



In-situ Remediation of Sulfate Contamination Using Low Molecular Weight Organic Compounds

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Abstract

Elevated levels of sulfates in soil and groundwater can pose an environmental challenge. Many locations in the world have elevated sulfates concentration as a result of human activity or natural processes, such as presence of certain naturally occurring minerals and soil types. While at high concentrations sulfates are not toxic per se, they can cause unappealing aesthetic effects in drinking water as well as to induce laxative effects in humans and animals. In this work we demonstrate an environmentally friendly technology for remediation of sulfate contamination from soil and groundwater using food-grade organic materials and naturally occurring sulfate reducing bacteria (SRB). Our results demonstrate essential elimination of sulfates from contaminated samples using low molecular weight organic compounds. In one set of experiments, a combination of sodium lactate and sodium acetate caused reduction of >90% in sulfates concentrations in two month, with final recorded concentration being below naturally occurring sulfate levels. In another experiment, we used a combination of ethanol and butanol to treat contaminated soil and groundwater, resulting in sulfate levels reductions >98% within two months. In addition, a highly unexpected observation has been made. While widely accepted view of sulfate remediation suggests that it should decrease the concentration of soluble metal, our results demonstrated a surprising opposite effect where sulfate remediation has lead to increases in soluble metals concentrations. We further demonstrate a successful use of oxidizers to reduce concentrations of these metals to their original background levels.

Keywords: Sulfate Remediation; Sulfate Reducing Bacteria; Groundwater; Metals Solubility; Biotechnology of Bioremediation

Introduction

Sulfate contamination of soil and groundwater is an abundantly occurring problem which can pose certain environmental and public health challenges [1]. While sulfates are designated as a secondary contaminant with a maximum suggested concentration limit of 250 mg/L in groundwater, at levels reaching 600 mg/L and above sulfates can cause laxative and other undesirable health effects in humans and animals. In addition, high sulfate concentrations can cause detrimental aesthetic effects with

drinking water taste and odor [2]. Many locations in the world have elevated sulfates concentration in soil and groundwater as a result of human activity or natural processes, and hence it is desirable to reduce sulfates concentrations in such locations below the recommended 250 mg/L [1,2].

Sulphate-reducing bacteria (SRB) are typically anaerobic microorganisms capable to utilize sulphates as terminal electron acceptor. SRBs are ubiquitous in a variety of ecological niches and are members of different genera such as *Desulfovibrio*,

Desulfobacterium, *Desulfococcus* and others [3,4]. During anaerobic respiration of SRBs, electrons are passed from the substrate to sulfate acceptor, leading to reduction of sulfate to hydrogen sulfide. In a sense, if compared to aerobic life forms, SRBs “breathe” sulfate similarly to how multicellular organisms breathe molecular oxygen. Notably, while most SRBs are anaerobic, there are some species that are relatively tolerant to oxygen and even some that can utilize it for metabolic purposes [5-7].

SRBs can be divided into organotrophs and lithotrophs, where organotrophs use organic compounds as substrates, while lithotrophs use molecular hydrogen as a substrate to oxidize [5,8]. As to electron acceptors, SRBs are not limited to sulfates and in certain instances are capable to reduce other types of sulfur compounds such as sulfite, dithionite, thiosulfate, trithionate and tetrathionate. The typical electron donors or substrates for organotrophic SRBs are low molecular weight organic compounds (LMWOC), which include but not limited to organic acids and their salts (ex. lactate and acetate), alcohols (ex. ethanol and butanol), volatile organic compounds, and other small molecular weight organic molecules [5,9,10]. Those are often fermentation products of other microorganisms within the ecosystem. In our work, we decided to use a combination of more than single compound in an attempt to make the substrate utilizable to as many different species of SRBs as possible. In one set of experiments we used a combination of sodium lactate and sodium acetate (“lactate/acetate combo”), and in another a combination of ethanol and butanol (“ethanol/butanol combo”).

Variety of sulfate remediation technologies, including those employing SRBs, have been established and known for many decades. They were mostly developed in industrial remediation settings, rather than in academia. In many of those, the particular emphasis was made not on sulfate removal, but for its use for supposed precipitation and remediation of heavy metals in groundwater and soil. For instance, ARCADIS technologies owns a number of patented inventions [11-14] where the use of SRBs for precipitation of heavy metals in soil is contemplated. Similar sulfate remediation technologies are described by Riensel [2] in Water Online publication. Remediation of sulfate rich mine runoff was also proposed [15]. Notably, the contemporary view of these and other works [16-18] suggests that sulfate remediation process leads to precipitation of metals due to formation of metal

sulfides and general reduction pH, leading to precipitation of metal hydroxides, as summarized in figure 1. However, our experimental results contradict this widely accepted notion, demonstrating highly unusual and unexpected results of sulfate remediation process, which leads to increases of soluble metals. We further demonstrate a solution for reduction of soluble metals back to pre-remediation levels using oxidizers.

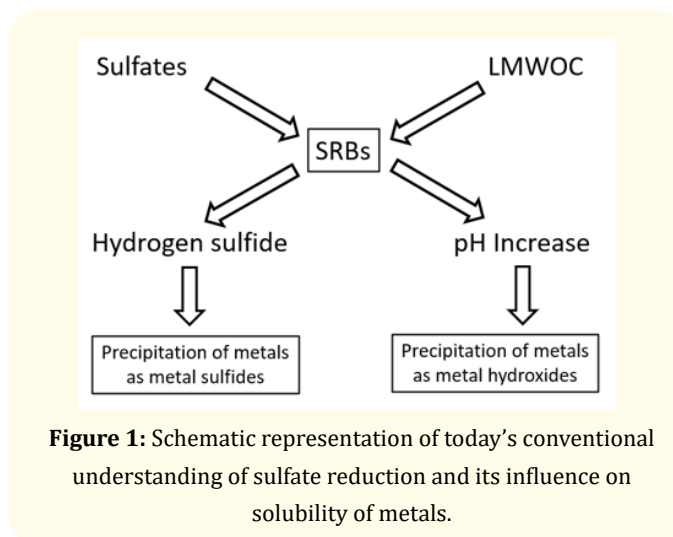


Figure 1: Schematic representation of today’s conventional understanding of sulfate reduction and its influence on solubility of metals.

Materials and Methods

Sample collection

On site samples collection was performed by a licensed drilling contractor under the supervision of a professional geologist. Sample collection was performed using a direct push method and a drilling rig. Soil samples were collected from two different intervals: (1) the base of the low permeability clayey silt layer (approximately 25 to 35 feet below ground surface); and, clay (2) the saturated sand and gravel alluvial layer (approximately 45 to 55 feet below ground surface). Groundwater samples were collected from existing monitoring wells on site.

Sample analysis

All sulfate, metal and other testing has been performed in a third party commercial environmental laboratory (Eurofins Test America) utilizing the standard EPA approved methods. Method numbers and specific parameters tested are shown in table 1.

Constituent	MCL	Method Detection Limit
Method 9056A		
Sulfate	250 mg/L	0.348 mg/L
Method 6020A		
Arsenic	10 µg/L	0.750 µg/L
Barium	2,000 µg/L	2.23 µg/L
Cadmium	5 µg/L	0.197 µg/L
Calcium	-	583 µg/L
Chromium	100 µg/L	0.980 µg/L
Iron	300 µg/L	47.0 µg/L
Lead	15 µg/L	0.450 µg/L
Magnesium	-	199 µg/L
Manganese	50 µg/L	2.06 µg/L
Potassium	-	216 µg/L
Selenium	50 µg/L	0.890 µg/L
Sodium	-	329 µg/L
Method 5310C		
Total Organic Carbon (TOC)	-	141 µg/L

Table 1: Baseline Groundwater Samples Characterization Methods, Maximum Contaminant Levels (MCL) and Detection Limits.

Materials

Technical grade sodium lactate (60% solution) and sodium acetate (anhydrous powder), ethanol and butanol, as well as hydrogen peroxide were purchased from commercial suppliers.

Experimental microcosms

Bench-scale testing has been performed in microcosms comprising on-site collected materials placed in laboratory-grade bottles. The following types of experimental microcosms were set up: 1) soil and groundwater with organic substrate (LMWOC) amendment including lactate/acetate (comprised of sodium lactate and sodium acetate) or ethanol/butanol (comprised of mixture of ethanol and butanol), as described below and 2) control samples with soil and groundwater only (no organic substrate added). Two types of soil were incorporated into the experimental design: 1) sand and gravel from the alluvial aquifer (“sand samples”), and 2) silty clay from the confining unit overlying the sand and gravel aquifer (“clay samples”).

Microcosms were set up using laboratory-grade 250 mL plastic bottles with screw-top caps, which were tightly closed and sealed with Parafilm to prevent oxygen entry. Each 250 mL microcosm consisted of 180 g (approximately 120 mL) of the relevant soil type and the remaining volume in the bottle (approximately 170 mL to account for voids in the soil) was made up with groundwater (amended with the substrates or not, in the case of the control samples). Prior to placing in the bottles, soils were screened to remove particles greater than 10 mm in diameter and homogenized.

Substrate combinations were prepared by mixing sodium lactate with sodium acetate (lactate/acetate combo), and ethanol with butanol (ethanol/butanol combo) into the corresponding microcosms. In case of lactate/acetate, the final substrate concentration refers specifically to lactate and acetate concentrations, and not to sodium salts, due to the fact that other types of lactate and acetate salts might be employed and it is most useful to have a baseline concentration of active compounds. For lactate/acetate combo, the final concentrations in the microcosms were designed to contain 10mM of each component, with approximately 900 mg of lactate and 600 mg of acetate per 1 liter of solution, resulting in approx. 1,500 mg/L concentration of total substrate materials. Ethanol/butanol combo was prepared in a similar manner, wherein the final concentration ethanol and butanol in the microcosms was 5 mM and 2.5 mM, respectively, or approximately 227 mg of ethanol and 185 mg of butanol per 1 liter solution resulting in approx. 410 mg/L of total substrate concentration. Summary of microcosms set up is shown in table 2.

Microcosm Type	Mass of Soil (g)	Volume of Groundwater (mL)	Final Substrate Concentration (mg/L)
Silty Clay / Groundwater Control (No Substrate)	180	170	0 mg/L
Sand / Groundwater Control (No Substrate)	180	170	0 mg/L
Mixture of Sodium Lactate and Sodium Acetate (lactate/acetate), for Each Soil Type	180	170	1,500 mg/L (10 mM of each compound)
Mixture of Ethanol and Butanol (ethanol/butanol), for Each Soil Type	180	170	410 mg/L (5 mM of ethanol and 2.5 mM of butanol)

Table 2: Microcosms Setup.

A number of each type of microcosm was prepared in order to conduct testing with 30 days intervals. Initial sulfate concentration, similar in all microcosms since all were derived from the same drilled cores, was found to be around 620 mg/ml. For the duration of the study, microcosms were gently shaken about once a week to ensure adequate contact between the aqueous and solid phase. Samples were held in a temperature controlled environment, approximating ambient subsurface temperature.

Results

Selection of the test site

An industrial location, with a history of high sulfate concentration in soil and groundwater has been selected for the experiments. As an initial step to confirm existence of high levels of sulfate contamination, baseline groundwater sample analysis was performed using samples collected from monitoring wells. Samples were analyzed for sulfate, total organic carbon (TOC) and a number of metals as shown in table 1. Results confirmed presence of high sulfate concentrations and demonstrated typical sulfate levels ranging between 500-1,000 mg/L, and more frequently between 600-800 mg/L.

Following initial groundwater characterization, a drilling contractor has been dispatched to the location to collect soil samples for further laboratory study. Due to specifics of local geology, the samples contained sections of sand and silty clay, found at different depth. Clay and sand samples were treated separately to determine remediation effects in these two distinct matrices. Further in this work we'll refer to those two types of soil as "sand samples" and "clay samples". All collected materials were immediately sealed on site upon extraction, and the collection containers were filled to the very top to prevent sample oxidation or change in redox potential.

Bench scale studies of sulfate reduction

Experimental microcosms, containing site collected samples with or without LMWOC substrates, have been prepared as described in Material and Methods section and summarized in table 2. Sulfate reduction results in microcosms treated with lactate/acetate and ethanol/butanol mixtures are shown in figure 2 and 3, respectively. As figure 2 demonstrates, after approximately 60 days of sample incubation with lactate/acetate mixture, sulfates were essentially eliminated from the microcosms. A low concentration of sulfates (about 9% of the original amount) still remained in silty clay sample treated with lactate/acetate, however the remaining

concentration of approximately 53 mg/ml was significantly below the maximal concentration limit (MCL) limit of 250 mg/ml, and even lower than natural sulfate concentration typical to this region of the country, which is estimated to be around 60-80 mg/ml.

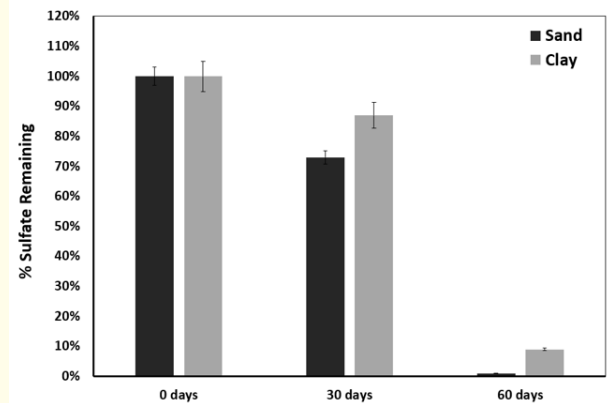


Figure 2: Sulfate reduction over 60 days period using lactate/acetate substrate combination.

While microcosms with ethanol/butanol substrate combination showed slightly superior results at lower concentrations, as compared to lactate/acetate and is shown in figure 3, virtually eliminating sulfate in both sand and clay samples, ethanol and butanol are likely not the best option for practical use in the field due to flammability and general inconvenience of storage and handling. On the other hand, lactate/acetate substrate combination showed comparable results, differing from ethanol/butanol only by a few percentage points in the clay sample. Lactate and acetate, two food-grade compounds, are non-flammable and are easy to store and handle, hence are likely to be the preferred choice for practical large scale applications.

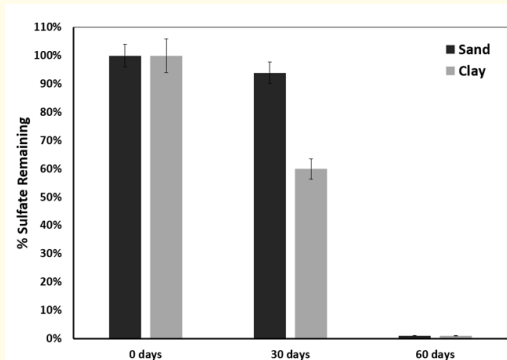


Figure 3: Sulfate reduction over 60 days period using ethanol/butanol substrate combination.

To summarize results of figures 2 and 3, the reduction in sulfate concentrations after the first 30 days was relatively modest as compared to a full plunge after 60 days. This is likely due to the fact that the initial amount of SRBs in the soil is not very high, and it takes some time for the microbial population to start proliferating. A growth lag in bacterial SRB population development, before it enters logarithmic growth phase, likely contributes to the observed timeframe. Once logarithmic SRB population growth commenced, the exponential increase in SRB numbers allows faster and more robust utilization of both substrates and sulfates, leading to a drastic reduction in sulfate concentration in the second month of the experiment.

Treatment of soluble metals

One of the major findings in our work went against the grain of the common wisdom. As noted previously, it is generally anticipated that soluble metal concentrations are to go down as a result of sulfate remediation activities due to formation of metal sulfides and hydroxides. However, highly unexpectedly, our experimental results showed a completely opposite effect, with significant increases in concentrations of various metals in LMWOC treated samples. Increases in soluble metal concentrations included barium, manganese, iron, magnesium, and others. For the sake of brevity, exemplar results for barium and manganese from the samples incubated for 60 days with lactate/acetate combo are shown in figures 4 and 5.

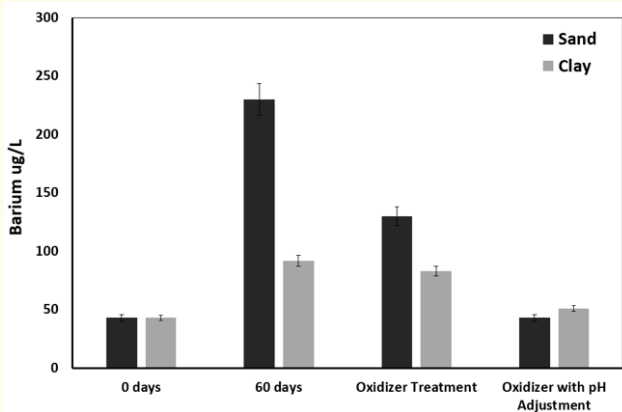


Figure 4: Concentrations of barium, elevated in experimental microcosms after 60 days of LMWOC treatment, reduced to pre-treatment levels with oxidizer and pH adjustment.

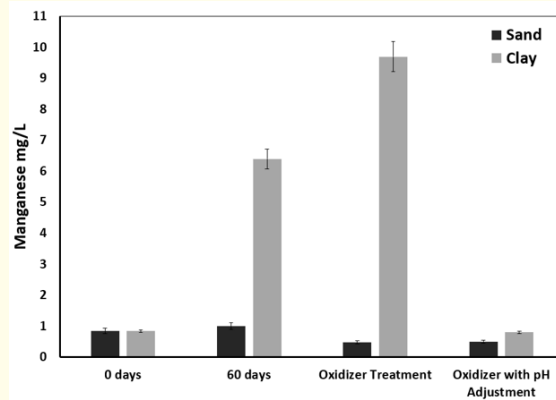


Figure 5: Concentrations of manganese, elevated in experimental microcosms after 60 days of LMWOC treatment, reduced to pre-treatment levels with oxidizer and pH adjustment.

Figure 4 shows changes in barium concentrations over the course of lactate/acetate treatment. As can be noted, after 60 days of incubation a several fold increase in soluble barium concentrations was noted in both sand and clay samples, with concentration rise more pronounced in clay samples. While these elevated concentrations are still significantly lower than the recommended barium MCL of 2 mg/L, it is still of great environmental benefit to reduce barium levels closer to the naturally occurring initial experimental concentrations, which in this case were around 40 ug/L. A number of environmental technologies exist today for precipitation of metals and thus reduction of their concentrations in soil and groundwater. One such method is the use of oxidizers, with the most commonly used agent being hydrogen peroxide (H₂O₂).

Initial oxidation experiments were carried out at pH = 6.8-7.4. Hydrogen peroxide stock solution of 3% was diluted, directly prior to the application, to approx. 0.3% and 0.05% working concentrations which were slowly titrated into the microcosm samples. The titration started with 0.05% solution, steadily increasing oxygenation levels, followed by titration of higher concentration hydrogen peroxide of 0.3%. For clay samples, additional titration with 3% stock solution of hydrogen peroxide has been performed, until sample oxygenation reached and stabilized at 3 mg/L as determined by a dissolved oxygen (DO)

probe, at which point the samples were considered fully oxidized. The oxygenation titration was performed within 30-60 minutes timeframe and samples were analyzed for metal concentrations within a few days after the oxidation experiment.

Hydrogen peroxide titration produced a very significant reduction in soluble barium concentration as shown in Figure 4, column entitled "oxidizer treatment". However, barium levels still remained somewhat higher than the initial background concentrations and further reduction of soluble barium was desirable. To this end, we repeated the oxidation experiment at higher alkalinity of pH=8.0-8.3. Samples were first normalized to the higher pH of 8.0-8.3, using one molar sodium hydroxide solution (1M NaOH), followed by hydrogen peroxide titration process as described above. Sodium hydroxide was also co-titrated with hydrogen peroxide as needed to maintain pH in the 8.0-8.3 range to prevent increase in acidity due to precipitation of metal hydroxides. As Figure 4 shows, in column entitled "oxidizer with pH adjustment", sample oxidation at higher pH indeed caused further precipitation of barium with its concentrations returning to initial background levels.

As an additional example, results for manganese concentrations are shown in figure 5. Notably, in case of manganese, sulfate reduction and application of LMWOC did not significantly affect manganese levels in the sand samples, but did cause a sizeable increase of soluble manganese in clay. The nature of this difference is not known. Furthermore, oxidizer treatment at lower pH=6.8-7.4 did not remedy the problem in clay samples, however pH increase to 8.0-8.3 caused manganese levels to drop back to their original background levels.

Optimization of substrate concentrations

Previous sections demonstrated that LMWOC substrates are capable to significantly reduce or completely eliminate sulfate from soil and groundwater. It would be further beneficial to establish the optimal concentration for LMWOC, at which the maximum desired effect is achieved by application of minimum amount of substrates. This information would be of particular importance for field applications, where large amounts of substrates might be used and have financial impact on remediation.

To determine optimal LMWOC substrate concentration range, we performed a titration of lactate/acetate by exposing

microcosms to three different concentrations over a course of 120 days. Concentration of 750 mg/L, 1,500 mg/L and 3,000 mg/L, or 5 mM, 10 mM and 20 mM of each substrate, respectively, have been tested. Figure 6 shows that lactate/acetate concentration of 1,500 mg/L, or 10 mM of each substrate, appears to provide the optimal balance between amount of substrate, the desired outcome and a reasonable remediation timeframe of two months. After four months of incubation of the microcosms the amount of the remaining sulfate was essentially the same as to what was found in samples after two months incubation, suggesting that after two months most if not all of the organic substrate is depleted and further sulfate reduction is not occurring unless additional substrate to be injected into the samples.

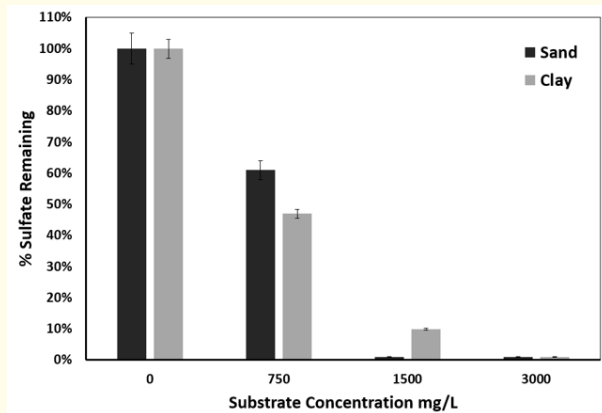


Figure 6: Determining optimal LMWOC substrate concentrations range.

As further shown in figure 6, lower substrate concentrations of 750 mg/L have failed to sufficiently remediate sulfate contamination, with over 50% of sulfate still remaining in place after four months of treatment. It is likely that by this time the organic substrate has been completely depleted and no further sulfate reduction will occur. Even if the process still continues, it is clear that it will take significantly longer time to achieve any additional reduction of sulfate. Alternatively, additional reduction may require follow up substrate injection, which will significantly increase the cost of remediation *in-situ*. For these reasons concentrations lower than 1,500 mg/L appear to be suboptimal.

While higher concentration of 3,000 mg/L shows similar, or somewhat superior results as compared to 1,500 mg/L, with sulfate being completely removed after the four months of incubation

time, the increased cost of substrate materials will likely not justify the use of this concentration instead of 1,500 mg/L. Hence concentrations higher than 1,500 mg/L appear also to be inferior to the selected 1,500 mg/L for financial reasons.

It should be noted that optimal concentration of LMWOC substrates may fluctuate depending on site geology, sulfate concentration, presence of contaminants, availability of other organic materials existing in the soil and other conditions. Also, in some locations optimal remediation time can be longer or shorter as compared to the two months timeframe the location selected for this study.

Discussion

In this work we, for the first time to our knowledge, experimentally showed that *in-situ* sulfate reduction is feasible in a bench-scale study and determined optimal concentrations of substrates for its technological application. In addition, our results demonstrate highly surprising and unusual phenomena where sulfate reduction leads to increases, rather than decreases, of soluble metals in the treated samples (see schematic Figure 7), and provide solutions to resolve these unexpected challenges. The common prior wisdom teaches that during sulfate reduction a beneficial side effect is decrease in soluble metals due to their precipitation in the form of sulfides and hydroxides, however, strikingly, our experiments demonstrate otherwise. We don't yet have explanation for this newly discovered phenomenon. It could involve, for example, actions of other microorganisms in soil or inorganic chemical processes [19], or novel aspects of SRB biology. Or perhaps precipitation occurs with certain types of metals and not others. These questions will be the subject of our future investigations. We also describe a solution for the unexpected challenge of increased metal concentrations by adding an additional step of using oxidizer and pH adjustment, which eventually leads to soluble metal concentrations reduction to the background levels.

In the field conditions and practical *in-situ* applications, the use of oxidizers must be carefully timed with completion of sulfate remediation, and preferably when the sulfate has been reduced to the desired levels. This is due to the fact that, in addition to precipitating metals, oxidizer will likely eliminate or significantly reduce SRB population. If additional sulfate removal is required at a later date, exogenous SRBs will need to be introduced prior to, at the time of or immediately after LMWOC substrate administration in order to replace those annihilated by the oxidizer. Another option would be to wait until the endogenous SRB populations are restored, which can take some time and negatively impact project completion timelines.

Further development and augmentation of this technology can be anticipated. For instance, locations that might have limited or non-existent SRB populations may require co-injection of SRB species together with substrates to ensure commencement of sulfate reduction. Cultured SRBs, which are co-injected or otherwise delivered into soil or groundwater, can also be used to enhance the activity of indigenous species. Another potential avenue for further investigation is co-delivery of microorganisms capable to enhance SRB activity or to improve SRB performance. For instance, organisms capable to ferment long chain hydrocarbons into LMWOC which can be utilized by SRBs. As an example, yeast can be delivered with various types of sugars or other high molecular weight organic compounds, such as molasses, which cannot be utilized by SRBs directly. These auxiliary organisms will facilitate *in-situ* fermentation of the high molecular weight compounds and generation of LMWOC, such as ethanol in case of yeast, which can be used directly as substrates by SRBs. This approach of using high molecular weight compounds with auxiliary organisms capable to turn those into low molecular weight compounds can be beneficial when the project is sensitive for substrate price. Some of the higher molecular weight substrates - such as molasses - can often be purchased at very low cost.

Our next step is expected to be a pilot *in-situ* study on a small to medium scale to expand this technology from the lab into the field. The pilot will involve limited substrate injections at a site historically known to contain elevated sulfate levels, and testing the results via sampling of monitoring wells for a number of months. If successful, this technology could become an affordable and effective solution for resolving sulfate remediation challenges in multiple locations throughout the world.

Conclusion

In this work we have developed a novel method for treatment of environmental sulfate contamination. It is expected to be a

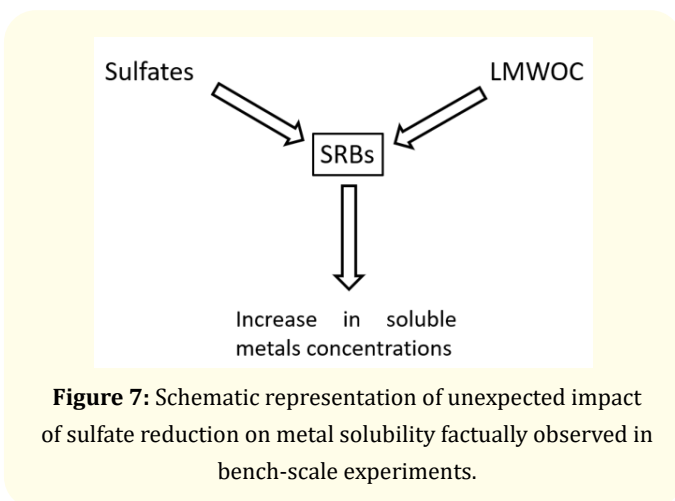


Figure 7: Schematic representation of unexpected impact of sulfate reduction on metal solubility factually observed in bench-scale experiments.

highly desirable technology in the near future due to increase in environmental regulations and tightening of drinking water standards around the world.

In the United States, this technology is likely to be of utmost importance for power generating utility companies. For many decades a large percentage of electricity in the US was generated in coal-powered plants, which as a result accumulated millions of tons of coal ash. One of the hallmarks of coal ash is relatively high amounts of sulfur compounds, which under native conditions and rain convert into sulfates and find their way into the groundwater. Our technology can assist in completely resolving these environmental challenges at low cost and within a very short timeframes. It is expected to become indispensable for managing coal ash impounds in many locations across the United States.

We also hope to see the implementation of sulfate remediation technologies in other countries, where sulfate contamination - either man-made or natural - detrimentally affects drinking water. This may be particularly relevant for communities with highly industrialized economies generating sulfates as byproducts of manufacturing or power generation. Ultimately, we hope that this new technology will be able to enhance the quality of human life in various locations around the globe.

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Conflict of Interest Statement

Phoenix Environmental Research is an inventor and a party to a patent application relating to sulfate remediation technology described in this manuscript.

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