



**Natural Resources
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**SMART BIOREMEDIATION TECHNOLOGY TO
ACHIEVE HIGH SULFATE REDUCTION IN MINING
WATERS OF NE MINNESOTA**
Phase I Report



by

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EXECUTIVE SUMMARY

There exists a significant need in northeastern Minnesota to provide a viable solution to the current challenge of maintaining existing iron ore and developing non-ferrous mining industries while simultaneously protecting watersheds from elevated aqueous sulfate concentrations that could prove detrimental to biota, especially wild rice. "Smart Bioremediation Technology to Achieve High Sulfate Reduction in Mining Waters of NE Minnesota – Phase I" focuses on proof of concept early-stage development of a realistic solution to the aqueous sulfate issue potentially threatening Minnesota's existing \$3 billion/year ferrous mining industry as well as Minnesota's projected \$4 billion/year non-ferrous mining industry (Skurla, 2012). Initial funding was provided for this Phase I work by the Natural Resources Research Institute (NRRI) and an Innovation Grant from the Laurentian Vision Partnership through the East Range Joint Powers Board.

The design of the technology included smart sensors and controls which enabled remote operation and monitoring of the pilot scale system. Solar panels mounted on the systems floating bioreactor modules provided DC power to operate embedded pumps, sensors, controls, and data transmitters. The system was designed to enable stand alone, year round remote operation in environmental conditions encountered in either operating or legacy mining operations across the U.S. The modular nature of the technical design allows for practical scale up to accommodate flow requirement needs for the mining industry.

The robust system design combined biological sulfate reduction with remediation hydrogeology approaches to remove sulfur from mining impacted waters (Reinsel, 2015). Sulfate reducing bacteria (SRB) from local stream sediments were utilized to provide the sulfate reduction. Preliminary analytical results indicate that the smart bioremediation technology is capable of producing aqueous sulfate reduction in the mining waters flowing through the bioreactor systems.

The Phase I project has provided a proof of concept design for remediation of sulfate in mining impacted waters. Additional studies (Phase II study and MN Drive study) are currently under way and will be delivered during summer, 2016. The purpose of these future studies is to deliver a smart technology bioremediation water treatment system that is capable of being commercialized and that can effectively decrease aqueous sulfate levels in impacted waters in a cost-effective manner to concentrations that can be further decreased by other technologies so that stringent aqueous sulfate concentrations can be achieved. Additional work for Phase II has been funded by a second Minnesota Laurentian Vision Partnership Innovation Grant. As well, the University of Minnesota has recently funded a two year \$500,000 MnDRIVE Transdisciplinary Research Grant providing for system optimization and commercialization. The MnDRIVE project work will optimize the biological sulfate reduction component of the technology by optimizing the natural SRB bacterial communities employed as well as the SRB nutrient feed rates and nutrient compositions. The hydrogeology component will be optimized by evaluating the optimum sulfur precipitation chemistry. Research work on both of these system components will focus on environmental design, flow enhancement, and cost effectiveness.

The Phase I project has created a pilot scale bioremediation test facility that provides a vehicle for future collaborative research work for optimization and commercialization by scientists and engineers from the Natural Resources Research Institute, University of Minnesota Duluth, the University of Minnesota Twin Cities, and from Clearwater Layline, LLC, Circle Pines, MN.

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INTRODUCTION

Problem Statement

Sulfate is a natural anionic chemical compound commonly found in soil and water. Aqueous sulfate concentrations near current and past mining activities have been identified as a critical area of concern that affects Minnesota. Aqueous sulfate can be found at varying concentrations in discharges from permitted facilities such as mining operations, municipal wastewater treatment plants, and other industrial facilities. The primary factors controlling sulfate concentrations in surface water include the local geology adjacent to the surface water body (unconsolidated sediments and rock mineralogy and geochemistry) and local man-made geological features such as lean ore stockpiles and tailings piles. Importantly, iron sulfide minerals (for example, pyrite and pyrrhotite) are present in the rocks mined as iron ore. As well, sulfide minerals comprise the bulk of the ore minerals that may be mined and processed for copper, nickel, and platinum group metals for several projects currently in development in northeastern Minnesota. Dissolution of sulfur from these sulfur-bearing minerals in unconsolidated and lithified geological materials by means of exposure to water and the atmosphere contributes to increased sulfate levels in lakes and streams. The majority of sulfate entering streams on the Minnesota Iron Range is generated when iron sulfide present in waste rock and mine tailings are exposed to water and air and the iron sulfide is converted to soluble sulfate and insoluble ferric oxy-hydroxide phases (Berndt and Bavin, 2009).

In particular, the mining industry in Minnesota is increasingly concerned over the potential enforcement of strict, low part-per-million (ppm) regulatory limits for aqueous sulfate concentrations in point-source discharges that flow into areas where wild rice is growing. Wild rice is a culturally significant and important component of aquatic communities in parts of Minnesota, particularly in the northern part of the state. It provides food for waterfowl and shelter for animals and fish. Wild rice is also an important cultural resource to many Minnesota Tribal Communities and is economically important to those who financially benefit from its harvest and marketing (Minnesota Pollution Control Agency (MPCA), 2014). Discharges of water from northeastern Minnesota iron mining operations into streams that eventually flow to areas where wild rice is growing are regulated. Current Minnesota statute limits for aqueous sulfate discharges into wild-rice growing areas are 10 mg/liter (10mg/l, 10 parts per million (ppm)). The threat to municipal facilities could negatively affect the quality of life for individuals living in cities that could be required to reduce sulfate discharges from their wastewater treatment plants. Ongoing research is working to identify and set a valid level based on scientific studies that indicate the impact of aqueous sulfate on wild rice production (MPCA, 2014).

Currently, the Minnesota Department of Natural Resources (MDNR), Minnesota Pollution Control Agency (MPCA), and the U.S. Forest Service (USFS) are reviewing public comments related to a Supplementary Draft Environmental Impact Statement (SDEIS) for a proposed copper nickel mine at the NorthMet Deposit, which is being developed by PolyMet Corporation. Major topics of concern include acid rock drainage (ARD) and aqueous sulfate levels. Further, there are ongoing discussions related to ARD and sulfate levels for currently operating iron mines in northeastern Minnesota and a proposed iron mine in the Gogebic Range of northwestern Wisconsin. Since aqueous sulfate levels related to mine pit lakes and tailings ponds discharges, tailings pond water seepage, industrial process water, and municipal wastewater treatment

plants commonly exceed the current MPCA 10mg/l limit, there is an immediate need to reduce aqueous sulfate levels to comply with sulfate limits associated with these water systems. Currently accepted technologies such as reverse osmosis and ion exchange exist to achieve these reductions, but the cost of implementation and operation are often prohibitively expensive.

Sulfate Reduction: What Is Known

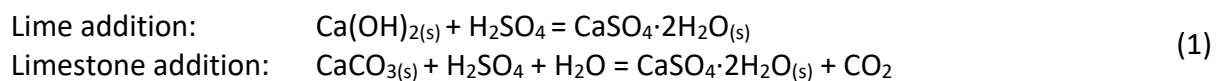
Surface waters obtain their chemistry by interaction with minerals and microbes on earth. When minerals containing iron sulfide are exposed to oxygen in the air, water soluble sulfates are produced which enter natural waters as well as insoluble ferric oxy-hydroxide phases (Berndt and Bavin, 2009). Depending on the mineralogy of the unconsolidated or lithified geological materials, these waters can be buffered in a variety of ways such that near-neutral to acidic waters with elevated sulfate concentrations may occur. Elevated aqueous sulfate concentrations can exist in the range of 500-4,000 ppm (mg/l) sulfate in neutral pH mine drainage waters where sufficient soluble carbonate ions are available to neutralize the sulfuric acid produced in typical acid rock drainage (ARD) discharges (Miller, 2005). Near neutral pH mining impacted waters are found throughout the Mesabi Iron Range of Northeastern Minnesota. Problems associated with high aqueous sulfate ARD treatment systems are likely to also be the case with treatment systems that occur in near neutral conditions.

Many of the methods used to reduce or eliminate acidic or neutral pH ARD pollution have various concerns (Johnson and Hallberg, 2005) because they:

1. Are only effective for short periods of time since they plug up and overflow or dry up;
2. Are too expensive to apply;
3. Cause channeling of water flow, which results in only part of the flow being treated;
4. Create other undesirable chemical products with their application; and
5. Are unable to function in sub-freezing conditions in Minnesota.

Limitations of sulfate water treatment systems in near-neutral settings are similar to limitations in acidic settings with acidic systems additionally combining neutralization processes prior to sulfate reduction (Miller, 2005). Existing solutions to reduce sulfate and ARD are classified in Figure 1.

The simplest and most cost-effective methods to reduce high sulfate levels are often through chemical reaction using limestone. These systems can effectively reduce aqueous sulfate concentrations in the range of 5,000 mg/l to less than 1,500 mg/l but have great difficulty achieving sulfate levels below 500 mg/l (Lorax Environmental, 2003). The limiting factor here is the solubility of calcium sulfate which is precipitated when limestone or lime is added to the high sulfate containing mine impacted waters. Calcium sulfate is precipitated, but is somewhat soluble leaving 500 mg/l to 1500 mg/l sulfate in solution depending on the prevailing water chemistry:



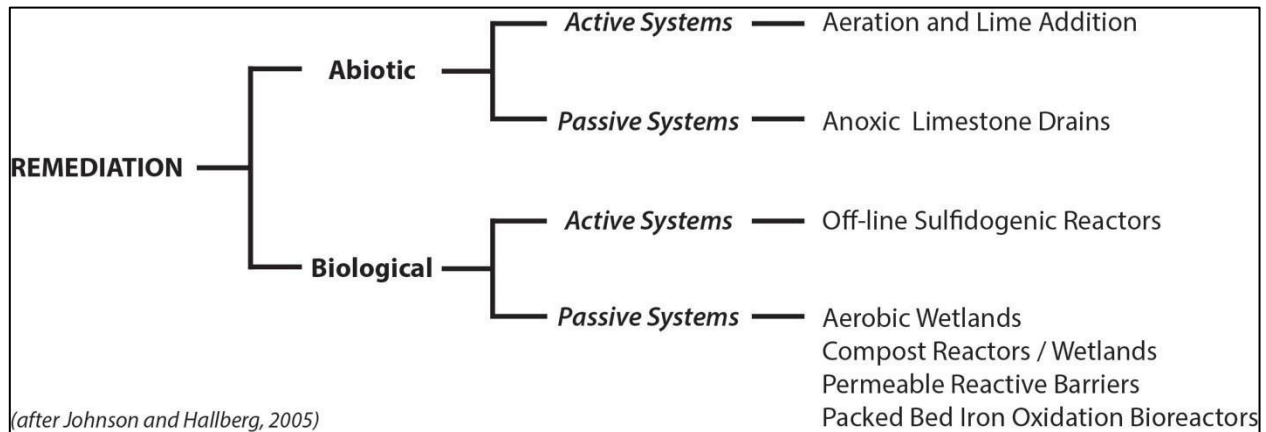


Figure 1. Abiotic and biological methodologies used to reduce sulfate and acid rock drainage (Johnson and Hallberg, 2005).

Biological SRB bioreactors treat sulfate-laden waters with the biological processes found in nature. The SRB are found in anaerobic environments that have excess sulfate and an available carbon source. They have been successfully used in passive or semi-passive systems for mitigation of mining-influenced water (Lorax Environmental, 2003; Johnson and Hallberg, 2005; Gusek, 2002). These bioreactors are used in many applications to reduce acidity, precipitate dissolved metals, and remove sulfate. Due to the slow biological activity of SRB, these systems have generally been utilized for low flow applications (Johnson and Hallberg, 2005). The largest currently existing SRB bioreactor processes up to 1,200 gallons per minute (gpm) (Reinsel, 2015). SRB systems are capable of greatly reducing the concentration of sulfate with low operating costs when implemented correctly (Miller, 2005). This technology is commonly accepted to have great promise as a potentially low cost, semi-passive method of treating mining-influenced water to remove or reduce acidity and/or aqueous sulfate concentrations. SRB systems have been proven to work effectively at relatively low cost in many situations where effective reduction of higher sulfate levels is required at lower flow conditions (Miller, 2005). The difficulty with currently designed SRB bioreactor systems is that they are not easily scaled up to allow for handling water flows exceeding ~1,900 l/m (500 gpm) in a cost effective and controlled manner. The systems are also often difficult to monitor and control because sites associated with the treatment activity are often located in remote areas (Neculita et al., 2007).

Reverse Osmosis (RO) is an established water treatment process. It is commonly used in the treatment of drinking water and the other industrial process waters, for example, at mining operations where metal sulfide minerals are processed and/or concentrated. An example of such a system that has recently been deployed occurs at the Eagle Mine in northern Michigan (<http://eaglemin.com/wp-content/uploads/WaterTreatmentProfile1.pdf>). RO can be used for sulfate removal or concentration and, when operational, creates highly concentrated sulfate brine. RO technology and water treatment systems are commonly accepted to be expensive to install and operate. Although RO can produce water with low levels of sulfate, it is not considered to be economically viable for most of the large-scale existing mining applications or for closed

facilities where less labor- and utility-intensive processes (semi-passive or passive) are preferred (Lorax Environmental, 2003; Miller, 2005).

Ion exchange technologies are evolving and show promise to be more cost effective than RO. These systems require active monitoring and operation with reactive resins and reagent regeneration. These are active, complicated systems that are not yet well developed for neutral mine water application (Lorax Environmental, 2003). Ion exchange technologies are currently being utilized at the Eagle Cu-Ni-PGM mine water treatment plant in Upper Michigan (<http://eaglemin.com/wp-content/uploads/WaterTreatmentProfile1.pdf>).

A comprehensive review of sulfate removal technologies utilized to treat mine effluents was published by Lorax Environmental (2003). Twelve treatment processes were evaluated and subsequently organized into four categories, including:

1. Chemical treatment with mineral precipitation (Table 1);
2. Membranes (Table 2);
3. Ion-exchange (Table 2); and
4. Biological sulfate removal (Table 3).

Data in Tables 1, 2, and 3 describe pretreatment requirements, feed water sulfate concentrations, product water sulfate levels achieved, brine production, sludge production, monitoring requirements, maintenance requirements, capital cost estimates, operating cost estimates, technology advantages, technology disadvantages, and technology improvements required.

A summary of the sulfate reduction results of these 12 technologies (Lorax Environmental, 2003) indicates that:

1. Chemical treatment processes allow mines to treat waters with maximum concentration of sulfates up to 29,000 ppm and reduce water sulfate concentrations down to 69 ppm;
2. Membranes treatment processes allow mines to reduce sulfates concentration in mine waters from 6,900 ppm to 113 ppm;
3. Ion-exchange treatment processes allow mines to treat water with maximum concentration of sulfates up to 3,353 ppm and reduce water sulfate concentrations down to 50 ppm; and
4. Biological sulfate removal processes allow mines to reduce sulfate concentrations in mine discharges from 8,342 ppm to 198 ppm.

Biological sulfate removal technologies have total operating costs that are considerably lower than reverse osmosis or ion exchange primarily because they do not generate a liquid waste stream containing a high aqueous sulfate concentration. This waste stream incurs high capital and operating costs because it typically must be evaporated (Hydrometrics, 2001).

As shown in Table 3, biological sulfate removal technologies utilizing bioreactors have been proven to achieve operating treatment costs of less than \$1.00/1000 gal. treated (\$0.27/cubic meter), can reduce mine discharge waters from >8,000 ppm sulfate down to 198 ppm sulfate, and can also remove trace metals from mine discharge waters. These and other attributes listed

below provided the driving force to utilize active biological remediation treatment as a key component of the NRR/Clearwater Layline smart bioremediation sulfate reduction technology:

Attributes for Using Active Biological Remediation Treatment:

1. Provides a low cost solution for reducing aqueous sulfate;
2. Provides the capability of reducing aqueous sulfate concentrations to levels approaching 200 ppm (mg/l);
3. Provides the capability of reducing aqueous trace metal concentrations besides reducing aqueous sulfate concentrations;
4. Provides low maintenance costs with minimal need for daily on-site operators;
5. Is capable of functioning in warm and cold climates;
6. Eliminates production of 'brine' solutions having massive sulfate concentrations and needing special disposal considerations; and
7. Mimics processes occurring in nature.

Tables 1-3 sulfate removal processes described include:

- Lime/Limestone addition to remove sulfate as calcium sulfate (gypsum);
- BaS: Barium Sulfide precipitation to remove sulfate as barium sulfide;
- SAVMIN: South Africa Veolia/ MINTEC corporations method to remove sulfate as ettringite (hydrated aluminum sulfate compound);
- CESR: Cost Effective Sulfate Removal process to remove sulfate as ettringite;
- RO: Reverse Osmosis: Membrane technology to remove sulfate;
- SPARRO: Slurry precipitation and recycle reverse osmosis technology to remove sulfate;
- EDR: electrodialysis reversal technology by GE to remove sulfate;
- GYP-CIX: Gypsum Chemeffco ion exchange removal of sulfate; and
- Bioreactor, Constructed Wetlands, Alkalinity Production Systems, Permeable Reactive Barriers: see Lorax Environmental (2003) detailed descriptions for the biological sulfate removal systems listed.

	Lime/Limestone	BaS	SAVMIN	CESR
	2001	1990	1999-2001	2001?
Pretreatment	no	no	no	no
Feed water	SO ₄ :3,000 ppm	SO ₄ :27,500 ppm	SO ₄ :649 ppm	SO ₄ :29,100 ppm
Product water	SO ₄ :1,219 ppm	SO ₄ :190 ppm	SO ₄ :69 ppm	SO ₄ :190 ppm
Brine production	no	no	no	no
Sludge production	low-moderate	low-moderate	moderate-high	high-very high
Monitoring	moderate-high	high	high	high
Maintenance	low	low	low	low
Capital cost*	unknown (low)	USD 0.48M per 103 m ³ /day	USD 0.31M per 103 m ³ /day	unknown
Operating cost*	USD 0.10 / m ³	USD 0.36 / m ³	USD 0.17 / m ³	USD 0.79 / m ³
Advantages		low levels of sulfate	low levels of sulfate	low levels of sulfate
	very cheap	recycling of expensive BaS	recycling of ettringite	
	also trace metal removal		also trace metal removal	also trace metal removal
Disadvantages	limited sulfate removal	little trace metal removal		
	production of sludge	production of sludge	production of sludge	production of sludge
Improvements	recycling of sludge	recycling of sludge	recycling of sludge	recycling of sludge

Table 1. Summary of case studies on chemical treatment processes with mineral precipitation (Lorax Environmental, 2003).

	RO	SPARRO	EDR	GYP-CIX
	2001	1992-1994	2001	2001
Pretreatment	yes	yes	yes	no
Feed water	SO ₄ : 4,920 ppm	SO ₄ : 6,639 ppm	SO ₄ : 4,178 ppm	SO ₄ : 1,200-2,800 ppm
Product water	SO ₄ : 113 ppm	SO ₄ : 142 ppm	SO ₄ : 246 ppm	SO ₄ : 50 ppm
Brine production	yes	yes	yes	yes
Sludge production	low	low	low	low-moderate
Monitoring	low-moderate	low-moderate	low-moderate	low
Maintenance	high	high	high	moderate
Capital cost*	USD 0.44-0.53M per 10 ³ m ³ /day	USD 0.52M per 10 ³ m ³ /day	USD 0.56-0.67M per 10 ³ m ³ /day	USD 0.33-0.37M per 10 ³ m ³ /day
Operating cost*	USD 0.88 / m ³	USD 0.22 / m ³	USD 0.48 / m ³	USD 0.60 / m ³
Advantages	drinking water quality	drinking water quality	drinking water quality	drinking water quality
		improved membrane life		
Disadvantages	scaling problems		scaling problems	
	short membrane life	short membrane life	short membrane life	production of sludge
Improvements	not suitable for scaling waters	membrane life	not suitable for scaling waters	recycling of sludge

Table 2. Summary of case studies on treatment processes using membranes and ion-exchange (Lorax Environmental, 2003).

	Bioreactor	Constructed Wetland	Alk. Prod. Systems	Permeable React. Barrier
	2001	1993/1999	1999	1999-2002
Pretreatment	yes	yes	yes	no
Feed water	SO ₄ : 8,342 ppm	SO ₄ : 1,700 ppm	SO ₄ : 3,034 ppm	SO ₄ : 2,500-5,200 ppm
Product water	SO ₄ : 198 ppm	SO ₄ : 1,540 ppm	SO ₄ : 1,352 ppm	SO ₄ : 840 ppm
Sludge production	low-moderate	no	no	no
Monitoring	moderate-high	low	low	low
Maintenance	moderate	low	low	low
SO₄ reduction rate	12-30 g/L, day	0.3-197 g/L, day	0 g/L, day	Max. 10.5-15.3 g/L, day
Capital cost*	USD 0.24M per 10 ³ m ³ /day	unknown (low)	unknown (low)	USD 65,000
Operating cost*	USD 0.27 / m ³	unknown (low)	unknown (low)	USD 30,000/yr
Advantages	also trace metal removal	also trace metal removal	also trace metal removal	also trace metal removal
	recycling of H ₂ S and CO ₂	passive treatment	gypsum precipitation	passive treatment
Disadvantages	cost of C + energy source	little sulfate reduction	no sulfate reduction?	long-term performance?
	production of sludge			
Improvements	recycling of sludge	specific design required	specific design required	alternative reactive media
	cheap C + energy source			

Table 3. Summary of case studies on treatment processes using biological sulfate removal (Lorax Environmental, 2003).

Advancing the Technology

In order to advance bioremediation sulfate reduction technology beyond its current state and avoid problems inherent in many current bioremediation bioreactor designs, Phase I in the development cycle of the NRRI/Clearwater Layline smart bioremediation technology focused on designing a system to:

1. Provide flow control of mining waters through bioreactors to avoid channeling seen in many large-scale SRB bioremediation systems;
2. Provide a modular design for the bioreactor units which would be scalable to handle larger flow requirements (600 gpm and greater);
3. Provide a floating modular sulfate reduction system capable of being operated in future cold months;
4. Provide water sulfate reductions down to 200 ppm sulfate or lower for system feed waters as high as 1200 ppm sulfate;
5. Provide aqueous sulfate reduction technology that is capable of being operated and monitored remotely; and
6. Provide an aqueous sulfate reduction technology with operating treatment costs below \$1.00/1,000 gallons water treated (<\$1.00 per ~3,785 liters treated).

Partners Involved with Advancing the Sulfate Reduction Technology

Key University Partners Included:

Natural Resources Research Institute, University of Minnesota Duluth

Roles and Responsibilities: UMD's NRRI is the primary University partner providing research funding and scientific expertise to advance the development, design, and operation of the smart sulfate reduction bioreactor systems described, as well as to provide research and design for the hydrogen sulfide removal component of the technology. Iron precipitation methodology is currently being evaluated for hydrogen sulfide removal from SRB bioreactor discharge waters at NRRI's Coleraine Minerals Research Laboratory. This technology is subsequently field-tested at the mine pit lake site in Hoyt Lakes, MN. David Hendrickson, Director of Strategic Development at NRRI's Coleraine Minerals Research Laboratory, is the Principal Investigator for the sulfate reduction research.

Key Contributing Partners Included:

Clearwater Layline, LLC

Roles and Responsibilities: Clearwater Layline, LLC is the primary industrial partner and the Minnesota small business that is working with UMD NRRI's Principal Investigator to design, develop, operate, and evaluate the smart sulfate reduction bioreactor systems described. A Circle

Pines, MN-based small business, Clearwater Layline LLC (Clearwater) provides technical and market advice, guidance, and support to address critical factors related to water/wastewater treatment. The company principal, Mr. Jeffrey Hanson, possesses expertise in sulfate-reducing bioreactor equipment design, development, and operation. He also provides experience working and interfacing with state natural resource and environmental regulatory personnel to evaluate aqueous sulfate limits and effects of ARD in Minnesota. Clearwater also provides expertise in on-site activities that include system construction, installation, operations, and water sampling, as well as system evaluations designed to improve system optimization.

PolyMet Mining Corporation

Roles and Responsibilities: PolyMet Mining Corporation (PolyMet) is a primary industrial partner that is the first mining company in northeastern Minnesota to apply for environmental and other permits necessary to enable future mining of copper-nickel-cobalt and precious metal resources located in the Duluth Complex near Hoyt Lakes, Minnesota. The company's personnel are responsible for providing the test site where the NRRI/Clearwater Layline SRB technology is installed. PolyMet has provided in-kind support of the project through use of a test location, security of the system, and other human resources to assist in the commercialization of the technology. They have also provided office space and facilities near the existing test site to conduct meetings, culture sulfate-reducing bacteria, and conduct laboratory water chemistry testing during this Phase I study.

Cliffs Natural Resources

Roles and Responsibilities: Cliffs Natural Resources (CliffsNR) is a primary industrial partner that owns and manages three large-scale iron ore mining operations in northeastern Minnesota. They have expressed strong support for development of the proposed technology since it represents a low-cost environmental remediation technology that may be capable of economically reducing aqueous sulfate concentrations in northeastern Minnesota watersheds. CliffsNR provided valuable expertise in environmental remediation and experience working with state and federal regulatory agencies for mining and in environmental permitting. CliffsNR is currently responsible for maintaining water permits for the Cliffs Erie site in Hoyt Lakes where NRRI's pilot scale sulfate reduction equipment is located in a legacy iron ore mine pit lake.

East Range Joint Powers Board (ERJPB) and Laurentian Partnership

Roles and Responsibilities: The ERJPB administers the towns of Aurora, Hoyt Lakes, and White that are impacted by elevated sulfate levels associated with mining impacted waters. As a result of this impact, ERJPB applied for, and received, an Innovation Grant of \$125,000 from the Laurentian Vision Partnership of the IRRRB (Iron Range Resource and Rehabilitation Board) to help support this sulfate reduction project. This funding was justified on the basis that biological sulfate reduction would not only allow for increased economic activity with mining but also

improve municipal water quality. The Laurentian Vision Partnership Innovation Grant was submitted in July, 2012 and was awarded to NRRI and Clearwater Layline, LLC in September, 2012.

Broader context partners providing support, funding, and/or equipment donations for the technology include:

1. U.S. Environmental Protection Agency (Duluth, MN);
2. MN Department of Natural Resources (Division of Lands and Minerals);
3. Iron Range Resources and Rehabilitation Board (IRRRB);
4. Mining Minnesota;
5. Premier Plastics; and
6. Silicon Energy, LLC.

Project Funding

In August, 2012, David Hendrickson (Project PI, UMD NRRI) and Jeffrey Hanson (Project PI, Clearwater Layline, LLC President) submitted a proposal that called for the development of a methodology to reduce aqueous sulfate concentrations from 1,200-1,500 ppm to 300 ppm or lower using a combined biological and chemical technique that duplicated natural processes known to reduce sulfate. The objective of this early stage research was to conduct an on-site field test to determine whether a biological sulfate reduction system could be designed and deployed in a largely automated manner to address the volume of sulfate reduction needed, while also being lower cost and more economically viable than other potential treatment systems. On September 1, 2012, the proposal funded a two year \$369,500 Innovation Grant Program grant from UMD NRRI, the Laurentian Partnership of the Iron Range Resources and Rehabilitation Board (IRRRB), PolyMet Mining, and Cliffs Erie, Inc.

Contributions from the individual groups are listed as follows:

NRRI :	\$130,000
IRRRB:	\$125,000
Clearwater/Layline:	\$ 54,000
Cliffs/Polymet:	\$ 30,500
Premier Plastics:	\$ 30,000
Total:	\$369,500

The project funding for this Phase I Sulfate Reduction Project was used for design, construction, installation, operation and evaluation of the initial floating bioremediation raft system containing four floating bioreactors with an integral nutrient addition system, and a separate iron dosing/ iron precipitation tank system located on the shoreline and receiving effluent from the floating bioreactors. Phase II Sulfate Reduction Project funding would be requested after the Phase I research was completed to provide Proof of Concept results supporting the bioremediation system design.

METHODOLOGY

Test Objective and Water Quality Goals

The objective of the pilot testing was to provide proof of concept results for the design of the bioremediation technology by:

- Evaluating the effectiveness of the SRB bioreactor system for sulfate reduction under actual site conditions; and
- Evaluating whether implementation of the SRB bioreactor in the Hoyt Lakes mine pit lake was practical and cost effective.

Based on successful results of the initial testing, a potential full-scale SRB bioreactor system could be evaluated. An evaluation of treatment efficiency and cost effectiveness for the SRB bioreactor test could then be completed and thus compared to other potential sulfate reduction technologies.

A full-scale SRB bioreactor treatment system would need to treat a flow rate on the order of 400 gpm (~1,500 lpm) and would need to reduce the existing sulfate concentration of mine pit lake water from approximately 1,100 mg/L to a concentration of about 200-300 mg/l (ppm). Given the potentially wide range of applicable water quality targets for sulfate discharge to Spring Mine Creek, a value of 250 mg/L was used in this evaluation. Additionally, the discharge of the bioreactor treatment system could not affect the discharge water quality in a way such that other permit discharge parameters no longer met regulatory compliance.

These SRB bioreactor flow and sulfate reduction design criteria were based on requests presented from Polymet Mining Company to provide a bioremediation sulfate reduction technology to meet their operational flow needs and reduce mining water sulfate concentrations down to about 250 ppm (mg/l). This 250 ppm (mg/l) product water could then be fed into a reverse osmosis system to reduce sulfate concentrations to 10 ppm (mg/l) or lower, if necessary, prior to being discharged into Spring Mine Creek.

Proposed System Design

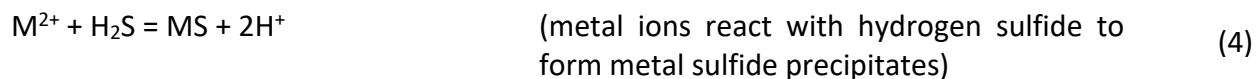
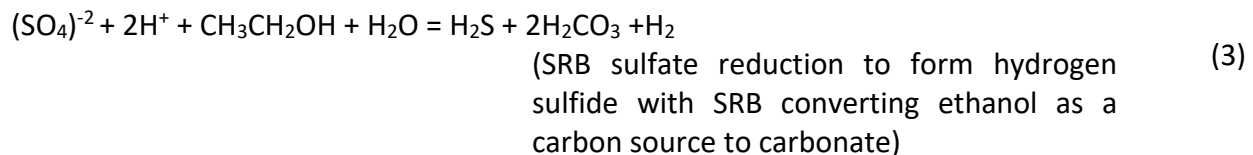
The project proposes to develop and validate a uniquely designed bioremediation system that:

1. Is efficient at removing sulfate, trace metals, and aqueous sulfur species associated with ARD;
2. Controls water flow through the reactors to avoid channeling, providing a method of effectively treating the entire flow;
3. Is divided into a series of bioreactor modules allowing for effective and efficient treatment of high volumes of water flow through replicated, parallel flow systems;
4. Offers a lower-cost solution to providing aqueous sulfate reduction technology;
5. Utilizes recycled plastic materials for various components in its construction;

6. Utilizes a renewable energy, direct current (DC) power supply provided by efficient solar panel technology;
7. Duplicates biological and chemical reactions occurring in nature to provide for efficient bioremediation of sulfate pollution;
8. Is designed to operate year-round in both hot and cold (sub-freezing) seasons;
9. Utilizes embedded, solid state sensors and control equipment to monitor the operating condition of the system and remotely transmits operating data to allow for remote computer control of the system;
10. Potentially reduces trace metal concentrations in mine impacted water such as copper, lead, and zinc, and forms precipitated metal sulfides in the range of pH 5.0 to pH 7.0 (Reisman et al., 2008, 2009); and
11. Has been proven at small pilot-scale in a modular system that is capable of being scaled up with additional duplicate modules to handle the flow rate required.

Design of the Sulfate Reduction Technology

The sulfate reduction technology utilizes SRB grown in modular floating bioreactors to convert aqueous sulfate concentrations to dissolved hydrogen sulfide gas (H₂S). A carbon source and nitrogen and phosphorus nutrients are added to the bioreactors to maintain SRB growth. The dissolved hydrogen sulfide gas produced by the SRB is then reacted with various minerals and/or chemicals to precipitate out the sulfur into various chemical forms as shown in the following reactions (Johnson and Hallberg, 2005):



The bioremediation process design is illustrated in Figures 2, 3, and 4.

The conceptual design shown in Figure 2 illustrates the basic components of the system. Mine pit lake water is pumped from a constant depth of 10 meters (30 ft.) into floating bioreactor modules containing sulfate-reducing bacteria grown on fiber media in the modular tanks. Bioreactor product water then flows into a precipitation tank where minerals or chemicals added cause the sulfur present to precipitate out. Sulfur precipitates are then removed. The bioreactor modules are equipped with solar panels to provide power to various sensors and transmitters positioned on the top of the modules. Transmitters transmit operating data wirelessly to laptop computers and/or cell phones to monitor operating conditions and control the bioreactors remotely. Wireless data is password protected to maintain the security of the data and the system.

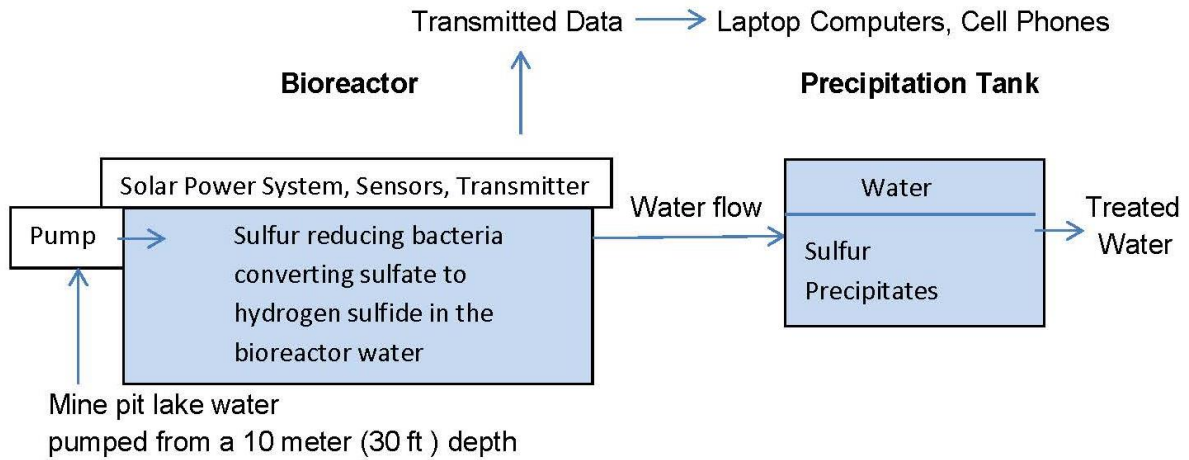


Figure 2. Conceptual system design.

The modular system design shown in Figure 3 illustrates the grouping together of bioreactor modules in a 3 X 3 configuration using the center module as a precipitation tank. The current design utilizes a 2 X 2 configuration having 4 bioreactor modules which each have a 4,000 gallon (15,142 liters) capacity. The grouping together of the bioreactor modules in the figure below is referred to as a 9 module ‘raft’ system. Four solar panels are shown providing the power for this raft configuration. The center white module can serve as a precipitation tank for removing sulfur species created by the SRB.



Figure 3. Modular system design.

The system installation shown below in Figure 4 illustrates the preliminary Phase I floating bioremediation raft system which incorporated a 2 x 2 design housing a total of four modular units. Wooden platforms were built on top of the floating modules to facilitate construction, sampling, plumbing, and electrical work on the units. A nutrient feed tank, solar panels, battery packs, water pumps, sensors, and other devices were installed on the top of the modules. A dock assembly is shown providing access to the raft from the pit lake shoreline.



Figure 4. Preliminary system installation in an iron ore mine pit lake, Hoyt Lakes, Minnesota.

Modular Design of the Floating Bioreactor Modules

Many of the difficulties with scalability and consistent operation of SRB bioreactors are related to geometry (Gusek, 2002). SRB bioreactors have been typically installed in tanks, trenches, mine shafts, or whole mine pit lakes using a wide variety of mixed substrates and carbon sources. All of these reactor types run at low flow rates due to the hydraulic residence time (HRT) required. The combination of low flows and less than optimal geometries results in difficulties such as preferential flow paths, packing, substrate depletion and plugging. All of these concerns and limitations are addressed with the design concept adopted in the NRRI/Clearwater Layline LLC SRB system. The design is based on the use of a submerged, non-biodegradable, growth substrate with a large exposed surface area which is optimal for microbial growth. The substrate bio-carrier material consists of recycled carpet fiber which does not contribute to the biological process as a carbon source and is therefore not consumed over time. It is non-biodegradable providing for an indefinite substrate life for the bioreactor.

In many of the mining applications in Minnesota there are high levels of sulfates in neutral waters with low levels of dissolved metals. In these cases, a two-stage SRB bioreactor system is

required and was installed. The initial biological reactor converts sulfate to hydrogen sulfide. The second stage precipitation tank comprises a processing tank to settle out the sulfur as a precipitate or as elemental sulfur. Material settling out in the processing tank can then be pumped out and handled by appropriate means and/or disposed of using other methods approved by state or federal regulators. These are design concepts addressed in this Stage 1 study, and such concepts will be optimized in future studies comprising in the Phase II research. By having the sulfur precipitation occurring in this second stage, downstream of the bioreactor, any potential plugging problems caused by the precipitation are isolated from the biologically active first stage bioreactor. This design will greatly reduce the need for periodic back-flow purging of the bioreactor.

The bioreactors are constructed of high density polyethylene (HDPE) plastic and each have a volume of about 14,400 liters (3,800 gal.). The system is constructed by attaching multiple floating bioreactors together to create a larger floating platform upon which one can walk on. This floating platform serves a dual role as: 1) a structure to walk on; and 2) a structure to maintain existing equipment in the bioreactors, and to install new equipment in the bioreactors. The separate precipitation tank system is located on the shoreline, per the requirements of the MPCA, where water treated in the bioreactor modules was pumped to remove hydrogen sulfide and form a precipitate containing sulfur. Chemical characterization of the precipitate is currently being conducted as part of the Phase II project research.

Bioreactor Remote Electric Power Generation Design

Since no AC power is available at the remote pit lake site, solar panels were chosen as the most practical method to provide DC power to charge battery packs positioned on top of the floating bioreactor modules. This system was powered by two Model SiE 190 solar panels loaned to NRRI by Silicon Energy Corporation, Mt. Iron, MN. The solar panels charge two deep cycle batteries which provide DC power to pumps, automated valves, sensors, and communication equipment as shown in Figures 5 and 6.

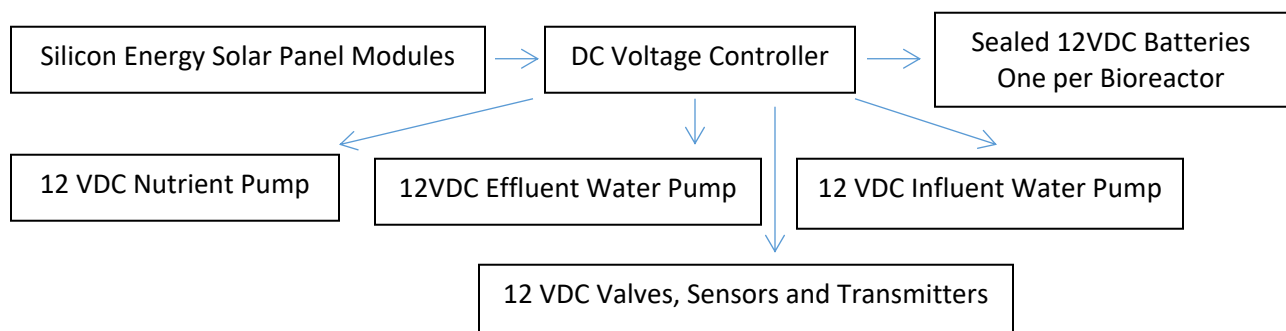


Figure 5. Solar power supply block diagram.



Figure 6. Silicon Energy SiE195 Cascade PV solar panels mounted on the bioreactor raft.

System Water Flow Rate Design

Each module was designed to accommodate a DC pump which pulls pit water from a depth of 9.1 meters (30 ft.). Water flows vertically down through a homogeneous floating substrate in a design that does not favor the formation of preferential flow paths and minimizes plugging. It also allows for agitation and provides an open pathway for excess biomass or precipitate buildup to slough off, or be back-flow purged, from the bioreactor. The preferential flow path problems that some horizontal flow bioreactors have encountered are likely related to variation in substrate densities and the hydraulic pressure gradient that varies from top to bottom within a tank, trench, pond, or mine pit lake (Lorax Environmental, 2003). The design used in this work addresses the substrate issue with a homogeneous, vertical flow substrate. By using this vertical flow bioreactor submerged in a mine pit lake, the hydraulic pressure across a cross-section of the flow pattern is exactly equal across the whole profile for one bioreactor or a large number of replicated bioreactors. This replicable, constant and consistent hydraulic pressure is a critical feature that allows for scalability to handle large flow projects of 3,800 lpm (1,000 gpm) or more. The first Phase I System was designed to house four bioreactor units linked together. It was expected that these bioreactor modules would allow a water flow of between 1 and 4 gpm, which was adjusted to verify that the expected sulfate conversion and reduction was occurring.

In many mining-influenced water applications where SRB bioreactors are used, acidic conditions with high levels of dissolved metals are present. In those cases the hydrogen sulfide (H_2S) generated by the SRB reacts readily with the dissolved metals to form a metal sulfide precipitate. Both the biomass growth and the precipitate will therefore accumulate within the bioreactor accomplishing the full process in a single stage. The proposed vertical flow reactor

handles this accumulation easily by allowing for periodic back-flow purging of the excess biomass and precipitate, downward and out of the bioreactor. The natural gravitational movement of this purged biomass and metal sulfide would be towards the bottom of the mine pit lake that, if permitted, would allow for sub-aqueous, anaerobic storage. Since the MPCA did not allow disposal of the sulfur precipitates to the bottom of the mine pit lake during this Phase I investigation, precipitate was captured and held in the iron dosing tanks positioned on the shore of the pit lake.

Bacterial Bioremediation Design for Sulfate Reduction

The chemical reactions in the SRB are facilitated by the bacteria *desulfovibrio* (Gusek, 2002). The SRB found in nature and bioreactors often thrive in temperatures in the 2° to 6° C range. One of the keys to consistent SRB growth is to maintain a consistent temperature even if it is cold. This design concept therefore calls for submerging the whole SRB bioreactor into a mine pit lake below the surface and insulated from the ambient air in a zone of year-around consistently cold water with low dissolved oxygen concentration.

A liquid carbon source, ethanol, was fed to the bioreactor as the carbon source for the SRB system to help assure complete conversion of the sulfate entering the system. Using a liquid carbon source material allows for accurate metering and eliminates the variability of substrate depletion. The initial carbon source supplied was ethanol. Potential, future, liquid carbon sources could include lactate, glycerol, paper industry wastes, sugar beet effluent, liquid manure or sewage treatment wastes. At present, the most successful and controllable SRB bioreactors utilize an ethanol carbon source (Miller, 2005).

Site Location for Installation of Bioreactor Modules

Testing of the SRB bioreactors was undertaken in a mine pit lake at the former LTV Steel Mining Company (LTVSMC). This test site was proposed by Cliffs Natural Resources and PolyMet and is located in Area 5, east of PolyMet on the Dunka Road very near the western perimeter of Northshore Mining properties. This small lake is part of a series of pit lakes that flow sub-surface from one to the other and eventually discharge to the headwaters of Spring Mine Creek. Spring Mine Creek is a tributary of the Embarrass River. This mine pit lake is a portion of the Area 5NE pit that is isolated from the main section of Area 5NE by an in pit stockpile (see Figs. 7 and 8). The pit used for the test has no visible surface water inlet or outlets, but is hydraulically connected to the 5NE pit through the in pit stockpile. Water flows from the Area 5NE pits into the 5NW pit, which ultimately discharges to surface discharge station SD033. SD033 is the headwaters for Spring Mine Creek, which then flows into the Embarrass River and eventually into the St. Louis River and Lake Superior.

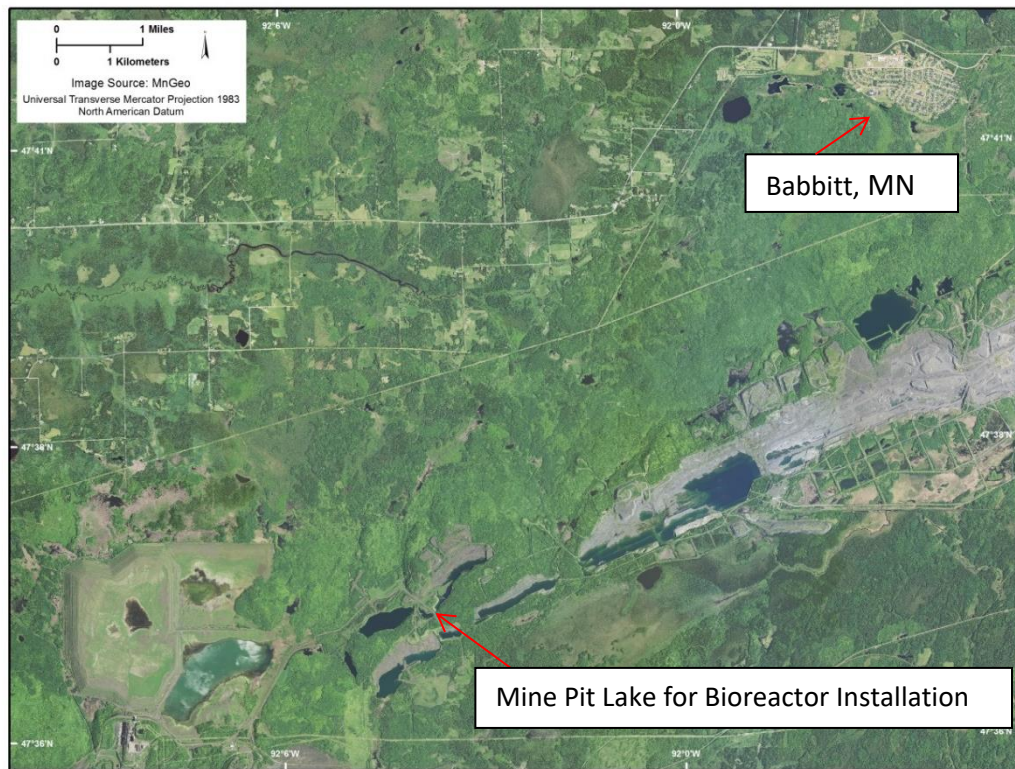


Figure 7. Aerial view shows the chain of pit lakes that flows from east to west and the test site mine pit lake for the bioreactor installation



Figure 8. Aerial view of bioreactor mine pit lake site on Cliffs Erie site.

This Area 5NE pit lake had all of the requirements for an appropriate field test site. Although electrical power is absent, this site has the following advantages:

1. Small pit with depths of well over 20 feet;
2. A two-basin layout for bioreactor and precipitation in one pit lake;
3. Well protected from the wind;
4. Road access to work area and launch area;
5. Level work area on shore; and
6. Sulfate levels over 1,200 ppm.

Installation of Floating Bioreactor Modules

This first phase of testing was initiated in mid-to-late May 2013 with the first set of field data obtained on May 25, 2013. After completion of the inoculum development, liquid-phase material from the 55-gallon drums was transferred into the four pilot bioreactors and allowed to recirculate and homogenize between the modules. Placement of the bioreactors in the pit lake is shown in Figure 9. The four bioreactor modules each have an approximately 3,800 gallon capacity (at dimensions of 9'-10" W by 9'-10" L by 10'-11" D) and are submerged in the pit lake. A floating raft structure was constructed which surrounded the modules and provided a working surface for sampling and servicing the reactors. The structure also allows access to the units, the outer edge of which is located 30 feet from the shore. Each bioreactor is surrounded by a containment vessel constructed of flexible, impermeable HDPE sheet material. Following one-and-one-half weeks of homogenization, feeding of the bioreactors with pit water from a depth of approximately 40 feet was initiated. SRB bioreactors were then operated and maintained with carbon, nitrogen, and phosphorus nutrients feed being initiated. An aerial view of the test site is shown in Figure 10. A ramp was built to allow access from the land to the floating raft system (see Fig. 11).



Figure 9. Placement of bioreactors in the pit lake.



Figure 10. Overview of test site.

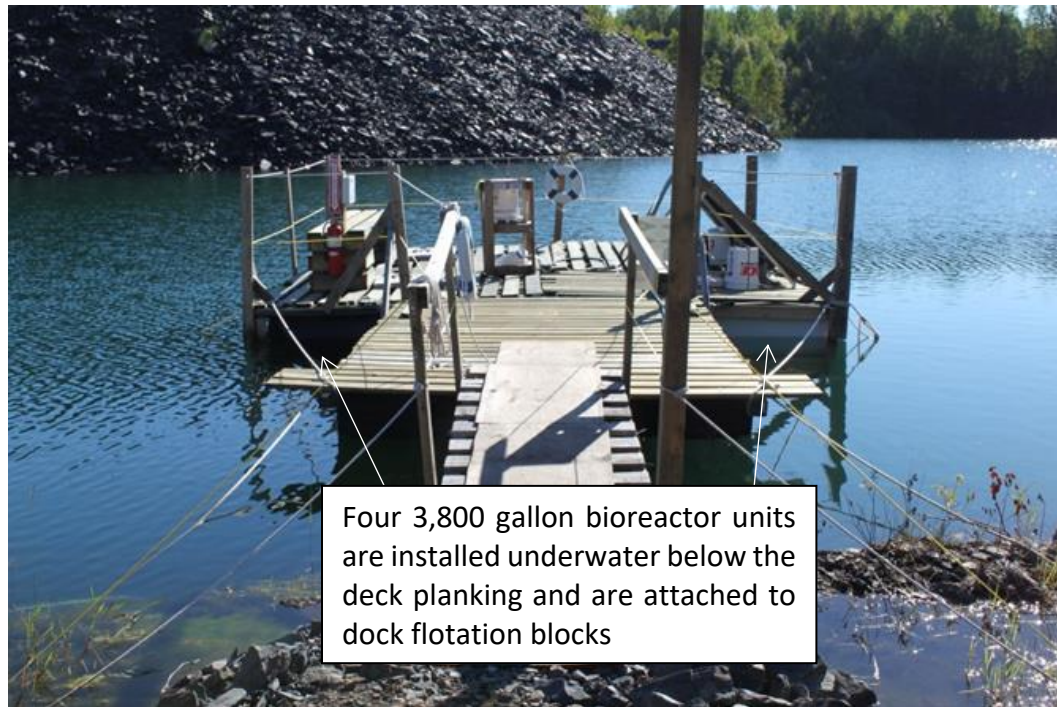


Figure 11. Initial floating raft system installed with four submerged bioreactor modules for Phase I.



Figure 12. Precipitation tanks initially located on the shoreline in Phase I.

METHODS FOR TESTING THE SYSTEM DESIGN

Sampling Protocols

SRB Bioreactor Sampling and Analysis

The design of the bioreactor modules allows for water sampling at several different locations including:

1. Pump inlet to the entire system;
2. Inlet to each bioreactor module;
3. Outlet of each bioreactor outlet; and
4. Outlet of the precipitation tank.

This design allows for sampling and monitoring of all steps in the sulfate reduction process. Collected data was used to demonstrate performance of each individual module as well as overall system performance. Floating bioreactor testing began May, 2013 and proceeded for approximately nine weeks. The sampling schedule is outlined in Table 4 with analyses specified in Table 5 and analytical methods and sample preparation methods described in Table 6.

Sampling of the SRB bioreactors was conducted according to the frequency outlined in Table 4. Field parameters were also measured at the time of sample collection. The first sampling event of the initial pilot testing period occurred on June 8, 2013 with eight weeks of sampling being completed by August 3, 2013. Sampling was terminated after August 3rd as work focused on preparing the site for winter operation. The feed water taken at 10 meters (30 ft.) of depth, each bioreactor effluent stream, and the combined effluent from the iron precipitation tanks positioned on the shoreline (Fig. 12) were sampled. The combined feed to the precipitation tank was also sampled for water quality analysis.

Phase II work will include making physical and chemical measurements on site with portable equipment including specific analysis probes and portable spectrometers. These Phase II sampling procedures are designed to minimize potential physical and/or chemical changes that can occur in water samples due to handling and analyzing the samples after significant periods of time have elapsed.

Week	Influent at Pump Manifold	Bioreactor Effluent	Prec. Tank Effluent	QC Duplicates
Week 1	1	4	1	1
Week 2	1	4	1	1
Week 3	1	4	1	1
Week 4	1	4	1	1
Week 5	1	4	1	1
Week 6	1	4	1	1
Week 7	1	4	1	1
Week 8	1	4	1	1

Table 4. SRB bioreactor sampling schedule 6-22-2013 to 8-17-2013 (# of samples).

Parameter	Matrix	Frequency	Detection Limit
Calcium (mg/L)	Liquid	Weekly	0.50 mg/l
Iron, Total ($\mu\text{g/L}$)	Liquid	Weekly	50 $\mu\text{g/l}$
Magnesium (mg/L)	Liquid	Weekly	0.50 mg/l
Hardness, Total (calculated) (mg CaCO_3/L)	Liquid	Weekly	1.0 mg/l
Iron, Dissolved ($\mu\text{g/L}$)	Liquid	Weekly	50 $\mu\text{g/l}$
Alkalinity, Bicarbonate (mg CaCO_3/L)	Liquid	Weekly	10 mg/l
TDS (mg/L)	Liquid	Weekly	10 mg/l
pH (SU)	Liquid	Weekly	0.1
Sulfide (mg/L)	Liquid	Weekly	5.0 mg/l
Sulfate (mg/L)	Liquid	Weekly	2.0 mg/l
NH_3 (mg N/L)	Liquid	Weekly	0.10 mg/l
NO_2+NO_3 (mg N/L)	Liquid	Weekly	0.10 mg/l
Phosphorus, Total (mg/L)	Liquid	Weekly	0.0040 mg/l
DOC (mg/L)	Liquid	Weekly	1.0 mg/l

Table 5. Analytical parameters (detection limits reported by Pace Analytical Services, Virginia, MN).

Analytical methods for each analysis are described in Table 6, along with descriptions of sample preparation methods.

Parameter	Analytical Method	Sample Prep. Method
Calcium	ICP, EPA 200.7	EPA 200.7
Total Iron	ICP, EPA 200.7	EPA 200.7
Magnesium	ICP, EPA 200.7	EPA 200.7
Total Hardness	APHA Standard Methods (SM) 2340B	APHA Standard Methods 2340B
Dissolved Iron	ICP, EPA 200.7	EPA 200.7
Bicarbonate Alkalinity	APHA Standard Methods 2320B	SM 2320B
Total Dissolved Solids	APHA Standard Methods 2540C	SM 2540C
pH	APHA Standard Methods 4500-H+B	SM 4500-H+B
Sulfide	APHA Standard Methods 4500-S F (2000)	SM 4500-S F (2000)
Sulfate	EPA 300.0	EPA 300.0
Ammonia	EPA 350.1	EPA 350.1
NO ₂ + NO ₃	EPA 353.2	EPA 353.2
Phosphorus	EPA 365.1	EPA 365.1
Dissolved Organic Carbon	APHA Standard Methods 5310C	SM 5310C

Table 6. Analytical methods and sample preparation methods.

Development of Inoculums of Sulfur-Reducing Bacteria (SRB)

An inoculum of SRB had to be developed and allowed to incubate prior to operation of the SRB bioreactors. This was necessary to develop a high concentration of SRB growing on the media provided. One milliliter of this inoculum contains on the order of one million SRB (Hicks, personal communication, August, 2013). Without establishing an SRB colony prior to operation, it is believed that the in-pit SRB treatment system would have developed very slowly.

The initial inoculum phase work began on March 19, 2013, with collection of sediment and water samples from Spring Mine Creek, which discharges (Fig. 13) from the pit lake that is being treated. Sediment samples from a wetland and a stream bed were also collected to provide an initial SRB source, and the samples were tested to confirm the presence of SRBs.



Figure 13. Sediment was sampled from the discharge stream of the pit lake to act as a source of natural sulfate-reducing bacteria for use in the bioreactor modules.

Inoculum development materials consisted of sixteen 55-gallon drums, each filled with 5 gallons of pit lake water. Pit water added to each drum was amended with an ethanol carbon source, as well as ammonium hydroxide (NH_4OH) and phosphoric acid (H_3PO_4), supplied by NRRI as reagent grade chemicals, to provide sources of bioavailable nutrients nitrogen (0.01M) and phosphorus (0.002M), respectively. A solid phase consisting of the sediment samples was added to each drum to provide initial SRB and floating SRB media. This provided a substrate for biofilm development. The drums were then sealed to prevent aeration of the water and left to sit for a nine-week incubation period (Fig. 14). Incubation ended on May 10, 2013 so that the material could be placed in the modules in the pit lake.



Figure 14. Sealed, vented inoculum tanks culturing sulfate-reducing bacteria sampled from stream sediment near the pit lake site.

Design of Nutrient Feed System for Sustaining SRB Growth in the Floating Bioreactors

Bioavailable organic carbon (OC), nitrogen (N), and phosphorus (P) were added to the floating bioreactor system in the form of ethanol, urea, and mono-ammonium phosphate (MAP). These nutrients were added to pit lake inflow water prior to distribution to the four bioreactors. Concentrations of these constituents were adjusted based on measurements of OC, N, and P in the individual bioreactors and precipitation tank outflows. Three gallons of ethanol, two pounds of urea, and 0.5 pounds of MAP were added daily to the bioreactors, six days per week. A single gravity feed nutrient tank was positioned on the floating raft bioreactor system to allow distribution of the combined nutrient fed solution to the four bioreactors (Fig. 15).



Figure 15. Bioreactor nutrient addition design.

Methods for Field Testing

Details of the field monitoring data and analytical results from the completed phases of the SRB bioreactor testing are shown below. All water samples collected from both the initial SRB inoculum verification sampling and the ongoing SRB bioreactor treatment testing were obtained by NTS via a nitrogen headspace sampling procedure to protect from atmospheric contact. Water samples were then processed (i.e., added to their respective preserved or non-preserved laboratory sample bottle) inside an air-tight glove box under nitrogen headspace. Field measurements were obtained using the Hach® HydroLab MS-5. Samples collected during these sampling events were analyzed for water quality parameters including calcium, magnesium, total iron, dissolved iron, bicarbonate alkalinity, pH, sulfate, sulfide, total hardness, total dissolved solids (TDS), total ammonia, phosphorus, and dissolved organic carbon (DOC) (Tables 9-15) by Pace Analytical Laboratories, Virginia, MN. Bioreactor effluent sampling events were completed by Northern Testing Services (NTS), Virginia, MN and analyzed by Pace. Select field parameters (specific conductance, temperature, pH, and turbidity) were measured in each bioreactor module prior to every sampling event with the Hach Hydrolab Minisonde 5 Water Quality Meter by NTS staff.

Task	Sept-Oct 2012	Nov-Dec 2012	Jan-Feb 2013	Mar-April 2013	May-June 2013	July-Aug 2013
Laurentian Vision Innovation Grant defined and background work begins	X					
Innovation Grant approved and project begins	X					
Secure materials and construct first 4 bioreactors and precipitation tanks	X	X				
Prepare for inoculum brewing			X	X		
Brew inoculum for 9 weeks with inoculum reactor tank monitoring and analysis				X	X	
Move the 4 assembled bioreactors to the site Launch bioreactors and transfer inoculums into the four bioreactors				X	X	
Begin water analysis testing on the pit water and bioreactor water samples					X	X
Suspend the operation of the 4 bioreactors to allow for Phase II work August 15, 2013						X

Table 7. Project timeline.

INOCULUM DEVELOPMENT AND VERIFICATION RESULTS

Background

Water in each of the sixteen 55-gallon drums used for the SRB inoculum development was monitored weekly for field parameters including: pH, temperature, dissolved oxygen (DO), specific conductance (SC), oxidation-reduction potential (ORP), and turbidity (Table 8). These parameters are indicators of sulfate reducing conditions and can be easily measured in the field for immediate results. Each of the 16 drums was also sampled on April 27 and May 11, 2013 and samples were submitted to an analytical laboratory for water analysis. A subset of the reactors (reactors 1, 11, 13, and 16) underwent sampling and analysis at a weekly frequency.

Characteristics of water sampled from these 55-gallon drums were indicative of chemically reducing conditions for biological sulfur reduction. Concentrations of sulfate in liquid samples from drums 1, 11, 13, and 16 decreased by 40-95% over the nine week incubation period (see Table 8). A corresponding increase in aqueous sulfide concentrations was also measured in each of these reactors over the nine-week period. Field measurements from liquid in all 16 of the 55-gallon drums were also indicative of conditions favorable to biological sulfate reduction, including a decrease in pH and lower DO and ORP values. Lab and field data from inoculum tanks 1, 11, 13, and 16 are included in Table 8.

Data Tables of Inoculum Samples

	pH (SU)							
	3/30/2013	4/6/2013	4/13/2013	4/20/2013	4/27/2013	5/4/2013	5/11/2013	5/20/2013
Reactor 1	8.5	8.4	8.2	8.0	7.9	7.8	7.6	7.6
Reactor 11	8.4	7.6	7.2	7.1	7.3	7.3	7.5	7.6
Reactor 13	8.4	8.0	7.6	7.4	7.4	7.1	7.2	7.2
Reactor 16	8.4	7.8	7.5	7.3	7.3	7.1	7.2	7.2

	Specific Conductance ($\mu\text{S} / \text{cm}$)							
	3/30/2013	4/6/2013	4/13/2013	4/20/2013	4/27/2013	5/4/2013	5/11/2013	5/20/2013
Reactor 1	3669	3787	3776	3936	3992	3966	4628	4537
Reactor 11	2929	2946	2938	2977	3100	3821	3829	3926
Reactor 13	3130	3157	3229	3247	3337	3867	3815	3357
Reactor 16	3249	3330	3359	3385	3584	4148	4030	4130

	Oxidation / Reduction Potential (mV)							
	3/30/2013	4/6/2013	4/13/2013	4/20/2013	4/27/2013	5/4/2013	5/11/2013	5/20/2013
Reactor 1	136	14	-231	-313	-370	-265	-168	-152
Reactor 11	-154	-211	-234	-390	-426	-276	-173	-181
Reactor 13	-176	-233	-323	-410	-433	-269	-156	-150
Reactor 16	-182	-261	-270	-412	-430	-266	-156	-155

	Dissolved Oxygen ($\text{mg O}_2 / \text{L}$)							
	3/30/2013	4/6/2013	4/13/2013	4/20/2013	4/27/2013	5/4/2013	5/11/2013	5/20/2013
Reactor 1	1.1	0.5	0.5	0.0	0.9	0.3	0.0	0.2
Reactor 11	0.5	0.6	0.7	0.3	0.6	1.2	1.2	0.5
Reactor 13	0.5	0.8	0.3	0.5	0.8	1.0	0.7	0.4
Reactor 16	0.7	0.3	0.1	0.3	0.7	1.1	1.0	0.5

Table 8. Weekly field parameters and laboratory analysis from initial SRB inoculum development (four reactors).

Table 8 (continued). Laboratory analysis from initial SRB inoculum development.

	Temperature (°C)							
	3/30/2013	4/6/2013	4/13/2013	4/20/2013	4/27/2013	5/4/2013	5/11/2013	5/20/2013
Reactor 1	15.3	16.4	17.1	18.1	17.0	13.2	14.6	14.2
Reactor 11	16.1	16.2	17.5	18.8	16.6	12.8	14.4	11.8
Reactor 13	15.8	16.6	17.8	19.1	16.5	12.7	14.2	11.8
Reactor 16	16.3	17.1	18.2	19.7	17.4	14.0	14.2	11.7

Inoculum reactor tank #1	Ca (mg/L)	Fe, Tot (ug/L)	Mg (mg/L)	Hard, Tot (mg/L)	Fe, Diss (ug/L)	Alk, Bicarb (mg/L)	TDS (mg/L)	pH (SU)	Sulfide (mg/L)	Sulfate (mg/L)	NH3 (mg N/L)	NO2+NO3 (mg N/L) (DL 0.1)	P, Tot (mg/L)	DOC (mg/L)
3/19/2013	101	7950	220	1160	1470	609	2460	8.4	6.7	1270	59.6	<DL	4.4	
3/30/2013	95.4	1500	214	1120	1260	594	2390	8.6	5	1260	97.3	<DL	4.3	350
4/6/2013	95.5	804	207	1090	512	549	2160	8.4	5.8	1230	107	<DL	5.5	356
4/13/2013	96.3	395	209	1100	224	688	2570	8.4	8.2	1180	125	<DL	6.6	359
4/20/2013	95.9	299	213	1120	141	704	2510	8.4	39	1130	125	0.15	8	399
4/27/2013	96.2	445	209	1100	136	729	2340	8.3	59.8	1080	132	0.23	10	409
5/4/2013	96.4	264	210	1110	100	793	2540	8.3	74	1000	141	<DL	10.7	448
5/11/2013	98.3	245	196	1050	65	1150	2490	8.4	76	871	243	<DL	11.1	735
5/20/2013	105	274	205	1110	50	1290	2390	8.2	67.2	771	266	<DL	13.8	13.8

Inoculum reactor tank #11	Ca (mg/L)	Fe, Tot (ug/L)	Mg (mg/L)	Hard, Tot (mg/L)	Fe, Diss (ug/L)	Alk, Bicarb (mg/L)	TDS (mg/L)	pH (SU)	Sulfide (mg/L)	Sulfate (mg/L)	NH3 (mg N/L)	NO2+NO3 (mg N/L) (DL 0.1)	P, Tot (mg/L)	DOC (mg/L)
3/19/2013	102	3770	228	1190	574	381	2060	7.8	5	1160	10.8	<DL	4.9	
3/30/2013	101	1350	237	1230	306	444	1940	8.4	5.8	1130	45.6	<DL	20.2	240
4/6/2013	97.9	314	227	1180	150	466	1860	7.9	7.6	1070	39.4	<DL	18.9	206
4/13/2013	97.3	176	224	1170	105	534	2040	7.9	43.4	993	49.4	<DL	19.1	224
4/20/2013	99.1	111	226	1180	65.7	611	1940	8.1	58.8	927	51	<DL	18.6	236
4/27/2013	97.7	90.4	221	1160	54.4	690	1930	8.2	82.6	818	71.1	<DL	18.7	268
5/4/2013	96.8	179	205	1090	50	1200	2000	8.1	83	478	208	0.14	18.8	614
5/11/2013	90.4	54.7	202	1060	50	1330	2020	8.3	83.6	306	203	<DL	20.6	545
5/20/2013	93.4	90.8	208	1090	50	1550	1990	8.2	33.8	47.8	205	<DL	15.2	484

<DL refers to values below-detection levels.

Table 8 (continued). Laboratory analysis from initial SRB inoculum development.

Inoculum reactor tank #13	Ca (mg/L)	Fe, Tot (ug/L)	Mg (mg/L)	Hard, Tot (mg/L)	Fe, Diss (ug/L)	Alk, Bicarb (mg/L)	TDS (mg/L)	pH (SU)	Sulfide (mg/L)	Sulfate (mg/L)	NH3 (mg N/L)	NO2+NO3 (mg N/L)	P, Tot (mg/L)	DOC (mg/L)
3/19/2013	99	3410	222	1160	1130	458	2230	7.1	5	1170	13.8	<DL	3.6	
3/30/2013	100	1340	226	1180	150	511	2620	8.5	7	1110	65.1	<DL	11.8	320
4/6/2013	93.9	269	217	1130	100	589	1930	8.4	32.4	1020	63.4	<DL	13.7	265
4/13/2013	95.4	135	224	1160	54.8	676	2070	8.4	58.2	961	77.5	<DL	15.4	285
4/20/2013	92.1	89.4	224	1150	50	740	2030	8.4	77.4	881	79.9	<DL	15.7	305
4/27/2013	91.7	242	214	1110	50	786	2020	8.1	86.6	770	102	<DL	14.9	341
5/4/2013	87	230	213	1090	50	1300	2090	7.7	72.8	463	209	<DL	26.8	718
5/11/2013	83.9	175	210	1070	50	1300	2160	7.9	94.2	446	196	0.12	25	637
5/20/2013	85.9	385	211	1080	50	1260	2070	7.9	76.4	401	186	<DL	22.1	557

Inoculum reactor tank #16	Ca (mg/L)	Fe, Tot (ug/L)	Mg (mg/L)	Hard, Tot (mg/L)	Fe, Diss (ug/L)	Alk, Bicarb (mg/L)	TDS (mg/L)	pH (SU)	Sulfide (mg/L)	Sulfate (mg/L)	NH3 (mg N/L)	NO2+NO3 (mg N/L)	P, Tot (mg/L)	DOC (mg/L)
3/19/2013	102	2300	234	1220	1080	484	2360	8	5	1200	26	<DL	3.9	
3/30/2013	97.8	890	226	1170	279	561	2080	8.6	7.4	1140	62.3	<DL	9.9	320
4/6/2013	95.4	537	216	1130	170	589	1950	8.3	37.2	1070	65.9	<DL	12.9	272
4/13/2013	99	229	226	1180	119	658	2240	8.3	55.2	990	79.8	<DL	15.6	289
4/20/2013	98.6	151	226	1180	97.3	723	2100	8.3	74.6	928	77.4	0.11	15.6	310
4/27/2013	98.4	129	218	1140	61.8	806	2180	8	94.8	823	112	<DL	16.4	410
5/4/2013	111	87.7	209	1140	54.2	1920	2300	7.9	86.8	501	223	0.11	27.9	812
5/11/2013	109	84.3	211	1140	50	1290	2260	7.9	81.2	489	207	<DL	28.5	742
5/20/2013	112	94.1	219	1180	50	1360	2320	8.1	76.2	422	205	<DL	28.3	740

<DL refers to values below-detection limits.

Graphs of Inoculum Tank Sulfate and Sulfide Nutrient Data

Sulfate reduction in the SRB inoculum reactor tanks

Plots of sulfate reduction occurring in the four SRB inoculum tanks that were sampled and tested weekly are shown in Figure 16.

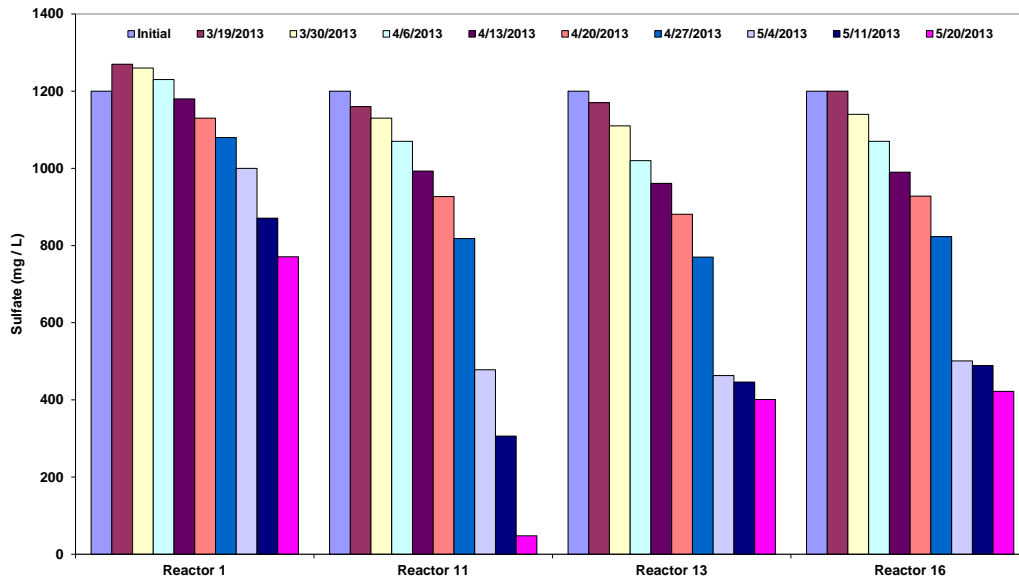


Figure 16. Sulfate reduction during initial SRB inoculum development.

Interpretation of sulfate reduction results

The initial sulfate level in all 16 inoculum reactor tanks was 1,200 ppm. Over the two-month period of inoculum development, sulfate concentrations were reduced in the vented inoculum reactor tanks by an average of 64% and, in some cases, by as much as 99% (reactors 8 and 15), i.e., from 1,200 ppm to 12 and 11 ppm, respectively (see Table 9). Of the four inoculum reactor tanks tested weekly for sulfate, all achieved significant sulfate reduction, while Inoculum Reactor Tank 11 finished with less than 100 ppm sulfate (see Table 8). Of the total of 16 inoculum reactor tanks, 25% achieved reduced sulfate to below 100 ppm. Although the monitoring of the inoculum reactor tanks demonstrated varied results, the potential to achieve sulfate levels below 100 ppm was not the primary purpose of this stage. The primary purpose of this inoculum development stage was to provide viable, active and robust sulfate reducing bacteria colonies to populate the bioreactors to be launched in the mine pit lake.

Inoculum Reactor Tank Date >	Sulfate (mg/l) 4/27	Sulfate (mg/l) 5/11	Sulfate (mg/l) 5/20	Sulfide (mg/l) 4/27	Sulfide (mg/l) 5/11	Sulfide (mg/l) 5/20
#1	1080	871	771	60	76	67
#2	542	82	96	77	218	86
#3	965	879	635	69	85	74
#4	816	503		91	68	
#5	792	510		86	92	
#6	1190	1170	1070	28	35	57
#7	900	772		77	73	
#8	512	12		76	81	
#9	519	376		65	84	
#10	884	625		87	87	
#11	818	306	48	83	86	34
#12	788	554		86	94	
#13	770	446	401	87	94	76
#14	703	455		85	91	
#15	923	12	11	84	84	74
#16	823	489	422	95	81	76
Average	814	504	432	77	89	68

Table 9. Sulfate and sulfide laboratory analysis from initial SRB inoculum development (16 reactors).

Aqueous sulfide increases occurring in the SRB inoculum reactor tanks

Plots of aqueous sulfide increases occurring with time in the inoculum tanks are shown in Figure 17.

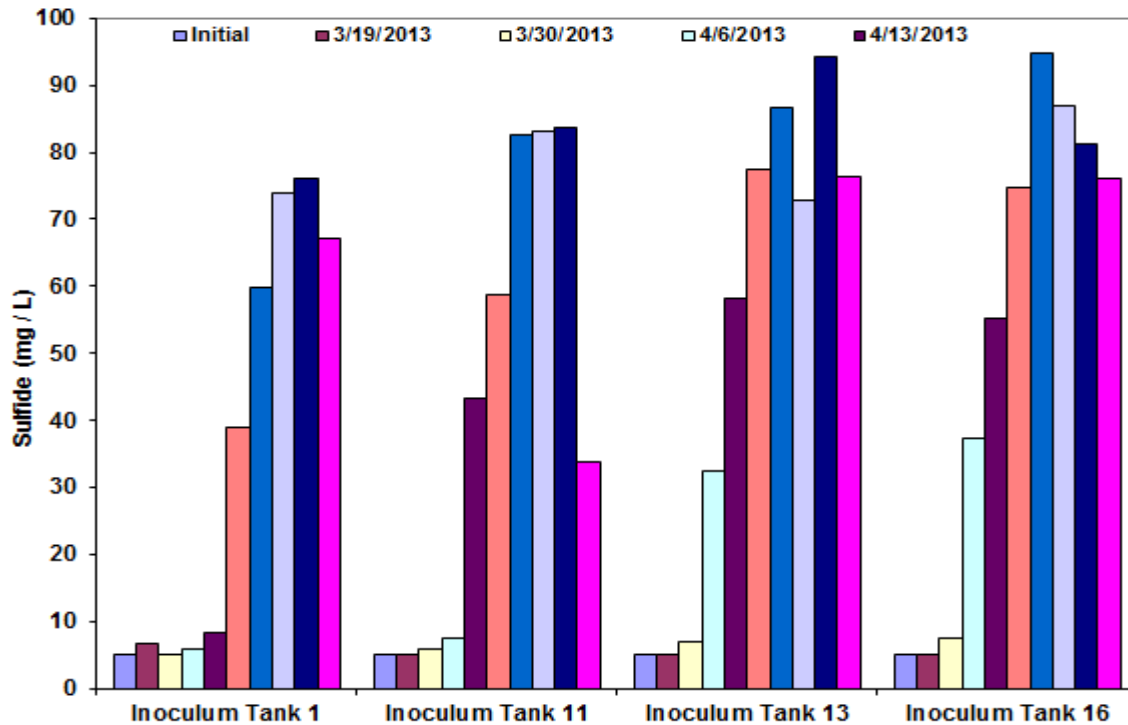


Figure 17. Sulfide production during initial SRB inoculum development.

Interpretation of sulfide increase results

Over the two-month period of inoculum development, sulfide concentrations increased from non-detect sulfide in the initial pit water to an average of 89 ppm across all 16 inoculum reactor tanks (see Table 9). This significant sulfide production is indicative of active and robust sulfate reducing bacterial development.

It is interesting to note that the sulfide concentration in Inoculum Reactor Tank 11 showed the most prominent decrease in the last round of sampling compared to the other three reactors that underwent weekly testing. At the same time, Inoculum Reactor Tank 11 achieved the lowest sulfate concentrations. This may indicate that the sulfide concentration in this stagnant tank was lower because the sulfate had been almost completely consumed, thereby limiting sulfide production. Although significant levels of sulfide were produced in all of the reactors, the amount of sulfide produced, even as the sulfate is nearly all consumed, is insufficient to account for the total sulfate reduction. This indicates that further analysis is recommended to detect and analyze other forms of sulfur that may be present. Some small specs of yellow material (possibly sulfur) were observed within the fiber mass inside the reactor tanks when they were transferred to the field bioreactors. Further investigation of the total sulfur mass balance of the system is required.

to best understand the data and is being completed as part of the Phase II and MnDRIVE studies that are currently in progress.

BIOREACTOR WATER QUALITY RESULTS

Background

Details of the field monitoring data and analytical results from the completed Phase I of the SRB bioreactor testing are shown below. All water samples collected from both the initial SRB inoculum verification sampling and the ongoing SRB bioreactor treatment testing were obtained by NTS via a nitrogen headspace sampling bottle to protect from atmospheric contact during the sample taking process at the field test site. Water samples were then taken back to the PolyMet administration building and processed (i.e., added to their respective preserved or non-preserved laboratory sample bottle) inside an air-tight glove box under nitrogen headspace. Field measurements were obtained using the Hach® HydroLab MS-5. Samples collected during these sampling events were analyzed for water quality parameters including calcium, magnesium, total iron, dissolved iron, bicarbonate alkalinity, pH, sulfate, sulfide, total hardness, total dissolved solids (TDS), total ammonia, phosphorus, and dissolved organic carbon (DOC) (Tables 10-16) by Pace Analytical Laboratories, Virginia, MN. Bioreactor effluent sampling events were completed by Northern Testing Services (NTS), Virginia, MN and analyzed by Pace.

Data Tables for Bioreactor Samples

	Detection Limit (DL)	6/8/13	6/15/13	6/22/13	6/29/13	7/6/13	7/13/13	7/20/13	7/30/13	8/3/13
Calcium (mg/L)	1.0	70.30	72.20	81.30	79.60	79.80	79.80	86.40	84.30	79.90
Iron ($\mu\text{g/L}$)	50	61.50	<50	<50	<50	<50	<50	<50	<50	<50
Magnesium (mg/L)	1.0	267.00	250.00	261.00	256.00	271.00	262.00	277.00	262.00	253.00
Total Hardness (mg CaCO_3/L)	20.0	1270.00	1210.00	1280.00	1250.00	1310.0	1280.00	1350.00	1290.00	1240.00
Iron, Dissolved ($\mu\text{g/L}$)	50	<50	<50	387.00	<50	<50	<50	<50	<50	<50
Alkalinity, Bicarbonate (mg CaCO_3/L)	5.0	299.00	348.00	278.00	275.00	272.00	274.00	268.00	279.00	274.00
TDS (mg/L)	20.0	1840.00	1960.00	1960.00	1960.00	1930.0	1920.00	1940.00	1930.00	1950.0
pH (SU)	0.10	7.90	7.70	7.80	7.80	7.80	7.70	7.80	7.90	7.90
Sulfide (mg/L)	2.0	<2.0	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Sulfate (mg/L)	20.0	1130.00	1200.00	1220.00	1200.00	1210.0	1220.00	1250.00	1220.00	1200.0
Total Ammonia (mg N/L)	0.10	<0.10	<0.10	<0.10	<0.10	0.11	0.14	<0.10	<0.10	0.20
Phosphorus (mg/L)	0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Dissolved Organic Carbon (mg/L)	1.0	1.20	1.80	2.50	1.70	1.60	2.00	1.50	1.90	1.60

Values with “<DL” represent below detection limit values; “--” indicates parameter not analyzed.

Table 10. Analytical results for **SRB Pilot Feed Water** (from Pit 5NEc, 30 ft. deep).

	Detection Limits (DL)	6/8/13	6/15/13	6/22/13	6/29/13	7/6/13	7/13/13	7/20/13	7/30/13	8/3/13
Calcium (mg/L)	1.0	70.20	66.40	69.90	74.00	89.40	89.30	93.30	87.40	87.00
Iron (µg/L)	50	395.0	126.0	<50	<50	<50	<50	<50	<50	<50
Magnesium (mg/L)	1.0	184.0	204.0	238.0	238.0	270.0	256.0	260.0	265.0	256.0
Total Hardness (mg CaCO ₃ /L)	20.0	932.0	1000.	1160.	1160.	1330.	1280.	1300.	1310.	1270.
Iron, Dissolved (µg/L)	50	354.0	86.20	<50	<50	<50	<50	<50	<50	<50
Alkalinity, Bicarbonate (mg CaCO ₃ /L)	5.0	352.0	370.0	346.0	374.0	385.0	743.0	787.0	523.0	1070.
TDS (mg/L)	20.0	1690.	1630.	1740.	1920.	1910.	1890.	1920.	1850.	1820.
pH (SU)	0.10	7.50	7.50	7.60	7.90	8.10	8.30	7.40	7.40	7.90
Sulfide (mg/L)	2.0	1.19	15.80	25.00	29.20	29.60	77.80	11.40	83.80	106.0
Sulfate (mg/L)	20.0	874.0	952.0	1000.	1110.	1020.	451.0	579.0	803.0	206.0
Total Ammonia (mg N/L)	0.10	1.20	2.10	9.10	7.30	0.10	21.50	19.00	3.30	3.30
Phosphorus (mg/L)	0.10	643.0	2.50	4.20	2.20	0.33	5.90	5.30	6.90	17.00
Dissolved Organic Carbon (mg/L)	1.0	135.0	90.70	57.60	--	179.0	254.0	299.0	203.0	315.0

Values with “<DL” represent below detection limit values; “--” indicates parameter not analyzed.

Table 11. Analytical results for **SRB Reactor 1 Effluent**.

	Detection Limit (DL)	6/8/13	6/15/13	6/22/13	6/29/13	7/6/13	7/13/13	7/20/13	7/30/13	8/3/13
Calcium (mg/L)	1.0	66.80	66.40	68.50	73.60	86.30	83.30	88.10	84.60	83.70
Iron (µg / L)	50	347.0	134.0	<50	<50	<50	<50	<50	<50	<50
Magnesium (mg/L)	1.0	186.0	194.0	234.0	254.0	268.0	252.0	263.0	250.0	255.0
Total Hardness (mg CaCO ₃ /L)	20.0	935.0	963.0	1130.	1230.	1320.	1250.	1300.	1240.	1260.
Iron, Dissolved (µg/L)	50	303.0	103.0	<50	<50	<50	<50	<50	<50	<50
Alkalinity, Bicarbonate (mg CaCO ₃ /L)	5.0	354.0	387.0	310.0	344.0	403.0	634.0	632.0	504.0	1130.
TDS (mg/L)	20.0	1650.	1660.	1770.	1890.	1860.	1720.	1670.	1800.	1850.
pH (SU)	0.10	7.60	7.60	7.60	7.80	7.80	8.20	7.80	7.50	8.00
Sulfide (mg/L)	2.0	1.44	20.60	17.60	29.40	14.20	74.80	85.60	83.40	101.0
Sulfate (mg/L)	20.0	903.0	912.0	1030.	1090.	1010.	600.0	689.0	779.0	184.0
Total Ammonia (mg N/L)	0.10	0.20	3.90	7.60	5.60	1.20	15.30	14.10	2.60	5.80
Phosphorus (mg/L)	0.10	4.30	3.20	3.70	2.00	0.70	4.40	4.30	6.80	12.70
Dissolved Organic Carbon (mg/L)	1.0	129.0	107.0	49.20	--	148.0	162.0	183.0	205.0	204.0

Values with “<DL” represent below detection limit values; “--” indicates parameter not analyzed;

Table 12. Analytical results for SRB Reactor 2 Effluent.

	Detection Limit (DL)	6/8/13	6/15/13	6/22/13	6/29/13	7/6/13	7/13/13	7/20/13	7/30/13	8/3/13
Calcium (mg/L)	1.0	79.9	70.00	70.30	76.00	90.70	82.50	87.00	81.30	81.10
Iron ($\mu\text{g/L}$)	50	336.	125.0	<50	<50	<50	<50	<50	<50	<50
Magnesium (mg/L)	1.0	191.	186.0	241.0	263.0	273.0	248.0	257.0	251.0	260.0
Total Hardness (mg CaCO_3/L)	20.0	985.	941.0	1170.	1270.	1350.	1230.	1280.	1240.	1270.
Iron, Dissolved ($\mu\text{g/L}$)	50	288.	94.10	<50	<50	<50	<50	<50	<50	<50
Alkalinity, Bicarbonate (mg CaCO_3/L)	5.0	370.	443.0	327.0	336.0	441.0	758.0	1070.	571.0	1120.
TDS (mg/L)	20.0	730.	1620.	1780.	1910.	1800.	1800.	1840.	1720.	1720.
pH (SU)	0.10	7.50	7.90	7.80	7.90	7.70	7.90	7.20	7.50	7.90
Sulfide (mg/L)	2.0	1.56	37.20	22.00	20.60	60.60	76.40	56.60	96.60	101.0
Sulfate (mg/L)	20.0	890.	857.0	1060.	1130.	932.0	366.0	282.0	576.0	128.0
Total Ammonia (mg N/L)	0.10	0.12	0.98	6.00	6.00	0.10	24.30	23.90	0.66	2.00
Phosphorus (mg/L)	0.10	4.40	3.60	3.30	1.60	0.14	6.50	6.30	5.70	13.20
Dissolved Organic Carbon (mg/L)	1.0	153.	114.0	37.60	--	156.0	260.0	287.0	149.0	184.0

Values with “<DL” represent below detection limit values; “--” indicates parameter not analyzed.

Table 13. Analytical results for SRB Reactor 3 Effluent.

	Detection Limit (DL)	6/8/13	6/15/13	6/22/13	6/29/13	7/6/13	7/13/13	7/20/13	7/30/13	8/3/13
Calcium (mg/L)	1.0	77.60	69.80	67.90	75.70	89.30	87.50	86.30	84.40	78.50
Iron (µg/L)	50	324.0	150.0	<50	<50	<50	<50	<50	<50	<50
Magnesium (mg/L)	1.0	190.0	187.0	237.0	261.0	270.0	263.0	255.0	260.0	252.0
Total Hardness (mg CaCO ₃ /L)	20.0	978.0	943.0	1140.	1260.	1330.	1300.	1270.	1280.	1230.
Iron, Dissolved (µg/L)	50	275.0	90.70	<50	<50	<50	<50	<50	<50	<50
Alkalinity, Bicarbonate (mg CaCO ₃ /L)	5.0	362.0	424.0	335.0	346.0	431.0	760.0	876.0	656.0	1100.
TDS (mg/L)	20.0	1660.	1730.	1720.	1960.	1740.	1850.	1660.	1720.	1530.
pH (SU)	0.10	7.50	8.10	7.90	8.00	7.50	8.10	7.20	7.30	8.20
Sulfide (mg/L)	2.0	1.35	37.00	19.20	21.00	63.60	85.40	74.80	58.00	114.0
Sulfate (mg/L)	20.0	882.0	850.0	1050.	1140.	931.0	370.0	303.0	578.0	133.0
Total Ammonia (mg N/L)	0.10	2.40	4.80	6.40	6.20	0.10	24.80	23.10	0.67	2.00
Phosphorus (mg/L)	0.10	3.90	3.50	2.80	1.70	0.17	6.60	6.30	6.00	13.00
Dissolved Organic Carbon (mg/L)	1.0	147.0	114.0	35.20	--	155.0	264.0	286.0	155.0	181.0

Values with “<DL” represent below detection limit values; “--” indicates parameter not analyzed.

Table 14. Analytical results for **SRB Reactor 4 Effluent.**

	Detection Limit (DL)	6/22/13	6/29/13	7/06/13	7/13/13	7/20/13	7/30/13	8/03/13
Calcium (mg/L)	1.0	68.80	74.10	91.40	86.50	94.50	83.80	82.80
Iron (µg/L)	50	<50	<50	<50	<50	<50	<50	<50
Magnesium (mg/L)	1.0	233.00	254.00	278.00	252.00	273.00	252.00	255.00
Total Hardness (mg CaCO ₃ /L)	20.0	1130.0	1230.0	1370.0	1260.0	1360.0	1250.0	1260.0
Iron, Dissolved (µg/L)	50	<50	<50	<50	<50	<50	<50	<50
Alkalinity, Bicarbonate (mg CaCO ₃ /L)	5.0	333.00	351.00	416.00	754.00	732.00	582.00	1130.0
TDS (mg/L)	20.0	1760.0	1890.0	1870.0	1790.0	1820.0	1810.0	1780.0
pH (SU)	0.10	7.90	7.90	7.00	7.60	7.10	7.30	7.30
Sulfide (mg/L)	2.0	22.60	25.40	48.00	61.80	9.40	92.00	53.60
Sulfate (mg/L)	20.0	1050.0	1120.0	977.00	424.00	447.00	686.00	181.00
Total Ammonia (mg N/L)	0.10	7.70	6.40	0.21	23.20	20.50	1.30	2.50
Phosphorus (mg/L)	0.10	3.60	2.00	0.18	6.20	5.60	6.40	14.50
Dissolved Organic Carbon (mg/L)	1.0	48.00	--	169.00	256.00	278.00	183.00	238.00

Values with “<DL” represent below detection limit values; “--” indicates parameter not analyzed.

Table 15. Analytical results for **Precipitation Tank Influent.**

	Detection Limit (DL)	6/22/13	6/29/13	7/06/13	7/13/13	7/20/13	7/30/13	8/03/13
Calcium (mg/L)	1.0	67.90	74.00	89.00	81.70	91.80	85.00	72.20
Iron (µg/L)	50	58.70	<50	<50	<50	<50	<50	<50
Magnesium (mg/L)	1.0	228.00	259.00	274.00	250.00	273.00	255.00	221.00
Total Hardness (mg CaCO ₃ /L)	20.0	1110.0	1250.0	1350.0	1230.0	1350.0	1260.0	1090.0
Iron, Dissolved (µg/L)	50	<50	<50	<50	<50	<50	<50	<50
Alkalinity, Bicarbonate (mg CaCO ₃ /L)	5.0	332.00	350.00	395.00	690.00	674.00	672.00	877.00
TDS (mg/L)	20.0	1680.0	1920.0	2040.0	1820.0	1830.0	1850.0	1550.0
pH (SU)	0.10	7.80	8.30	7.30	8.30	8.10	7.50	8.40
Sulfide (mg/L)	2.0	12.80	6.40	18.40	52.60	57.00	74.60	43.30
Sulfate (mg/L)	20.0	1070.0	1090.0	927.00	480.00	581.00	466.00	345.00
Total Ammonia (mg N/L)	0.10	7.90	6.10	<0.10	17.70	18.00	0.91	1.40
Phosphorus (mg/L)	0.10	3.70	1.80	0.19	4.90	5.10	7.00	7.60
Dissolved Organic Carbon (mg/L)	1.0	49.20	74.70	207.00	207.00	251.00	11.00	111.00

Values with “<DL” represent below detection limit values; “--” indicates parameter not analyzed.

Table 16. Analytical results for **Precipitation Tank Effluent.**

Graphs of Pit Water & Bioreactor Sulfate, Sulfide, pH, Bicarbonate Alkalinity and Nutrient Data

Aqueous Sulfate Concentrations Versus Time in the Pit Water and Bioreactors

Plots of aqueous sulfate concentrations versus time in the pit feed water and bioreactor modules are shown in Figure 18.

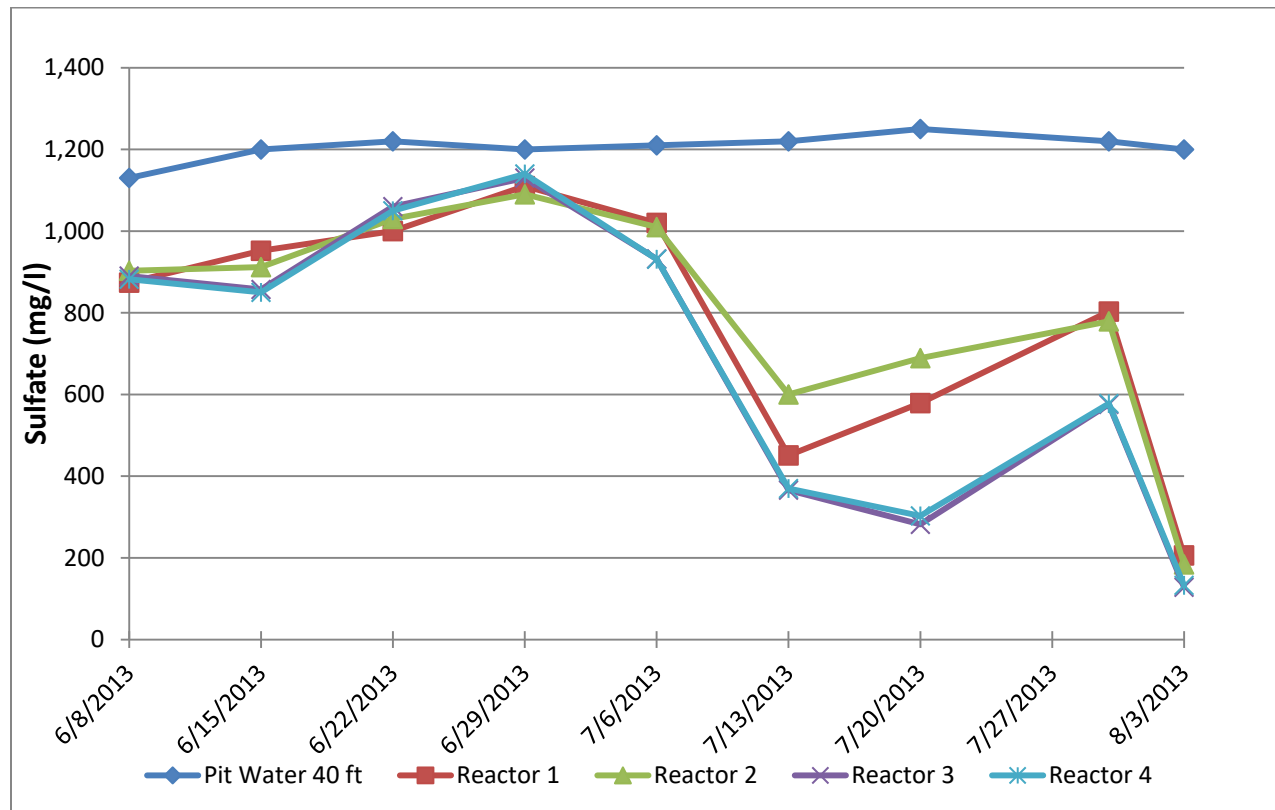


Figure 18. Sulfate reduction trend observed in Phase I pilot testing bioreactors.

Interpretation of Sulfate Results

The data indicates that the bioreactors are functioning properly and reducing aqueous sulfate. The data also indicates a degree of reproducibility in sulfate reduction. This preliminary data shows that the system is capable of significant sulfate reduction.

Aqueous Sulfide Concentrations in the Bioreactors

Plots of aqueous sulfide increases occurring with time in the bioreactor modules are shown in Figure 19.

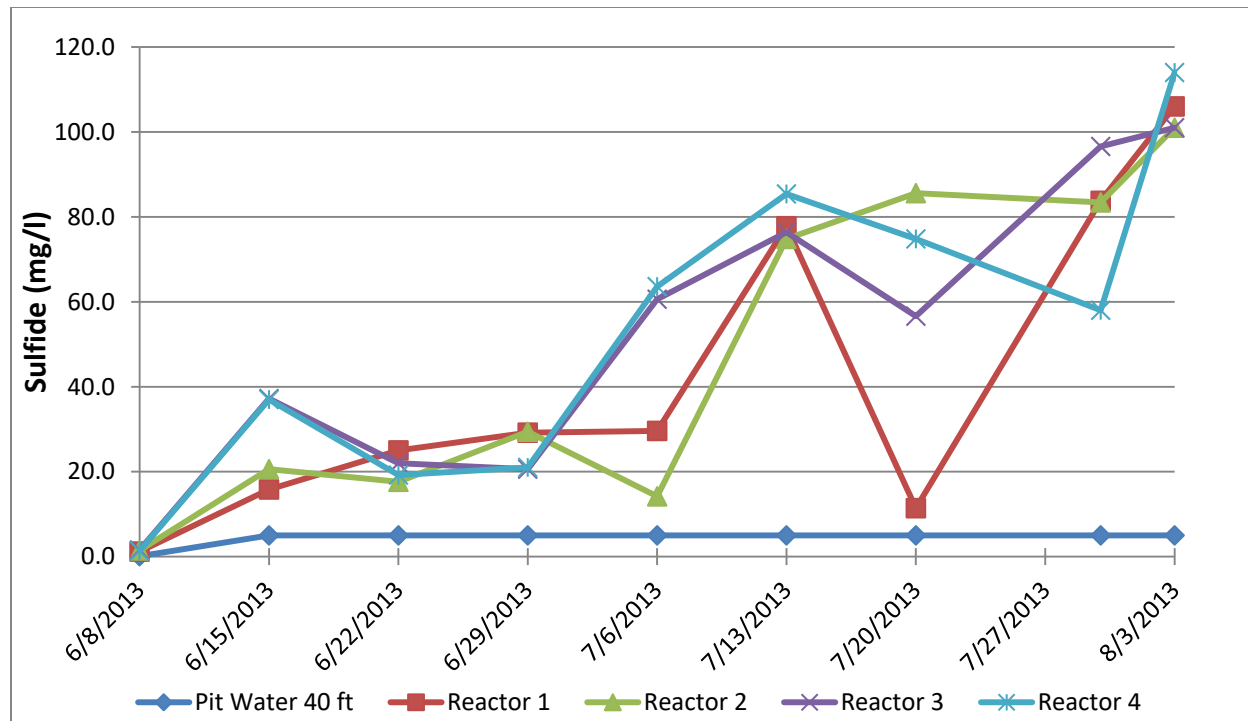


Figure 19. Sulfide increase trend observed in Phase I pilot testing bioreactors.

Interpretation of Sulfide Results

The water samples obtained from the SRB bioreactors show increases in sulfide concentration. This indicates that with time, chemical reactions within the SRB are converting aqueous sulfate to hydrogen sulfide. But it can also be noted that the amount of sulfide produced, although significant and increasing, is insufficient to account for the total sulfur in the sulfate reduction noted above. This same relationship was noted in the Inoculum Reactor Tank analysis. The sulfide measured for these tests is only aqueous sulfide. No attempt was made to quantify gaseous sulfide caused by the gassing off of aqueous sulfide. Both the inoculum and the field bioreactor results point towards the need for a better analysis of the sulfur speciation in the effluent. An objective in Phase II of this project will be to conduct a mass balance and describe speciation of the sulfur.

A variation in sulfide between bioreactors is noted and will be further evaluated in Phase II of the project.

pH Changes Measured in the Pit Water and Bioreactors

Plots of pH changes occurring with time in the pit water and bioreactor modules are shown in Figure 20.

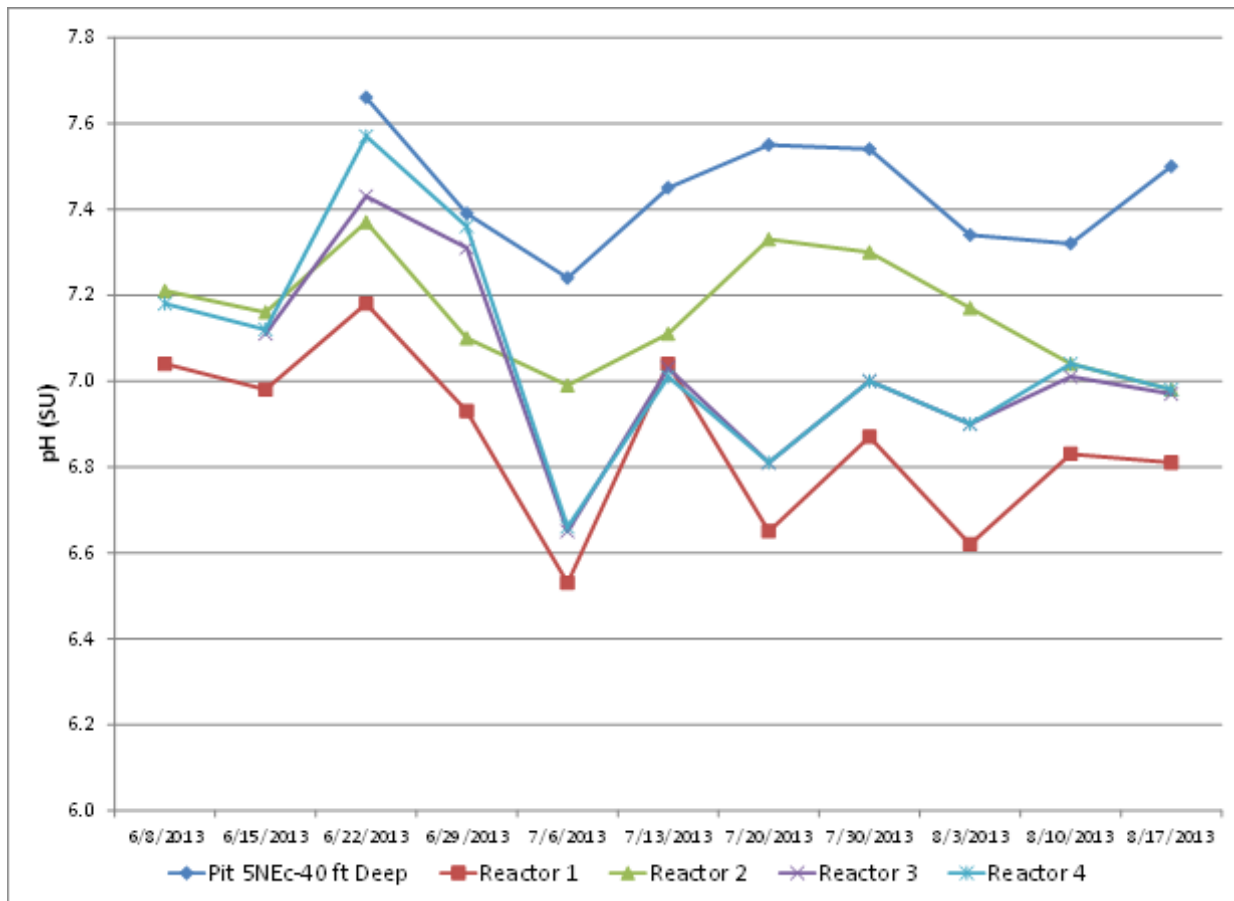


Figure 20. Plot of pH changes at various sampling dates in pilot testing bioreactors.

Interpretation of pH Results

The pH values varied by approximately ± 0.2 pH units in the influent feed water to the bioreactors, varying from pH 7.2 to pH 7.6, and averaging approximately pH 7.4. The pH in the bioreactor modules was reduced to an average of approximately pH 7.0. This slight drop in pH below the feed water pH average of pH 7.4 appears to have resulted by the SRB production of hydrogen ions during the sulfate reduction process.

A variation in pH between bioreactors is noted and will be further evaluated in Phase II of the project.

Bicarbonate Alkalinity Changes Measured in the Bioreactors

Plots of bicarbonate alkalinity changes occurring with time in the pit lake water, the bioreactor module discharge and the precipitation tank are shown in Figure 21 as follows:

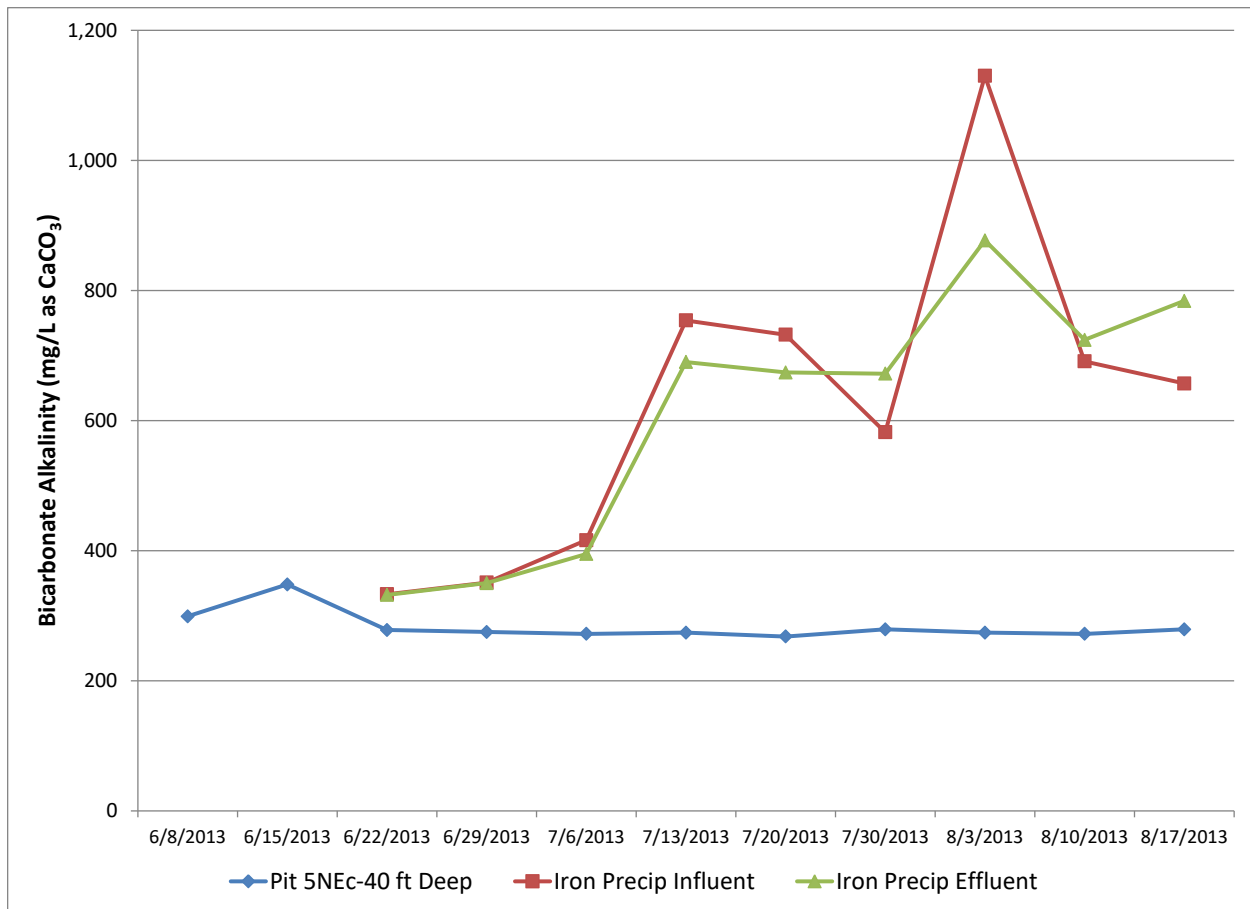


Figure 21. Bicarbonate alkalinity trend observed in pit water vs. precipitation tank influent and precipitation tank effluent.

Interpretation of Bicarbonate Alkalinity Results

Bicarbonate alkalinity is produced by the SRB as they convert organic carbon and sulfate to bicarbonate and hydrogen sulfide as shown in the following reaction:



Formaldehyde + Sulfate = Bicarbonate + Hydrogen Sulfide _(aq)

Bicarbonate alkalinity thus increased as a result of SRB conversion of organic carbon in the form of ethanol (converted microbially to formaldehyde) to form bicarbonate.

Nutrient Total Ammonia, Phosphorus, and Dissolved Organic Carbon Concentrations in the Bioreactors

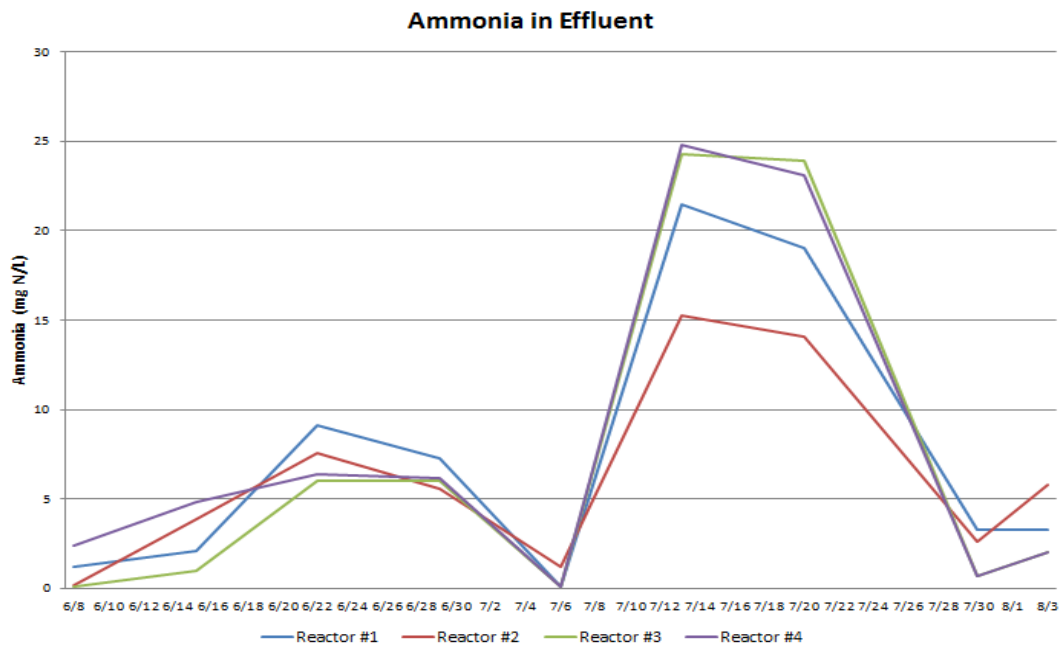


Figure 22. Ammonia concentration in effluent of four bioreactors.

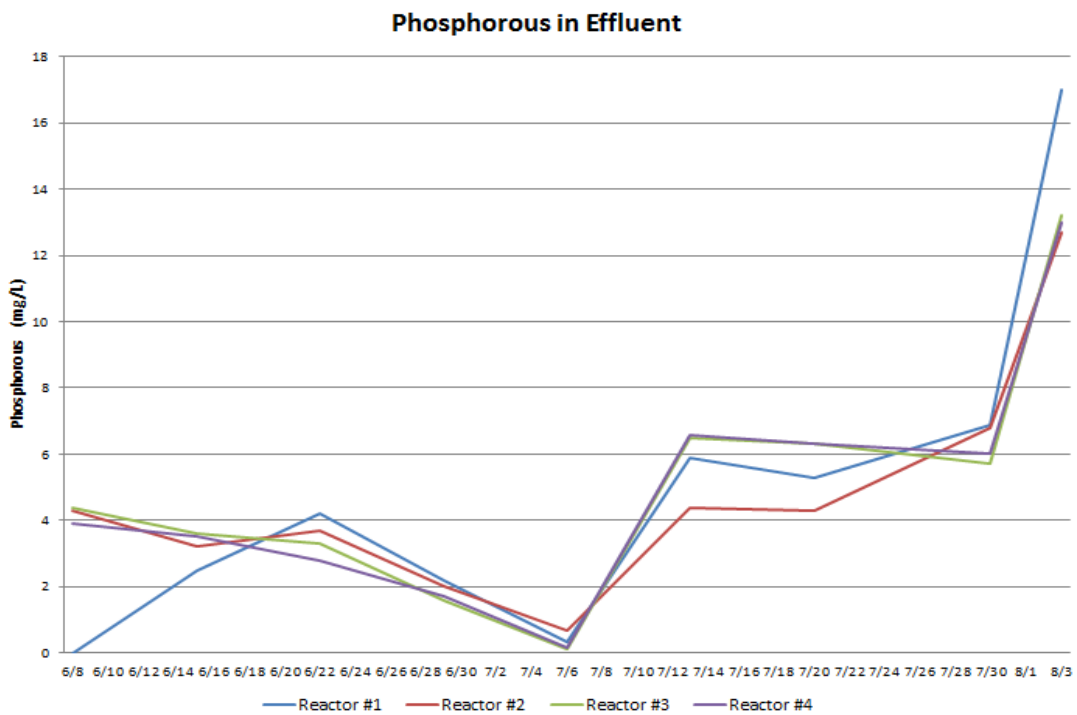


Figure 23. Phosphorous concentration in effluent of four bioreactors.

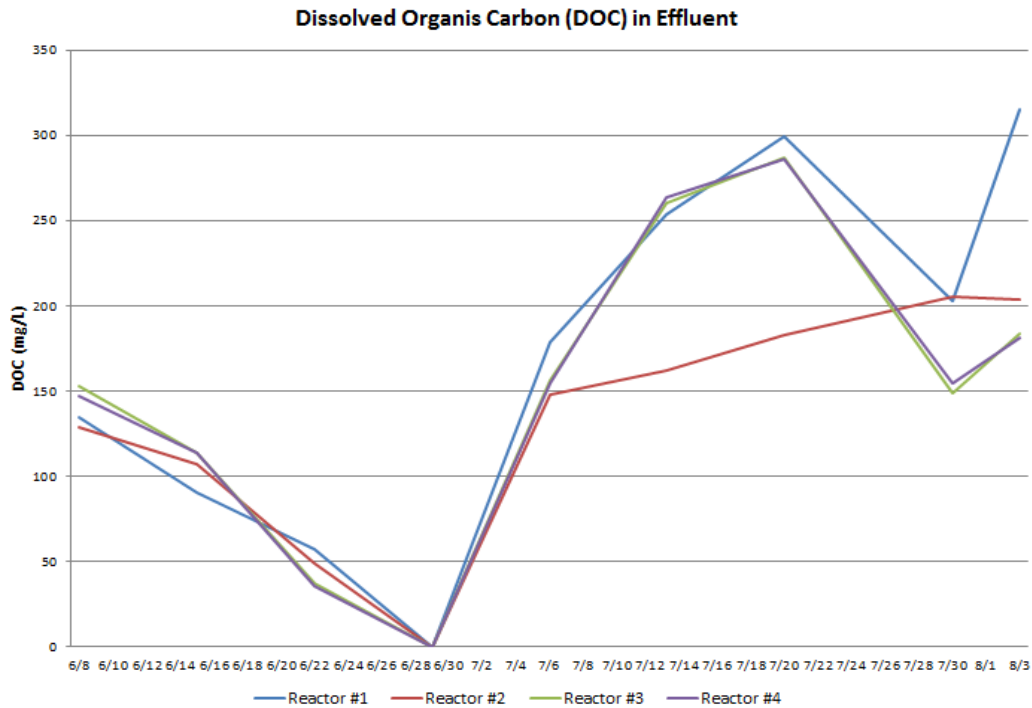


Figure 24. Dissolved organic carbon concentration in effluent of four bioreactors.

Interpretation of Nutrient Results

Total ammonia, phosphorus and dissolved organic carbon were monitored for the purpose of examining how much nitrogen, phosphorus, and carbon was left over from nutrient addition after the SRB consumed these growth limiting nutrients (see Tables 8-14 and Figs. 22-24 above). Results indicate that excess nitrogen, phosphorus, and dissolved organic carbon are present in the effluent. Therefore, it is assumed that these nutrients are not limiting SRB growth as they are present in excess of what is needed by the SRB.

Bioreactor System Water Flow

Summer Operation (2 x 2 raft design: 4 bioreactors)

DC pumps provide flow through the system from water that was being pumped from the 9.1 meter (30 ft.) level in the pit lake at a temperature of 4.4° C-7.2° C (40°-45° F). The Bioreactor system effluent flow data are summarized for 6/24 to 8/1 in Table 17. These data match the graphical data presented in Figure 25. Other data was lost due to an equipment failure.

Date	Time	Hours run	Gal. Pumped	Flow (gpm)
6/24/2013	11:30	50.0	4277	1.4
6/25/2013	9:45	22.2	1745	1.3
6/26/2013	10:15	24.5	1893	1.3
6/27/2013	9:48	23.6	1777	1.3
	11:00	0.7	27	0.6
6/28/2013	9:40	22.7	2618	1.9
	13:00	5.3	607	1.9
6/29/2013	9:30	18.5	2100	1.9
7/1/2013	9:30	48.0	5446	1.9
7/2/2013	9:30	24.0	2727	1.9
7/3/2013	9:30	24.0	2764	1.9
7/5/2013	9:10	47.6	5288	1.9
7/6/2013	9:40	24.5	2734	1.9
7/8/2013	9:40	48.0	5339	1.9
7/9/2013	9:05	23.5	2341	1.7
7/10/2013	13:40	28.5	423	0.2
7/11/2013	9:30	19.9	2198	1.8
7/12/2013	15:10	29.7	116	0.1
7/13/2013	9:55	18.5	575	0.5
7/16/2013	16:10	77.2	741	0.2
7/17/2013	8:00	16.9	1992	2.0
7/18/2013	10:00	26.0	3765	2.4
	12:35	0.2	15	1.3
7/19/2013	9:30	21.0	2785	2.2
7/20/2013	9:40	24.1	195	0.1
7/22/2013	13:00	51.2	4911	1.6
	14:40	1.7	61	0.6
7/23/2013	13:00	22.3	1505	1.1
7/24/2013	9:50	20.8	1455	1.2
7/25/2013	9:50	24.0	1648	1.1
	11:15	1.4	47	0.6
7/27/2013	10:00	46.8	3962	1.4
7/29/2013	12:15	45.0	3917	1.5
7/30/2013	10:40	22.5	1979	1.5
7/31/2013	9:30	22.8	2106	1.5
8/1/2013	9:30	24.0	2215	1.5

Table 17. Bioreactor system effluent flow data.

Graph of Water Flow Data for Raft Effluent (Total of Four Bioreactors)

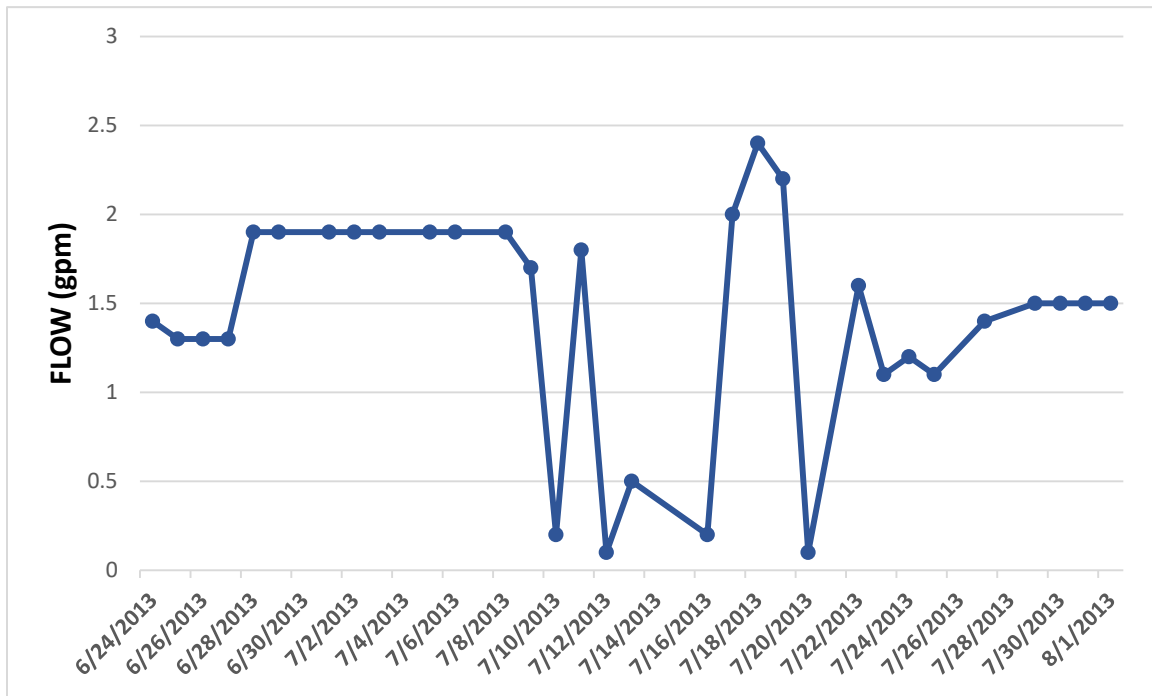


Figure 25. Cumulative system flow from the four bioreactors.

Interpretation of Summer Bioreactor Water Flow Results

The data graphed in Figure 25 was made from flow meter data which was transmitted by the wireless communications equipment positioned on the bioreactor modules. Data are collected continuously and transmitted wirelessly to a cellular gateway. Operational data are recorded and checked via the internet to track on-site conditions. The flow during the period of 7/10/13 to 7/20/13 was reduced to 0.0 gpm (reported as 0.1-0.2 gpm) when the system was shut down to better reconfigure the water distribution to the bioreactors and place the flowmeters in the optimal position to accurately measure system flow. If this period of no flow is taken out of the data, the cumulative system flow from the four bioreactors is generally between 3.8 and 7.6 lpm (1.0 and 2.0 gpm).

Modular System Design and Operation

The summer and winter 2013-2014 operation indicated that the existing design of the floating bioremediation system was uniquely engineered to maintain flow and water temperature as needed to avoid freezing during the harsh sub-zero weather conditions of northern Minnesota. System optimization is being further refined on an ongoing basis as part of the Phase II and MnDRIVE studies.

It is also noted that nutrient feed was not maintained through the winter season of 2013-2014. Nutrient feed was resumed in May, 2014. Resumption of bioreactor water sampling and analytical water analysis also took place at this time.

Precipitation of Sulfur

Various solid mineral forms of iron oxides were used as initial low cost iron containing materials to react with dissolved hydrogen sulfide produced in the bioreactors to form various forms of iron sulfide precipitates. These included: 1) crushed iron ore (taconite) pellets; 2) Minnesota taconite plant tailings; and 3) crushed direct reduced iron pellets. These solid materials were placed in the on-shore precipitation tanks which received bioreactor discharge water containing dissolved hydrogen sulfide. When little reduction in hydrogen sulfide was seen, ferric hydroxide was substituted as an iron source to react with the dissolved hydrogen sulfide to more efficiently react with hydrogen sulfide in an effort to produce an iron sulfide precipitate. Ferric hydroxide precipitate showed reaction with dissolved hydrogen sulfide as evidenced by the immediate formation of a black precipitate which is believed to be a form of iron sulfide. This test further demonstrated the ability to settle out this precipitate within the space of a 909 L (200 gal.) tank.

The black precipitate was analyzed using x-ray diffraction (XRD) and scanning electron microscope-based energy dispersive x-ray (EDS) analysis at the University of Minnesota Duluth and the University of Minnesota Twin Cities campuses to determine the chemical composition. These analyses were inconclusive due to the amorphous, non-crystalline nature of the precipitate. Complete analysis of the precipitate is ongoing as part of the Phase II and MnDRIVE studies.

Cost Effectiveness of the Technology for Sulfate Removal

The initial goal of smart bioremediation technology was to design a low cost sulfate reduction system whose operating cost would be less than \$1.00/1000 gallons of water treated. Because of smaller size of the initial pilot system, an operating cost incurred by the first raft system operating with four bioreactors was very much higher than a cost of \$1.00/1000 gallons.

The capital cost goal of the smart bioremediation technology being described is estimated at being less than the bioreactor system capital cost shown in Table 3 as \$0.24M/m³/day (\$240,000/264 gal./day) for bioremediation technology.

The primary objective of the Phase I stage of this project was to demonstrate effective sulfate reduction in a floating bioreactor configuration. With these results we believe that it is viable to

design a system that is compatible with the cost goals described above. Phase II operation is intended to more fully define the capital and operational costs of this system.

DISCUSSION

Sulfate Reduction Results

Figure 18 displays the trend in sulfate concentrations for each of the four reactors compared to the feed concentration (see Table 8) and demonstrates sulfate reduction in the bioreactors. As of early August, characteristics of the liquid sampled from each of the four bioreactors were indicative of chemically reductive conditions conducive to biological sulfate reduction. Over the course of eight weeks, measured aqueous concentrations of sulfate in the bioreactors are consistently less than measured aqueous sulfate concentrations in the untreated pit water. Since the July 13, 2013 sampling date, aqueous sulfate concentrations in the bioreactors have ranged from 33% to 84% of the aqueous sulfate concentrations of the untreated pit water.

Sulfate concentrations decreased by as much as 6.8 fold for all four bioreactors, reducing from about 1,260 ppm sulfate in feed waters to about 180 ppm sulfate in bioreactor discharge water over the two-month period from June 8, 2013 to August 3, 2013. These reductions appear to have resulted from consistent water feed rates and SRB feed nutrient mix. Work continues to optimize the activity of the SRB in each bioreactor.

A variation in sulfate between bioreactors is observed and will be further evaluated in Phase II of the project.

Sulfide Concentrations Remaining in System Effluent

The water samples obtained from the SRB bioreactors show increases in sulfide concentration. This is interpreted to indicate that with time, chemical reactions driven by SRB metabolic processes are converting aqueous sulfate to aqueous hydrogen sulfide that is partially off-gassing as gaseous hydrogen sulfide. This is supported by observations of a “rotten gas” (hydrogen sulfide) smell in the vicinity of the bioreactors. During the future Phase II work a mass balance of sulfur speciation within the system will be developed to estimate the gaseous portion of the hydrogen sulfide that is off-gassing. It should be noted here that a prime objective of the system as designed is to maintain as much hydrogen sulfide in its aqueous state to allow for removal of sulfur from the system. A mass balance calculation for sulfur will provide an estimate of the true sulfur removal through the system. A sulfur mass balance will be conducted in Phase II of the project. A variation in sulfide between bioreactors is also noted and will be further evaluated in Phase II of the project.

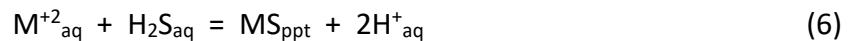
A corresponding increase in measured sulfide concentrations in the bioreactor outlets was also observed. The combined effluent sulfide, shown as “Precipitation Tank Influent” in Table 13, has generally continued to increase relative to the feed water (see Table 15) as testing progressed. The exception was the 7/20 sample, although the precipitation tank effluent (Table 16) remained higher, suggesting probable oxidation of the influent sample. Further review indicates that this temporary dip in sulfide measured in the precipitation tank influent (the same

water as the bioreactor composite effluent) was likely caused by the interruption for system flow between 7/10/13 and 7/20/13. Shortly after flow was restored, the sulfide levels in the precipitation tank influent continued to increase over time.

Performance of the precipitation tanks can also be gauged by comparing the precipitation tank influent and effluent sulfide concentrations. As shown in Tables 15 and 16, the effluent concentrations have at times been lower than the influent conditions, suggesting that sulfide precipitation had been occurring in the precipitation tanks as well as in the bioreactors. This apparently occurred due to the carryover of SRB from the bioreactors into the precipitation tanks. The SRB that were carried over likely attached themselves to the solid state iron materials in the precipitation tank and continued with their sulfate reduction activity. Water samples will be taken to measure SRB levels in the precipitation tank to evaluate SRB carryover. The exact composition of solids retrieved from the SRB bioreactors is currently being evaluated and will be discussed in upcoming reports.

pH Variations in the Pit Lake Water Versus the Bioreactor Effluents

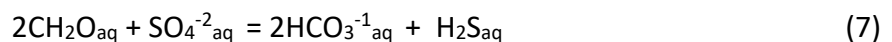
The pH values varied by about +/- 0.2 pH units in the influent feed water to the bioreactors, varying from pH 7.2 to pH 7.6, and averaging about pH 7.4. The pH in the bioreactor modules was reduced to an average of about pH 7.0. This slight drop in pH below the feed water pH average of pH 7.4 appears to have resulted from precipitation of metal sulfide in the bioreactor, with production of hydrogen ions as shown in the following equation:



A variation in pH between bioreactors is noted and will be further evaluated in Phase II of the project.

Bicarbonate Alkalinity Variations

Bicarbonate alkalinity is produced by the SRB as they convert organic carbon and sulfate to bicarbonate and hydrogen sulfide as shown in the following reaction:



Carbonate alkalinity thus increased as a result of SRB conversion of organic carbon to form bicarbonate.

Regarding the influence of the SRB bioreactor system on the other parameters of concern, only bicarbonate alkalinity showed an increase relative to the feed water (Fig. 21). Alkalinity is produced from the biological sulfate reduction reaction as SRB consume ethanol, which explains the upward trend in bicarbonate alkalinity concentration. The variation in bicarbonate alkalinity between bioreactors will be further evaluated in Phase II of the project.

Nutrient Results as Measured by Total Ammonia, Phosphorus, and Dissolved Organic Carbon

Total ammonia, phosphorus and dissolved organic carbon were monitored for the purpose of examining how much nitrogen, phosphorus, and carbon was left over from nutrient addition after the SRB consumed these growth limiting nutrients. Some variation is seen due to changes in nutrient addition rate and growth of SRB as seen in Tables 8-14. Most importantly, sufficient nutrients were added in the form of urea, mono-ammonium phosphate, and ethanol to provide an excess of N, P, and DOC so these nutrients did not become growth limiting (see Tables 8-14).

Results of the SRB pilot test data collected to this point show that biological sulfate reduction is occurring in the NRRI Smart Bioremediation System and that sulfate concentrations in the legacy iron mine pit lake discharge water can be significantly reduced with this technology.

Bioreactor System Water Flow

The summer bioreactor system flow data (Table 15) is summarized as follows:

1. Maximum target flow per bioreactor was 1.9 lpm (0.5 gpm) ;
2. Maximum recorded flow in 24 hours was 9.1 lpm (2.41 gpm) (four bioreactors); and
3. Average bioreactor flow was 1.3 lpm (0.34 gpm) including recycling and downtime.

Flow was then suspended for the Phase I (2 x 2) module raft in September, 2013 and construction of two new (3 x 3) module rafts was initiated for Phase II of the project.

CONCLUSIONS

Proof of Concept

The following general conclusions are made from Phase I of the project realizing that the purpose of the project was to provide a proof of concept review for use of this technology to achieve sulfate reduction in mining related water systems:

1. Preliminary results indicate that sulfate reduction can be achieved with the bioremediation technology described. Further research is needed to consistently achieve high sulfate reduction;
2. The sulfate reduction bioreactor systems are capable of being monitored remotely using on-board sensors, control valves and pumps, and cellular communications;
3. The solar panels mounted on the bioreactors provide relatively continuous power to DC equipment mounted on the bioreactor systems. Additional remote start generators will be evaluated to supplement the renewable energy systems;
4. Recycled poly-type materials of construction work well to provide durable, non-biodegradable bioreactor modules and substrates; and

5. The engineering system design proved functional for pumping mine pit water from a 10 meter depth and having it flow through biological sulfate reduction bioreactor modules as well as precipitation tanks during summer operations. Optimization of the design continues as operational learning continues.

Analysis of the ability for the SRB reactors to function during the winter season is currently ongoing with Phase II of this overall project and will be presented in a subsequent report.

Future Design Optimization/Next Steps

Phase II for the project includes the following design optimization work summarized in the following next steps:

1. Construct, install, operate, and optimize 2-3 more raft systems containing nine modules each with seven bioreactors, one iron dosing module and one nutrient feed module;
2. Add microbiologists to the team with expertise in sulfur reducing bacteria to optimize the type of SRB in the bioreactors and their sulfur reducing biological activity;
3. Develop a sulfur balance for the system identifying all forms of sulfur present in the system;
4. Quantify the different forms of mercury in the discharges from the bioreactors and the precipitation tanks;
5. Optimize the carbon source for the SRB;
6. Seek to further reduce the capital and operating costs of the technology with a focus on achieving an operating cost of \$1.00/1,000 gallons treated;
7. Create a third raft system containing an optimized population of SRB and compare its performance in reducing sulfate to the two Phase II bioreactor rafts each having nine modules;
8. Further optimize the reduction of H₂S discharged from the bioreactor modules through conversion to precipitated forms of iron sulfide and/or oxidation of hydrogen sulfide to form particulate elemental sulfur, both of which can be removed from the pit lake water system. Work to achieve near zero H₂S discharge levels from the system;
9. Optimize the DC power generation of the smart bioremediation system by optimizing the existing solar power system and/or adding additional low cost renewable energy systems;
10. Conduct chemical analysis work on bioreactor and precipitation tank discharge water at the field test site to quantitate sulfate, sulfide, and thiosulfate concentrations in 'fresh' water samples taken within 2-3 hours of the analysis work to assure that the chemical forms sulfur do not convert to other chemical forms; and
11. If new intellectual property is developed, submit a University of Minnesota patent application for the technology.
12. Develop a commercialization pathway to prepare a commercially ready system for the mining industry in northeastern Minnesota;

FINAL REPORT

A final report detailing the continuation of Phase II of this project is expected to be published late summer 2015. Further optimization efforts of the Minnesota Laurentian Vision grant team and the University of Minnesota MnDRIVE team working on the 'Smart Bioremediation Technology to Achieve high sulfate reduction in mining waters of NE Minnesota' project is expected to be published in 2016.

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