2016 Project Abstract
For the Period Ending June 30, 2019

PROJECT TITLE: Engineered Biofilter for Sulfate and Metal Removal from Mine Waters
PROJECT MANAGER: Sebastian Behrens
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 04p

APPROPRIATION AMOUNT: $ 440,000
AMOUNT SPENT: $ 417,575
AMOUNT REMAINING: $ 22,425

Sound bite of Project Outcomes and Results
In this project we produced and evaluated the heavy metal absorption properties of a new sorbent material made from pyrolyzed waste biomass. The developed material improves copper and nickel removal from water and constitutes an efficient, low-cost alternative to conventional sorbent materials for heavy metal removal from affected waters in Minnesota.

Overall Project Outcome and Results
Biochar is a stabilized, recalcitrant organic carbon material, created from biomass heated to temperatures between 300-1000°C, under low oxygen concentrations. Biochars can be produced from a variety of biomass feedstocks. Recently, biochars have found several applications in environmental remediation of heavy metals contamination. In this project we compared the sorptive properties of different biochars for soluble copper (Cu^{2+}) and nickel (Ni^{2+}) removal from contaminated waters. In order to enhance the sorptive properties of biochars, we pyrolyzed hardwood biomass in the presence of magnesium hydroxide or magnesium chloride. Using the newly produced biochar-composite material we compared its sorption isotherms for copper and nickel with an unmodified biochar. Copper and nickel sorption capacities were greatly improved for the biochars pyrolyzed in the presence of magnesium salts, indicating that biochar mineral supplementation can increase the efficiency of metal adsorption and removal from solution. Scanning electron microscopy (SEM) coupled to energy dispersive X-ray spectroscopy (EDS) revealed strong surface localization of both copper and nickel after sorption onto magnesium hydroxide treated biochar, with a lesser extent of copper surface localization on magnesium chloride than magnesium hydroxide treated biochar. A subsequent study was conducted to test the effects of post-pyrolysis mineral modification (added as soluble Mg) on unmodified biochar's sorption capacity for heavy metals. This part of the project revealed that metal-mineral surface complexation, rather than covalent modification, was the major driver for enhancing copper sorption in treatments amended with either magnesium hydroxide or magnesium chloride. However, similar effects were not observed for nickel sorption. In this project we develop a biochar-mineral composite material that promotes heavy metal adsorption. The new sorbent material made from waste biomass is an efficient, low-cost, environmentally-friendly alternative to conventional sorbent materials that can be used for mine water treatment in water filters or permeable reactive barriers.

Project Results Use and Dissemination
Three peer-reviewed manuscripts are expected to be published from this work; these will be submitted to the LCCMR when accepted for publication. Multiple presentations about the research have been given at both regional and national/international conferences.

Environment and Natural Resources Trust Fund (ENRTF) M.L. 2016 Work Plan Final Report

Date of Report: August 15, 2019

Final Report

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2019

Does this submission include an amendment request? NO

PROJECT TITLE: Engineered Biofilter for Sulfate and Metal Removal from Mine Waters

Project Manager: Sebastian Behrens

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Location: NE Minnesota, Aitkin, Carlton, Cook, Itasca, Lake, St. Louis

Total ENRTF Project Budget:	**ENRTF Appropriation:**	**$440,000.00**
	Amount Spent:	**417,575.00**
	Balance:	**$22,425.00**

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04p

Appropriation Language:
$440,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to develop an efficient, low-cost, biomass-derived adsorbent material for use in bioactive filters able to remove sulfate and metals from mining-impacted waters. This appropriation is subject to Minnesota Statutes, section 116P.10. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Engineered biofilter for sulfate removal from mine waters

II. PROJECT STATEMENT:
Minnesota is currently facing the challenge to balance the economic gain of ongoing and intended mining activities in the Northeast with the potential offset by environmental damage from sulfate and toxic metals in mine discharge waters. Iron mineral mining in the State of Minnesota produces waters with high sulfate concentrations. Currently proposed non-iron, copper-nickel mining activities can even produce waters that contain besides high sulfate concentrations also elevated concentrations of toxic metals. Existing and proposed mining operations in Minnesota are located within the Mississippi River, Lake Superior, and Rainy River watersheds that comprise many pristine wetlands, floodplains, streams and lakes. Elevated heavy metal and sulfide concentrations are toxic to many plants (e.g. wild rice) and animals living in these impacted areas. Biological, passive water filters with an efficient, low-cost, and environmentally-friendly absorbent material (biochar = biomass-derived, carbon-rich solids obtained by heating biomass with little to no oxygen in a process called pyrolysis) are a simple solution to remove sulfate and toxic metals from mining impacted waters.

The purpose of this research is to develop an efficient, bioactive filter to remove sulfate and metals from water. The outcomes of this work will be:

- A mineral-enriched, biomass-derived adsorbent material (composite biochar) optimized to stimulate biological sulfate reduction and adsorption of metal sulfides and heavy metals.
- An effective and low-cost biochar filter to remove sulfate and toxic metals from mine waters.
- Application guidelines for scale-up and field implementation of the new bio-filtration technology.

The treatment of mine waters by passive filtration systems is a low cost, broadly applicable approach for non-point source clean-up of sulfate and heavy metals from water. The availability of effective low cost water treatment methods for sulfate and heavy metal-rich waters will lower the environmental impact of mining in Minnesota. Results from this research will be key for the Minnesota Department of Natural Resources (MNDNR) and the Minnesota Pollution Control Agency (MPCA) to use in support of the decision making process on protecting wild rice from excess sulfate and promote the development of new technologies to remove sulfate and heavy metals from waters impacted by mining operations. Motivation for the project comes from Minnesota's need for an environmentally-friendly, sustainable water treatment technology that can be applied to lower the environmental impact of mining in order to protect Minnesota's water resources and pristine aquatic ecosystems.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January 1, 2017:
The objective of this project is the evaluation of metal modified biochar as an engineered material for environmental remediation of sulfate-rich mine drainage and wastewater. Due to its highly variable and customizable surface chemistry engineered biochar composites offer great potential as adsorbent media in a variety of remediation applications for metal removal from water. In the first phase of the project we successfully produced six different metal-enhanced biochars and two unmodified control biochars from woods chips. We began by characterizing the physico-chemical properties of the different biochars as an important prerequisite for the upcoming experiments in which we will quantify the metal sorption properties of the different biochars (*Activity 1*). We also performed experiments to evaluate the effect of biochar on microbial sulfate reduction. Therefore, we setup cell suspension and batch growth experiments with a pure culture of a sulfate-reducing model organism in the presence of biochar. In these experiments we found that microbial sulfate reduction and cellular growth are not affected by biochar. This was an important first results of this project because it means that the biochar will not negatively impact the performance or survival of sulfate reducing microorganism in the biofilters we plan to construct (*Activity 2*). In order to construct these biofilters we purchased the first pump and set of flow columns. Fed batch bioreactors were started to enrich for

lithoautotrophic sulfate reducing microbial communities for biofilter seeding (*Activity 3*). The project was presented to University researchers, mining companies, and environmental consultants at a symposium on "Frontiers in Mine Water Technology" on September 26, 2016.

Project Status as of July 1, 2017:
We began to measure the first sorption isotherms for copper and nickel on the produced "mineral-enhanced" biochars (*Activity 1*). The first results of these experiment show that the mineral-enhanced biochars have a significantly increased capability to sorb copper and nickel when compared to the unmodified ("plain") biochar. Cell suspension experiments with sulfate and iron-reducing bacteria were performed as part of *Activity 2* to evaluate the effect of biochar to enhance microbial electron shuttling between the bacteria and either a solid (iron oxide) or dissolved (sulfate) electron acceptor. We also obtained a new isolate of a lithoautotrophic sulfate-reducing bacterium *Desulfobacterium corrodens* strain IS4 that is capable of reducing sulfate just with hydrogen as electron donor and does not require an organic carbon substrate because it is capable of fixing CO_2 just like green plants. Further experiments are underway to evaluate the performance of the new isolate in batch arrangements and lab-scale biofilters (*Activity 3*) to study how the different biochars we produced affect microbial iron and sulfate reduction under different conditions (pH, sulfate/metal concentrations). A budget amendment request is submitted with this work plan update to enable the purchase of additional pumps required for the experiments under *Activity 3.*

Amendment Request (07/06/2017):
I am requesting a shift within the "Equipment/Tools/Supplies" budget to enable the purchase of pumps at $15,494 for the proposed construction of lab-scale biofilters in *Activity 3*, while staying within the approved budget category amount. All other item in the budget line "Materials to construct lab-scale biofilters" will be purchased from other funds available to me beyond the ENRTF. Funds from Activity 1 ($6500 from the initial $10,000) and Activity 2 ($3000 from the initial $12,500) in the budget category "Equipment/Tools/Supplies" have been moved to Activity 3 to increase the available budget from $7,500 to now $17,000. Since $1,500 had already been spend in this category for Activity 3 the remaining fund of $15,500 will enable us to purchase the required pumps for the proposed biofilter experiment in this Activity. The revised budget submitted with this work plan project status update contains the above described budget amendments as well as budget updates as of June 06, 2017. **-> Amendment approved by LCCMR 6/12/2017.**

Project Status as of January 1, 2018:
We continued to study the sorption behavior of copper ad nickel onto the mineral-enhanced and unmodified reference biochars (Activity 1). Experiments were conducted as described previously. More details are provided below in the project status update for Activity 1. Chemical equilibrium modeling was performed to better understand interactions between the heavy metals and magnesium salts used to produce the mineral-enhanced biochars. In Activity 2 we continued to study the effect of the produced biochar on microbial sulfate reduction with specific focus on hydrogen/CO_2 based autotrophic growth. Flow through experiments were performed to test different hydraulic residence times and cell retention in biochar-amended flow column reactors (Activity 3). In addition to biofilter experiments with microbial pure cultures we have started biofilter tests in the lab under simulated field condition with natural microbial communities. Results from this project were shared with the attendees of the Minnesota Water Technology Summit (September 28, 2017) and over a 1,000 elementary school students from the Twin Cities who attended the Metro Area Children's Water Festival (September 27, 2017).

Project Status as of July 1, 2018:
Results from Activity 1 were summarized in a manuscript intended for submission to scientific peer-review. For Activity 2 we obtained environmental water and sediments samples from aquatic ecosystems impacted by mining activities in Northeast Minnesota. The samples were used to start cultivation experiments with the aim to select for and enrich sulfate reducing bacteria from Minnesota mine sites that can be subjected to additional biofilter experiments proposed under Activity 3. Following identification of a range of successful operating

parameters for the biofilter experiments (Activity 3) we continued by constructing additional biochar-filled filter system in order to expand experiments to include the newly obtained enrichment cultures obtained from Minnesota mining sites. The project has been highlighted in the online journal 'Water Laws' published by Smith Partners on June 4th, 2018. The published news article features the work of a student of the Water Resources Science graduate program who received the 2017-18 Smith Partners Sustainability Fellowship for her research on water treatment and cleanup associated with this project.

Project Status as of January 1, 2019:
A manuscript summarizing the results from Activity 1 has been written and is currently still under revision by all authors who contributed to the study. A second manuscript has been prepared to summarize some of the interesting finding of the enhanced electrochemical properties of the designed biochar materials. We successfully obtained sulfate-reducing enrichment cultures from mining impacted field sites in Northeast Minnesota and demonstrated their high sulfate reduction activities in the presence of elevated heavy metal concentrations. Biochar-containing flow columns have been inoculated with the sulfate-reducing enrichment cultures and metal sorption studies have been performed under continuous flow condition under sulfate-reducing condition in an anaerobic chamber. Batch sorption test have been repeated to extend the current study to include more heavy metals relevant for mine water remediation.

Overall Project Outcomes and Results (June 30, 2019):
Biochar is a stabilized, recalcitrant organic carbon material, created from biomass heated to temperatures between 300-1000°C, under low oxygen concentrations. Biochars can be produced from a variety of biomass feedstocks. Recently, biochars have found several applications in environmental remediation of heavy metals contamination. In this project we compared the sorptive properties of different biochars for soluble copper (Cu^{2+}) and nickel (Ni^{2+}) removal from contaminated waters. In order to enhance the sorptive properties of biochars, we pyrolyzed hardwood biomass in the presence of magnesium hydroxide or magnesium chloride. Using the newly produced biochar-composite material we compared its sorption isotherms for copper and nickel with an unmodified biochar. Copper and nickel sorption capacities were greatly improved for the biochars pyrolyzed in the presence of magnesium salts, indicating that biochar mineral supplementation can increase the efficiency of metal adsorption and removal from solution. Scanning electron microscopy (SEM) coupled to energy dispersive X-ray spectroscopy (EDS) revealed strong surface localization of both copper and nickel after sorption onto magnesium hydroxide treated biochar, with a lesser extent of copper surface localization on magnesium chloride than magnesium hydroxide treated biochar. A subsequent study was conducted to test the effects of post-pyrolysis mineral modification (added as soluble Mg) on unmodified biochar's sorption capacity for heavy metals. This part of the project revealed that metal-mineral surface complexation, rather than covalent modification, was the major driver for enhancing copper sorption in treatments amended with either magnesium hydroxide or magnesium chloride. However, similar effects were not observed for nickel sorption. In this project we develop a biochar-mineral composite material that promotes heavy metal adsorption. The new sorbent material made from waste biomass is an efficient, low-cost, environmentally-friendly alternative to conventional sorbent materials that can be used for mine water treatment in water filters or permeable reactive barriers.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Production of mineral-biochar composite material for heavy metal and metal sulfide sorption.
Description:
The main objective of this activity is the study of engineered biochars (modified with iron sulfate, magnesium chloride, and magnesium hydroxide) as sorption materials for heavy metal and metal sulfide (copper and nickel) removal from solution. We will produce mineral oxide–enriched biochars by blending waste biomass with metals prior to thermochemical decomposition of the organic material at elevated temperatures in the absence of oxygen. Biochars will be produced from woods chips. Prior to pyrolysis the feedstock will be mixed with iron

sulfate, magnesium chloride, and magnesium hydroxide. Biochars will be produced at 400°C and 700°C in a PYREG pyrolysis reactor.

The modified sorbents will be rinsed three times in deionized water, dried, and sieved to a homogenous particle size (0.125-0.5 mm). The physico-chemical properties of the prepared biochar-based sorbent materials (pH, EC, metal leaching and mass loss at pH 3, 5, and 6.5) will be characterized. The sorption behaviors of the produced mineral-biochar composite materials will be studied in batch arrangements at different initial concentrations of copper and nickel (1-100 mg L^{-1}). Copper and nickel will be quantified by flame atomic absorption spectroscopy (FAAS) and/or inductively coupled plasma mass spectrometry (ICP-MS). The obtained equilibrium sorption data will be analyzed by mathematical equations of adsorption models. Parameters of adsorption isotherms will be calculated by non-linear regression analysis using the programs MATLAB (MathWorks, Inc. MA, USA) and SigmaPlot (Systat Software, Inc., CA USA).

Summary Budget Information for Activity 1:

ENRTF Budget:	**$ 136,610**
Amount Spent:	**$ 125,439**
Balance:	**$ 11,172**

Outcome	**Completion Date**
1. Production of mineral biochar composites	June 30, 2017
2. Optimal production parameters for most efficient contaminant sorption properties	June 30, 2017
3. Sorption properties of engineered biochars at various heavy metal concentrations	June 30, 2017

Activity Status as of January 1, 2017:

We successfully produced and characterized new "metal-enhanced" biochars by blending woods chips with metals prior to thermochemical decomposition of the organic material by pyrolysis. Prior to pyrolysis the wood chips have been mixed with either iron sulfate, magnesium chloride, and magnesium hydroxide. All biochars have been produced at 400°C and 700°C using a PYREG pyrolysis reactor. We also produced unmodified, plain biochar as control for the sorption and flow column experiments. We characterize some basic physico-chemical properties of the produced biochars, such as pH, EC, metal leaching and mass losses at pH 3, 5 and 6.5 (Table 1). The pH values of the biochars produced at 400°C were generally lower in comparison to the biochars produced at 700°C. In case of the control biochar produced at 400°C the pH value was < 6, what could indicate that the biochar contains condensates, tars or oils, which are residuals of the pyrolysis process. We will therefore mainly use the unmodified biochar produced at 700°C as control in the planned experiments. All produced biochars showed metal leaching < 5.5 mg g^{-1}, except for the iron sulfate enriched biochar produced at 400°C for which metal leaching was as high as 14.7 mg g^{-1} at pH 3. The mass loss of all biochars after rinsing with deionized water was always below 0.45 % (Table 1).

Currently we are performing the first copper and nickel sorption experiments with the produced "mineral-enhanced" and control biochars. These experiments are carried out in batch arrangements at different initial concentrations of metals (copper and nickel at 1-100 mg L^{-1}).

Table 1: Physico-chemical properties of the produced biochars before and after washing with deionized water.

Biochars	**pH**	**EC[c]**	**Metal content**			**Mass loss**		
			pH 3	**pH 5**	**pH 6.5**	**pH 3**	**pH 5**	**pH 6.5**
		[mS cm^{-1}]		**[mg g^{-1}]**			**[%]**	
Control 400°C	5.0[a]/5.4[b]	0.12/0.03	n.d.	n.d.	n.d.	0.19/0.17	0.1/0.09	0.09/0.06
Control 700°C	10.4/10.2	0.42/0.11	n.d.	n.d.	n.d.	0.19/0.14	0.08/0.08	0.08/0.07
Fe_2SO_4-enriched 400°C	3.2/3.1	4.09/2.88	14.7/11.6	14.6/11.5	12.5/10.3	0.4/0.32	0.29/0.15	0.28/0.14
Fe_2SO_4-enriched 700°C	4.1/3.9	1.03/0.97	5.5/4.7	4.2/3.9	4.2/2.5	0.2/0.16	0.18/0.12	0.16/0.11
$MgCl_2$-enriched 400°C	9.3/9.9	5.91/4.77	2.4/2.1	2.3/2.1	2.3/2.0	0.5/0.44	0.45/0.42	0.37/0.4
$MgCl_2$-enriched 700°C	9.9/9.9	1.66/1.41	2.4/2.3	2.3/2.2	2.3/2.2	0.46/0.43	0.39/0.32	0.37/0.28
$Mg(OH)_2$-enriched 400°C	10.1/10.1	1.21/0.89	2.2/2.0	2.2/2.2	2.1/2.0	0.57/0.05	0.39/0.04	0.38/0.04
$Mg(OH)_2$-enriched 700°C	10.5/10.3	0.55/0.34	1.9/1.6	1.9/1.5	1.8/1.4	0.56/0.00	0.37/0.00	0.36/0.00

[a]before washing; [b]after washing (24 h, deionized water, 115 min^{-1}); [c]EC electrical conductivity; n.d. not determined

Activity Status as of July 1, 2017:
We performed copper and nickel sorption experiments with the magnesium chloride ($MgCl_2$) and magnesium hydroxide ($Mg(OH)_2$)-modified biochars and the control biochar. The experiments were carried for different copper and nickel concentrations ranging from 1-100 mg L^{-1}. The mineral-enhanced biochar composites have been rinsed three times in deionized water (<0.5 µS cm^{-1}), dried (48 h at 60°C), and sieved to a homogenous particle size (0.125-0.5 mm). Sorption experiments have been carried out in the presence of a weak electrolyte (0.01 M potassium chloride solution) under the following conditions: s/l ratio: 1:2000 (0.5 g L^{-1}), pH 4.0-4.5, contact time: 48h, invert rotation: 45 rpm, temperature: 25°C ± 2°C. The solution pH has been adjusted at set times during the experiment with hydrochloric acid to minimize metal precipitation due to pH rise. After 48 h of shaking, the biochar-metal solution has been filtered through a 0.45-µm pore size membrane filter and amended with hydrochloric acid. Copper and nickel have been quantified by flame atomic absorption spectroscopy (FAAS). The obtained equilibrium sorption data has been analyzed by mathematical equations of adsorption models according to Langmuir and Freundlich.

Activity Status as of January 1, 2018:
An additional copper and nickel sorption study was constructed to determine metal localization onto modified and unmodified biochar surfaces and to provide a semi-quantitative analysis of total metal binding to the surface of each biochar preparation. This study was conducted in the same manner as listed previously, and copper or nickel concentrations were fixed within the previously tested range (40 mg/L). Post-sorption biochar particles were captured on 0.45-um pore size membrane filter, dried overnight at 70 ^{o}C, subjected to overnight vacuum at -30 mm/Hg, and were then submitted to the UMN Characterization Facility for scanning electron microscopy (SEM) coupled to energy-dispersive X-ray spectroscopy (EDS).

Chemical equilibrium modeling (Visual MINTEQ version 3.1) was performed to better understand potential complexation reactions between copper or nickel and added magnesium salts, as well as to determine any potential solid precipitates that may have formed during batch sorption at pH 4. Additional equilibrium modeling was performed to understand sorption characteristics of nickel, copper, and magnesium onto biochar during batch sorption. Solid phase humic acids (HA) were used as surrogate input parameters for biochar in the model.

Activity Status as of July 1, 2018:
Data obtained from experiments performed as part of Activity 1 have been statistically analyzed, tabulated and graphed according to standard for scientific publication. A first draft of a manuscript has been written and is currently been revised by all contributing co-authors on the project. The manuscript summarizes the experiments performed to produce the mineral enhanced biochars, the chemical and physical characterization of the different biochars, and their sorption properties for heavy metals such as copper and nickel under various environmentally relevant conditions. Once revisions on the manuscript are completed and all authors agree on publication of the data the manuscript will be submitted for peer-review to an international scientific journal.

Activity Status as of January 1, 2019:
A manuscript summarizing the results from Activity 1 has been written and is currently still under revision by all authors who contributed to the study. Since a master student and a postdoc who worked on the project have moved on to take up jobs in industry, timely revision of the manuscript has been delayed significantly. However, we are optimistic to complete the work on the joint publication soon. Once revisions on the manuscript are completed and all authors agree on publication of the data the manuscript will be submitted for peer-review to an international scientific journal. The three outcomes for Activity 1 have been met.

Final Report Summary:
The three outcomes for Activity 1 have been met. We successfully produced and characterized new “metal-enhanced” biochars by blending woods chips with magnesium salts prior to thermochemical decomposition of the organic material by pyrolysis. We performed copper and nickel sorption experiments with the magnesium

chloride ($MgCl_2$) and magnesium hydroxide ($Mg(OH)_2$)-modified biochars and unmodified control biochars. The obtained equilibrium sorption data has been analyzed by mathematical equations of adsorption models according to Langmuir and Freundlich. An additional copper and nickel sorption study was conducted to determine metal localization onto modified and unmodified biochar surfaces and to provide a semi-quantitative analysis of total metal binding to the surface of each biochar preparation. The additional experiments revealed that metal-mineral surface complexation, rather than covalent biochar modification, was the major driver for enhancing copper sorption in treatments amended with magnesium hydroxide and magnesium chloride. However, similar effects were not observed for nickel sorption. A manuscript summarizing the results from Activity 1 has been written and is currently under revision by all authors who contributed to the study. A draft version of the manuscript will be provided to LCCMR staff for reference. The new biochar sorbent material with enhanced complexation properties for heavy metals such as copper and nickel might provide a useful alternative to conventional sorbent material for the removal of metal ions from water.

ACTIVITY 2: Determine performance of microbial sulfate reduction in the presence of engineered biochar composites.
Description:
A pure culture of a sulfate-reducing model organism (*Desulfovibrio vulgaris*) and anoxic lake sediments from NE Minnesota containing natural sulfate-reducing microbial communities will be exposed to the engineered biochar composites (produced in *Activity 1*) in the presence of different organic carbon substrates (lactate, ethanol, molasses, organic solids such as hay, sugar beet waste, sawdust, compost) and heavy metal concentrations (iron, copper, nickel) in sulfate containing freshwater medium. Growth and activity of the batch cultures will be monitored. Batch experiments will be performed in triplicates. Sterile setups with the engineered biochars, peat, and microporous, aluminosilicate will serve as negative controls. Sulfate reduction rates will be determined by quantifying sulfate concentrations by ion chromatography (IC) and dissolved as well as precipitated sulfides using a spectrophotometric assay. Metal concentrations in solution will be quantified by flame atomic absorption spectroscopy (FAAS) and inductively coupled plasma mass spectrometry (ICP-MS). The objectives are to study the effect of the engineered biochars on microbial sulfate reduction and to identify the best practice to mix in biodegradable organic materials as food for the bacteria for efficient and prolonged microbial sulfate reduction performance. The most active sulfate reducing microbial populations will be identified by sequencing 16S rRNA gene amplicons and gene fragments of the dissimilatory sulfite reductase (the *dsrA* gene is a process-specific functional marker gene for microbial sulfate reduction). Quantitative PCR and sequencing of the *dsrA* gene fragments and mRNA transcripts will identify which sulfate reducing microbial populations are most active under the experimental conditions. Knowing which sulfate-reducing microbes perform best in the presence of the engineered biochar materials and elevated metal concentrations will be an important prerequisite to build and operate lab-scale bioactive filter for effective sulfate, heavy metal, and metal sulfide removal from mine waters (*Activity 3*).

Summary Budget Information for Activity 2:		
	ENRTF Budget:	**$ 153,438**
	Amount Spent:	**$ 142,185**
	Balance:	**$ 11,254**

Outcome	**Completion Date**
1. Determine efficiency of microbial sulfate reduction in the presence of mineral-biochar composites	June 30, 2018
2. Best practice to mix organic substrates (food for the bacteria) with the biochar adsorbent material to optimize bioactivity	June 30, 2018
3. Identification and quantification of most efficient sulfate reducing microbial populations	June 30, 2018

Activity Status as of January 1, 2017:

We successfully performed cell suspension and batch growth experiments with a pure culture of a sulfate-reducing model organism (*Desulfovibrio alaskensis* G20) in the presence of biochar. We chose *D. alaskensis* strain G20 and not *Desulfovibrio vulgaris* as originally planned because strain G20 was growing better under the conditions we have tested in our laboratory. *D. alaskensis* has been exposed to the unmodified woods chip biochar in sulfate containing freshwater medium. Microbial sulfate reduction activity has been determined by quantifying sulfate using IC and dissolved as well as precipitated sulfides using a spectrophotometric assay. Microbial growth has been quantified by quantitative polymerase chain reaction (qPCR) targeting the *dsrAB* gene (the dissimilatory sulfite reductase is a key functional genetic marker for microbial sulfate reduction). We found that microbial sulfate reduction is not affected by the unmodified biochar. This is an important step towards the successful completion of this project because it means that no toxic substances leach from the char and no essential nutrients are captured by the biochar that would impair microbial growth or sulfate reduction activity. We successfully established a new qPCR assays for the quantification of *dsrAB* genes using new primer sets with optimized coverage of the broad diversity of sulfate-reducing and sulfide-oxidizing functional microbial groups. This will be important further on in the project when we study the effect of biochar on more complex sulfate reducing microbial communities. We are currently performing experiments to evaluate if biochar can facilitate direct or indirect (via hydrogen) electron transfer to and/or from *D. alaskensis* under sulfate reducing and iron-reducing conditions. These experiments are performed in batch culture and in gradient tubes.

Activity Status as of July 1, 2017:

We performed cell suspension experiments with the sulfate reducing bacteria *Desulfovibrio alaskensis* G20 to evaluate if the presence of biochar affects the capabilities of the organism to reduce iron and sulfate with organic substrates as electron donor by enhancing electron shuttling between the microorganism and the solid (iron oxide) or dissolved (sulfate) electron acceptors. Control experiments are being performed with a model microorganism of the well characterized iron-reducing bacteria *Shewanella oneidensis* strain MR-1 for which we previously observed a stimulating effect of biochar on the microbial reduction activity of the iron oxide ferrihydrite. We also obtained a culture of a new isolate of an lithoautotrophic sulfate-reducing bacterium *Desulfobacterium corrodens* strain IS4. The interesting capability of this new strain is that it reduces iron and sulfate with hydrogen as electron donor while it is growing completely autotrophic by using bicarbonate (CO_2) for synthesis of biomass (similar to green plants). This could render the need to add any organic carbon substrates into biofilters with biochar unnecessary. Further, we began experiments to evaluate if the presence of biochar can lower the toxicity effects of elevated concentrations of heavy metals (copper, nickel) on the growth and activity of the sulfate-reducing bacterium *Desulfovibrio alaskensis* G20.

Activity Status as of January 1, 2018:

An additional cell suspension experiment using *Desulfovibrio alaskensis* G20 was performed to determine the effects of biochar on alternative electron donor metabolism for sulfate reduction. This study evaluated the use of an inorganic electron donor, hydrogen, which can be generated at a much lower cost than that of conventional feedstocks for sulfate reduction, such as lactate. Sulfate and headspace hydrogen samples were withdrawn periodically over the course of 48 hours. Headspace hydrogen consumption was measured using gas chromatography coupled to a reduction gas detector. This work was intended to evaluate any potential interference which biochar may have on the autotrophic sulfate reduction capacity of *Desulfovibrio alaskensis* G20.

Activity Status as of July 1, 2018:

Having performed experiments to address outcomes 1 and 2 proposed for Activity 2 we continued our work by setting up enrichments cultures for sulfate reducing bacteria in order to identify and quantify the most efficient sulfate reducing microbial populations (proposed outcome 3) for our biofilter experiments currently been performed as part of Activity 3. We obtained environmental water and sediments samples from aquatic ecosystems impacted by mining activities in Northeast Minnesota that were characterized by elevated concentrations of sulfate and heavy metals. The samples were brought to the laboratory and used to start cultivation experiments with the aim to select for and enrich sulfate reducing bacteria from Minnesota mine sites. We indent to use these natural enriched populations of sulfate reducers for the biofilter experiments proposed under Activity 3. The indigenous sulfate reducer populations should be better adapted to the harsh chemical conditions in mine waters and their performance in the biochar filters will be evaluated in comparison to the pure culture of *Desulfovibrio alaskensis* G20 that we have studied intensively with respect to rates of sulfate reduction in the presence of biochar and different concentrations of copper and nickel.

Activity Status as of January 1, 2019:
We successfully obtained sulfate-reducing enrichment cultures from water and sediments samples collected at aquatic ecosystems impacted by mining activities in Northeast Minnesota that were severely affected by elevated concentrations of sulfate and heavy metals. The obtained enrichment cultures demonstrate high sulfate reduction activities in the presence of elevated heavy metal concentrations. First experiments to use these natural enriched populations of sulfate reducers for the biofilter experiments proposed under Activity 3 are currently in preparation. Outcome 1 of Activity 2 has been met. Outcomes 2 and 3 have been completed using batch enrichment cultures of sulfate reducers. Ongoing experiments that are part of Activity 3 will continue to contribute results to satisfactorily meet outcomes 2 and 3 for mixed sulfate reducing communities under continuous flow conditions in the bioreactors constructed for Activity 3.

Final Report Summary:
We successfully performed cell suspension and batch growth experiments with a pure culture of a sulfate-reducing model organism (*Desulfovibrio alaskensis* G20) in the presence of biochar. We chose *D. alaskensis* strain G20 and not *Desulfovibrio vulgaris* as originally planned because strain G20 was growing better under the conditions we have tested in our laboratory. We successfully established a new qPCR assays for the quantification of *dsrAB* genes using new primer sets with optimized coverage of the broad diversity of sulfate-reducing and sulfide-oxidizing functional microbial groups. A manuscript has been prepared that summarizes to share the new assay conditions with the broader scientific community. A draft version of the manuscript is available to LCCMR staff for reference. We further obtained environmental water and sediments samples from aquatic ecosystems impacted by mining activities in Northeast Minnesota and successfully obtained sulfate-reducing enrichment cultures. The obtained enrichment cultures demonstrated high sulfate reduction activities in the presence of elevated heavy metal concentrations. Outcome 1 of Activity 2 has been met. Outcomes 2 and 3 have been completed using batch enrichment cultures of sulfate reducers. Results from Activity 2 established that microbial sulfate reduction can be sustain in in presence of biochar. Pure cultures and enrichment cultures showed an elevated tolerance to toxic metal concentrations in the presence of biochar. These findings were important prerequisites for Activity 3 which focused on the combined use of biochar as sorbent material in biologically active flow through filtration systems to further enhance metal removal from water by through microbially stimulated metal sulfide precipitation.

ACTIVITY 3: Construction of lab-scale bioactive filters for mine water remediation.
Description:
We will construct bench-scale upflow anaerobic packed bed reactors containing organic carbon substrates and the engineered biochars produced in *Activity 1* to study the removal of sulfate and heavy metals from mine waters under continuous flow conditions. Bioreactor will be constructed from columns (diameter 90 mm, height 400 mm, net empty working volume 2.54 L) and equipped with four sampling ports for liquid and solid material. The reactors will be packed with layers of acid washed silica and the reactive adsorbent mixture (engineered biochar + organic substrates + microbial enrichment cultures). The biochar composites will be compared to peat-based sorption materials and microporous, aluminosilicate minerals. Artificial sulfate-rich lake and mine water (based on the composition of leachate from sulfide-bearing rock formations from the Duluth Complex in NE Minnesota) will be pumped from a reservoir tank into the bottom inlet of each reactor. Duplicate column experiments for each column-bed-reactive-mixture type will be conducted in a non-parallel manner. Control experiments containing no sulfate-reducing microbial communities will be performed under the same conditions as the inoculated bioreactor tests. Sulfide concentrations, the amount of sulfate removed, and the

concentrations of the dissolved metals will be quantified using the analytical methods described above (*Activity 2*). Liquid and solid samples from each biofilter will also be taken for analyses of the microbial community composition using the molecular methods described in *Activity 2*. The objective of this activity is to optimize filter performance by quantifying filter efficiency at various flow rates, pH values, sulfate, and metal concentrations in order to derive critical parameters for biofilter scale-up and field implementation of the developed technology, e.g. as bioactive barriers. Results will be summarized in a user handbook for guidance on how to use the engineered biochars for the treatment of mine waters.

Summary Budget Information for Activity 3:

ENRTF Budget:	**$ 149,952**
Amount Spent:	**$ 149,952**
Balance:	**$ 0**

Outcome	Completion Date
1. Installation of bench-scale upflow sulfate-reducing packed bed biofilters	June 30, 2019
2. Quantification of filter efficiency at various flow rates, pH values, sulfate, and metal concentrations	June 30, 2019
3. Sorption data for effective use of engineered biochar in field scale bioactive barriers	June 30, 2019
4. Field application handbook on engineered biochars for water treatment	June 30, 2019

Activity Status as of January 1, 2017:
We are currently getting ready for our first biofilter experiments. The first peristaltic pump and set of flow columns have been purchased and we will set up the first sulfate-reducing biofilters containing mixtures of organic wastes and the produced engineered biochars soon. In addition, we constructed fed batch bioreactors with graphite electrodes (will be replaced by self-constructed biochar electrodes) to enrich for lithoautotrophic sulfate reducers that are capable to directly take up electrons from the cathode or that can use cathodic hydrogen as electron donor for sulfate reduction. Making use of the conductive properties of biochar and the electron uptake capabilities of lithoautotrophic sulfate reducers might enable us to adjust electron flow into sulfidic biofilters for better process control of sulfate and metal removal from mine waters.

Activity Status as of July 1, 2017:
We tested different pumps, column materials, and biofilter designs. Based on these preliminary test we decided on an experimental setup for the evaluation of the performance of the sulfate-reducing packed bed biofilters in the following experiments under this Activity. Using the optimized experimental setup we performed a first set of lab-scale biofilter experiments with the control biochar under sulfate-reducing conditions with strain *Desulfovibrio alaskensis* G20. In these experiments we successfully demonstrated sulfate removal via biological sulfate reduction in a biochar-filled flow column. Further experiments to optimize sulfate removal efficiencies and test different pH, metal and sulfate concentrations are currently in progress. For these experiments we need additional pumps and materials to construct more lab-scale biofilter. A budget amendment request has been submitted together with this work plan update to enable us to purchase the required pumps.

Activity Status as of January 1, 2018:
We have tested the effects of different hydraulic retention times on sulfate reduction in our *Desulfovibrio alaskensis* immobilized biofilters. As a supplement to this study, we have tracked cell washout using fluorescence microscopy and cell counts. The latter is important especially in determining optimal flow conditions for the bioreactor, as the biofilter activity will be diminished once cells are removed faster than they can replicate in the system. As we are completing biofilter optimization under pure culture conditions, we have also started the construction of additional biofilters using a mixed microbial community derived from environmental samples which are representative of in field conditions.

Activity Status as of July 1, 2018:
We have optimized biofilter design and operating conditions using a pure culture of *Desulfovibrio alaskensis* and successfully identified a range of operating conditions for our flow-through experiments that allow for simultaneous sulfate and heavy metal removal from polluted mine water in a biologically active filter system. Additional biofilters were constructed in order to expand these important experiments to include the enrichment cultures obtained from Minnesota mining sites described under Activity 2. Within the final year of this project we plan to systematically optimize the operating conditions of our biochar-filled filter systems to slowly adapt the enriched sulfate reducer communities to environmentally process parameters that a relevant, transferable, and scalable to actual in-field requirements.

Activity Status as of January 1, 2019:
We continued to run biochar flow columns under aerobic and anaerobic condition in the presence and absence of sulfate-rich artificial mine waters to quantify the sorption capacity of the different biochar filter materials for a diverse range of heavy metals. Flow columns have been inoculated with the sulfate-reducing enrichment cultures obtained under activity 2. Sorption studies under continuous flow condition were performed under sulfate-reducing condition in an anaerobic chamber. In order to complement the flow column studies we began to repeat some of the earlier batch sorption test as well, extending the study to include more heavy metals. Batch and flow column sorption test will continue in the spring and be completed by project end at the end of June.

Final Report Summary:
Following extensive testing and optimization of our experimental setup we performed lab-scale biofilter experiments with biochar under sulfate-reducing conditions using strain *Desulfovibrio alaskensis* G20 and the sulfate reducing enrichment cultures obtained in Activity 2. In these experiments we successfully demonstrated sulfate removal via biological sulfate reduction in a biochar-filled flow columns. We also constructed fed batch bioreactors with graphite and newly developed biochar electrodes to demonstrate that certain types of bacteria are capable to directly take up electrons from a carbon electrode (cathode) and use these eletrons to reduce alternative electron acceptors such nitrate. Making use of the conductive properties of biochar and the electron uptake capabilities of lithotrophic bacteria might enable us to adjust electron flow into pyrogenic carbon containing water filters to reduce sulfate and/or nitrate without the need of continuously supplying an external carbon source as food and electron source for anaerobic respiration of the microbial communities in biologically active water filters for the removal of sulfate (and/or nitrate) and metals from contaminated waters. Outcome 1 and 2 of Activity 3 have been completed. Due to the complexity of the experimental design and initial optimization work we ran out of time to complete outcomes 3 and 4 prior to the end of project funding. However, the first results from Activity 3 have been summarized in a draft manuscript for publication that is currently under revision by all co-authors. A draft version of the manuscript is available to LCCMR staff for reference and is expected to be submitted for publication in an international scientific journal soon following completion of this project.

V. DISSEMINATION:
Description: The target audience for results from this research will be professionals in the area of mine water treatment. Specific targets will be environmental engineers and scientists in academia, industry, state agencies such as the DNR and MPCA, and environmental consultants. Results will be disseminated through scholarly publications in peer-reviewed scientific journals and via a publically available final report. Results from the research project will also be presented at local/regional conferences.

Status as of January 1, 2017:
We presented the project on September 26, 2016, at a symposium on “Frontiers in Mine Water Technology”. The symposium was sponsored by the MnDrive Initiative Advancing Industry, Conserving our Environment and the University of Minnesota BioTechnology Institute. Focus of the meeting was on bioremediation for Minnesota’s mine waters. The project was presented during an open discussion session in form of a poster

entitled “Mineral-enhanced biochar for the removal of sulfate and heavy metals from aqueous solutions”. Symposium participants were University scientists, employees of the DNR and MPCA as well as scientific and management staff of the company Poly Met Mining, Inc. and environmental engineers from consulting companies (CH2M, Barr Engineering, Global Minerals Engineering).

Status as of July 1, 2017:
We presented the project and our first results on April 25, 2017 at a symposium at BARR Engineering in Bloomington. The symposium was organized by the MnDrive Initiative Advancing Industry, Conserving our Environment and the University of Minnesota BioTechnology Institute. The event brought together environmental engineers from BARR and researchers from the University of Minnesota to discuss the current status and progress of ongoing bioremediation research and field demonstration project with emphasis on new innovative water treatment technologies.

Status as of January 1, 2018:
Results from this project were presented at the Minnesota Water Technology Summit on September 28, 2017 held at Target Field. Participants in this event included several representatives from Minnesota-based corporations, environmental consulting firms, and embassy experts from multiple foreign markets. Furthermore, we have prepared a technical manuscript draft on our results generated from **Activity 1** (production of mineral-biochar composite material for heavy metal and metal sulfide sorption) for which we intend to submit for peer review in the near future. In addition to the dissemination of these data, our group has participated in the Metro Area Children’s Water Festival (September 27, 2017) for which we constructed displays and performed scientific demonstrations on the topic of microbial interactions with pyrogenic carbon in water and wastewater treatment processes for over 1,000 elementary students from the Twin Cities.

Status as of July 1, 2018:
The research funded by this project has been highlighted in the online journal Water Laws published by Smith Partners on June 4th, 2018. Smith Partners is a Minneapolis law practice serving local governments, public-private partnerships and other community institutions. The online report features the work of graduate student Gloria Thomas, who is a PhD candidate in the Water Resources Science Program at the University of Minnesota and received the 2017-18 Smith Partners Sustainability Fellowship for her work on the project. The article can be found at URL: https://www.waterlaws.com/news/. Furthermore, we continued to make revisions to a scientific manuscript on our results generated from **Activity 1** (production of mineral-biochar composite material for heavy metal and metal sulfide sorption) that we plan to submit for peer review in the next months.

Status as of January 1, 2019:
A second draft manuscript summarizing the laboratory protocol of a new assay developed as part of the research conducted under **Activity 2** has been prepared for publication. The new molecular assay allows for the detection of quantification of environmental populations of sulfate-reducing bacteria. The assay will be very useful for applied biotechnology and remediation strategies that need to monitor the presence and growth of sulfate-reducing bacteria in engineered and natural ecosystems impacted by elevated sulfate concentrations.

Final Report Summary:
Three peer-reviewed manuscripts are expected to be published from this work. The three manuscripts will summarize the results obtain for each of the three project activities. The manuscripts will be submitted to the LCCMR when accepted for publication. Draft manuscripts currently under revisions by all co-authors are available to LCCMR staff for reference upon completion of the project funding period. Multiple presentations about the research have been given at both regional and national conferences.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 360,772	1 project manager at 15% FTE for each of the 3 years (6 weeks summer salary each year) ($68,518); 1 postdoctoral researcher at 100% FTE for each of the 3 years ($171,938); 1 graduate student at 50% FTE for each of the 3 years ($120,316)
Professional/Technical/Service Contracts:	$ 46,000	University of Minnesota Genomics Center (UMGC) - DNA sequencing, quantitative PCR ($12,000); Research Analytical Laboratory at the University of Minnesota - Inorganic chemical analyses for water, biochar composites, and filter material ($34,000)
Equipment/Tools/Supplies:	$ 30,000	Materials and costs to produce biochars ($10,000); Materials to construct lab-scale biofilters including pumps, tubing, fittings, valves and machining ($15,000); Chemicals, gases, glass ware for anaerobic cultivation and filter operation ($5,000)
Travel Expenses in MN:	$ 3,228	Travel to collect water and sediment samples from aquatic ecosystems in NE Minnesota impacted by high sulfate and heavy metal concentrations
TOTAL ENRTF BUDGET:	**$ 440,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 4.95 FTEs

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0.1 FTEs

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
State			
In-kind: The University of Minnesota does not charge the State of Minnesota its typical overhead rate of 52% of the total modified direct costs (graduate tuition and academic fringe are excluded).	$ 201,020	$ 33,503	General office and laboratory support during the project period
University of Minnesota - MnDrive Start-Up funds to Sebastian Behrens	$ 80,210	$ 13,368	Graduate student salary at 50% FTE for 2 years
TOTAL OTHER FUNDS:	**$ 281,230**	**$ 46,871**	

VII. PROJECT STRATEGY:

A. Project Partners:

The project team consists of the project manager Dr. Sebastian Behrens (Dept. of CEGE, University of Minnesota) and project partner Dr. Kurt Spokas (USDA-ARS; St. Paul, MN). Behrens is an expert on the microbiology of mineral-metal-biochar interactions and Spokas is an expert on the physical sorption/desorption characteristics of engineered biochars. Since Spokas is a federal employee, his participation comes at no cost to the project. The proposed research will be conducted in collaboration with Michael Berndt and Zach Wenz who are geochemists at the Minnesota Department of Natural Resources (MNDNR). The MNDNR will support the proposed project by helping with the selection and facilitating the access to sampling sites at no direct cost to the study.

B. Project Impact and Long-term Strategy:

Results from this research will be key for the MNDNR and the Minnesota Pollution Control Agency (MPCA) to use in support of the decision making process on protecting wild rice from excess sulfate and promote the development of new technologies to remove sulfate and heavy metals from waters impacted by mining operations. The long-term strategy of the project is to lower the environmental impact of mining in Minnesota and to protect Minnesota's water resources and pristine aquatic ecosystems.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
German Science Foundation "Iron cycling in freshwater sediments under oxic and anoxic conditions"	2012-2015	$ 185,000
German Science Foundation "Microbial processes and iron-mineral formation in household sand filters used to remove arsenic from drinking water in Vietnam"	2011-2014	$ 307,859
German Science Foundation "Abundance, activity, and interrelation of phototrophic and chemotrophic microbial iron oxidation in freshwater sediments "	2012-2015	$ 300,775
LGFG Fellowship, State of Baden-Württemberg Germany "Biochar effects on microbial nitrous oxide formation in soils - composition and activity of the nitrous oxide-forming microbial community"	2013-2014	$ 113,620
German Science Foundation Research Unit: "Natural halogenation processes in the environment - Direct and indirect formation of organohalogens by microorganisms"	2011-2014	$ 380,644

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS:

A. Parcel List: N/A

B. Acquisition/Restoration Information: N/A

IX. VISUAL COMPONENT or MAP(S): "See attached figure"

X. RESEARCH ADDENDUM: "See attached Research Addendum"

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 1, 2017; July 1, 2017; January 1, 2018; July 1, 2018, and January 1, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.

Environment and Natural Resources Trust Fund
M.L. 2016 Final Project Budget

Project Title: Engineered Biofilter for Sulfate and Metal Removal from Mine Waters
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04p
Project Manager: Sebastian Behrens
Organization: University of Minnesota - Department of Civil Environmental, and Geo-Engineering
M.L. 2016 ENRTF Appropriation: $440,000
Project Length and Completion Date: 3 Years, June 30, 2019
Date of Report: August 15, 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 06/06/2017	Amount Spent	Activity 1 Balance	Revised Activity 2 Budget 06/06/2017	Amount Spent	Activity 2 Balance	Revised Activity 3 Budget 06/06/2017	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	**Production of mineral biochar composite material for heavy metal and metal sulfide sorption.**			**Determine performance of microbial sulfate reduction in the presence of engineered biochar composites.**			**Construction of lab scale bioactive filters for mine water remediation.**				
Personnel (Wages and Benefits)	$115,010	$115,010	$0	$119,610	$119,610	$0	$126,152	$126,152	$0	$360,772	$0
Sebastian Behrens, Assoc. Professor, 9 month appointment, Dept. CEGE, Project manager, 6 weeks summer salary for each of the 3 years (15%), fringe rate at 33.7% (total $68,518)											
Postdoctoral researcher, full time (100%) for each of the 3 years, fringe rate 22.4% (total $171,938)											
Graduate student, 50% position, full time (100%) for each of the 3 years, fringe rate at 92.89% (includes tuition) (total $120,316)											
Professional/Technical/Service Contracts											
University of Minnesota Genomics Center (UMGC) - DNA sequencing, quantitative PCR -> identification and enumeration of sulfate reducing bacteria; 1680 samples; 210 samples per run = 8 runs x $1500 per run	$3,000	$3,000	$0	$9,000	$9,000	$0				$12,000	$0
Research Analytical Laboratory at the University of Minnesota - Inorganic chemical analyses for water, biochar composites, filter material - Ion Chromatography and Flow Injection Analysis: ammonia, nitrate, nitrite, phosphorus, sulfate and chloride; Total C/Total N Analysis: TOC, TIC, TN, ICP-OES: metals; average $ 16 per sample per analysis; 425 samples x 5 = 2125 x $ 16	$13,600	$3,929	$9,672	$13,600	$3,929	$9,672	$6,800	$6,800	$0	$34,000	$19,343
Equipment/Tools/Supplies (total costs $30,000)	$3,500	$3,500	$0	$9,500	$9,500	$0	$17,000	$17,000	$0	$30,000	$0
Materials and costs to produce biochars (total $3,500)											
Materials to construct lab-scale biofilters including pumps, tubing, fittings, valves and machining (total $17,000)											
Chemicals, gases, glass ware for anaerobic cultivation and filter operation (total $9,500)											
Travel expenses in Minnesota											
Travel to collect water and sediment samples from aquatic ecosystems in NE Minnesota impacted by high sulfate and heavy metal concentrations	$1,500	$0	$1,500	$1,728	$146	$1,582				$3,228	$3,082
COLUMN TOTAL	**$136,610**	**$125,439**	**$11,172**	**$153,438**	**$142,185**	**$11,254**	**$149,952**	**$149,952**	**$0**	**$440,000**	**$22,425**

2016 Graphical Project Abstract
For the Period Ending June 30, 2019

PROJECT TITLE: Engineered Biofilter for Sulfate and Metal Removal from Mine Waters
LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 04p
PROJECT MANAGER: Sebastian Behrens
AFFILIATION: University of Minnesota