3	P	X
Indianola Medrad	20	3	3	P	Y
Indianola Medrad	21	3	3	P	O
Indianola Medrad	22	3	3	S	X
Indianola Medrad	23	3	3	S	Y
Indianola Medrad	24	3	3	S	O
Indianola Medrad	25	3	3	L	X
Indianola Medrad	26	3	3	L	Y
Indianola Medrad	27	3	3	L	O
Indianola TP	28	0	3	No substrate control	X
Diamonville Upper	29	0	3	No substrate control	Y
Indianola Medrad	30	0	3	No substrate control	O
Total	30	81	90		

Parameters measured

The following chemical and microbiological analyses were performed on each incubation vessel. See Appendix for more detailed information.

Physico-chemical parameters. Oxidation–reduction potential (ORP/Eh), pH, dissolved sulfide concentration and sulfate concentration were measured in each incubation vessel. ORP values were measured by using a Platinum Ag/Ag Cl electrode (Accumet). pH values were measured with a pH electrode (Oakton pH 11 Meter Kit). Dissolved sulfide and sulfate were measured by using commercial kits (LaMotte 3322 and 7778 respectively).

Metal analysis. Total and dissolved aluminum, manganese and iron concentrations were chosen to be monitored at the beginning and end of the incubation process.

SRB-by Most Probable Number (MPN). SRB-MPNs followed a modified protocol based on Fortin et al., 1998. Culture tubes were inoculated anaerobically and kept at 30 ºC in the dark.
Total counts. Numbers of total bacteria were assayed by microscopy with DAPI and Acridine orange standard staining. Water samples were preserved with a fixative solution and stored at 4

ºC for further analysis. Digital images were subsequently taken to obtain a more precise count of cells.

Results and Discussions

In the incubation experiments, we investigated the efficiency of sulfate reduction, production of hydrogen sulfide, metal removal and effect on pH. The three mine effluents were incubated anaerobically with a reactive mixture consisting of an inert porous support, a carbon source, a nutrient source and three different lime amendments. Sulfate, sulfide, Eh, pH, total bacteria and SRB were monitored at four sampling points (See Appendix I through IV). The incubation lasted 171 days.

The initial chemical/biological composition of the three mine waters is shown in Table 3. Initially Indianola mine waters (TP and Medrad) had a rusty color while Diamondville water was colorless. In the non substrate control vessels a yellow precipitate was formed immediately after mixing with the solid matrix.

Table 3. Average and standard deviation of initial chemical and biological composition of AMD waters from three mines.

	Sulfide		**Sulfate**		**pH**		**Eh**		**SRB-MPN**	**Total counts**
	ppm		**ppm**				**mV**		**#cells/ml**	**#cells/ml**
Mine	AV.	SD	AV.	SD	AV.	SD	AV.	SD		
Indianola TP	0.00	0.00	163.33	5.77	6.75	0.05	-17.6	4.0	< 0.3	1.1E+06
Diamondville Redrick Upper	0.00	0.00	170.00	0.00	2.95	0.49	225.0	1.7	< 0.3	8.5E+05
Indianola Medrad	0.00	0.00	170.00	0.00	6.69	0.06	-7.4	1.8	2.3	2.1E+06

SRB and carbon sources

SRB are anaerobic bacteria that inhabit anoxic environments. In the mine environment, SRB survive because they inhabit the sediment and not the water (Lyew & Sheppard, 2001). In fact, SRB in soils and sediments congregate on surfaces and particles as a mechanism of physical protection. However, for practical purposes, published column experiments, reactors and batch experiments are set up to monitor the water instead of sediments or soils. The data generated by these methods are representative.

As reported in Lyew & Sheppard, 2001; Chang et al., 2000; Luptakova & Kusnierova, 2005 and several related studies which evaluated treatment of AMD, the establishment of SRB was indicated with the following criteria: the presence of black precipitates, sulfurous odor (generation of hydrogen sulfide as a product of metabolism) and the generation of FeS (and other metal sulphides) according to the following equation:

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

In this experiment, the reactive mixture was not given an acclimation period [as described by Costa & Duarte, 2005] by which SRB adapt to the matrix conditions because the implemented design included sampling at time 0 right after the initial set up. In contrast, there was a three hour

period before initial sampling in the current study. During the incubation process, the formation of black precipitates was observed. Regardless of the lime treatment (X, Y, 0), Indianola mine (TP and Medrad) waters with P substrate showed more vessels with black precipitates (see Table 4). Due to the experimental vessel construct, it was not possible to detect if the black precipitate was formed at the bottom (i.e. in contact with the porous support). A black formation was spread out up to the top of the vessels. The sensorial detection of hydrogen sulfide [strong odor] during sampling was observed for all Indianola TP and Indianola Medrad vessels with P substrate. Indianola TP vessels with substrate S and Diamondville vessels with substrate P showed black precipitates from end to end.

Table 4. Presence/absence of sulfidogenesis in incubation vessels following AMD treatment.

Mine	Substrate	Lime addition	# of vessels with black precipitate	# of vessels with strong H_2S odor
Indianola TP	P	X	9	9
		Y	2	3
		0	0	0
	S	X	3	3
		Y/0	0	0
	L	X/Y/0	0	0
Diamondville	P	X	3	3
		Y	0	3
		0	1	3
	S	X/Y/0	0	0
	L	X/Y/0	0	0
Indianola Medrad	P	X/Y/0	9	9
	S/L	X/Y/0	0	0

SRB were counted by the Most Probable Number (MPN) technique. A three tube serial dilution was performed at each sampling point anaerobically. A growth of >110 MPN/ml was detected. This is the maximum quantifiable growth which can be determined using the statistical table for MPN assays. Thus, an increase of SRB numbers beyond that value could not be assessed.

Substrate P showed the highest SRB population of the three evaluated substrates [Figure 1]. This trend was observed in each mine regardless of the lime treatment. The vessels with substrate P were also the first to turn black (approximately after a week of the initial incubation)]. Indianola TP and Indianola Medrad waters treated with substrate P showed the fastest growth of SRB at each sampling point regardless of the lime treatment (2 days to reach maximum growth) followed by substrate S and substrate L with the least growth. Similarly for Diamondville, waters treated with substrate P for all lime treatments showed the fastest growth at each sampling point (~ 4 days to maximum growth) followed by substrate S with substrate L displaying the least growth.

The formation of black precipitates and the sensorial detection of hydrogen sulfide were not observed in the non substrate controls and in most of the vessels containing substrate L. Based on the analysis, substrate L appeared to be the least desirable both in terms of sulfidogenesis and as carbon source although some SRB growth was observed. This result agrees with Prasad et al., 1999 who established that sludges in general are poor substrates since a major portion of the degradable organic compounds are removed during waste treatment processes..

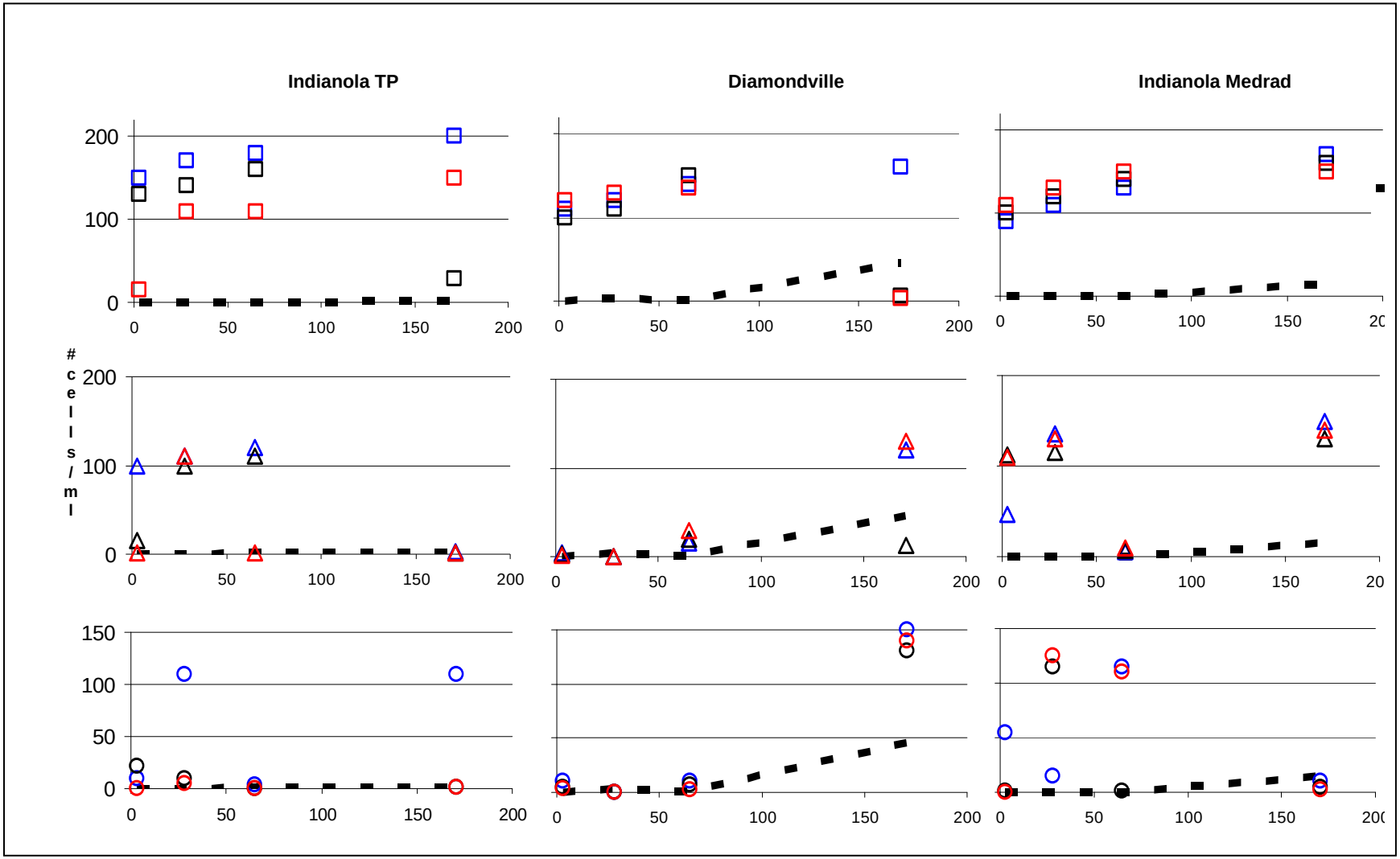

Figure 1. Mean values [n = 3] of SRB-MPN [#cells/ml] during the length of the incubation [171 days] for the three mine effluents evaluated.

Total bacteria counts

There was no trend over time observed for total bacterial counts for any of the treatments (Figure 2). However, mine water treated with substrate P appeared to have more bacterial cells overall.

Sulfate removal

Temporal changes in the incubation vessels were observed during the 171 days of incubation. Besides the presence of black precipitates in the vessels, sulfate measurements indicated that sulfate reduction did occur. Indianola TP mine waters mixed with substrate P regardless of the amount of lime displayed nearly 100 percent sulfate removal (Figure 3). Indianola TP waters amended with substrate S and L showed inconsistent results [data not graphed].

Diamondville waters treated with substrate P and X amount of lime or no lime displayed similar levels of sulfate removal (~ 95%). Sulfate removal was negligible for Diamondville waters treated with both substrates S and L (data not graphed).

Indianola Medrad waters mixed with substrate P displayed approximately 95% sulfate removal regardless of the amount of lime. Substrates S and L had no positive effect on sulfate removal.

The non substrate control vessels for each mine showed no sulfate removal. These results are comparable to the 98% efficiency that Prasad et al., 1999 found when ethanol was used as carbon source. Ethanol is a very well documented substrate for SRB growth but its relatively high cost is not justified for AMD treatment on a large scale.

Overall, the efficiency of sulfate removal was higher than the removal efficiency obtained by other studies with organic substrates (e.g. Christensen et al., 1996 reported only 27% sulfate removal with whey; Prasad et al., 1999 98% with ethanol). In the case of substrates S and L, loss of sulfate may have taken place at the beginning of treatment due to absorption onto ferric hydroxides [Fe_3SO_4] (see Appendix data). It is also possible that some additional sulfate originated in the carbon sources (Zagury et al., 2006). However, the concomitant production of hydrogen sulfide (see below) verifies that sulfate reduction occurred.

Sulfide production

Sulfide production is the result of metabolic activity of SRB. These bacteria catalyze the dissimilatory reduction of sulfate to sulfide and generate alkalinity by converting strong acid [sulfuric acid] to weak acid [sulfide]. During the incubation process a very strong odor of hydrogen sulfide was detected, indicating that sulfide was produced in high rates. The results obtained by using commercial kits likely underestimated the total amount of hydrogen sulfide that was generated because some sulfide most likely escaped when vessels were opened for analysis.

For Indianola TP waters, amendment with substrate P yielded high concentrations of hydrogen sulfide. The sulfide concentrations varied with the amount of lime addition. The highest to lowest concentration of hydrogen sulfide produced was observed for X, 0 and Y lime addition, respectively [75, 67, 50 ppm]. Substrates L and S barely increased sulfide from 0 to 5 ppm.

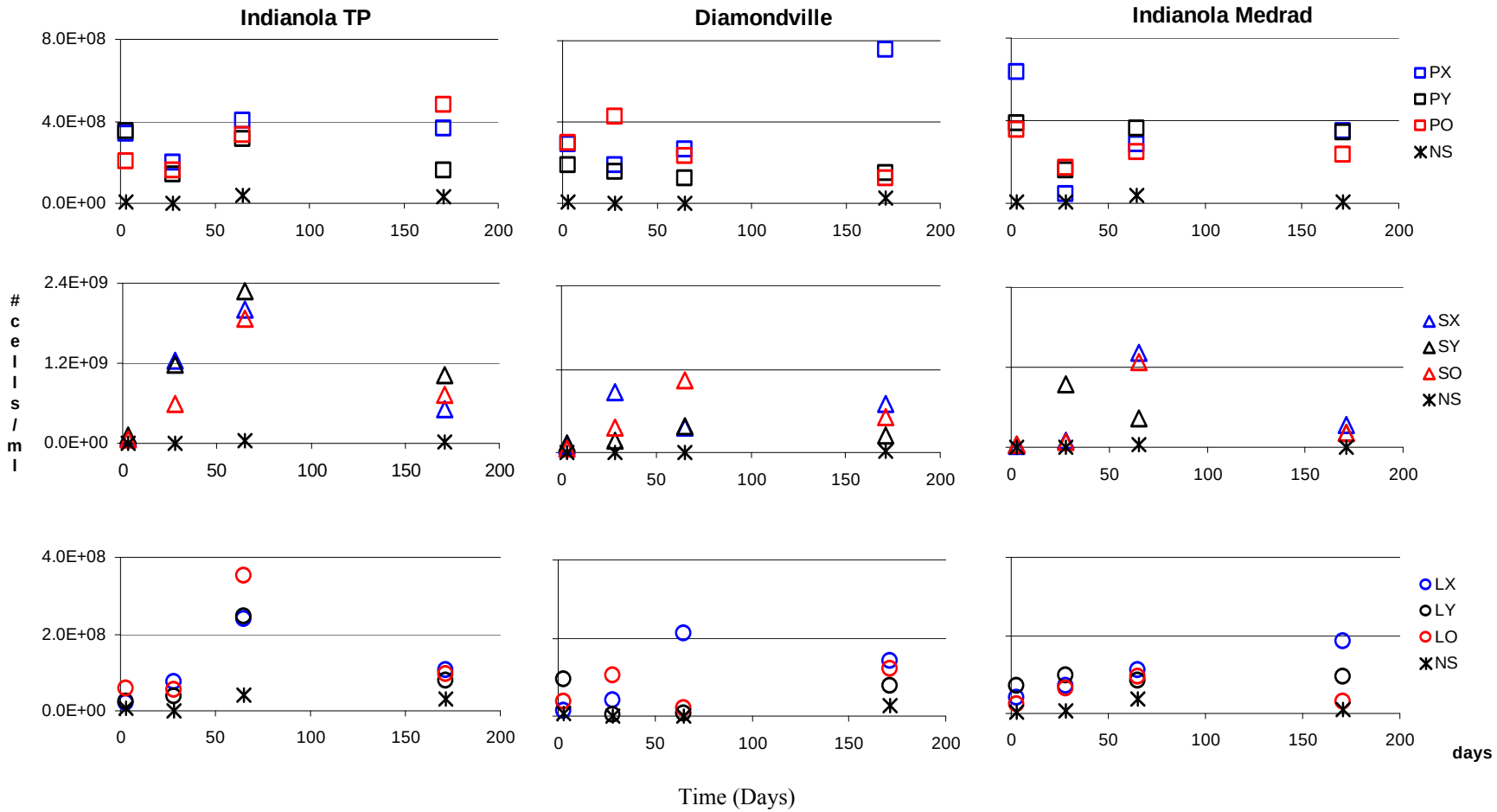

Figure 2. Mean values [n = 3] of total bacterial counts [#cells/ml] enumerated during the length of the incubation [171 days] for the three mine effluents evaluated. Most relevant treatments are shown.

For Diamondville waters, amendment with substrate P yielded the highest concentration of hydrogen sulfide. The sulfide concentrations varied with the amount of lime addition, with a high of 62 ppm for X amount of lime. Waters treated with substrate L and S showed an oscillating production of sulfide during the incubation process.

Indianola Medrad waters mixed with substrate P also generated high concentrations of hydrogen sulfide [ranging from 57 ppm for X amount of lime to 75 ppm for Y and 0 amount of lime]. Substrates L and S showed very little increase in sulfide production.

For the no substrate control treatments, no significant change in sulfide production was observed. The concentrations remained as 0 ppm or as low as 15 ppm.

As shown in Figure 3 for waters treated with substrate P, sulfide production appears to be correlated with sulfate reduction. The highest amount of sulfide generated was reached around day 60. We hypothesize that the subsequent decrease in sulfide concentration is because SRB growth was inhibited by competition with fermenting bacteria.

pH

Measured pH values obtained during the incubation experiments did not increase as expected [Figure 4]. Instead, an oscillatory pattern was observed. At the end of the incubation period pH values increased slightly in comparison to the initial values at time 0. Interestingly, when treated with substrate P, Diamondville waters showed a significant increase of pH from ~3 prior to incubation [see Table 3, initial values of mine effluents] to 5.7 in average after 171 days[see Appendix III]. Indianola TP and Medrad waters showed only a slight increase in pH.

Both Lyew & Sheppard, 2001 in a 28- day batch experiment using conductivity for monitoring AMD and Cheong et al., 1998 in a pilot reactor system for AMD treatment documented pH fluctuations. However, they did not explain why this phenomenon occurred. Other studies (Zagury et al., on line; Christensen et al., 1996; Kulnieks et al., 2005) concluded that at the beginning of treatments acidic pH and oxidizing conditions (+Eh) are commonly observed. Our pH results may reflect just that beginning intermittent period.

La and co-workers, 2003 reported a drop in pH after 120 days of performance of a bioreactor. They explained that the low values occurred because alkalinity was not generated. In the same manner, Chang and collaborators (2000) observed low pH values at the beginning of treatment of AMD with solid waste (35 weeks incubation period). Longer experiments have shown a pH increase in spite of the organic donor tested. For example, Costa & Duarte, 2005 evaluated bioremediation of AMD with SRB for 180 days. They reported that pH increased after 40 days. Gibert et al., 2004 measured biological mitigation of AMD with SRB for 210 days. They described that pH rose in some cases after 60 days of operation. These studies showed increases in pH (from 5 to 7). A longer experiment appears to be required to generate more conclusive results regarding impact on pH in the current study.

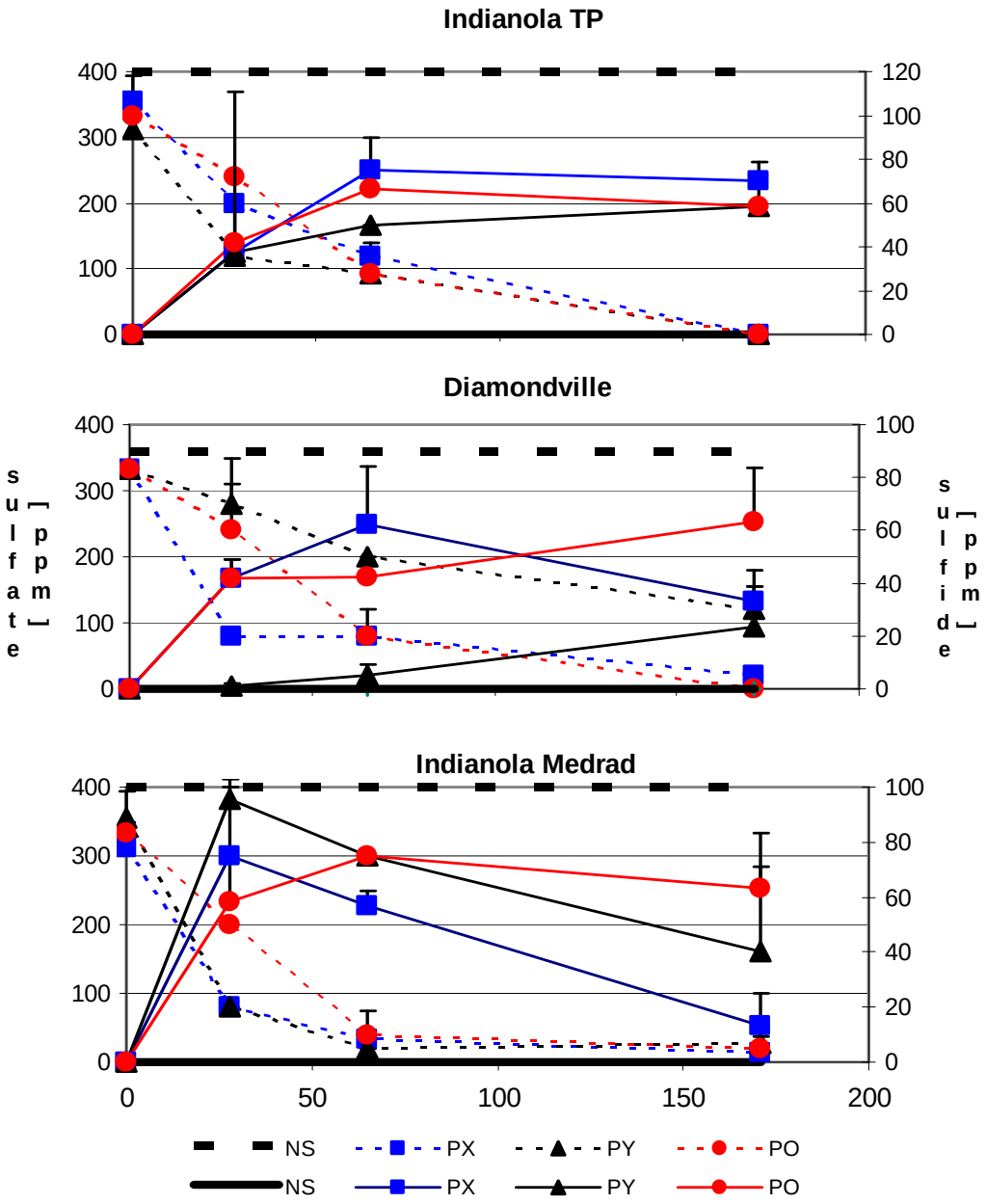

Figure 3. Mean values [n=3] in ppm and standard deviation of sulfate left axis (dashed lines) and sulfide right axis (solid lines) over time (days). The most relevant treatments are shown: mine water treated with substrate P and X amount of lime [■], substrate P and Y amount of lime [▲], and substrate P and 0 amount of lime [●]. No substrate controls for sulfate shown in black thick broken line and for sulfide shown in black thick solid line.

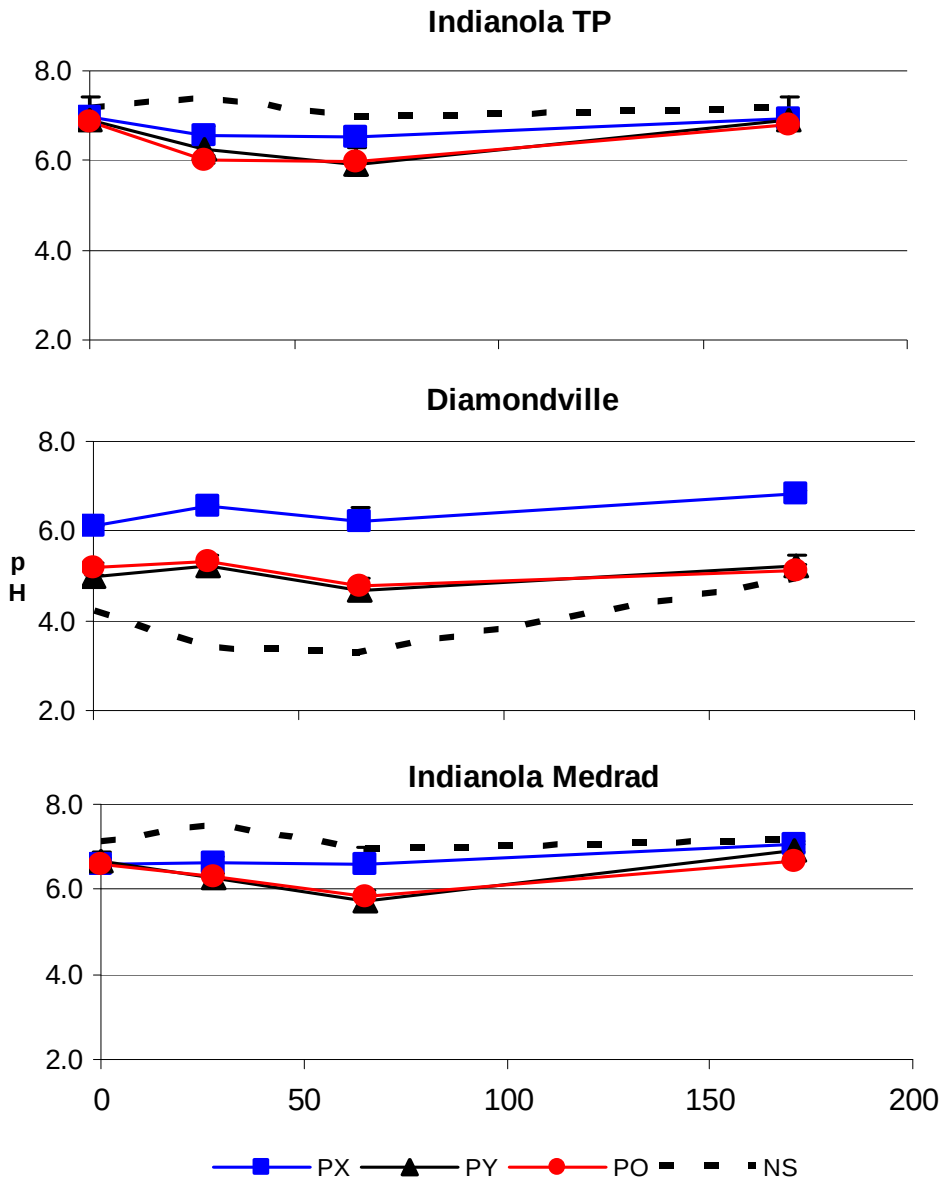

Figure 4. Mean values [n=3] and standard deviations of pH measured for mine effluents over time (days). Most relevant treatments are shown: mine water treated with substrate P and X amount of lime [■], substrate P and Y amount of lime [▲], and substrate P and 0 amount of lime [●]. No substrate controls shown in black thick broken lines.

Eh

It is important to note that during the length of the study the Eh values measured were relative and not absolute. In general, mine effluents treated with substrate P and X amount of lime had the least oxidizing environments [more reducing relatively] (Figure 5). Measured Eh values in vessels with L and S substrates were far more oxidizing (data not graphed). Among the three mine effluents evaluated, Indianola TP displayed the most reducing environment when treated with substrate P and X amount of lime, followed by Indianola Medrad and Diamondville respectively. The oxidation-reduction potential for both Indianola TP and Medrad effluents became more oxidizing around Day 30 of the incubation followed by a decline and stabilization. In contrast, Diamondville's Eh was more reducing during the first 30 days of the incubation, after which it remained stable.

Decomposition in anaerobic environments tends to decrease Eh (more negative). We suspect that because Eh readings were performed in bulk samples, reducing microenvironments and gradients were not detectable. In addition, the nature of the reactive mixture (abundant suspended solids and biofilms) might have obstructed the redox sensor. Garcia et al., 2001 indicated that sulfate removal and SRB growth are possible only if reducing conditions are met (specifically -300 mV). In contrast, however, our data show that even under slightly oxidizing environments, SRB growth, sulfide production and sulfate removal can occur.

Sulfidogenesis, pH and fermentation

In passive biological systems where a mixture of a carbon source, porous support, and a neutralizing agent are provided, SRB oxidize organic carbon [electron donor] into bicarbonate and reduce sulfate [electron acceptor] to hydrogen sulfide as follows:

$$SO_4^{2--} + 2\ CH_2O \rightarrow H_2S + 2\ HCO_3^- \qquad \text{Eq. 1}$$

Theoretically the bicarbonate neutralizes the acidity in the environment, increases alkalinity and favors the precipitation of metal carbonate minerals (Zagury et al., 2006).

$$H_2S + M^{2+} \rightarrow MS\downarrow + 2H^+ \qquad \text{Eq. 2}$$

Microbial systems that use organic substrates as carbon sources also conduct fermentation where organic molecules serve as both electron acceptors and donors. Anaerobic fermentative bacteria degrade high molecular weight compounds into more easily degradable compounds. Volatile fermentative byproducts (such as methanol, ethanol and acetate) generated by fermentative anaerobic bacteria can be used by SRB as carbon sources (Chang et al., 2000).

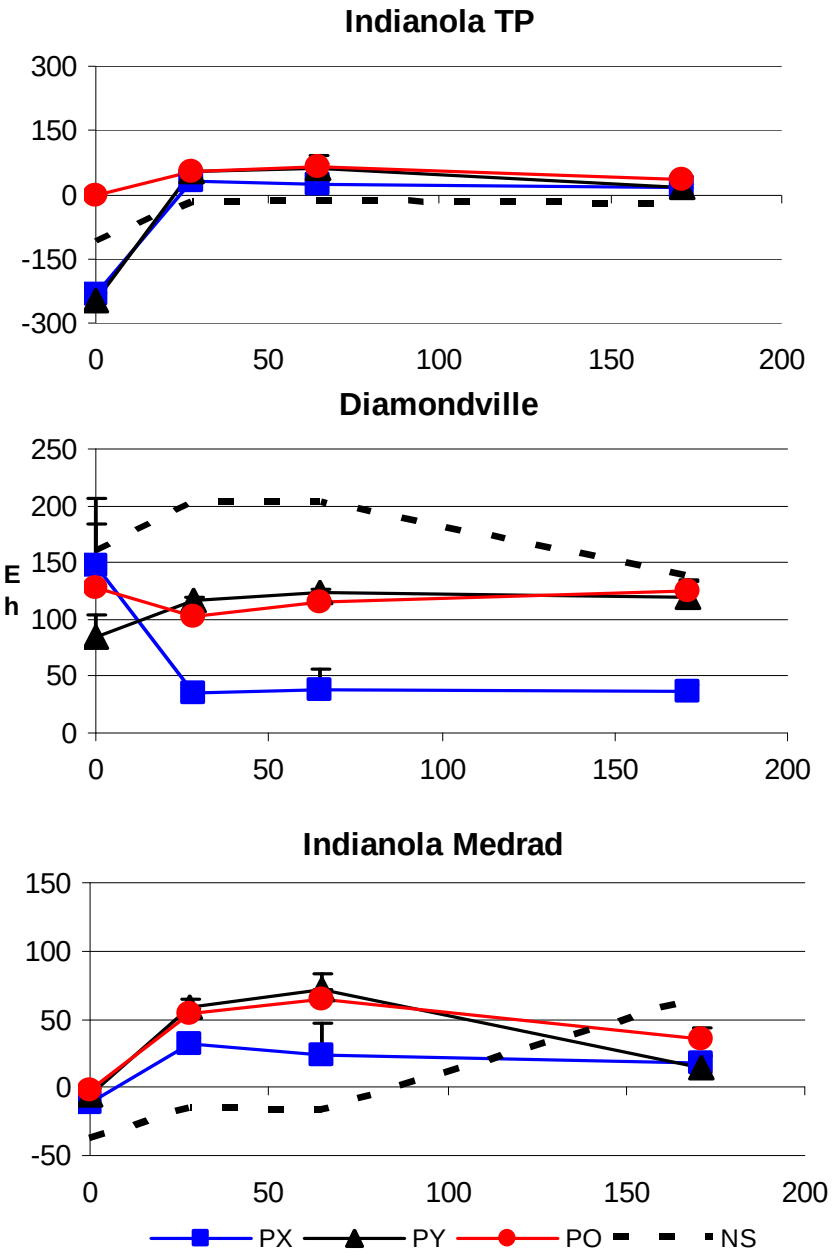

Figure 5. Mean values [n=3] and standard deviations of Eh measured for mine effluents. Most relevant treatments are shown: mine water treated with substrate P and X amount of lime [■], substrate P and Y amount of lime [▲], and substrate P and 0 amount of lime [●]. No substrate controls shown in black thick broken lines.

In our study, nutrients might have also supported growth of fermenting bacteria. We hypothesize that due to the high amount of carbon source and other compounds (proteins, amino acids, polysaccharides) in the substrates, fermentative anaerobes grew faster than SRB. Fermentative bacteria are able to outcompete the slower growing SRB for both nutrients and easily degradable substrates. Initially, organic compounds are converted to fatty acids by fermenters faster than they are consumed by SRB (Chang et al., 2000; Prasad et al., 1999). Our data suggests that in some cases the nature of the substrate [P] did enhance the growth of SRB; in others fermentation became the predominant biological process. One of the problems with fermentation occurring at higher rates than SRB activity is the associated increase of hydrogen ions [H^+]. Prasad et al., 1999 indicated that pH tends to decrease when acids and alcohols are being excreted and not utilized immediately by SRB. Even so, SRB can grow at pH 5 – 8 (Willow & Cohen, 2003) and can be adapted to pH ~ 5 without problems (Garcia et al., 2001). SRB create their own niches and regulate their microenvironments (Baker & Banfield, 2003). Kimura et al., 2006 indicated that most SRB are neutrophilic and not active below pH 5.

Our experiment showed that sulfidogenesis occurred at low pH. For most treatments, SRB growth was enhanced by the addition of a carbon source. Some reactive mixtures showed an imbalance between carbon degradation and sulfate reduction. The presence of residual sulfate (in most treatments with substrate L and S) indicates that carbon was added in excess or in disproportion producing an incomplete substrate oxidation with the generation of acetic acid (Castro et al., 2000). In our study, carbon depletion likely took place but was not measured. Most treatments showed positive MPN indices, sulfate removal, and production of hydrogen sulfide at the same time.

Metal analysis

Total and dissolved metals were analyzed at the beginning and end of the incubation period (see Appendix I for details).

For Indianola TP, effluent waters treated with substrate P and X amount of lime displayed a decrease in dissolved aluminum, iron and manganese concentrations over time (33%, 82% and 95% respectively). Similar results were observed when the effluents were mixed with substrate P and Y amount of lime (decreases of 45%, 70% and 86% respectively). Effluents mixed with substrate P and 0 amount of lime showed incongruent results. Total aluminum concentrations in Indianola TP effluents treated with substrate P and X, Y and 0 amount of lime showed similar decreases (93%, 92%, 84% respectively). Total iron concentrations in effluents treated with substrate P and X amount of lime were reduced 88%. When effluents were treated with substrate P and either Y or 0 amount of lime, the decrease in total iron was 75% for both treatments. The only significant decrease in total manganese concentrations for Indianola TP effluents occurred with substrate P and X amount of lime (88%). Surprisingly, total aluminum concentration in the effluents treated with substrate S regardless of the amount of lime decreased 80%. Total and dissolved metal concentrations (other than total aluminum for substrate S) in Indianola TP effluents treated with substrate S and L displayed negligible increases or decreases.

For the Diamondville treatment groups, with the exception of a decrease measured in total and dissolved aluminum in all substrate P treatments, metal concentrations (total and dissolved) in effluents treated with any of the substrates examined (P,S,L) did not decrease.

For Indianola Medrad, effluent waters in all substrate P treatments (X,Y,0 lime) displayed a decrease in dissolved iron and dissolved manganese concentrations over time. Total aluminum, iron and manganese concentrations in these effluents treated with substrate P and X amount of lime decreased significantly (97%, 95%, 97% respectively). When treated with substrate P and Y amount of lime, effluents showed a significant decrease in total aluminum and iron (93% and 89%). Metal concentrations did not show any decrease in the substrate S or L treatments.

In order to verify metal precipitation due to SRB activity, three treatments were randomly chosen for metal analysis of the solid phase (mixture in the bottom of the incubation vessels). Total aluminum, iron and manganese were measured by ICP at the beginning and the end of the incubation. As shown in Table 5 Indianola TP waters treated with substrate P and X amount of lime showed the highest levels of metal precipitation for the three metals. There was no metal precipitation for both Indianola Medrad effluents treated with substrate L and 0 amount of lime and Diamondville waters treated with substrate S and Y amount of lime. Although we did not measure precipitation for each treatment due to budgetary constraints it is apparent that substrate P plays an important role in modifying the biochemistry of the incubation vessels In vessels containing Indianola TP effluent treated with substrate P and X amount of lime metals in dissolved or total status in the liquid portion indeed precipitated (leading to the increase in metal concentration in solid phase).

Table 5. Mean values and standard deviations [n = 3] for total metal analysis: Results for solid phase analysis.

			Initial concentrations [ppm]					
			Aluminum		**Iron**		**Manganese**	
Mine	Substrate/Lime	Sample ID	AV.	SD	AV	SD	AV	SD
Indianola TP	**PX**	PX1	5591.3	1360.9	1980.3	264.7	71.7	18.5
Diamondville	**SY**	SY2	408.0	37.5	387.7	65.8	42.3	6.1
Indianola Medrad	**LO**	LO3	8365.3	3908.3	3962.7	1575.5	42.7	23.5
n = 3			**Final concentrations [ppm]**					
			Aluminum		**Iron**		**Manganese**	
			AV.	SD	AV	SD	AV	SD
Indianola TP	**PX**	PX1	10402.3	4462.4	3345.3	1551.8	140.7	60.3
Diamondville	**SY**	SY2	421.3	249.6	395.7	17.1	28.0	13.7
Indianola Medrad	**LO**	LO3	4027.3	2492.5	1485.3	696.1	14.9	6.2

Conclusions

Research indicates that sulfide production, sulfate reduction, and SRB growth occur under reducing conditions. In our experiment, absolute reducing conditions were not achieved. However, hydrogen sulfide production and sulfate removal as a result of the metabolic activity of

SRB indeed occurred. SRB growth occurred throughout the incubation period (confirmed by MPN data), and sulfate reduction fostered metal precipitation. This effect was not observed in all treatments, only those amended with substrate P and X amount of lime. The oscillatory behavior of pH likely resulted from an excessive production of fatty acids, alcohols and other fermentative products. It could also be the result of a disproportionate amount of carbon source added.

This study generated information to conclude that substrate P proved to be a good carbon source which promoted SRB growth, sulfate removal, hydrogen sulfide production and metal precipitation. Metal concentrations (aluminum, iron and manganese) in effluents treated with an appropriate carbon source (substrate P) decreased in the liquid portion and therefore precipitated. Vessels that had no addition of substrate (controls) showed little or no change in the parameters measured throughout the experiment. This work demonstrates that the use of indigenous SRB for the treatment of AMD at a large scale (i.e. flow bioreactor) is promising.

Recommendations

The following considerations should be noted for the next experiment:

1. The amount of organic compounds added to the mine water should be optimized in order to stimulate SRB activity [no fermentation], metal precipitation and alkalinization.

2. Nitrogen and phosphorus should be added since most AMD sites receive minimal inputs of fixed nitrogen from external sources (Baker & Banfield, 2003).

3. Since most SRB are facultative anaerobes (no obligate anaerobe has been cultured from AMD habitats, Baker & Banfield 2003), anaerobic conditions should be provided but not enforced.*

4. Sulfide precipitation of metals should be monitored because some metals become toxic to SRB over the long term.

5. Mineralogy analysis should be included as part of a larger scale project.

6. In order to obtain robust data, a set of chemical and biological parameters including TOC, total nitrogen, concomitant sulfide production and sulfate reduction rates, and microbial identification should be constantly monitored.

* In the mine environment (*in situ)*, water filled mines and open pits do not provide 100 % anaerobiosis.

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Appendix I: Mean values [n=3] and standard deviations of total and dissolved metal concentrations

				Initial												Final											
Parameter				METALS DISSOLVED [ppm]						METALS TOTAL [ppm]						METALS DISSOLVED [ppm]						METALS TOTAL [ppm]					
				Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.
Mine	**Substrate/Lime**	**ID**	**#**	Aluminum		Iron		Manganese		Aluminum		Iron		Manganese		Aluminum		Iron		Manganese		Aluminum		Iron		Manganese	
Indianola TP	PX	PX1	3	0.22	0.02	0.83	0.31	0.69	0.01	12.33	0.95	6.02	0.12	0.82	0.02	0.14	0.02	0.14	0.03	0.03	0.01	0.78	0.49	0.69	0.38	0.14	0.06
Indianola TP	PY	PY1	3	0.25	0.07	0.74	0.15	0.70	0.05	9.52	3.15	5.06	0.55	0.80	0.02	0.14	0.02	0.22	0.09	0.09	0.06	0.71	0.13	1.24	1.12	0.74	0.08
Indianola TP	PO	P01	3	0.21	0.05	0.88	0.33	0.69	0.03	8.63	1.69	4.64	0.61	0.80	0.03	0.21	0.05	0.22	0.03	0.27	0.36	1.35	0.64	1.13	0.57	0.80	0.13
Indianola TP	LX	LX1	3	0.36	0.14	0.65	0.64	0.40	0.04	4.63	1.22	3.50	0.24	0.44	0.03	0.27	0.07	26.67	1.15	0.52	0.03	0.38	0.07	28.00	2.00	0.55	0.02
Indianola TP	LY	LY1	3	0.27	0.17	0.27	0.13	0.39	0.03	4.55	1.81	3.92	0.58	0.42	0.04	1.05		27.50		0.54		1.10		29.00		0.56	
Indianola TP	LO	L01	3	0.29	0.12	0.30	0.02	0.41	0.03	5.30	0.40	3.39	0.38	0.43	0.02	1.60	0.40	30.67	7.23	0.51	0.02	1.70	0.46	30.67	7.23	0.54	0.03
Indianola TP	SX	SX1	3	0.16	0.06	1.26	0.24	0.24	0.01	0.22	0.04	1.84	0.28	0.27	0.00	0.19	0.05	12.40	5.39	0.30	0.09	0.26	0.04	18.67	3.51	0.37	0.03
Indianola TP	SY	SY1	3	0.12	0.08	1.14	0.02	0.23	0.02	0.27	0.04	1.72	0.01	0.27	0.00	0.45	0.12	18.67	1.15	0.44	0.06	0.47	0.12	21.00	3.00	0.45	0.06
Indianola TP	SO	S01	3	0.06	0.01	0.95	0.06	0.24	0.00	0.18	0.04	1.48	0.13	0.26	0.01	0.41	0.01	16.67	1.15	0.41	0.01	0.42	0.02	17.33	0.58	0.41	0.01
Indianola TP	NSX	NSX1														0.03		0.03		0.03		0.03		0.09		0.03	
Indianola TP	NSY	NSY1														0.03		0.03		0.03		0.03		0.03		0.03	
Indianola TP	NSO	NS01														0.03		0.03		0.03		0.03		0.14		0.03	
Baseline values				0.03		9.99		0.48		0.03		10.70		0.48													
Diamondville Redrick Upper	PX	PX2	3	0.83	0.94	0.64	0.53	1.69	0.12	12.13	4.82	4.75	1.08	1.75	0.11	0.14	0.02	0.18	0.10	0.04	0.02	1.01	0.11	0.89	0.36	0.87	0.72
Diamondville Redrick Upper	PY	PY2	3	0.77	0.96	0.46	0.40	1.63	0.11	8.80	2.08	4.27	0.51	1.75	0.06	0.16	0.10	19.67	4.04	1.80	0.61	1.86	1.90	27.33	4.93	2.13	0.12
Diamondville Redrick Upper	PO	P02	3	5.46	0.35	3.52	0.64	1.73	0.03	9.83	0.74	4.56	0.63	1.79	0.07	0.26	0.09	32.33	8.33	2.20	0.10	1.33	1.01	34.67	10.97	2.23	0.15
Diamondville Redrick Upper	LX	LX2	3	2.05	0.40	0.17	0.05	1.53	0.08	8.77	0.55	3.16	0.36	1.58	0.11	1.83	0.31	27.67	3.06	1.47	0.12	2.20	0.60	28.67	5.13	1.57	0.06
Diamondville Redrick Upper	LY	LY2	3	10.80	1.31	1.51	0.41	1.46	0.05	16.63	0.74	4.79	0.18	1.57	0.01	9.27	6.13	25.33	5.69	1.63	0.21	11.27	7.18	28.67	3.21	1.73	0.06
Diamondville Redrick Upper	LO	L02	3	12.97	0.25	1.87	0.65	1.62	0.02	19.87	3.48	6.35	0.77	1.64	0.03	18.67	4.62	24.67	2.89	1.80	0.00	35.33	11.37	28.00	5.29	1.83	0.06
Diamondville Redrick Upper	SX	SX2	3	0.25	0.03	0.60	0.05	0.97	0.03	5.63	0.46	2.83	0.07	1.02	0.01	2.50	0.26	9.60	1.23	1.37	0.06	2.87	0.23	12.07	2.53	1.43	0.06
Diamondville Redrick Upper	SY	SY2	3	0.47	0.08	0.70	0.15	0.91	0.01	6.00	0.21	2.89	0.12	1.08	0.01	2.90	1.23	7.20	3.14	1.12	0.40	3.80	0.44	9.77	1.97	1.43	0.06
Diamondville Redrick Upper	SO	S02	3	0.48	0.13	0.84	0.12	0.99	0.10	5.38	0.53	2.56	0.26	1.09	0.03	3.27	0.45	9.67	1.35	1.40	0.00	3.37	0.49	10.73	1.62	1.37	0.06
Diamondville Redrick Upper	NSX	NSx2																									
Diamondville Redrick Upper	NSY	NSY2																									
Diamondville Redrick Upper	NSO	NS02																									
Baseline values				19.80		20.60		12.50		13.10		2.11		2.16													
Indianola Medrad	PX	PX3	3	0.17	0.05	1.26	0.15	0.74	0.11	8.86	2.96	7.79	0.63	0.85	0.10	0.54		0.81		0.18		0.23		0.32		0.03	
Indianola Medrad	PY	PY3	3	0.27	0.16	1.88	0.55	0.74	0.06	8.89	0.76	7.77	0.61	0.88	0.06	0.29	0.14	0.42	0.24	0.40	0.42	0.53	0.05	0.85	0.23	0.72	0.19
Indianola Medrad	PO	P03	3	0.09	0.04	1.41	0.27	0.69	0.07	4.70	1.96	6.76	0.41	0.81	0.10	0.19	0.01	0.35	0.20	0.14	0.13	1.01	0.86	1.39	0.18	0.91	0.06
Indianola Medrad	LX	LX3	3	0.14	0.08	0.25	0.31	0.47	0.03	2.96	1.61	7.28	2.26	0.53	0.02	0.36	0.30	15.63	23.73	0.59	0.43	0.70	0.34	29.20	23.09	0.69	0.36
Indianola Medrad	LY	LY3	3	0.16	0.05	0.26	0.16	0.49	0.02	3.34	0.61	5.05	0.90	0.52	0.04	1.97	0.23	28.67	4.16	0.77	0.10	3.07	0.60	34.33	2.52	0.89	0.03
Indianola Medrad	LO	L03	3	0.20	0.11	0.55	0.31	0.55	0.06	3.89	1.11	6.64	0.84	0.58	0.03	2.13	0.42	26.33	0.58	0.71	0.03	2.80	0.66	28.67	2.52	0.77	0.05
Indianola Medrad	SX	SX3	3	0.05	0.01	1.83	0.31	0.30	0.02	0.28	0.07	3.91	0.61	0.36	0.01	0.30	0.13	11.67	7.09	0.37	0.30	0.35	0.07	17.00	3.00	0.55	0.05
Indianola Medrad	SY	SY3	3	0.06	0.01	2.08	0.02	0.31	0.01	0.25	0.06	3.38	0.16	0.34	0.01	0.39	0.10	14.00	6.24	0.51	0.10	0.46	0.04	18.00	2.65	0.57	0.01
Indianola Medrad	SO	S03	3	0.03	0.00	1.88	0.04	0.29	0.01	0.26	0.04	3.60	0.22	0.37	0.01	0.26	0.03	7.93	5.43	0.45	0.06	0.38	0.12	14.00	2.65	0.50	0.02
Indianola Medrad	NSX	NSX3																									
Indianola Medrad	NSY	NSY3																									
Indianola Medrad	NSO	NS03																									
Baseline values				0.025		6.09		0.387		0.025		6.11		0.39													
No total of samples				81		81		81		81		81		81													

Appendix II: Mean values [n=3] and standard deviations of sulfide and sulfate concentrations

			SULFIDE								SULFATE							
Date processed			**2-Nov-07**		**30-Nov-07**		**7-Jan-08**		**23-Apr-08**		**3-Nov-07**		**2-Dec-07**		**8-Jan-08**		**24-Apr-08**	
			Time 0		Time 1		Time 2		Time 3		Time 0		Time 1		Time 2		Time 3	
Mine	**S/L**	**ID**	**Aver.**	**S.D.**	**Aver.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**
Indianola TP	PX	PX1	0.07	0.12	37.50	0.00	75.00	0.00	70.0	8.7	355.2	38.5	200.0	0.0	120.0	0.0	0.0	0.0
Indianola TP	PY	PY1	0.00	0.00	37.50	0.00	50.00	0.00	58.3	14.4	310.8	38.5	120.0	69.3	93.3	46.2	0.0	0.0
Indianola TP	PO	P01	0.20	0.00	41.67	7.22	66.67	14.43	58.3	14.4	333.0	0.0	240.0	69.3	93.3	23.1	0.0	0.0
Indianola TP	LX	LX1	0.00	0.00	5.00	4.33	5.33	0.58	0.0	0.0	333.0	0.0	240.0	69.3	333.3	23.1	373.3	23.1
Indianola TP	LY	LY1	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	333.0	0.0	93.3	100.7	266.7	230.9	240.0	207.8
Indianola TP	LO	L01	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	333.0	0.0	266.7	115.5	373.3	23.1	373.3	23.1
Indianola TP	SX	SX1	0.07	0.12	58.33	14.43	42.50	4.33	56.7	31.8	333.0	0.0	200.0	120.0	200.0	0.0	80.0	34.6
Indianola TP	SY	SY1	0.00	0.00	45.83	7.22	41.67	7.22	43.3	49.3	333.0	0.0	160.0	69.3	200.0	0.0	80.0	20.0
Indianola TP	SO	S01	0.07	0.12	50.00	21.65	40.00	4.33	15.0	5.0	333.0	0.0	120.0	69.3	200.0	0.0	133.3	23.1
Indianola TP	NSX	NSX1	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	400.0	0.0	400.0	0.0	400.0	0.0	400.0	0.0
Indianola TP	NSY	NSY1	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	333.0	0.0	400.0	0.0	400.0	0.0	400.0	0.0
Indianola TP	NSO	NS01	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	333.0	0.0	400.0	0.0	400.0	0.0	400.0	0.0
Diamondville Redrick Upper	PX	PX2	0.00	0.00	41.67	7.22	62.50	21.65	33.3	11.5	333.0	0.0	80.0	0.0	80.0	0.0	20.0	0.0
Diamondville Redrick Upper	PY	PY2	0.00	0.00	0.83	1.44	5.33	3.75	23.3	15.3	333.0	0.0	280.0	69.3	200.0	0.0	120.0	34.6
Diamondville Redrick Upper	PO	P02	0.00	0.00	41.67	7.22	42.50	4.33	63.3	20.2	333.0	0.0	240.0	69.3	80.0	40.0	0.0	0.0
Diamondville Redrick Upper	LX	LX2	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	333.0	0.0	373.3	46.2	213.3	23.1	333.3	23.1
Diamondville Redrick Upper	LY	LY2	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	333.0	0.0	320.0	0.0	293.3	23.1	293.3	83.3
Diamondville Redrick Upper	LO	L02	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	333.0	0.0	320.0	0.0	306.7	23.1	320.0	0.0
Diamondville Redrick Upper	SX	SX2	0.00	0.00	13.33	10.41	5.33	4.51	0.0	0.0	310.8	38.5	400.0	0.0	300.0	0.0	200.0	0.0
Diamondville Redrick Upper	SY	SY2	0.00	0.00	12.50	21.65	1.67	2.89	0.0	0.0	333.0	0.0	346.7	46.2	226.7	23.1	320.0	0.0
Diamondville Redrick Upper	SO	S02	0.00	0.00	3.67	5.51	0.33	0.58	0.0	0.0	344.1	19.2	400.0	0.0	266.7	46.2	320.0	0.0
Diamondville Redrick Upper	NSX	NSx2	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	360.0	0.0	360.0	0.0	360.0	0.0	360.0	0.0
Diamondville Redrick Upper	NSY	NSY2	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	266.4	0.0	480.0	0.0	360.0	0.0	360.0	0.0
Diamondville Redrick Upper	NSO	NS02	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	266.4	0.0	480.0	0.0	360.0	0.0	360.0	0.0
Indianola Medrad	PX	PX3	0.00	0.00	75.00	25.00	56.67	5.77	13.3	11.5	310.8	38.5	80.0	0.0	33.3	11.5	13.3	11.5
Indianola Medrad	PY	PY3	0.00	0.00	95.83	7.22	75.00	0.00	40.0	31.2	355.2	38.5	80.0	0.0	20.0	0.0	26.7	11.5
Indianola Medrad	PO	P03	0.00	0.00	58.33	14.43	75.00	0.00	63.3	20.2	333.0	0.0	200.0	0.0	40.0	34.6	20.0	0.0
Indianola Medrad	LX	LX3	0.00	0.00	0.00	0.00	0.00	0.00	28.3	40.7	355.2	76.9	240.0	69.3	333.3	23.1	266.7	92.4
Indianola Medrad	LY	LY3	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	399.6	0.0	346.7	46.2	326.7	30.6	266.7	46.2
Indianola Medrad	LO	L03	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	321.9	134.6	266.7	115.5	300.0	34.6	320.0	0.0
Indianola Medrad	SX	SX3	0.00	0.00	15.00	19.84	5.33	4.51	1.7	2.9	399.6	0.0	400.0	0.0	293.3	46.2	266.7	46.2
Indianola Medrad	SY	SY3	0.00	0.00	0.83	1.44	0.00	0.00	0.0	0.0	532.8	0.0	400.0	0.0	373.3	46.2	320.0	0.0
Indianola Medrad	SO	S03	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	488.4	76.9	346.7	46.2	320.0	0.0	320.0	0.0
Indianola Medrad	NSX	NSX3	0.00	0.00			0.00	0.00	0.0	0.0	400.0	0.0	400.0	0.0	400.0	0.0	400.0	0.0
Indianola Medrad	NSY	NSY3	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	399.6	0.0	400.0	0.0	340.0	0.0	400.0	0.0
Indianola Medrad	NSO	NS03	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	399.6	0.0	480.0	0.0	340.0	0.0	360.0	0.0

Appendix III: Mean values [n=3] and standard deviations for oxidation reduction potential [Eh] and pH

Date processed			pH								Eh							
			3-Nov-07		30-Nov-07		7-Jan-08		24-Apr-08		3-Nov-07		30-Nov-07		7-Jan-08		24-Apr-08	
			Time 0		Time 1		Time 2		Time 3		Time 0		Time 1		Time 2		Time 3	
Mine	**S/L**	**ID**	**Av.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**
Indianola TP	PX	PX1	7.0	0.1	6.6	0.1	6.5	0.1	6.9	0.0	-233.2	16.2	32.6	5.8	23.9	9.0	15.1	0.9
Indianola TP	PY	PY1	6.9	0.1	6.3	0.0	5.9	0.5	6.9	0.2	-247.3	18.2	52.8	1.7	62.2	27.9	17.4	6.8
Indianola TP	PO	P01	6.9	0.0	6.0	0.1	6.0	0.3	6.8	0.1	-244.0	4.2	62.3	4.2	58.4	19.6	23.3	1.3
Indianola TP	LX	LX1	6.8	0.1	5.3	0.2	4.9	0.1	5.2	0.1	-145.1	15.6	104.2	13.0	115.1	6.5	114.9	8.0
Indianola TP	LY	LY1	6.6	0.0	4.7	0.3	4.4	0.1	4.7	0.0	-113.1	2.9	134.2	3.3	142.7	4.3	142.0	0.9
Indianola TP	LO	L01	6.6	0.1	4.9	0.2	4.4	0.0	4.7	0.1	-95.5	4.5	138.4	8.3	146.3	3.2	147.6	3.6
Indianola TP	SX	SX1	6.8	0.1	5.6	0.4	5.0	0.1	5.1	0.1	52.5	154.4	104.2	20.9	119.3	8.4	117.3	6.4
Indianola TP	SY	SY1	6.9	0.0	5.5	0.5	4.7	0.4	4.7	0.1	-159.5	39.4	108.7	25.2	131.5	26.4	143.3	12.4
Indianola TP	SO	S01	7.0	0.1	5.2	0.1	4.4	0.0	4.6	0.1	-227.6	2.6	124.6	6.0	143.4	1.4	149.0	4.3
Indianola TP	NSX	NSX1	7.2		7.4	0.0	7.0	0.0	7.2	0.0	-100.8	29.2	-24.6	1.2	-13.9	0.3	-11.8	0.0
Indianola TP	NSY	NSY1	7.3		7.3	0.0	6.9	0.0	7.4	0.0	-111.3	7.5	-19.3	0.7	-12.4	0.5	-12.7	0.0
Indianola TP	NSO	NS01	7.2		7.3	0.0	7.0	0.0	7.6	0.0	-111.3	4.2	-17.4	0.5	-14.3	2.0	-20.7	0.0
Diamondville Redrick Upper	PX	PX2	6.1	0.0	6.5	0.1	6.2	0.3	6.8	0.1	147.9	35.4	34.4	3.8	38.0	18.0	36.0	6.0
Diamondville Redrick Upper	PY	PY2	5.0	0.0	5.2	0.1	4.7	0.0	5.2	0.3	84.1	20.4	116.5	3.4	123.4	2.9	118.9	16.3
Diamondville Redrick Upper	PO	P02	5.2	0.1	5.3	0.1	4.8	0.1	5.1	0.1	128.1	78.8	102.3	7.4	115.3	8.1	125.4	7.6
Diamondville Redrick Upper	LX	LX2	5.3	0.1	4.6	0.1	4.4	0.1	4.8	0.1	214.6	8.3	138.1	5.6	142.4	6.9	149.5	10.7
Diamondville Redrick Upper	LY	LY2	4.2	0.0	4.6	0.2	4.1	0.2	4.3	0.2	223.2	12.9	139.5	13.2	156.0	13.2	176.3	14.7
Diamondville Redrick Upper	LO	L02	3.7	0.0	3.6	0.0	3.6	0.2	4.2	0.0	244.4	4.4	195.4	0.2	192.6	10.9	183.3	0.9
Diamondville Redrick Upper	SX	SX2	6.3	0.0	4.9	0.2	4.2	0.1	4.5	0.0	20.7	42.6	131.2	12.8	148.5	4.0	162.2	4.9
Diamondville Redrick Upper	SY	SY2	5.3	0.2	4.5	0.3	3.9	0.1	4.2	0.1	51.7	6.4	148.9	17.0	167.9	8.8	181.8	8.6
Diamondville Redrick Upper	SO	S02	5.2	0.2	4.6	0.1	4.1	0.2	4.3	0.0	45.9	11.6	142.7	8.5	153.6	9.0	172.2	4.7
Diamondville Redrick Upper	NSX	NSx2	3.8		6.2	0.0	6.9	0.0	6.6	0.0	180.2	45.9	-6.5	0.3	-35.8	1.7	72.6	0.0
Diamondville Redrick Upper	NSY	NSY2	5.2		4.2	0.0	4.5	0.1	4.9	0.0	143.9	1.4	143.4	1.0	151.4	1.9	144.1	0.0
Diamondville Redrick Upper	NSO	NS02	4.2		3.4	0.0	3.3	0.1	4.9	0.0	159.8	9.8	203.0	2.0	203.0	2.6	139.2	0.0
Indianola Medrad	PX	PX3	6.6	0.3	6.6	0.1	6.6	0.4	7.1	0.1	-11.1	9.7	31.6	5.5	24.1	23.2	18.0	4.7
Indianola Medrad	PY	PY3	6.7	0.1	6.3	0.1	5.7	0.2	6.9	0.2	-5.9	4.7	58.9	5.4	72.0	11.2	14.4	9.8
Indianola Medrad	PO	P03	6.6	0.1	6.3	0.1	5.8	0.1	6.7	0.2	-2.4	4.3	53.6	3.7	64.4	7.3	35.8	7.4
Indianola Medrad	LX	LX3	6.5	0.0	5.1	0.1	4.8	0.1	5.9	1.5	0.4	8.4	119.2	5.7	124.6	3.3	74.3	74.3
Indianola Medrad	LY	LY3	6.4	0.1	4.6	0.1	4.3	0.0	4.7	0.0	2.1	6.0	148.8	2.3	153.7	1.2	149.5	7.7
Indianola Medrad	LO	L03	6.5	0.0	4.5	0.0	4.3	0.1	4.6	0.0	3.2	5.2	151.0	2.8	153.1	4.2	148.2	10.8
Indianola Medrad	SX	SX3	7.0	0.0	5.3	0.3	4.7	0.2	4.7	0.1	-13.8	2.9	112.1	18.0	129.2	11.7	147.2	5.4
Indianola Medrad	SY	SY3	6.9	0.0	4.8	0.0	4.3	0.0	4.4	0.0	-8.1	3.6	139.2	1.8	148.8	1.6	155.9	5.5
Indianola Medrad	SO	S03	6.9	0.0	4.8	0.1	4.3	0.1	4.4	0.0	-8.2	2.6	144.1	5.6	150.7	3.1	155.7	8.8
Indianola Medrad	NSX	NSX3	6.9		6.7	0.2	6.8	0.0	7.3	0.0	-37.4		-5.8	0.4	-21.8	0.4	47.1	0.0
Indianola Medrad	NSY	NSY3	7.0		7.3	0.1	6.8	0.0	6.9	0.0	-37.0		-11.1	0.9	-14.4	0.7	46.5	0.0
Indianola Medrad	NSO	NS03	7.1		7.5	0.0	6.9	0.0	7.2	0.0	-36.9		-14.6	0.1	-15.8	0.9	67.4	0.0

Appendix IV: Mean values [n=3] and standard deviations for total bacterial counts

Date processed			**2-Nov-07**		**30-Nov-07**		**7-Jan-08**		**23-Apr-08**	
			Time 0		Time 1		Time 2		Time 3	
Mine	**S/L**	**ID**	**Aver.**	**S.D.**	**Aver.**	**S.D.**	**Av.**	**S.D.**	**Av.**	**S.D.**
Indianola TP	PX	PX1	3.4E+08	2.2E+08	2.0E+08	4.4E+07	4.0E+08	2.0E+08	3.6E+08	1.2E+08
Indianola TP	PY	PY1	3.5E+08	1.8E+07	1.4E+08	3.8E+07	3.1E+08	4.7E+07	1.6E+08	5.5E+07
Indianola TP	PO	P01	2.0E+08	9.5E+07	1.6E+08	3.8E+07	3.3E+08	7.5E+07	4.8E+08	4.8E+07
Indianola TP	LX	LX1	2.1E+07	1.1E+07	7.7E+07	1.9E+07	2.4E+08	1.4E+08	1.1E+08	6.9E+06
Indianola TP	LY	LY1	2.4E+07	1.3E+07	3.7E+07	7.3E+06	2.5E+08	6.8E+07	7.9E+07	3.2E+07
Indianola TP	LO	L01	5.8E+07	4.9E+07	5.6E+07	2.3E+07	3.5E+08	9.9E+07	9.6E+07	8.7E+07
Indianola TP	SX	SX1	7.5E+07	3.3E+07	1.2E+09	1.4E+09	2.0E+09	1.2E+09	5.2E+08	5.6E+08
Indianola TP	SY	SY1	1.1E+08	6.9E+07	1.2E+09	8.3E+08	2.3E+09	7.1E+08	1.0E+09	2.7E+08
Indianola TP	SO	S01	6.2E+07	1.7E+07	5.9E+08	3.2E+08	1.9E+09	1.5E+09	7.3E+08	7.6E+08
Indianola TP	NSX	NSX1	6.5E+06	7.9E+06	9.0E+05	4.2E+05	4.1E+07	2.4E+07	2.9E+07	2.6E+07
Diamondville Redrick Upper	PX	PX2	2.9E+08	6.5E+07	1.9E+08	7.5E+07	2.6E+08	2.2E+07	7.6E+08	1.6E+08
Diamondville Redrick Upper	PY	PY2	1.9E+08	7.3E+07	1.6E+08	2.1E+07	1.2E+08	1.6E+07	1.5E+08	7.7E+07
Diamondville Redrick Upper	PO	P02	2.9E+08	2.4E+08	4.3E+08	1.7E+08	2.3E+08	7.2E+07	1.2E+08	3.5E+07
Diamondville Redrick Upper	LX	LX2	1.3E+07	6.2E+06	4.2E+07	2.2E+07	2.1E+08	1.8E+07	1.4E+08	8.6E+07
Diamondville Redrick Upper	LY	LY2	9.4E+07	1.4E+08	2.5E+06	4.5E+05	5.4E+06	5.1E+06	7.9E+07	6.2E+07
Diamondville Redrick Upper	LO	L02	3.7E+07	3.0E+07	1.1E+08	1.6E+08	1.9E+07	1.4E+07	1.2E+08	1.2E+08
Diamondville Redrick Upper	SX	SX2	8.5E+07	3.6E+07	8.8E+08	1.1E+09	3.5E+08	4.7E+08	6.9E+08	1.9E+08
Diamondville Redrick Upper	SY	SY2	1.3E+08	9.8E+07	1.7E+08	1.4E+08	3.7E+08	3.3E+08	2.4E+08	1.6E+08
Diamondville Redrick Upper	SO	S02	6.4E+07	1.7E+07	3.5E+08	3.4E+08	1.0E+09	5.5E+08	5.1E+08	4.2E+08
Diamondville Redrick Upper	NSX	NSx2	5.4E+06	1.4E+06	1.4E+06	4.2E+05	1.1E+06	1.3E+06	2.8E+07	1.4E+07
Indianola Medrad	PX	PX3	6.4E+08	2.7E+08	4.7E+07	2.5E+07	2.8E+08	8.7E+07	3.5E+08	1.7E+07
Indianola Medrad	PY	PY3	3.9E+08	1.1E+08	1.6E+08	1.9E+07	3.6E+08	5.2E+07	3.5E+08	7.3E+07
Indianola Medrad	PO	P03	3.6E+08	3.2E+08	1.7E+08	2.0E+07	2.5E+08	8.5E+06	2.3E+08	3.2E+07
Indianola Medrad	LX	LX3	4.2E+07	3.5E+07	7.2E+07	1.1E+07	1.1E+08	4.4E+07	1.9E+08	5.9E+07
Indianola Medrad	LY	LY3	7.0E+07	4.5E+07	9.6E+07	9.2E+05	8.3E+07	4.7E+07	9.5E+07	2.7E+07
Indianola Medrad	LO	L03	2.5E+07	9.0E+06	6.5E+07	9.4E+07	9.5E+07	2.8E+07	2.9E+07	3.1E+07
Indianola Medrad	SX	SX3	2.9E+07	3.2E+06	9.8E+07	7.5E+07	1.4E+09	4.7E+08	3.3E+08	7.3E+06
Indianola Medrad	SY	SY3	3.8E+07	1.7E+07	9.4E+08	4.3E+08	4.4E+08	2.2E+08	2.2E+08	8.5E+07
Indianola Medrad	SO	S03	4.0E+07	9.7E+06	8.2E+07	2.4E+06	1.3E+09	8.2E+08	2.2E+08	1.1E+08
Indianola Medrad	NSX	NSX3	3.2E+06	3.4E+05	6.1E+06	7.8E+06	3.7E+07	1.5E+07	8.8E+06	6.5E+06