Final Report

Enhancing Montana's Energy Resources: Research in Support of the State of Montana Energy Policy Goals

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Objective 1

Develop methods for creating mineral seals for leaky wells at greater depths (> 5000 feet bgs) and higher ambient temperatures (>35 °C) than current Montana State University's Energy Research Institute (ERI) biomineralization technology.

A potential concern for oil and gas injection or extraction wells is the mechanical integrity. Small aperture leaks, such as fractures or delaminations between cement, rock and casing can provide pathways for hydrocarbon or other fluid seepage. Montana State University (MSU), in partnership with Montana Emergent Technologies, Inc. (MET) (Butte, MT), pursued novel methods of biochemical and chemical mineralization to "grow new rock" in small aperture leakage pathways using low viscosity solutions. In situ mineral precipitation has the potential to reduce permeability of "thief zones" in enhanced oil recovery (EOR) which divert injected fluids and reduce production efficiency. The objective of this research was to develop methods for creating mineral seals at greater depths (> 5000 feet bgs) and higher ambient temperatures (>35 °C). These innovative strategies can impact the Montana economy by improving the environmental integrity of unconventional oil and gas development, developing technologies to improve oil extraction, and educating engineers for the future Montana workforce. In addition, the research benefited MET by expanding the biomineralization technology's range for commercialization.

Accomplishments

To accomplish the objective MSU: (1) performed laboratory bench experiments to extend the temperature range for mineral precipitation, and thief zone plugging for EOR; (2) generated preliminary data that was used to form decisions about the field and prepare appropriate proposals to federal funding agencies; and (3) obtained federal funds and partnered with a Montana company (MET) to initiate and plan a mineral precipitation well sealing field test. Thus, MSU has accomplished the objective listed in the original proposal.

More specifically, methods were developed to extend the temperature and pressure range of the microbially induced calcite precipitation (MICP) based sealing technology by studying the use of the urease enzyme from microbial or plant based sources and studying the thermally induced production of mineral. In the enzyme based work it was determined that the temperature range could be extended to 80 °C and still seal fractures. Ureolytic activity was observed up to approximately 80 °C as demonstrated by sealing a 200 µm gap between cement and steel in a wellbore analog reactor (Figure 1). MET performed a test at 80 °C to show that the technology is capable of sealing at temperatures associated with deep oil and gas wells. The test protocol consisted of heating the fixture to either 70 or 80 °C and injecting S. pasteurii followed by a pulse of urea and calcium to initiate the mineralization process. Permeability was reduced three orders of magnitude in both temperature studies.

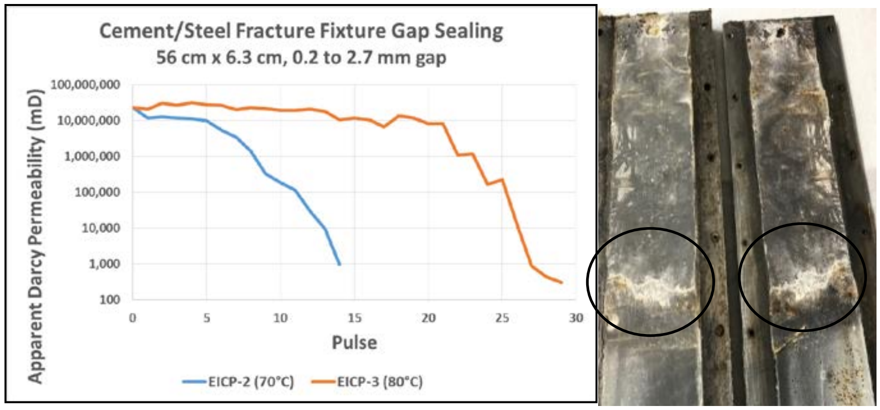

Figure 1. Left. Permeabilty decreased in the fracture fixture experiment as the number of calcium pulses increased. Right, a bridge of calcium carbonate was observed to form at the cement and steel interface where the gap size decreased from 500 µm to 200 µm (circled areas).

In the thermally induced preciptation work, it has been determined that the rate of urea hydrolysis increases with increasing temperature above 110 °C (Figure 2). The data suggested that in the presence of calcium the ureolysis rate from thermal induction is slower than without calcium. This difference has been observed now up to 130 °C. Observing the ureolysis rates at elevated temperature was important for planning field demonstrations to determine how fast reactions might occur in the subsurface.

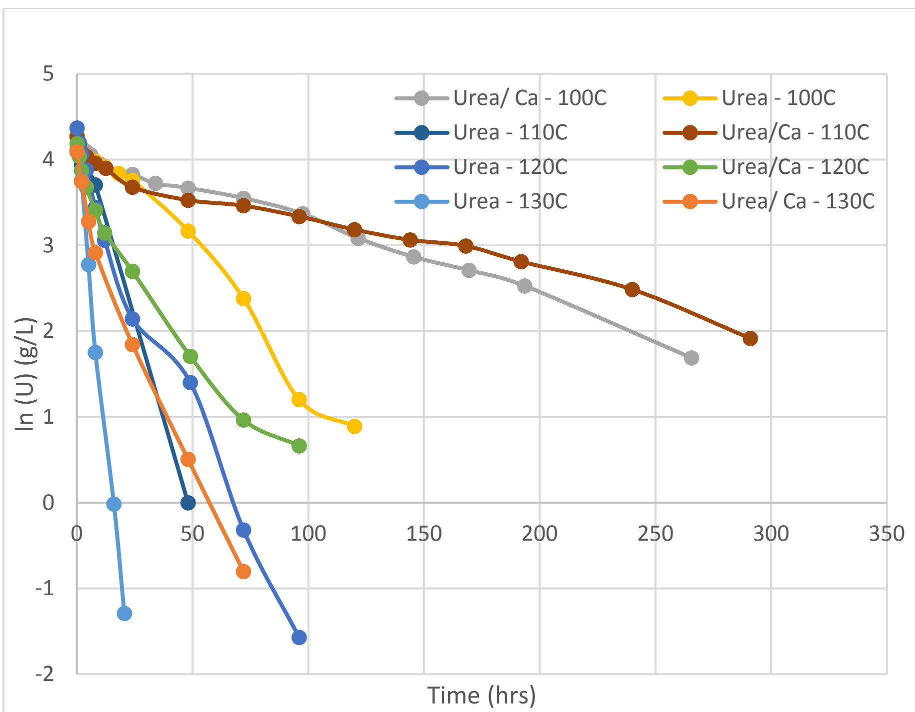

Figure 2. Thermally induced urea hydrolysis rates were observed to be influenced by the presence of calcium. In the presence of calcium, the rate of urea hydrolysis was observed to decrease as compared to the system without calcium.

Carbon steel pipes, 2.5 cm (~1 inch) in diameter, were used to observe the impacts of heating an equimolar (3M) urea/Ca^{2+} solution in sand-filled pipes. Triplicate pipe batch reactors were heated to 130 °C and fluids were exchanged three times over the course of three days. Significant binding of the sand was observed (Figure 3).

Figure 3. Thermally induced calcium carbonate precipitation cemented together particles of sand after removal from the steel pipe batch reactor.

Finally, a well was identified that was used to perform water flooding to increase oil recovery. A channel formed in the cement resulting in the water traveling through the channel into a thief zone above the targeted oil formation. Plans are to mobilize to this well in the fall of 2017 and perform a mineralization sealing test. The success of the experiment will be analyzed by evaluating the pressure required to inject. Success will be determined if the injection pressures are increased to the original pressures required during the water flood and if the well can be returned to injection status to continue oil recovery.

In addition to the successful experimental work and plan for the field, interested stake holders (Shell, Star of Texas, Gallagher Drilling, Warner Oil and Gas, Schlumberger) were identified and shared relevant results (see publications and presentations section below). Proposals were prepared to federal funding agencies to promote additional development of the technology. A significant return on investment was made to the MSU research enterprise and a Montana company by using data gathered in this project. Key proposals that were awarded are: (1) a U.S. Department of Energy (DOE) project through the Office of Fossil Energy ($2,522,425) to demonstrate advanced mineral precipitation strategies in the field and (2) an addendum to a current DOE project to build a mobile laboratory ($142,930) to promote commercialization and the technology readiness level of the mineralization sealing treatments. Finally, the preliminary data was used to prepare a proposal submitted April 2017 which was recently recommended for funding through the DOE Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) ($1,000,000) through a partnership with MSU and MET. The recommended project advances the commercial potential for this technology.

Impact

The research benefits MET, a Montana company (Butte, MT, http://www.mt-emergent.com/), by expanding the biomineralization technology's range for commercialization. MET and MSU are continuing to pursue the development of biomineralization-based technologies on a variety of research and development projects. MET is also a collaborator on a project which supports the design and construction of a mobile mineralization unit and field laboratory. The mobile laboratory will be used for upcoming field demonstration projects, advances the technology readiness level, and thus the commercialization potential of mineral-based sealing technologies.

This research does not only directly benefit the MSU partnership with MET, but also has direct implications for Montana’s oil and gas industry. The research advances the potential to extract oil and gas from Montana’s subsurface, an interest of Montana’s oil and gas service providers and well owners. This will directly affect Montana’s economy by providing jobs and creating tax revenue. Through improvements in environmental stewardship of the oil and gas extraction process, this research also maintains Montana’s natural resources and helps make Montana a safer place to live and work.

Metrics

Total additional grants received

Three additional grants have been received.

A project funded by the DOE started on October 1, 2015. The $2,522,000 project titled "Wellbore Leakage Mitigation using Advanced Mineral Precipitation Strategies" was funded to explore advanced and alternate mineral precipitation strategies in both laboratory and field settings.

An additional budget request was submitted and approved for DOE Project DE-FE0024296 "Methods to enhance wellbore cement integrity with microbially-induced calcite precipitation (MICP)". The additional funds ($142,930) are being used for the design and construction of a mobile mineralization unit and laboratory. Montana Research & Economic Development Initiative (MREDI) funds were leveraged as cost share in the amount of $27,000 for the construction of the unit. This mobile laboratory will be used in upcoming field demonstrations and will advance the technology to a higher readiness level, thus improving the commercialization potential of the mineral sealing method.

Total additional grants in progress

Two grants are in progress.

A proposal submitted April 2017 which advances the commercial potential for this technology was recently recommended for funding through the DOE SBIR and STTR ($1,000,000) through a partnership with MSU and MET.

A proposal (€255,500) has been recommended for funding "Advancing model concepts for engineered calcium carbonate precipitation with focus on multiple driving processes, temperature influence, and two-phase flow". The funding comes from the German Resarch Foundation (Deutsche Forschungsgemeinschaft (DFG)) to advance the modelling of mineralization technologies. The Principle Investigator (PI) is Johannes Hommel (University of Stuttgart). Robin Gerlach and Al Cunningham of MSU are collaborators. The modelling work enhances the commercial potential for mineral sealing in the subsurface by providing predictive capabilities prior to field deployment.

A proposal for a major piece of instrumentation has also been recommended for funding through the National Science Foundation (NSF) Major Research Instrumentation program. The proposal ($150,000) titled "MRI: Acquisition of Optical Coherence Tomography at Montana State University" will allow researchers at MSU to study microbe-mineral interactions that influence the success of engineered applications of mineralization. MREDI funds ($25,000) were used to support the purchase of this instrument.

Number of partnerships formed (private and public sector)

Seven partnerships were formed.

Existing partnerships were strengthened and new industry partnerships formed during this project. Industry and small business partners include: MET, CALX, Shell Canada, Star of Texas, Gallagher Drilling, Warner Oil and Gas, and Schlumberger.

Number of new Montana businesses created

One new business was created.

Montana Emergent Technologies, a small business based in Butte, Montana, recently developed a subsidiary company called CALX dedicated to commercial application of mineralization sealing in the subsurface.

Patents awarded or in progress

One patent is in progress.

A patent application has been submitted titled "Methods for increased hydrocarbon recovery through mineralization sealing of hydraulically fractured rock and refracturing".

Commercial products developed

The technology readiness level of mineralization sealing technologies in the subsurface has been advanced significantly through this project. Significant interest by oil companies has been expressed and MET has received calls demonstrating interest in commercial sealing projects.

Jobs created/supported:

- Private sector: 2 engineer positions will be created at MET with the newly recommended SBIR/STTR project
- Students: 5
- Faculty: 2
- Post-docs: 1

Publications

Phillips AJ, Cunningham, A, Gerlach, R, Hiebert, R, Hwang, C, Lomans, B, Westrich, J, Mantilla, C, Kirksey, J, Esposito, R, and Spangler, L. (2016) Fracture sealing with microbially-induced calcium carbonate precipitation: A field study. Environmental Science and Technology, 50 (7), pp 4111–4117 http://pubs.acs.org/doi/abs/10.1021/acs.est.5b05559.

Beser, D, West C, Daily, R, Cunningham, A, Gerlach, R, Fick, D, Spangler, L and Phillips, AJ. Assessment of ureolysis induced mineral precipitation material properties compared to oil and gas well cements. American Rock Mechanics Association 51st Annual Meeting Proceedings, June 25-28, 2017, San Francisco, CA. (Paper # 588) (Accepted).

Kirkland, CM, Zanetti, S, Grunewald, E, Walsh, DO, Codd, SL, Phillips, AJ. Detecting microbially induced calcite precipitation (MICP) in a model well-bore using downhole low-field NMR, Environmental Science and Technology, Published Dec. 20, 2016 http://pubs.acs.org/doi/abs/10.1021/acs.est.6b04833 DOI: 10.1021/acs.est.6b04833.

Feder, M, Morasko, V, Gerlach, R, Phillips, AJ. Plant-based ureolysis kinetics and urease inactivation at elevated temperatures for use in engineered mineralization applications (in preparation).

Presentations

Phillips, AJ, Gerlach, R, Cunningham, AB, Hommel, J, Helmig, R, Hiebert, R, Kirksey, J, Rowe, W, Esposito, R, and Spangler, L. "Biomineralization: A Strategy to Modify Permeability in the Subsurface". 9th International Conference on Porous Media & Annual Meeting, May 8-12, 2017, Rotterdam, Netherlands.

Phillips, AJ, Gerlach, R, Hiebert, R, Cunningham, AB, Spangler, L. "(Bio)mineralization for Permeability Modification and Wellbore Sealing" Society of Petroleum Engineers Annual Spring Symposium, April 21, 2017, Montana Tech, Butte, Montana.

Frieling Z, Akyel A, Gerlach R, Phillips, AJ. “Urease Transport and Distribution to Better Understand its Subsurface Behavior”. MSU Student Research Celebration, April 21, 2017, Bozeman, Montana.

Gerlach, R, Phillips, AJ, Cunningham, AB, Spangler, L. “Controlling Fluid Flow in the Subsurface through Ureolysis-Controlled Mineral Precipitation”, American Geophysical Union Fall Meeting, December 2016, San Francisco, California.

Gerlach, R., Al B. Cunningham, Lee Spangler, Adrienne Phillips, Randy Hiebert, Jim Kirksey, Wayne Rowe, Richard Esposito, Bart Lomans, Andreas Busch (2016): Biocementation for Wellbore Integrity Restoration and Enhanced Resource Recovery. Platform Presentations at the RMF2016: 22nd Reservoir Microbiology Forum, November, 15/16, 2016, London, United Kingdom.

Phillips, AJ, Gerlach, R, Cunningham, AB, Troyer, E, Norton, D, Hiebert, R, Kirksey, J, Rowe, W, Esposito, R, and Spangler, L. “Biomineralization: A Strategy to Modify Permeability in the Subsurface”. Geologic Society of America Annual Meeting, September 25-28, 2016, Denver, Colorado.

Norton, D, Gerlach, R, Eldring, J, Thane, A, Hiebert, R, Cunningham, A, Spangler, L, Phillips, AJ “Visualizing and Quantifying Biomineralization in a Wellbore Analog Reactor”. Geologic Society of America Annual Meeting, September 25-28, 2016, Denver, Colorado.

Phillips, AJ, Gerlach, R, Cunningham, AB, Spangler, L. “Wellbore Leakage Mitigation Using Advanced Mineral Precipitation Strategies” Department of Energy, Mastering the Subsurface through Technology Innovation & Collaboration: Carbon Storage & Oil & Natural Gas Technologies Review Meeting, August 17, 2016, Pittsburgh, Pennsylvania.

Phillips, AJ, Gerlach, R, Cunningham, AB, Spangler, L. “Methods to Enhance Wellbore Cement Integrity with Microbially-Induced Calcite Precipitation (MICP)” Department of Energy, Mastering the Subsurface through Technology Innovation & Collaboration: Carbon Storage & Oil & Natural Gas Technologies Review Meeting, August 17, 2016, Pittsburgh, Pennsylvania

Phillips, AJ, Gerlach, R, Cunningham, AB, Spangler, L. “Biomineralization Sealing Technology: A Promising Technology Developed in Montana”. Invited Presentation. Montana Energy Conference, March 30, 2016, Billings, Montana.

Feder, M, Phillips, AJ. Gerlach, R. “Advancing ureolysis driven mineral sealing strategies for environmental engineering applications.” Platform Presentation. Goldschmidt Conference. June 26-July 01, 2016, Yokohama, Japan.

Gerlach R, Phillips., AJ; Cunningham, AB, Spangler, L. “Biofilm-Mediated Mineral Precipitation Technology – From the Microscale to the Field-Scale.” Platform Presentation. Goldschmidt Conference. June 26-July 01, 2016, Yokohama, Japan.

Gerlach, R. “Biocementation as an Advanced Well Remediation Technology –Technology Development from the Microscale to the Field-Scale.” Invited Presentation. June 13, 2016, Los Alamos National Laboratory, New Mexicao.

Zambare, N.; Lauchnor, E.; Gerlach, R. (2016): Optimizing microbially induced calcite precipitation under radial flow conditions. Platform Presentation. Division of Environmental Chemistry, Session: Division of Environmental Chemistry: Carbonate & Sulfate Minerals: Nucleation, Growth & Control of Scale Formation. 251st American Chemical Society National Meeting. March 13-17, 2016, San Diego, California.

Hommel, J., Ebigbo, A., Gerlach, R., Cunningham, A.B., Helmig, R., Class, H (2015): Finding a balance between accuracy and effort for modeling biomineralization. Platform Presentation. NUPUS annual meeting 2015. September 8-12, 2015, Freudenstadt, Germany.

Hommel, J, Ebigbo, A, Gerlach, R, Cunningham, AB, Helmig, R, Class, H. Finding a balance between accuracy and effort for modeling biomineralization. Poster Presentation. NUPUS annual meeting 2015. September 8-12, 2015, Freudenstadt, Germany.

Hommel, J., Ebigbo, A., Gerlach, R., Cunningham, A.B., Helmig, R., Class, H (2015): Finding a balance between accuracy and effort for modeling biomineralization. Platform Presentation. IAMG 2015, 17th Annual Conference of the International Association for Mathematical Geosciences. September, 05-13, 2015, Freiberg, Germany.

Additional Dissemination of Information

News Stories

The project funding was announced on the DOE's website: http://www.energy.gov/fe/articles/doe-selects-nine-projects-receive-funding-carbon-storage-intelligent-monitoring-and-well

The field demonstration was highlighted by the:

- MSU News (http://www.montana.edu/news/16313/msu-team-shows-biofilm-and-mineral-producing-bacteria-have-potential-for-plugging-oil-and-gas-leaks)
- Bozeman Daily Chronicle (http://www.bozemandailychronicle.com/news/montana_state_university/msu-research-shows-bacteria-could-plug-oil-and-gas-leaks/article_7d9bef62-08c9-5649-8e96-b24cf77592e9.html) and KBZK.

Objective 2

Test use of microbially induced calcite precipitation (MICP) to remediate fly ash storage to comply with a new federal regulation (40 CFR Parts 257 and 261 Hazardous and Solid Waste Management System; Disposal of Coal Combustion Residuals (CCR) From Electric Utilities).

Recent regulations (40 CFR Parts 257 and 261 Hazardous and Solid Waste Management System; Disposal of Coal Combustion Residuals (CCR) From Electric Utilities) set forth requirements to prevent contaminants from CCR storage areas leaking into ground water and prevent the formation of contaminated dust. These requirements improve environmental conditions surrounding the CCR storage impoundments yet come with considerable costs to the operators of the facilities. One such facility impacted by the new rule is the Colstrip, MT coal-fired electricity generating facility (Colstrip). Other plants in other areas of the country including Alabama and Georgia are also impacted by the rules. Southern Company, one of the largest US energy utilities, has significant interest in novel methods to remediate or retrofit the ponds to cap them, seal them, or improve structural integrity of the banks and meet the goals of the new regulations. Thus, collaborations have been formed during the project with plant engineers at Colstrip and with a research and technology development engineer at Southern Company.

One proposed method to remediate CCR storage pond concerns in situ is the use of MICP. MICP has been shown to reduce permeability and seal fractures in rocks. Recently, it has been shown in the laboratory to cement together fly ash particles improving the structural integrity. The objective of this research was to investigate the use of MICP toward beneficial applications for the CCR storage ponds. The project had two main goals: (1) to help industry meet their regulatory requirements of reducing groundwater contamination from CCRs, and (2) mitigate dust of stored CCRs. The objectives of this project were to (1) collect samples of bottom ash, fly ash, and pond water to perform laboratory studies to assess the feasibility of MICP for CCR pond remediation, and (2) assess and plan a field demonstration of MICP in CCR ponds by working with MET, a Montana company, to implement the MICP technology in the field.

Accomplishments

Samples of ash and water were collected from Colstrip's coal combustion residual storage ponds on two different occasions. First, one-gallon bottle sized samples were used for bench scale laboratory experiments. Secondly, five-gallon bucket sized samples were collected for upscale lab experiments. Samples were also obtained from Southern Company including fly ash, scrubber wastes, and powder activated carbon waste materials.

Experiments were performed in coal combustion residual materials at laboratory scale to determine the parameters needed in the design of a field test. Two of the experiments are highlighted, the first showing the potential to mitigate dust and the second to reduce permeability and infiltration.

A cap was created over CCR material using a spray system pointing toward MICP as a potential method for dry disposal of CCRs to mitigate dust (Figure 4). If CCR materials can be stored dry, the risk of leaching contaminants from the CCR into groundwater is reduced. Producing a cap over the CCR has the potential to (1) reduce the dust formation of materials after evaporation of the water and (2) reduce the potential for infiltration of precipitation (rainwater or snowmelt) into closed ponds.

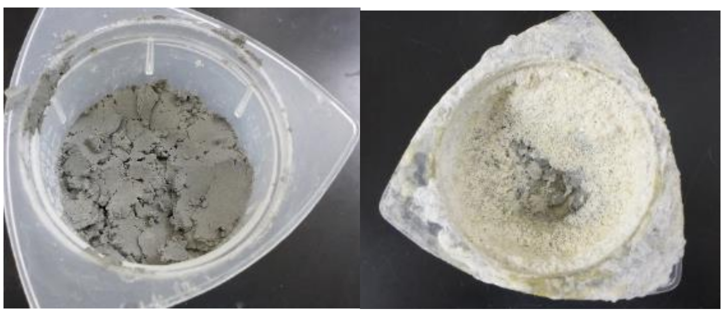

Figure 4. Left. Paste materials in a beaker. Right. Cap of biomineral formed on the top surface of the CCRs (whitish layer).

Column experiments (Figure 5) were preformed to assess permeability reduction in fly ash. Significant permeability reduction was achieved pointing to the potential to use MICP treatment to create an in situ seal at the bottom or top of an unlined pond to mitigate infiltration of pond water to aquifers below.

Figure 5. Experimental column set up. A syringe pump was used to inject MICP promoting solutions into a column loaded with sand and fly ash (column of the right). Permeability of the fly ash/sand mixture was observed to decrease over the course of the 20 injections of MICP promoting solutions.

During the project, MSU collaborated with MET, Colstrip, and Southern Company to plan field demonstrations. One planned pilot scale demonstration is designed to inject MICP promoting solutions at Colstrip into a ~1m^2 area of ponded material. The area to evaluate the reduction of permeability and infiltration of water through the material will then be monitored. A second field demonstration was planned and would take place at a plant of interest to Southern Company where MICP promoting solutions would be sprayed onto the surface of the materials to form a cap and reduce dust formation.

Stakeholders are very interested in the technology and were consulted throughout the project. Stakeholders include MET, Colstrip, and Southern Company. During the project, Southern Company collaborators commented on the feasibility of MICP in ponded CCR materials, stating in regard to the successful permeability reduction: “As our fleet transitions to dry ash handing, our ash ponds will be closed, which will require a final cover system which protects the CCR from direct contact and rain infiltration. The biomineralization could target the pore space of the ponded CCR for the purpose of reducing flow and decreasing leaching rate.” In addition, they commented on the use of MICP as a dust suppression agent, including the following comment: “Once placed in the landfill, the CCR material must be protected from dust formation. Biomineralization could be better suited to this task than chemical spray binders, due to the low viscosity of the biomineralization ingredients. Spray on binders seem to have difficulty penetrating sufficiently to form a durable travel surface.” Colstrip engineers have expressed equal interest and have provided samples, tours, and discussions with plant engineers.

To summarize, met the objectives of this project were met by demonstrating in the laboratory that MICP has the potential to bind together CCRs with the goal of permeability and infiltration reduction and dust suppression. Finally, two field demonstrations are planned with the help of collaborators and stakeholders.

Impact

Collecting the data during this project has led to dissemination of our results at the 2017 World of Coal Ash Conference. Four proposals were submitted, totaling $328,000 to industrial and private funding agencies. Of the proposed, $80,000 has been awarded. Commercialization plans have been developed with MET, and field tests have been planned contingent on the pending additional requested funding.

Metrics

Total additional grants received

One grant was received.

MSU received industrial testing funding from Southern Company in 2015 and 2016 for a total of $80,000.

Total additional grants in progress

Two grants are in progress.

MSU submitted a proposal to Southern Company for 2017 funding at a request level of $23,000. In addition, a pre-proposal was submitted to the Environmental Research and Educational Foundation (EREF) requesting funding to perform a dust suppression field demonstration ($225,000).

Number of partnerships formed (private and public sector)

Three partnerships were formed.

Partnerships between MSU and MET were strengthened during this project. In addition, MSU partnered with engineers at Colstrip and with engineers in the Southern Company organization.

Commercial products developed

During the project, the data suggested that commercial application of biomineralization to remediate leaking CCR disposal ponds or mitigate dust formation was possible. This project enhanced the technology readiness level of the technology to the point where field demonstrations are planned. MET is developing a mobile mineralization laboratory (trailer) that will be available to perform the field demonstrations commercially.

Jobs created/supported:

- Technicians: 2
- Students: 4
- Faculty: 1
- Post-docs: 1

Publications

Thane, A, Troyer, E, Gallagher, B, Phillips, AJ. Remediation of coal combustion residuals using microbially-induced calcite precipitation. World of Coal Ash Conference 2017 Proceedings (accepted)

Presentations

Thane A, Phillips, AJ, Troyer, E, Gallagher, B, Lee Spangler. “Remediation of Coal Combustion Residuals Using Microbially-Induced Calcite Precipitation” Poster presented at the Montana Biofilm Meeting, July 19, 2016, Bozeman, Montana.

Thane A, Troyer E, Cunningham A, Spangler L, Gallagher B, Phillips A. “Remediation of Coal Combustion Residuals Using Microbially-Induced Calcite Precipitation” World of Coal Ash Conference, May 2017, Lexington, Kentucky.

Filanoski, B, Phillips, AJ. “Microbial Induced Calcium Carbonate Precipitation of Coal Combustion Residuals”. 31st Annual National Conference on Undergraduate Research, University of Memphis, April 6-8, 2017, Memphis, Tennessee.

Filanoski, B, Troyer, E, and Phillips AJ. “Microbial Induced Calcium Carbonate Precipitation of Coal Combustion Residuals”. Montana State University Undergraduate Research Celebration, April 2016, Bozeman, Montana.

Filanoski, B, Troyer, E, and Phillips AJ. “Microbial Induced Calcium Carbonate Precipitation of Coal Combustion Residuals”. Montana State University McNair Scholars Program Research Celebration, November 2015, Bozeman, Montana.

Objective 3

Assess the potential to use bacterially driven mineral formation for removal of heavy metals, such as cadmium, arsenic and selenate from water produced by coal mining operations, coalbed methane, and enhanced oil recovery; and water contaminant remediation related to natural resource extraction and energy generation.

This objective initially aimed to assess the potential to use bacterially driven mineral formation for removal of heavy metals from contaminated water produced by mining operations, coalbed methane, and enhanced oil recovery. The bacterially driven process of interest for most of the research is MICP, driven by the bacterially catalyzed hydrolysis of urea. The project assessed whether MICP could also co-precipitate other metals within calcium carbonate ($CaCO_3$) or precipitate as other carbonate minerals, thus remediating heavy metal contaminated waters. The project ultimately focused on three key aspects of wastewater associated with these activities: (1) Laboratory studies of MICP with model bacterium *Sporosarcina pasteurii* in porous media and batch reactors to assess co-precipitation of selenium, strontium, and barium, common contaminants of produced water; (2) Laboratory studies of MICP in mine influenced water from a Montana superfund site and cultivation of native bacteria from mine sediments capable of promoting MICP; and (3) Assessment of the presence of selenium-reducing bacteria in selenium contaminated groundwater at the Colstrip power plant and the potential for selenium reduction in situ.

Accomplishments

Accomplishments of the objective

Laboratory studies of MICP in batch reactors to assess co-precipitation of selenium
Selenium (Se) is a contaminant that can leach out from coal mining waste when exposed to groundwater under aerobic conditions causing release into surrounding water streams. The most common forms of aqueous Se are selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}), which are oxyanions. Therefore, co-precipitation of Se with MICP would involve these oxyanions potentially replacing the carbonate anion. However, initial batch tests of MICP with selenate and selenite did not indicate removal of either anion. Thus, reduction of these oxidized forms of selenium to elemental Se was determined to be a more effective pathway and is discussed below.

Laboratory studies of MICP in porous media and batch reactors to assess co-precipitation of strontium and barium
MICP batch experiments also focused on co-precipitation of strontium (Sr) and barium (Ba). In addition to being common contaminants in produced water from hydraulic fracturing, the radionuclide forms of strontium and barium are the product of uranium fission. Initial batch tests demonstrated successful removal of both metals from solution during microbially driven MICP with a model bacterium, *S, pasteurii.*

A 2-D porous media flow cell system with spatial sampling ports, shown in Figure 6. *Schematic of spatial flow cell reactor.*, has been utilized to evaluate spatial and temporal dynamics of strontium removal from artificial groundwater. As demonstrated in Figure 7, analysis of precipitates indicates spatial gradients of strontium co-precipitation. While greater total removal of strontium is observed nearer to the flow cell effluent due to more overall precipitation, the ratio of Sr:Ca in the precipitate decreases with distance from the inlet. Sr:Ca ratio in $CaCO_3$ precipitate formed by MICP has been shown to correlate with the rate of precipitate formation. Additional experiments with the spatial flow cell and strontium co-precipitation are ongoing.

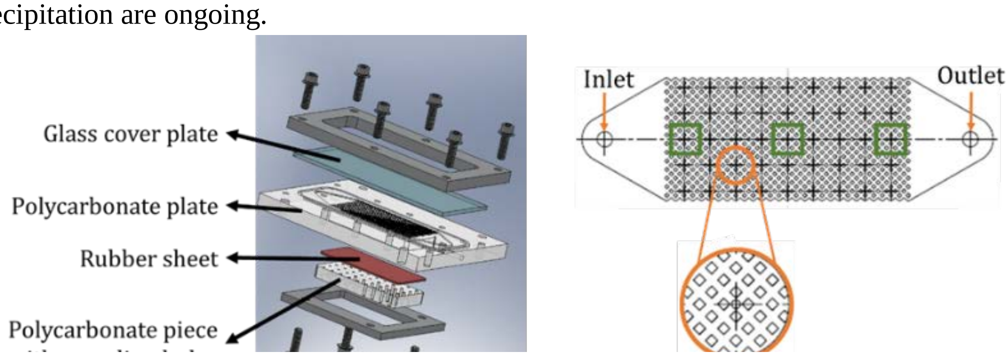

Figure 6. *Schematic of spatial flow cell reactor.*

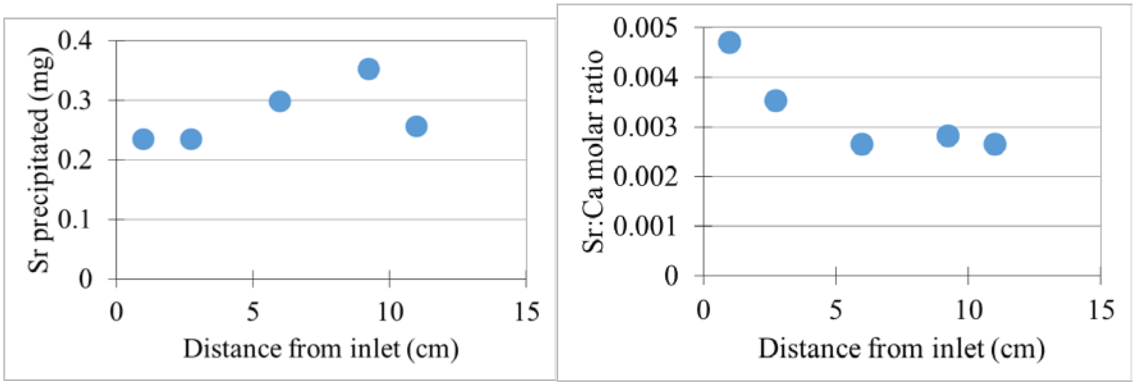

Figure 7. Left: Mass of strontium precipitated in spatial flow cell along flow path. Right: Ratio of strontium to calcium in precipitate from spatial flow cell. Data from single experiment conducted over three days.

MSU collaborated with a local Bozeman company, New Wave Research, recently acquired by Electro Scientific Industries, to analyze solid phase mineral composition using laser ablation of the minerals and analysis via inductively coupled plasma mass spectrometry (ICP-MS). A sample analysis of the Sr:Ca ration in minerals formed within the spatial flow cell reactor during the first continuous flow experiment is shown in Figure 8. The technology has immense potential for spatial analysis of mineral composition. This work and collaboration has led to a proposal for an updated instrument at MSU (ICP-MS) for elemental analysis coupled with laser ablation. If funded, the proposal will lead to installation of this powerful instrument and capability at MSU, where it currently is not available.

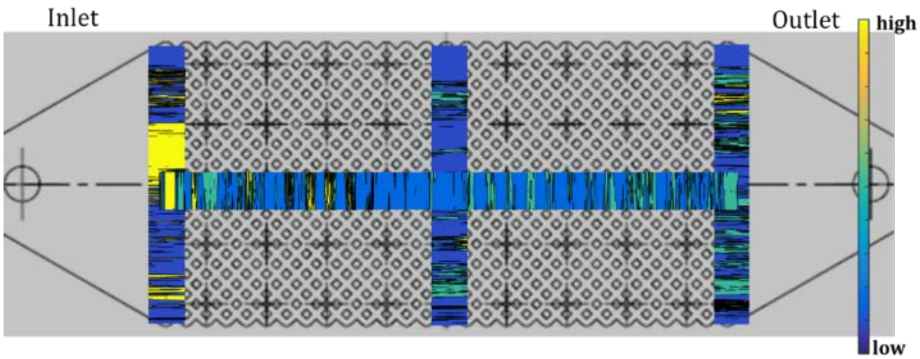

Figure 8. Sr:Ca ratio of mineral precipitate in spatial flow cell reactor, as determined by laser ablation and ICP-MS Yellow indicates higher relative strontium concentration.

Laboratory studies of MICP in mine influenced water from a Montana superfund site and cultivation of native bacteria from mine sediments capable of promoting MICP

The Carpenter-Snow Creek Mining District (CSCMD) site near Neihart, Montana, experienced silver, lead, and zinc mining from 1882 through 1929. Since 2001, it has been listed as an U.S. Environmental Protection Agency (EPA) superfund site. Byproducts of the mining activity still influence the soil, sediment, surface water, and groundwater on the site. Surface water and sediment samples were collected from three of the adits releasing mine influenced water (MIW) contaminated with heavy metals: Compromise, Moulton, and Evening Star. MIW samples from each of the three adits were evaluated for the feasibility of MICP and the potential for heavy metal co-precipitation.

Figure 9. Left: Mine adit with water flowing out of shaft at Evening Star in Carpenter-Snow Creek Mining District. Right: Graduate student Emily Stoick sampling sediments from the adit discharge area at Evening Star to cultivate native bacteria capable of metals remediation.

Batch experiments were conducted for all three mine adit water samples in bottles shown in Figure 10. Triplicates of each treatment were used. Filtered and unfiltered MIW treatments were used to determine if there were any native bacteria contributing to or inhibiting urea consumption and metal precipitation. Bacterial enrichments were also performed to cultivate potential native bacteria capable of promoting MICP.

Figure 10. Photo of Batch Experiment Bottles

In the MIW from the Evening Star and Compromise adits, ureolysis occurred successfully and both saw significant levels of manganese, nickel, zinc, and barium removed from solution (Table 1). MICP was unsuccessful in water from the Moulton adit. Further studies focused on water from the Evening Star adit, due to the accessibility of the site and removal of several key heavy metals in water.

Table 1. Average Percent of Metals Removed from Solution

	Compromise	**Evening Star**
Manganese	92.3	99.0
Nickel	59.7	39.4
Zinc	95.3	59.7
Barium	39.3	73.3
Cadmium	BQL	----
Copper	----	BQL
Cobalt	----	BQL

Bacterial growth studies with sediments from the Evening Star site were able to cultivate an organism that showed ureolytic activity and was able to grow in the MIW. The organism was enriched in liquid cultures and isolated with plating and bacterial colony selection. Utilizing this native organism in the MIW, MICP studies were performed with 0.5 and 2 g/L urea at room temperature and 10 °C, which is closer to the actual water temperature at the site. As shown in Figure 11, urea was hydrolyzed in all cases and several heavy metals were removed via MICP by the native organism (Table 2). Identification of the native bacteria is currently underway utilizing DNA (deoxyribonucleic acid) sequencing technology. The ability to use a native bacteria present at the site for remediation provides an advantage as the organism is accustomed to the site conditions, including temperature and water chemistry, and it eliminates the need to bioaugment with a non-native bacteria, which can require additional permissions and steps during approval of a remediation technology.

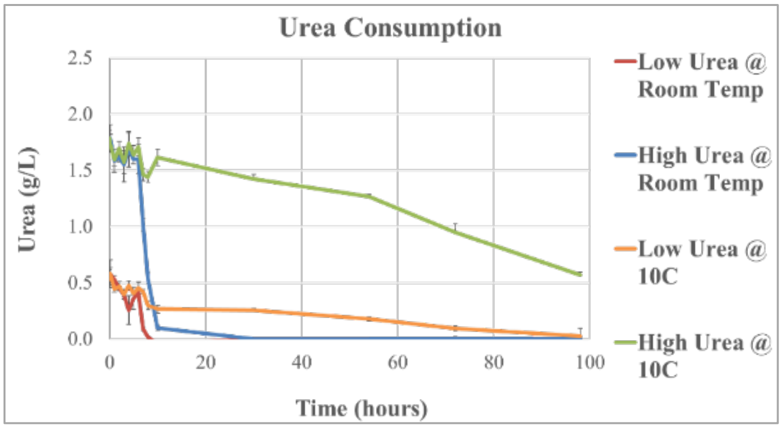

Figure 11. Urea hydrolyzed in batch tests using native bacteria at the Evening Star mine site.

As shown in Table 2, MICP treatment of the key heavy metals of concern in the MIW may not yet achieve the Montana Department of Environmental Quality (DEQ) water quality standard for aquatic life, but additional optimization of the microbial process has the potential to increase removal to sufficient levels.

Table 2. Removal of four heavy metal contaminants by model bacterium S. pasteurii and the native isolate.

Metal	***S. pasteurii* at Room Temp**	**Native Bacteria at Room Temp**	**Native Bacteria at 10 C**	**MT DEQ Water Quality Standard for Aquatic Life (μg/L)**	**Average Conc. Discharged into creeks without treatment (μg/L)**
Zinc	60%	52%	29%	37	697
Cadmium	None	41%	60%	0.097	0.634
Copper	None	100%	79%	2.85	4.45
Nickel	40%	13%	14%	16.1	25.5

Assessment of the potential for selenium reduction in contaminated groundwater at Colstrip

Bioremediation of selenium in the groundwater at Colstrip was assessed by sampling for native selenium-reducing bacteria in two wells containing selenate and performing laboratory tests with carbon additions to stimulate growth of bacteria and promote selenate reduction. Wire mesh sampling containers filled with coarse sand were placed in two sampling wells, 366S and 633M, at Colstrip (Figure 12, left). The samplers remained in the wells for one month to allow time for biofilm to form on the sand prior to sampling. The sand from each sampler was used for molecular analysis of the biofilm and microcosm experiments. DNA was extracted from sand and groundwater at each well for analysis of microbial communities. Four different additions of carbon for microbial growth and selenium removal were tested for each well in batch bottles (Figure 12, right): no carbon (control), methanol, glycerol, and molasses. Each treatment was run in duplicate. Oxygen sensitive dye was used in the bottles to indicate when oxygen was depleted, as selenium reduction is an anaerobic process.

Figure 12**.** Left: Deployment of microbial sampling chambers into wells at Colstrip. The chambers contained sand for attachment of bacteria present in the aquifer. Right: Set of batch bottles with sand as inoculum for growth and selenium removal studies. Pink indicates microbial activity has depleted the oxygen in the bottle.

Microbial Community Results

The biofilm communities from both wells were similar at the phylum level (Figure 13). The well water communities were more varied, with no phylum dominating the communities and a large proportion (15-20%) of unclassified bacteria. Genus-level classification of the native microbial community showed capability for selenium reduction; species of Pseudomonas, Polaromonas, Duganella, and Dechloromonas are known to reduce selenate and/or selenite. Future work may include sequencing of 16S ribosomal RNA (16S rRNA) from the microcosm experiments to observe the effect of the different carbon amendments on microbial community composition and to compare the post- and pre-experiment microbial communities.

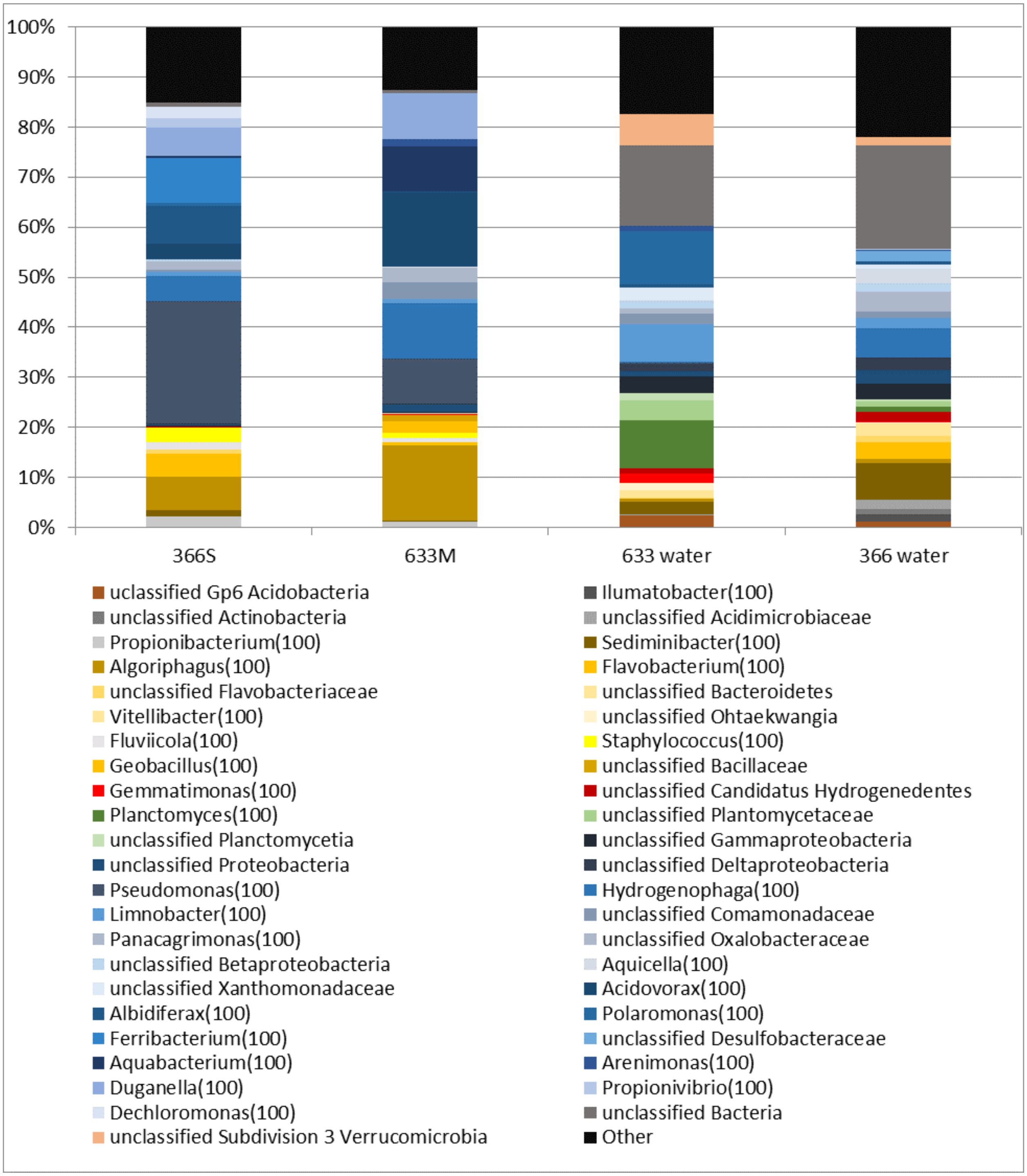

Figure 13. Molecular results of bacteria from groundwater and biofilms from sampling chambers in two Colstrip wells.

Batch Microcosm Bacterial Enrichments

Organisms had to utilize the supplemented carbon sources to consume oxygen and nitrate prior to reducing selenate in the groundwater. Oxygen removal was observed via color-change in the bottles (Figure 12, right). Molasses-amended microcosms were most active and removed both oxygen and nitrate at the highest rate.

In the 366S microcosms, the methanol bottles reduced nitrate at about the same rate as the molasses. The glycerol-amended bottles reached the same concentration around day 100. In contrast, the molasses-amended 633M microcosms reduced nitrate significantly more rapidly than any of the other amendments (Figure 14). Glycerol-amended 633M microcosms performed comparably to the controls, while methanol-amended bottles reduced nitrate only slightly faster than the controls.

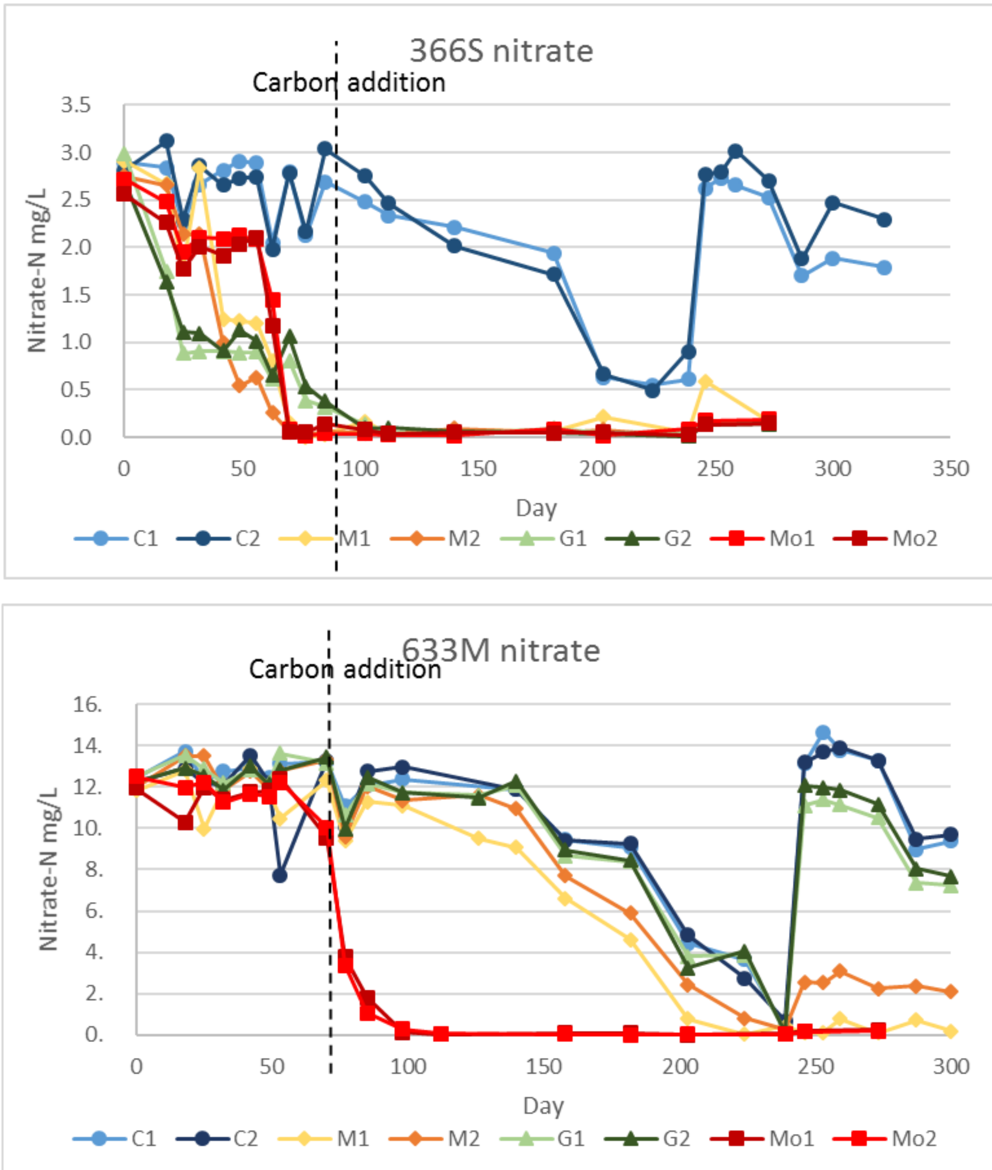

Figure 14. Nitrate concentrations in microcosm experiments. C – control, M – methanol, G – glycerol, Mo – molasses. The cause of the spike in the control and 633M glycerol experiments at day 246 is unknown.

Selenate concentrations for all carbon-amended 366S microcosms were below the detection limit at day 281 (Table 3). While the molasses-amended microcosms removed selenate most quickly – selenate was below the detection limit by day 100 – at day 281 approximately twice as much selenite was present in the molasses bottles as in either of the other carbon-amended microcosms. Selenite (SeO_3^{2-}) is a more toxic anion of selenium than selenate (SeO_4^{2-}). Thus, methanol and glycerol may be better carbon sources to achieve removal of both selenate and selenite.

Only the molasses-amended 633M microcosms showed significant reduction of selenate, which was expected based on the nitrate reduction discussed above. As with the 366S molasses-amended microcosms, the amount of selenite produced may be a concern.

Table 3. Average selenate and selenite concentrations in parts per billion, or µg/L, in batch microcosms on day 281.

	Well 366S				**Well 633M**			
ppb as Se	control	methanol	glycerol	molasses	control	methanol	glycerol	molasses
selenate	934	BDL	BDL	BDL	910	826	882	48
selenite	BDL	91	142	253	BDL	78	BDL	379

BDL = below detection limit of 10 µg/L.

The enrichments of selenium-reducing bacteria and characterization of the process are ongoing. Thus far, results indicate that the potential for selenate removal is present in the groundwater and possible with addition of molasses.

Comparison to Objectives in Original Proposal

Objectives in original proposal:

This objective will assess the potential to use bacterially driven mineral formation for removal of heavy metals, such as cadmium, arsenic and selenate from water produced by coalbed methane, enhanced oil recovery and coal mining operations. The proposed work will direct efforts towards demonstrating the ability to remediate a diversity of heavy metals, particularly those of prevalence in water generated from fossil fuel production. The project aims to inform potential field-scale development of biological remediation technologies to aid energy development while protecting water resources in Montana and enhancing cutting edge research at MSU.

A key difference between the performed work and proposed work is the focus on mine remediation that was absent from the original proposal. While the focus had been intended to be more on produced water from hydraulic fracturing and coal-bed methane, water was not obtain from those operations and the extent of our research towards that objective included laboratory studies on removal of Sr and Ba. Instead, when an interesting collaboration was started with officials at the CSCMD abandoned mine sites, remediation strategies to address the contaminated water at those sites were pursued. The ultimate goal of the project, to develop biological remediation strategies for waste water generated by resource extraction in Montana was still pursued with this slightly altered focus. Additionally, while the initial intention of the project was to focus on wastewater associated with coal mining and energy extraction, the water quality issues at Colstrip became a greater focus due to the concerns of stakeholders and the collaborative efforts of the plant Environmental and Compliance Director, Gordon Criswell.

Impact

The studies on strontium and barium removal via MICP will continue as part of Neerja Zambare's Ph.D. research and will be published in addition to contributing to her dissertation. These studies contribute to a longer-term effort by a collaborator now in Aberystwyth, UK (Andrew Mitchell), who has performed extensive research on MICP.

The research on mine influenced water will continue through Emily Stoick's Master's degree and will be published in peer-reviewed literature as part of her graduation requirements. Collaboration with the EPA on this project is ongoing, although limited in scope due to current budget restraints. Work will continue with the EPA superfund site manager and the possibility of scale-up to a field demonstration of the process at the mine site will be pursued. Additionally, collaboration with MET will result in submission of a SBIR proposal, likely to the EPA SBIR program during the next funding cycle.

The selenium remediation work at Colstrip has the potential to lead to further collaborations with Talen Energy, including groundwater modeling studies and design of potential in situ remediation strategies for selenium removal. Hannah Koepnick will continue her graduate studies in this area and will continue to characterize the microbial activity and selenium behavior in the Colstrip groundwater. The project has helped to develop a collaboration with Dr. Brent Peyton (MSU) and Lisa Kirk at Enviromin Consulting, which will result in a proposal to NSF for further support to continue research on biogeochemical processes and pathways leading to selenium reduction. Dr. Peyton's group has other ongoing research on selenium bioremediation and this collaboration will help the Colstrip study to contribute to and benefit from the larger body of current selenium research at MSU.

Metrics

Total additional grants received

Threes grant were received.

Neerja Zambare, graduate student on the project received the Montana Water Center Graduate Student Fellowship, 2016-2017 for "Removal of selenium by co-precipitation with microbially induced calcite precipitation"

Emily Stoick, graduate student on the project received the Montana Water Center Graduate Student Fellowship, 2017-2018 for "Microbially induced mixed metal precipitation and co-precipitation in mine influenced water"

Kevin Burt, undergraduate student received an Undergraduate Scholars Program award, Fall 2016, for "Calcium precipitation and trace metal co-precipitation during fluid flow and mixing"

Total additional grants in progress

Four grants are in progress.

NSF Major Research Instrumentation and Murdock Foundation proposal submitted for acquisition of new ICP-MS and laser ablation instruments. Lead: Robin Gerlach

NSF Geobiology and Low Temperature Geochemistry program – proposal in preparation on "Microbially driven selenite reduction pathways". Collaborators: Brent Peyton, Lisa Kirk (Enviromin Consulting)

NSF Geotechnical Engineering and Materials program – proposal in preparation on "Microbially driven precipitation in porous media under radial flow". Collaborators: Robin Gerlach and Brian Wood (Oregon State University)

Submission of an SBIR proposal for development of MICP based remediation of mine influenced water is anticipated to the EPA SBIR program during the next funding cycle. Collaborator: Randy Hiebert (Montana Emergent Technologies)

Number of partnerships formed (private and public sector)

Three partnerships were formed.

New partnerships were formed with Colstrip, Talen Energy, Gordon Criswell, Environmental and Compliance Director; Environmental Protection Agency, Roger Hoogerheide, superfund project manager; and Tetra Tech, Inc., Engineer Colin McCoy.

Jobs created/supported:

- Students: 2
- Faculty: 1

Publications

N. Zambare, E. G. Lauchnor, R. Gerlach. Effects of Flow Rates and Calcium to Urea Ratios on Microbially Induced Calcium Carbonate Precipitation under Radial Flow. In preparation for Environmental Science and Technology.

Presentations

* Indicates student author

Lauchnor, E. G., *N. Zambare, R. Gerlach. "Heavy metal remediation via biologically driven calcium carbonate precipitation." *American Chemical Society National Meeting*, August 20-24, 2016, Philadelphia, Pennsylvania.

*Stoick, E., and E. Lauchnor. Microbially Induced Calcium Carbonate Precipitation and Metal CO-Precipitation in Mine Influenced Water at the Carpenter-Snow Creek Mining District NPL Site. National Association of Abandoned Mine Lands Programs Annual Conference, September 25-28, 2016, Bozeman, Montana.

*Zambare, N., E. Lauchnor, and R. Gerlach. Heavy metal contaminants from mine tailings – Remediation by co-precipitation. National Association of Abandoned Mine Lands Programs Annual Conference, September 25-28, 2016, Bozeman, Montana.

*Stoick, E., and E. Lauchnor. Microbially Induced Metal Precipitation in Mine Influenced Water. MSAWWA & MWEA 2017 Annual Joint Conference, April 18-20, 2017, Great Falls, Montana.

Objective 4

Assess geologic carbon sequestration potential via EOR in oil and gas fields and storage in saline formations near Colstrip, MT, utilizing fine-resolution geospatial methodologies to estimate storage potential, source to sink infrastructure, and enhanced oil production from fields that meet screening criteria. This objective is also known as the CO2-EOR Suitability Assessment.

The CO_2-EOR Suitability Assessment Study conducted by ERI examined the potential for using carbon dioxide enhanced oil recovery (CO_2-EOR) as a means to utilize and reduce emissions from the coal-fired electricity generating facility in Colstrip, Montana while also stimulating economic development in other energy sectors in the state. To do this, ERI assessed the geologic carbon sequestration potential via EOR in oil and gas fields and storage in saline formations near Colstrip. Fine-resolution geospatial methodologies were used to estimate storage potential, source to sink infrastructure, and enhanced oil production from fields that meet screening criteria.

Accomplishments

Accomplishments of the objective

ERI reviewed well data from nearly 11,000 oil and gas wells within the regional vicinity of Colstrip and identified the producing reservoirs that warranted further screening to assess suitability for CO_2-EOR. The screening process identified a total of 28 reservoirs with CO_2-EOR potential within the regional vicinity of Colstrip.

A fine-resolution geospatial model was developed to calculate oil production and carbon storage estimates for each of the 28 reservoirs based on three different types of CO_2-EOR production methods: Conventional CO_2-EOR, Advanced CO_2-EOR, and Maximized CO_2-EOR. Results from this analysis were used to evaluate potential emission reduction scenarios for Colstrip.

In addition, ERI used a least cost analysis (LCA) model to identify preliminary carbon dioxide (CO_2) pipeline routes between Colstrip and the potential CO_2-EOR reservoirs. The routes were modeled using a LCA geoprocessing tool in ArcGIS[1]. The LCA modeling effort generated several preliminary route options ranging from 85-130+ miles in length that link the potential CO_2-EOR fields or hubs to Colstrip.

Lastly, to make this study available to a broader stakeholder audience, ERI developed an interactive web-based Story Map application titled "A Story Map for Carbon Utilization and Storage Potential in Montana". The Story Map is an interactive presentation that guides the user through the entire study from start to finish with interactive maps and graphics that provide opportunities to delve deeper into the study analysis. ERI also prepared a white paper titled "CO_2-EOR Suitability Assessment for the Colstrip Coal-Fired Electricity Generating Facility in Colstrip, Montana. A White Paper Prepared for Objective 4 of the Montana Research and Economic Development Initiative". This paper provides a more thorough description of the study's methods, results, and conclusions.

Results as compared to the original objectives

The first study objective of the study was to assess the potential for CO_2-EOR in oil fields near Colstrip. MSU-ERI reviewed information from 11,000 oil and gas wells within the study area and used data at both the well and field-scale to characterize existing oil fields and identify those suitable for CO_2-EOR. A screening process was developed to narrow the number of potential CO_2-EOR reservoirs within the study area from 471 to 28 reservoirs. The remaining 28 reservoirs were grouped into three clusters based on their spatial distribution and retained for further analysis to estimate their oil production and CO_2 storage potential. These clusters are referred to as the Northwest cluster, Northeast cluster, and Southeast cluster and are located about 85-130+ miles from Colstrip.

The second objective was to estimate the regional CO_2-EOR carbon storage capacity and oil production potential. A fine-resolution geospatial model was developed for the final 28 reservoirs using field-specific and literature-based information on oil field porosity, permeability, fracture pressure, residual oil saturation, formation volume factor, net pay thickness, oil gravity, temperature, and oil viscosity. Estimates for oil production and carbon storage through CO_2-EOR were then calculated for each qualifying reservoir using three models: Conventional CO_2-EOR, Advanced CO_2-EOR, and Maximized CO_2-EOR. The cumulative oil production estimates for the final 28 reservoirs are approximately 141 million barrels (BBL) for Conventional, and 283 million BBL for the Advanced and Maximized CO_2-EOR methods. The cumulative CO_2 storage potential for the final 28 reservoirs are approximately 42 million tons (Mt) for Conventional, 170 Mt for Advanced, and 255 Mt for Maximized CO_2-EOR. Of this, reservoirs in the Northeast cluster make up approximately 69% of the cumulative oil production and CO_2 storage capacity, while the reservoirs in the Northwest and Southeast clusters make up the remaining

[1] http://desktop.arcgis.com/en/arcmap/10.4/tools/spatial-analyst-toolbox/creating-a-least-cost-corridor.htm

16% and 15% of the capacity, respectively. Figure 15 provides a summary of the potential CO_2-EOR capacity of each CO_2-EOR clusters.

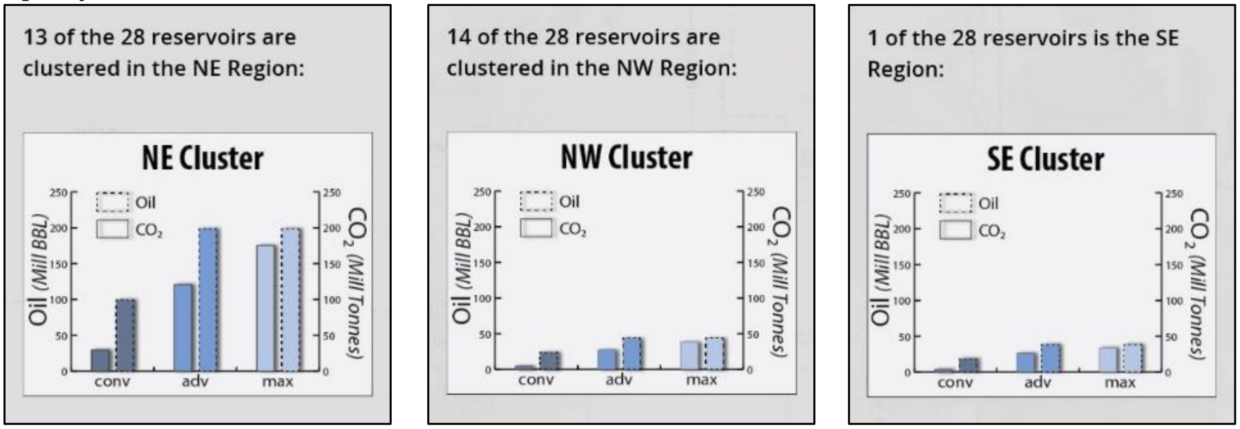

Figure 15. CO_2-EOR capacity estimates for the reservoirs in the Northwest, Northeast and Southeast field clusters.

ERI used the results of the CO_2-EOR capacity analysis to evaluate how the CO_2 storage estimates compare to Colstrip emissions. Potential carbon emission reduction rates were calculated using an annual emission rate of 15 Mt of CO_2 (based on Colstrip's emission rates from 2010-2015) over a hypothetical 20-year time period. Reductions were calculated on a cumulative and individual CO_2-EOR cluster basis to assess the upper and lower limits of the emission offsets. Individually, the Northeast cluster appears to have the greatest carbon emissions reduction potential for Colstrip at 59% maximum reduction, while the Northwest and Southeast clusters offer only a maximum reduction potential of 13% and 12%, respectively. Figure 16 compares the potential carbon emission reductions that could be achieved with each CO_2-EOR cluster based on an average 15 Mt/yr emission rate over a hypothetical 20 year period.

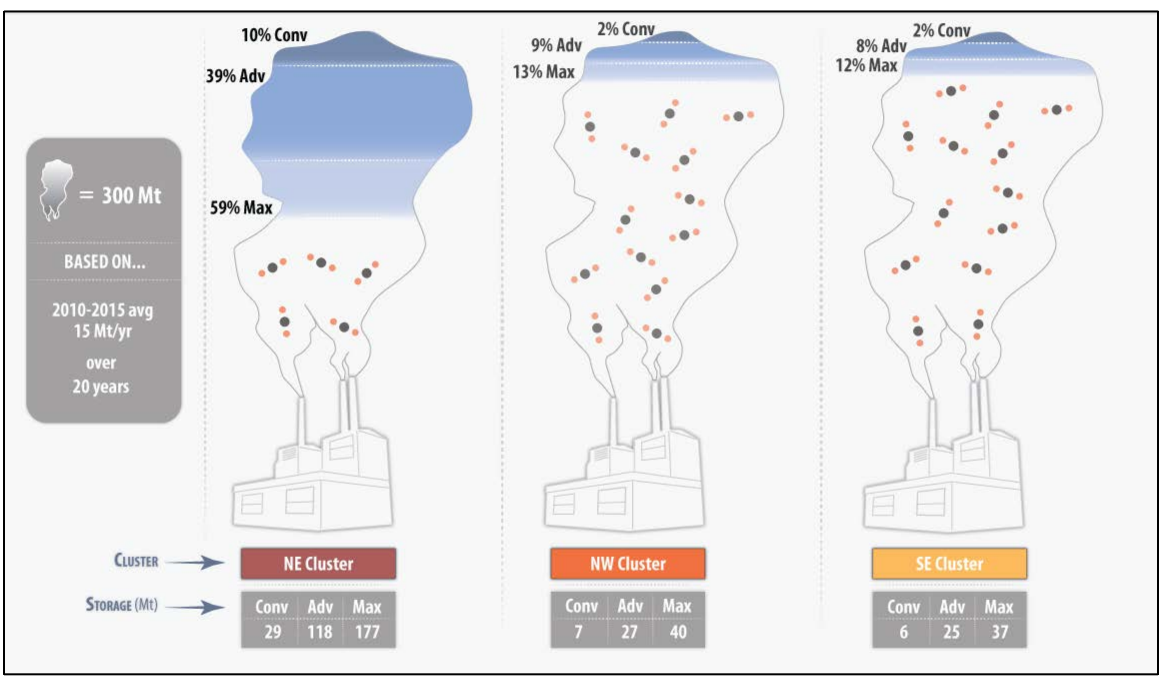

Figure 16. CO_2 storage potential emissions reduction at Colstrip over a hypothetical 20 year period

Based on ERI's estimates, Advanced or Maximized CO_2-EOR would need to be deployed in order to achieve even moderate emission reductions. This would require infrastructure upgrades to the existing oil fields that are using Conventional EOR methods. It would also require field operators to change the way they are operating their wells to allow for phased injections, additional produced water handling and treatment and CO_2 separation and recycling. Additional permitting and regulatory requirements would also likely apply to those fields.

The third objective was to determine preliminary routes to link Colstrip with potential CO_2-EOR fields for CO_2 transport. ERI made an initial assumption that carbon emissions would be captured from the Colstrip coal-fired generating facility and transported via buried pipeline to the Northwest, Northeast, and Southeast CO_2-EOR clusters. The preliminary routes between Colstrip and the CO_2-EOR clusters were modeled using a LCA geoprocessing tool in ArcGIS using five variables: land cover type, landowner type, slope, proximity to roads, and soil depth. The model analysis was performed by assigning cost factors on a scale of 1 to 5 to five model variables; developing a discreet cost surface for each model variable; generating a cumulative cost surface; and then calculating a path of least resistance across the cumulative surface. The resulting preliminary pipeline routes illustrated in Figure 17 provide a sense for the distance and level of effort needed to transport CO_2 from Colstrip to the CO_2-EOR clusters.

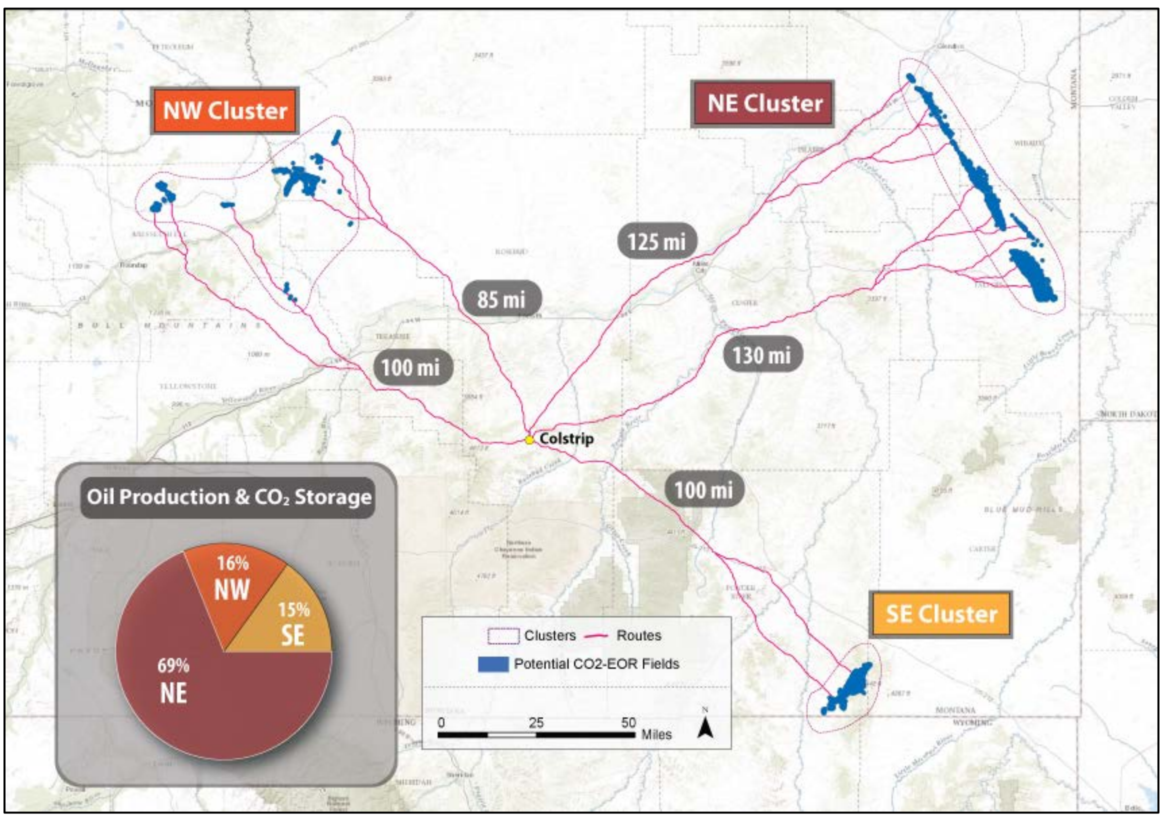

Figure 17. Regional CO_2-EOR production and storage comparison with primary pipeline routes and distances.

While the cumulative estimates for oil production, CO_2 storage, and emissions reduction for Colstrip are promising, the capacity and efficiency of the individual CO_2-EOR clusters illustrated in Figure 16 is an important consideration. The loss of any one of the CO_2-EOR regions significantly lowers the impact of the technology on Colstrip's emissions. For example, the Southeast cluster may not be an option because it is already an active CO_2-EOR project that pipes CO_2 from a source in Wyoming. Furthermore, extending the pipeline that already supplies the Southeast cluster to the Northeast cluster may be more cost-effective than developing a new network from Colstrip. Removing the Southeast and Northeast clusters from the assessment reduces the cumulative amount of carbon potentially stored by 84% (Figure 16).

Other factors such as oil market periodicity and price fluctuations will have a significant influence on field operator negotiations to secure CO_2-use agreements, as well as Colstrip's ability to capture and deliver CO_2 to EOR fields on a consistent, long-term basis.

Finally, all of these factors must be weighed against the expected operational life of the Colstrip facility and whether the costs associated with the design, construction, and operation of the CO_2 capture facility, pipeline infrastructure, and CO_2-EOR fields align with Colstrip's operational and economic goals for the remaining operational life of the facility.

Impact

Long-term Impact

The CO_2-EOR Suitability Assessment Study provides industry and stakeholders with an initial understanding of the scale and effort involved in achieving CO_2 emission reductions at Colstrip via regional CO_2-EOR. It also provides insight into which reservoirs in the region have the greatest capacity for carbon storage and which CO_2-EOR methods would need to be used in order to effectively reduce carbon emissions Colstrip.

This information will help industry and stakeholders discuss future options for the Colstrip facility. It may motivate private industry to conduct a detailed CO_2-EOR feasibility analysis to more fully understand the benefits, costs, and risks associated with developing a regional CO_2-EOR program with Colstrip; or it may compel stakeholders to seek other options for the facility. These decisions cannot be made, however, without some initial understanding of what this effort might look like and how effective emission reductions could be with CO_2-EOR. The CO_2-EOR Suitability Assessment Study provides that information and will help facilitate an informed discussion about whether this technology is a viable option for the Colstrip facility.

Future activities extending beyond the life of the original MREDI grant

The study suggests that if a significant investment was made to install capture technology at Colstrip and develop pipeline networks, substantial CO_2 emission reductions could be achieved if all three CO_2-EOR clusters utilize Advanced or Maximized CO_2-EOR methods. However, the distance between Colstrip and the CO_2-EOR clusters, pipeline requirements, CO_2-EOR deployment and operational costs, and CO_2 capture facility costs pose significant barriers.

As a next step, Colstrip may consider running this same analysis using more detailed data available from the private sector. The well and geologic data that was used for this study were drawn from publically available datasets, some of which had incomplete records. As a result, ERI's analysis may underestimate the number of suitable reservoirs and fields sites that could be used for CO_2-EOR. A more complete and accurate data set might include measured fracture pressures for the field-reservoir combinations; measured formation volumetric factors; and reservoir characteristics such as oil gravity, depth, temperature, etc. More details on the physical rock or lithology properties could show that there are additional reservoirs that are miscible than ERI has estimated.

To take this a step further, Colstrip may consider doing a detailed impact analysis and economic, engineering, and environmental feasibility study to assess whether CO_2-EOR is truly a viable option. Viability hinges on many factors including: domestic and international coal, oil, and gas economies; environmental regulations; land use policies; pipeline permitting; and Colstrip's ability to forge business partnerships and right-of-way access with the owners of the EOR fields and property owners along pipeline routes. All of these factors must be weighed against the expected operational life of the Colstrip facility and whether the costs associated with the design, construction, and operation of the CO_2 capture facility, pipeline infrastructure, and CO_2-EOR fields fall in line with the strategic objectives for the remaining operational life of the facility.

Metrics

Jobs created/supported:

- Research scientists/engineers: 3
- Technicians: 1
- Students: 1

Publications

Fairweather, Stacey; J. Blank, B. Winkelman and G. Nicholas. 2017. "A Story Map for Carbon Utilization and Storage Potential in Montana". Developed by Montana State University, Energy Research Institute and funded by the Montana Research and Economic Development Initiative.

Spangler, Lee, S. Fairweather, J. Blank, B. Winkelman, and G. Nicholas. June 2017. "CO_2-EOR Suitability Assessment for the Colstrip Coal-Fired Electricity Generating Facility in Colstrip, Montana. A White Paper Prepared for Objective 4 of the Montana Research and Economic Development Initiative". June 30, 2017. Prepared by the Montana State University Energy Research Institute for the Montana Board of Regents, Office of the Commissioner of Higher Education under Agreement Number: 51040-MUSRI2015-05. http://www.montana.edu/energy/mredi/documents/MREDI_CO2-EOR_WhitePaper.pdf

Presentations

Winkelman, Brandt. "A Story Map for Carbon Utilization and Storage Potential in Montana." ESRI UC, July 10-14, 2017, San Diego Convention Center, San Diego, California.

Objective 5

Develop methods to integrate phototrophic microbe based air capture of CO_2 and evaluate potential byproducts.

This objective was performed in collaboration by three research groups. Martha Apple's group at Montana Tech gauged the potential of a strain of microalgae (*Neospongiococcum sp*., known as PW-95) isolated from the coal bed methane ponds and a non-toxic strain of nitrogen-fixing cyanobacteria (*Anabaena cylindrica*) to serve as bio-fertilizers of three economically important crop plants (Wheat, Potatoes, and Flax) of Montana.

Brent Peyton's group at MSU is developing strategies to overcome the current limitations of using cyanobacteria as a large scale biofertilizer. Anabaena cylindrica strain 16 was grown in liquid medium without added nitrogen. This culture is highly resistant to contamination, and has served as an excellent fertilizer for enhancing plant growth in past small scale tests with wheat and other crops.

Brent Peyton's group also researched the potential of nitrogen fixing cyanobacteria (NFC) populations in developing and applying sustainable biofilm fertilizers. While most work on cyanobacterial fertilizers are focused on root system interactions, preliminary observations indicate that applying a suspension of NFC as a biofertilizer to the soil surface promotes plant growth equivalent to that observed with petrochemical based nitrogen fertilizer. While applied as an aqueous NFC suspension, a photosynthetic biofilm rapidly forms on the soil surface. Some researchers have studied cyanobacterial crusts in deserts, drylands, deserts, polar, and other inhospitable environments but little is known about the structure, activity, and benefits resulting from NFC photosynthetic biofilms in agricultural systems other than rice paddies.

Matthew Fields group at MSU researched enabling the utilization of Montana's vast coal reserves in an environmentally sound manner by developing methods to stimulate repeated methane production in coal bed methane (CBM) projects with nutrients produced by native algal species that can grow in CBM production water.

Accomplishments

Accomplishments of the objective

Experiments were conducted on the addition of PW-95 to wheat, potatoes, and flax and it was determined that it had a great potential as a biofertilizer, especially for wheat. Wheat fertilized with PW-95 produced more grain per plant, and more grain-producing tillers (side shoots) per plant. PW-95 was also tested for nutrient content. In addition, A. cylindrica, and Nanochloropsis gaditana are in the process of being tested for the same.

Figure 18. Cultures of microalgae and cyanobacteria for MREDI biofertilizer experiments

Experiments on the addition of the non-toxic, nitrogen-fixing cyanobacteria Anabaena cylindrica to wheat, potatoes, and flax were conducted and it was determined that it has a great potential as a biofertilizer, especially for wheat. Because A. cylindrica fixes atmospheric nitrogen into a biologically useful form, the addition of exogenous nitrogen is not required. This is important because nitrogen is a necessary plant nutrient that generally has to be added to the soil through relatively expensive commercial sources.

Methods were developed of culturing PW-95, A. cylindrica, and Nanochloropsis gaditana, an additional microalga from the coal bed methane ponds, at large bench-scale quantities. A lighting system was also created for culturing these light-requiring, photosynthetic microbes throughout the winter in the laboratory.

Figure 19. Culture of Nanochloropsis gaditana, a microalgal species isolated from a coal bed methane pond

Small biofilm incubators were devised in which soil was placed in the bottom half of a petri dish, and H_2O, nutrients, and A. cylindrica cells were added. The incubators were covered with the top of a petri dish and the competed incubators were placed under light. After approximately two weeks, the biofilm was sufficiently established so that approximately two-thirds of the biofilm could be transferred to the soil surface of experimental potted wheat plants and use the remainder to replenish the culture.

Figure 20. Petri dish incubator for Anabaena cylindrica soil biofilm

Micrographs of all three species of microalgae were taken. A series of experiments were also conducted to determine effective means of concentrating the microalgae via gravity-collection in funnels and along a gradient of alkalinity to induce flocculation.

Hyperspectral and Raman spectrographic signatures were tested for the three species of microalgae in collaboration with Xiaobing Zhou and graduate student Zhaoming Zhou (Montana Tech).

In collaboration with Xiaobing Zhou, graduate student Zhaoming Xhou, and undergraduate student Joseph Natale (Montana Tech) tests were conducted on wheat germ. The tests aimed to determine whether wheat grown with biofilms of the cyanobacteria, A. cylindrica, have different soil temperatures, moisture contents, and electrical conductivity than those of wheat grown with standard nutrient solutions or with only the addition of water.

Figure 21. Wheat seedling with cyanobacterial biofilm and a soil moisture, temperature, and electrical conductivity sensor

Cyanobacterial experiments were developed in collaboration with Brent Peyton (MSU).

This project also resulted in the completion of one master's degree in Environmental Engineering at Montana Tech for Olakunle Ogunsakin, thesis title: Evaluation of the Fertilization Properties of Microalgal Biomassa and Assessment of KOH-Induced Flocculation of PW95 Microalgal Cells. As part of the requirements for his degree, Olakunle presented seminar titled "Induced Flocculation of PW95 Cells and Biofertilization Effect of its Biomass" to the Environmental Engineering Department, Montana Tech.

Dr. Peyton's group cultured Anabaena cylindrica strain 16 in liquid medium without added nitrogen at multiple scales including 12L and 200L (~3 and 54 gallons) raceway ponds. The cell density was determined via dry weight for determination if enough biofertilizer had been produced. The 200L (~53 gallons) raceway test unfortunately failed due to very high temperatures and solar irradiance during the initial growth period. Cultures were restarted in three identical 12L (~3 gallons) reactors and soon developed enough biomass to transfer to Martha Apple (Montana Tech) for recently started growth tests on potatoes. Dr. Peyton and Dr. Apple developed collaborations on the use of cyanobacteria as a means of fertilizing economically important crop plants of Montana.

Isolation and characterization of algal strain PW95 that can grow in CBM production water

Nutrients (algal cell extracts) grown in CBM water perform at the same level as commercially available cell extracts. CBM production resulted in thousands of ponds in the Powder River Basin of low-quality water in a water-challenged region. A green alga isolate, PW-95, was isolated from a CBM production pond, and analysis of a partial ribosomal gene sequence indicated the isolate belongs to the Chlorococcaceae family. Different combinations of macro- and micronutrients were evaluated for PW-95 growth in CBM water compared to a defined medium. A small level of growth was observed in unamended CBM water (0.15 g/l), and biomass increased (two-fold) in amended CBM water or defined growth medium. The highest growth rate was observed in CBM water amended with both nitrogen (N) and phosphorus (P), and the unamended CBM water displayed the lowest growth rate. The highest lipid content (27%) was observed in CBM water with nitrate, and a significant level of lipid accumulation was not observed in the defined growth medium. Growth analysis indicated that nitrate deprivation coincided with lipid accumulation in CBM production water, and lipid accumulation did not increase with additional phosphorus limitation. The presented results show that CBM production wastewater can be minimally amended and used for the cultivation of a native, lipid-accumulating alga.

The native algal species, PW-95, not only grew in CBM water with minimal additions while utilizing CO_2, but also produced almost 30% w/w (weight per weight) of lipids. The lipids could have value as pre-cursors to bio-diesel, but also as nutrient additions to stimulate methane production from existing production wells in the Powder River Basin in Montana (see Objective 6).

Microbial characterization of algal aggregation

Dr. Field's group also performed microbial characterization of algal aggregation. Preliminary bacterial community analysis of algal isolate PW-95 growth in non-sterile CBM production water was conducted. The team had observed that algal biomass would aggregate or "clump" when in non-sterile CBM water (Figure 22 and Figure 23). This behavior could be advantageous for biomass collection. The population dynamics were tracked for 1 day, 7 days, and 14 days of water alone and water inoculated with the algal isolate. Both water and algal aggregates have distinct bacterial communities that shift upon inoculation with the algal culture. Subsequently, the bacterial community shifts further from Day 1 to Day 7, with a more stable community between Week 1 and Week 2. The CBM water is predominated by Flavobacterium, Lutibacter, Sediminibacterium, Methylophilales, Methylosoma, and Luteolibacter. In Day 1 post-inoculation, the water continues to be predominated by Flavobacterium, Lutibacter, and Methylosoma. After one week of algal growth, the CBM water becomes predominated by Sediminibacterium. The algal clumps appear to become predominated by Flexibacter, Flavobacterium, and Lewinella.

The data indicate that particular bacterial populations are associated with PW-95 and could promote aggregation of algal biomass that could impact biomass collection.

Figure 22. Algal PW95'clumping' in non-sterile CBM production water.

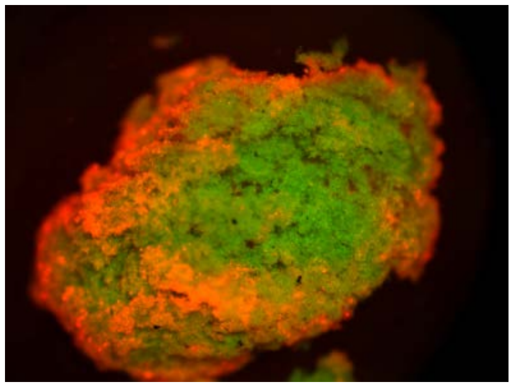

Figure 23. Algal aggregate imaged with Stereomicroscope.

Outdoor algal growth under alkaline conditions

A mixture of algal species (PW-95, WC-1, and X) was tested for growth in outdoor conditions (open raceway pond with natural light and atmospheric CO_2. Growth experiments were initiated indoors to slowly acclimate the mixed algal culture (strain PW95, strain WC-1, and strain X) to outdoor conditions (Figure 24).

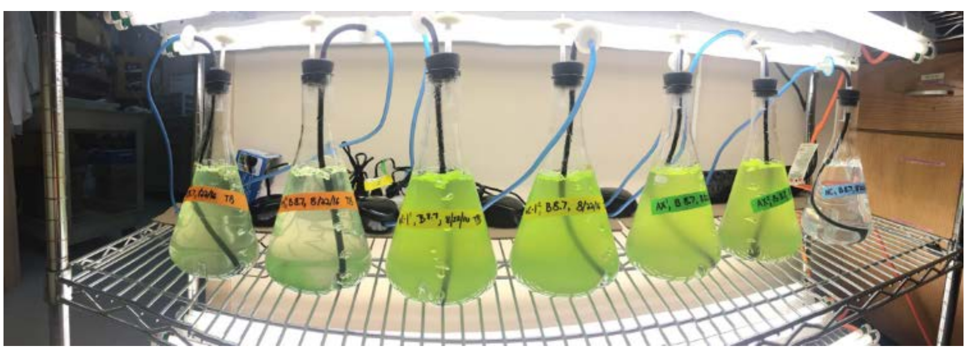

Figure 24. Cultivation of individual algal strains in preparation for scale-up of inoculum. Cultures are being slowly acclimated to outdoor conditions.

The mixed algal culture (strain PW95, strain WC-1, and strain X) was grown to test for outdoor growth (natural light and temperature fluctuations) along with biomass and lipid accumulation. In September 2016, two 200L (~53 gallon) raceways with a consortium of the three alkaliphilic alga taxa (Alga X, WC-1, and PW-95) were inoculated, and the cultures were allowed to grow for two weeks before the weather turned colder and cloudier (Figure 25). The mixed population grew for approximately 12 days with concomitant depletion of nitrate (Figure 26 and Figure 27).

The results demonstrate that the mixed, algal culture could be cultivated outdoors with natural sunlight under alkaline conditions that promoted CO_2 uptake.

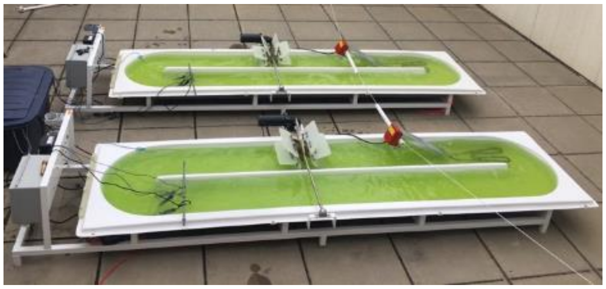

Figure 25. Cultivation of alga consortia that contains strains PW-95, WC-1, and X. Each respective culture was grown individually in the laboratory and then combined into a single inoculum. The consortia were grown outdoors for two weeks in 200L (~53 gallon) raceway ponds. A paddle wheel insured mixing and a modified livestock trough heater maintained a water temperature >15 °C.

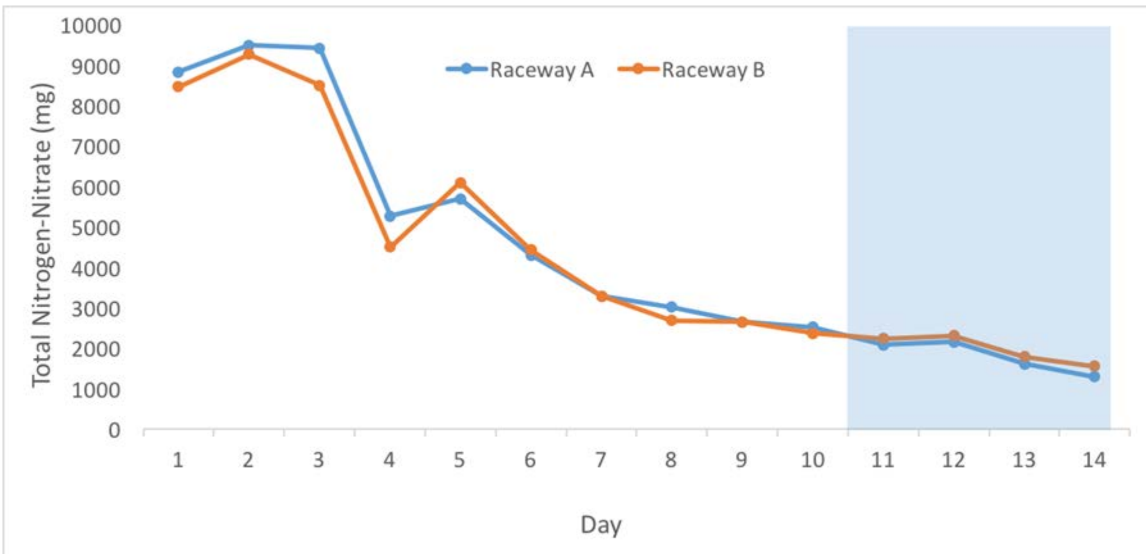

Figure 26. Total nitrogen for raceways A and B, as determined by a NAS-Szechrome assay. Blue box denotes change to cooler weather.

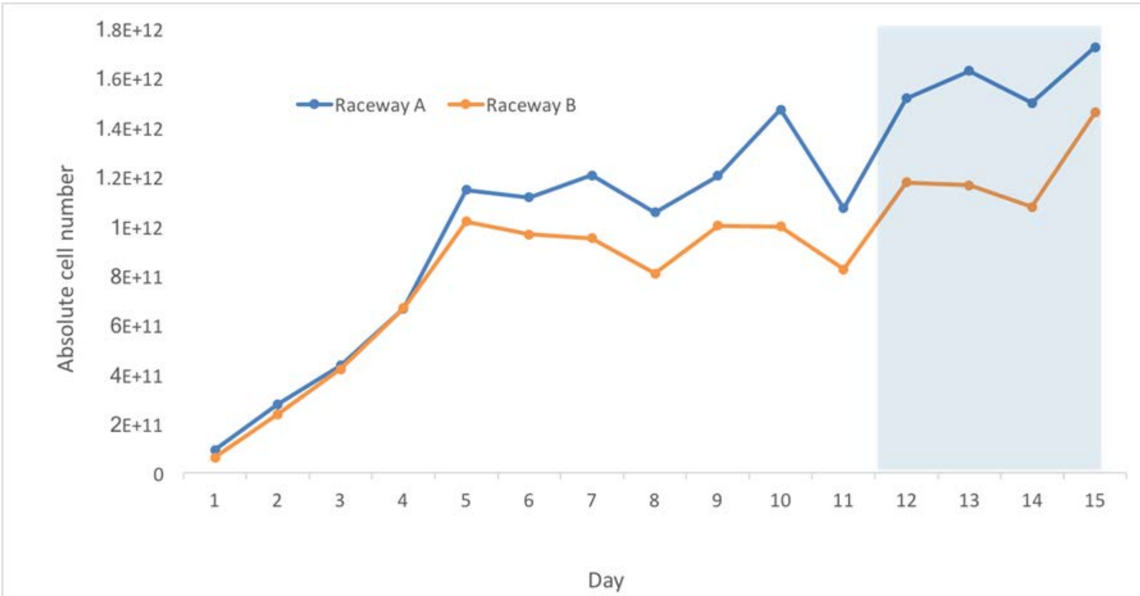

Figure 27. Absolute cell number for raceways A and B, as determined by daily cell counts. Blue box denotes change to cooler weather.

Collection of algal biomass after growth in CBM production water

Dr. Field's group collected algal biomass after growth in CBM production water. The native PW-95 strain was grown in CBM water, and initial experiments for biomass collection were tested after grinding the algal biomass. The native algal PW-95 strain was grown in CBM water supplemented with nitrate. The biomass was harvested, ground in a mortar and pestle, and re-suspended (0.5 g/l) in water. The biomass was then allowed to settle over a 24-hour time period. For test 1 (ground 1x), most of the biomass settled within one to two hours. Upon a second re-suspension (test 2), a small amount of additional biomass settled.

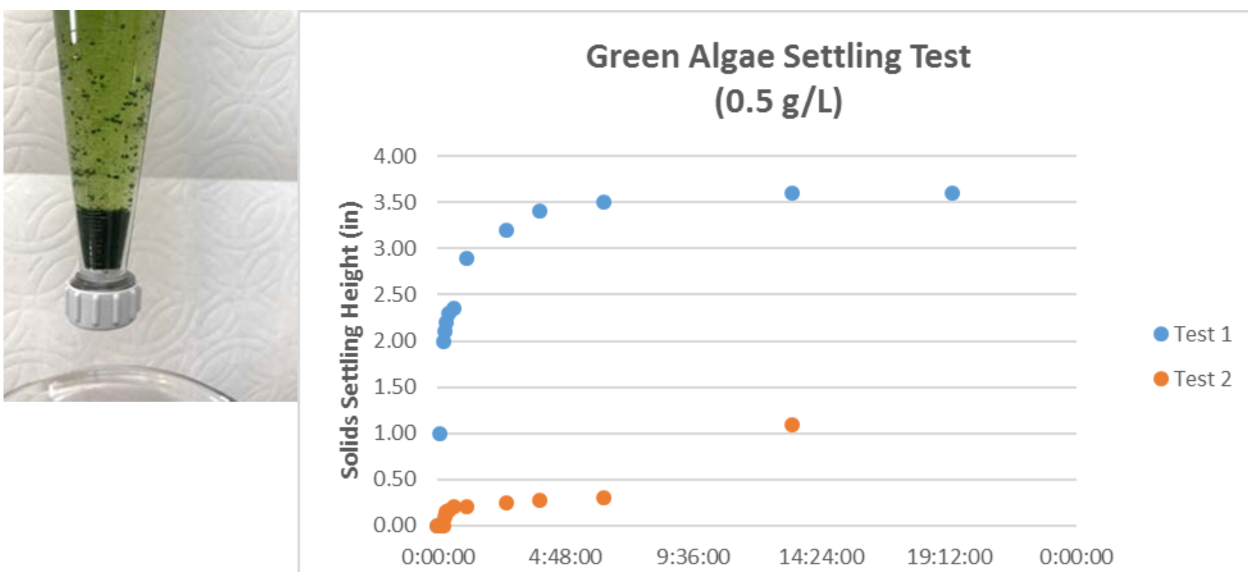

Figure 28. Green Algae Settling Test Comparison. Algal strain PW95 was grown to a slurry concentration of 0.5 g/L. In Test 1 the green alga was ground, mixed in DI water for 20 minutes and shook for 1 minute. In Test 2, the algae slurry was stirred and mixed a second time approximately 12 hours later.

Further tests were conducted for the collection of algal biomass produced in CBM production water and compared to growth in defined medium (Bold's): whole cells and sheared cells (cells sheared in a commercial blender). Whole cells are easier to collect, but could impact hydrology and mass transport if injected in the subsurface to stimulate CBM production. Sheared fractions could increase stimulatory effect as well as have less impact on hydrology, but would be more expensive to process. Therefore, whole cell fraction and sheared fractions were compared. It should be noted that Bold's medium was included for a growth comparison to amended CBM production water. Due to limited field inoculum and to keep relevant to potential application, only biomass grown in CBM production water was tested for processing.

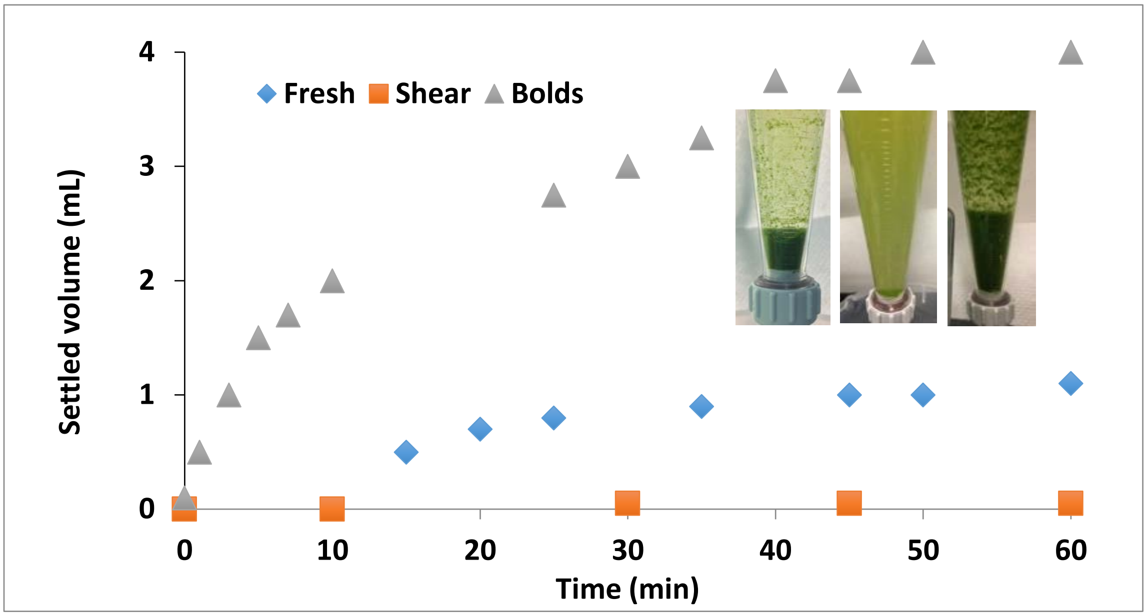

Figure 29. Settling of algal biomass as whole cell (fresh) and sheared after growth in CBM production water. Bold's sample represents whole cells grown in Bold's medium.

The whole cell and sheared fractions from cells grown in CBM production water were comparable in protein content, but non-purgable organic carbon (NPOC) was much higher in the sheared fraction.

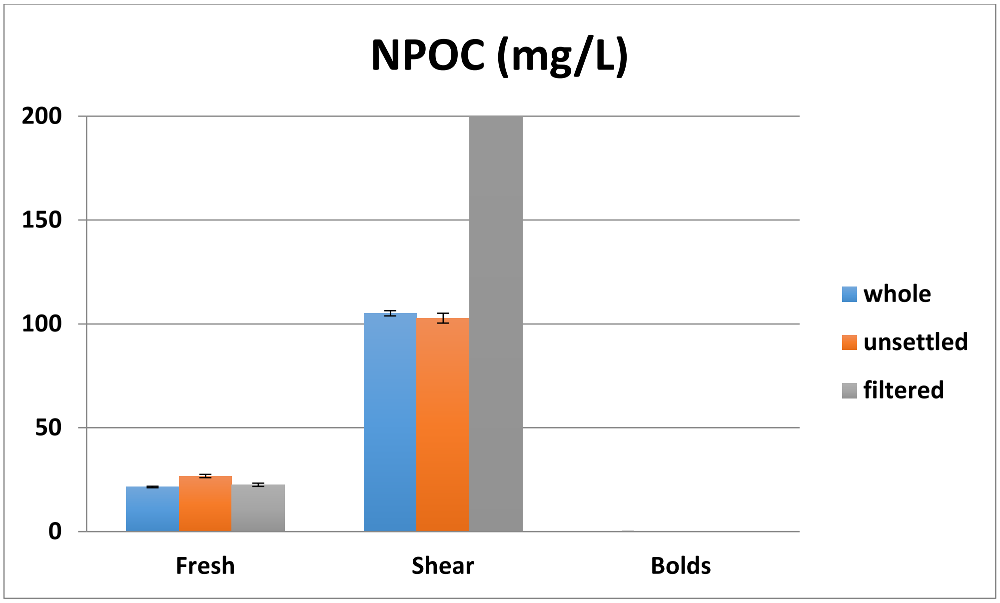

Figure 30. NPOC for the whole, unsettled, and filtered fractions from fresh (whole) algal cells and sheared algal cells.

The results show that whole cells are easier to collect than sheared fractions, but the sheared fractions may contain more organic carbon that could be used as a stimulatory nutrient for coal-dependent methanogenesis. Collected sub-samples are being tested for the ability to stimulate coal-dependent methanogenesis.

Results as compared to the original objectives

The results of this research were very similar to the objectives in the original proposal in that Dr. Apple's group tested microalgal bio-fertilizers on different crop plants from Montana and measured the resulting biomass and other parameters of productivity. The results differed from what was proposed in that Montana Tech did not scale up to larger, outdoor studies as there was only one complete growing season for the project. Still, Montana Tech was able to conclude that both PW-95 and Cyanobacteria are effective as bio-fertilizers, especially for wheat.

Impact

Long-term Impact

The knowledge that PW-95, a microalga from the coal bed methane ponds and A. cylindrica, a nitrogen-fixing cyanobacteria, have strong potential to be used as biofertilizers for crop plants of Montana, and especially for wheat will likely have an impact in two key areas. One is that the microalgae will be a renewable product from the water of the coal bed methane ponds and another is that microalgae and cyanobacteria can be developed into relatively inexpensive and essentially self-sustaining, sources of biofertilizers. Importantly, use of the nitrogen-fixing A. cylindrica as fertilizer will preclude the need for adding extra nitrogen to crops. This has the possibility of major environmental and economic impacts.

A current experiment on the influence of cyanobacterial biofilms on soil temperature, moisture, and electrical conductivity will likely provide useful data that can be used to determine whether and how biofilms influence soil geochemistry.

Future activities extending beyond the life of the original MREDI grant

If funded, a proposal submitted to the National Science Foundation with Matthew Fields as PI will provide valuable support for the continuation of this and related research on biofilms, microalgae, bio-fertilizers, and crop plants.

A new NSF project titled "Sustainable socio-economic, ecological, and technological scenarios for achieving global climate stabilization through negative CO_2 emission policies" will continue this work in Dr. Peyton's group. The proposed work provides much needed insight into scientific, policy, and stakeholder requirements to better develop greenhouse gas mitigation scenarios for Montana that can be designed to prevent 'dangerous' climate change without creating conflicts with food security and clean energy. Our proposed research provides the first comprehensive evaluation of the effects that a global bio-energy with carbon capture and storage (BECCS) economy may have in a region where rapid land-use transitions and CCS activities are underway. New sustainable technologies, including the potential use for biofertilizer, will be evaluated in field experiments and the results scaled regionally. Outcomes from this new scientific research will lead to new insights into how biogeochemical modeling, agricultural economics, carbon-capture and sequestration, water, and biodiversity research can be used within an integrated framework.

Metrics

Total additional grants received

Two additional grants were received.

An undergraduate student, Joe Natale, was award the Montana Tech Summer Undergraduate Research Fellowship (SURF).

National Science Foundation Award Number:1632810; "Sustainable socio-economic, ecological, and technological scenarios for achieving global climate stabilization through negative CO_2 emission policies", Principal Investigator: Paul Stoy; Senior Faculty: Peyton and Spangler. Start Date: 08/01/2016; Award Amount: $6,000,000. Focused on sustainability in Montana's upper Missouri River Basin.

Total additional grants in progress

One additional grant is in progress.

National Science Foundation: Food, Energy, Water Nexus, Matthew Fields (PI)

Number of partnerships formed (private and public sector)

Three partnerships were formed.

Plant material for the experiments were obtained from the Potato Lab of MSU and Northern Seed Company.

A partnership with Summit Energy Company was formed to study the coal bed methane ponds.

Jobs created/supported:

- Students: 5
- Faculty: 4

Publications

Zhou, X., Zhou, Z., Miao, J., Apple, M., and Spangler, L. 2017. Extraction of surface water area of coal-bed methane water ponds from Google Earth images by a novel multi-component algorithm. International Society for Photogrammetry and Remote Sensing (ISPRS) Journal of Photogrammetry and Remote Sensing. Submitted for review.

Presentations

Ogunsakin, O. and Apple, M. 2017. Evaluation of the fertilization properties of PW95 - a green alga isolate from the CBM ponds in Montana. Abstract accepted for presentation at the National Society of Black Engineers Conference, March 2017, Kansas City, Missouri.

Ogunsakin, O., Apple, M., Zhou, X., Peyton, B., and Spangler, L. 2016. Green Algae from Coal Bed Methane Ponds as a Source of Fertilizer for Economically Important Plants of Montana. American Geophysical Union Fall Meeting, December 12-16, 2016, San Francisco, California.

Ogunsakin, O., and Apple, M. 2016. Use of Microalgal biomass from coal bed methane ponds as a biofertilizer for economically important crops of Montana. PNWIS, Air and Waste Management, October 5-7, 2016, Juneau, Alaska.

Zhou, X., Zhou, Z., Apple, M, and Spangler, L. 2016. Monitoring of coalbed water retention ponds in the Powder River Basin using Google Earth images and an Unmanned Aircraft System. American Geophysical Union Fall Meeting, December 12-16, 2016, San Francisco, California.

Objective 6

Develop methods to stimulate repeated methane production in coal bed methane (CBM) projects.

This objective was performed by Dr. Matthew Fields (MSU) and Dr. Xiaobing Zhou (Montana Tech). Dr. Fields group performed repeated stimulations of coal-dependent methanogenesis with algal extract produced from algal biomass grown in CBM production water. Dr. Zhou’s group using true-color images, developed a maximum uniformness/isotropy based multi-component algorithm for feature extraction such as water and the exposed lined area of CBM ponds.

As the byproduct of coal-bed methane production in the Montana Powder River basin, CBM water is extracted and stored in lined CBM ponds to prevent it from infiltrating into ground water because of its relatively high sodium adsorption ratio. Refilling of the CBM water in these ponds depends on the water surface level that changes due to evaporation, rainfall, or snowfall events (assuming no infiltration). These ponds have native microalgae that can be stimulated for CBM gas production, and microalgae growing in the ponds can be used for biofuel and biofertilizer, etc. Dr. Zhou’s group developed a maximum uniformness/isotropy based multi-component algorithm for feature extraction such as water and the exposed lined area of CBM ponds extraction from true-color images. This algorithm was validated using in situ field Global Positioning System (GPS) measurements. The total water area of all CBM ponds in the Montana Powder River Basin is estimated to be 172,807.34 m2, as extracted from high-resolution (resolution of 0.20~0.34 m) Google images and regular color images collected by a camera drone. Hyperspectral reflectance and Raman scattering spectra were measured using a laser beam of 457 nm wavelength as the excitation light of coal bed methane algal isolates PW-95 (Neospongiococcum sp.), Nanochloropsis gaditana (Chlorella vulgaris), and Anabaena cylindrica (nitrogen-fixing cyanobacteria). The algae were isolated from CBM water by Montana State University researchers (Dr. Robin Gerlach and Dr. Brent Peyton’s groups) and cultured in the laboratory by Dr. Martha Apple’s group (Montana Tech). These spectral data can be used to characterize the different algal species.

Accomplishments

Accomplishments of the objective

Repeated stimulations of coal-dependent methanogenesis

Comparison of nutrient amendments to stimulate coal-dependent methanogenesis

Slow rates of coal-to-methane conversion limit biogenic methane production from coalbeds. The studies presented here show that rates of coal-to-methane conversion can be increased by the addition of small amounts of organic amendments. Algae, cyanobacteria, yeast cells, and granulated yeast extract were tested at two concentrations (0.1 and 0.5 g/L), and similar increases in total methane produced and methane production rates were observed for all amendments at a given concentration. In 0.1 g/L amended systems, the amount of carbon converted to methane exceeded the amount of carbon added in the form of amendment, indicating enhanced coal-to-methane conversion through amendment addition. The amount of methane produced in the 0.5 g/L amended systems did not exceed the amount of carbon added. While the archaeal communities did not vary much, the bacterial populations appeared to be strongly influenced by the presence of coal when 0.1 g/L of amendment was added; at an amendment concentration of 0.5 g/L, the bacterial community composition appeared to be affected most strongly by the amendment type. Overall, the results suggest that small amounts of amendment are not only sufficient but possibly advantageous if *in situ* coal-to-methane production is to be promoted.

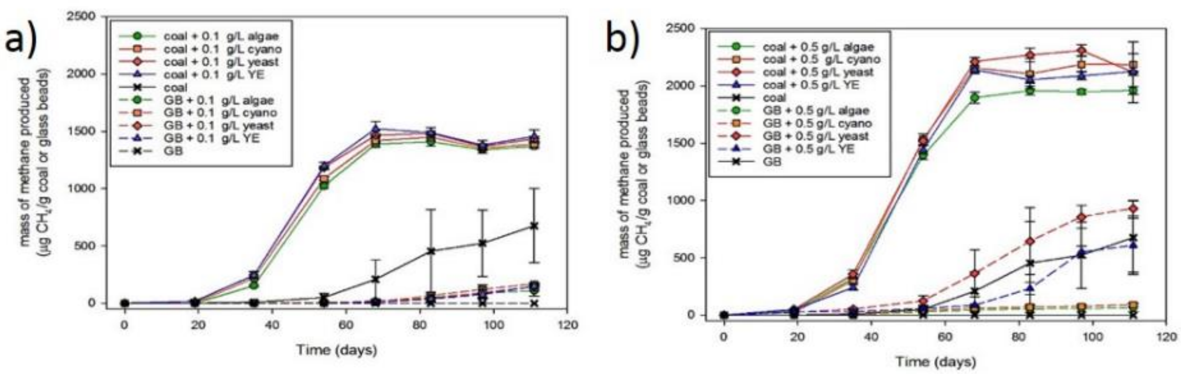

Figure 31. Methane production over time for (a) unamended and 0.1 g/L amended treatments and (b) unamended and 0.5 g/L amended treatments showing the cumulative methane produced at each sampling day. The methane produced is the sum of methane measured in the headspace and what can be assumed to be dissolved according to Henry's law. It does not include methane that may be sorbed to the coal or glass beads. Error bars represent 1 standard deviation for triplicates of each treatment.

Results demonstrated that algal and cyanobacterial extracts could stimulate coal-dependent methanogenesis to a similar extent as yeast extract.

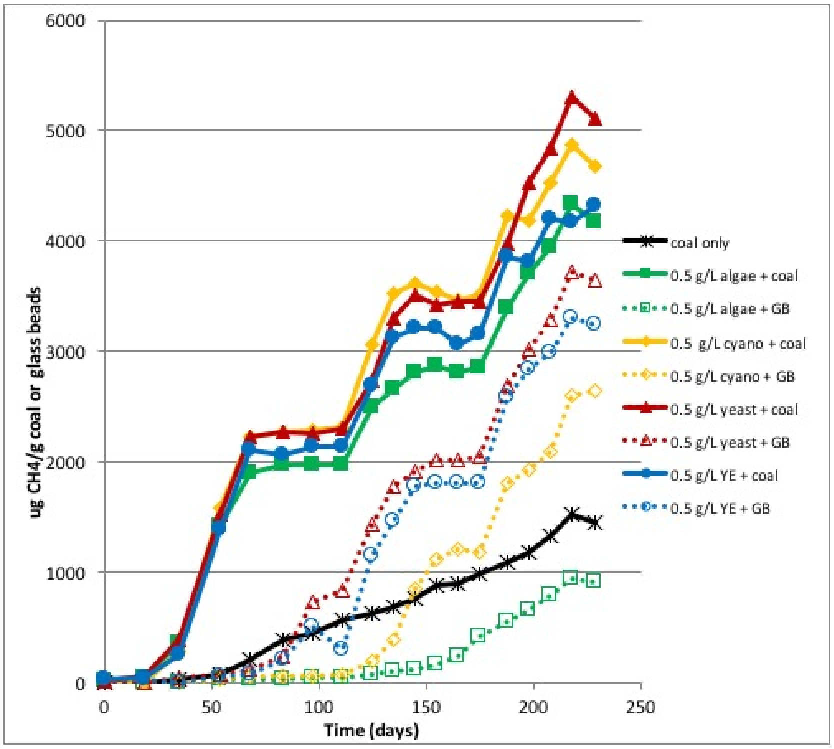

Figure 32. Four nutrient sources were tested for successive stimulations: algae, cyanobacteria, yeast, and yeast extract (all at 0.5 g/l). All nutrients were able to re-stimulate the same coal, but the amount of additional methane produced per amount of nutrient added decreased. In addition, there was a large difference between the ability of algae extract to stimulate successive coal-dependent methanogenesis over yeast or yeast extract.

Sequential stimulation of methane production.
Sequential stimulations were then tested with the same coal. Results suggest that coal-dependent methanogenesis can be sequentially stimulated; however, the coal-dependent nature of the methane production appears to subside (*i.e*., the evolving community begins to utilize more of the added nutrients and less of the coal). Algal and cyanobacterial extract appears to perform better in terms of subsequent stimulations for coal-dependent methanogenesis, as indicated by the calculated extent of carbon from coal versus added nutrient. Algal extract performs the best in these regards, after two re-stimulations the amount of methane from algal extract plus coal is more than yeast extract plus coal.

The results demonstrate that sequential stimulations under batch conditions are possible, and that algal extracts perform better in terms of promoting continued coal-dependent methanogenesis.

Algorithm for feature extraction such as water and exposed lined area of CBM ponds

Characterization of CBM ponds in the Montana Powder River basin using high-resolution true color satellite or airborne images

A novel feature extraction algorithm was developed that is based on color space transformation, k-means clustering analysis, connectivity, and uniformness and isotropy to extract water body and the exposed lined area of CBM ponds. In a field campaign in July 2016 to the research site, a drone was flown over four ponds to collect regular images over the ponds, along with GPS in situ measurements along the edges of the ponds. The algorithm was applied to high-resolution (0.20~0.34 m) Google (red, green, and blue (RGB)) images and the images collected from an unmanned aircraft system (UAS) to extract the water area and exposed lined area. This was done to so that water surface level and the liner conditions could be monitored using true color images from a regular camera or true color images formed from visible (RGB) bands of satellite images. Figure 33 shows an example of the intermediate and final outputs of the algorithm from an input image covering the John Wayne pond. The final output can include the extracted water body and the area of exposed liner. Figure 34 shows the ten CBM ponds in the Montana Powder River basin that are actively maintained by Summit Gas Resources, Inc. of Wyoming. These images are the input images for water and exposed lined area extraction. Figure 34 shows the ten extracted water bodies. The area of water, the exposed lined area extracted for each pond, and in situ measurements are shown in

Table 4. The algorithm was validated using in situ GPS measurements along four out of the ten CBM ponds in the Montana Powder River basin. The difference between the image-extracted water area and the measured area is within 3.0%. The total water area of all CBM ponds in the Montana Powder River basin is estimated to be 172,807.34 m2. A manuscript was formed based on this accomplishment and has been submitted to an international photogrammetry and remote sensing journal.

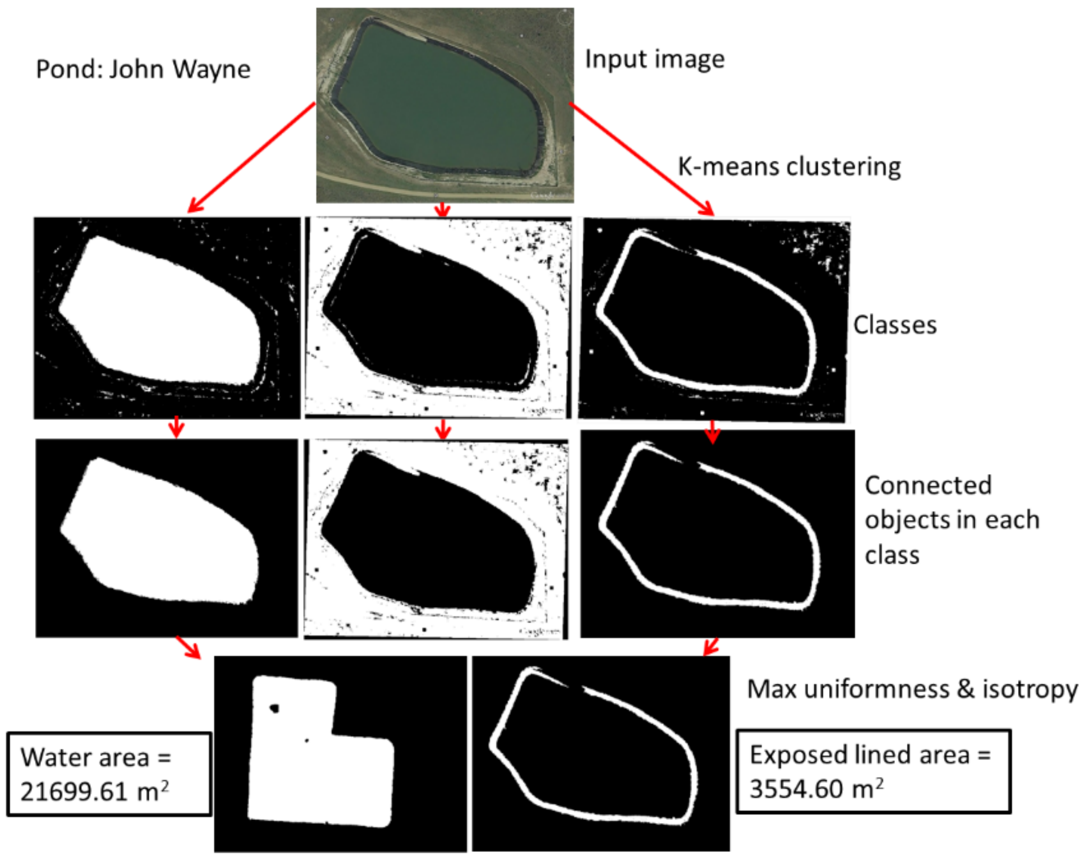

Figure 33. A sequence of intermediate and final output images during the extraction of water area and the exposed lined area using the Google Earth image of the John Wayne pond as the input.

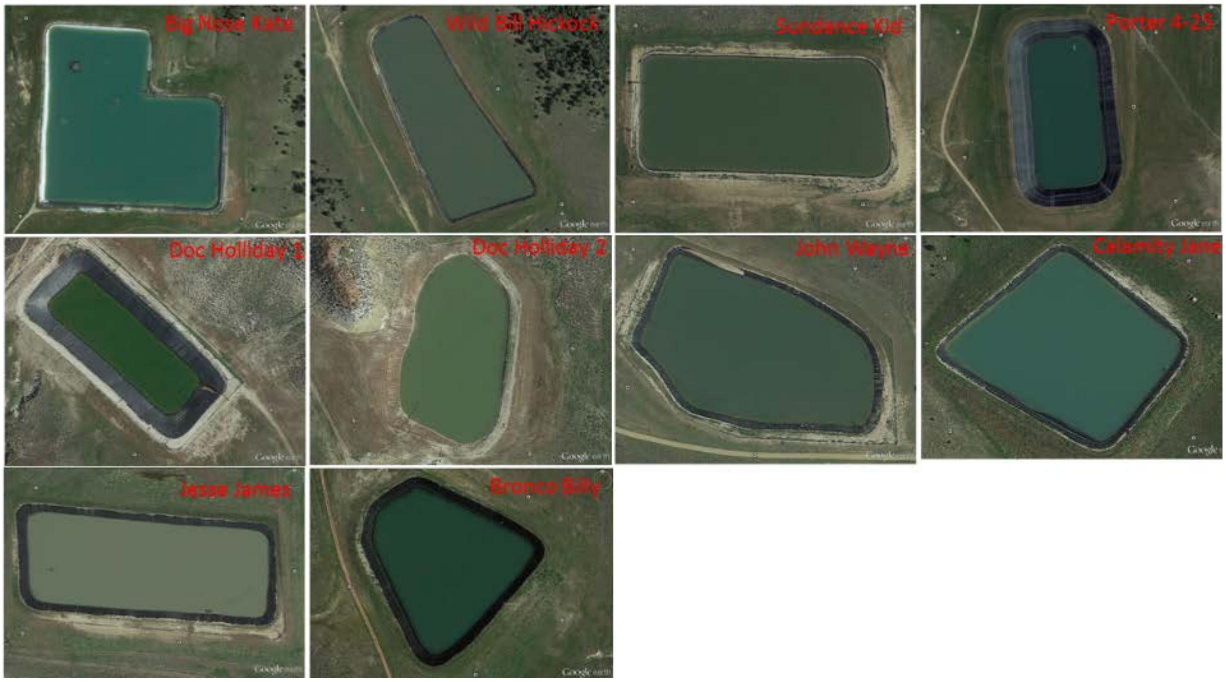

Figure 34. The ten georeferenced input images (0.20~0.34 m). The original images are in JPG format. After georeferencing, the files were converted to GeoTiff format.

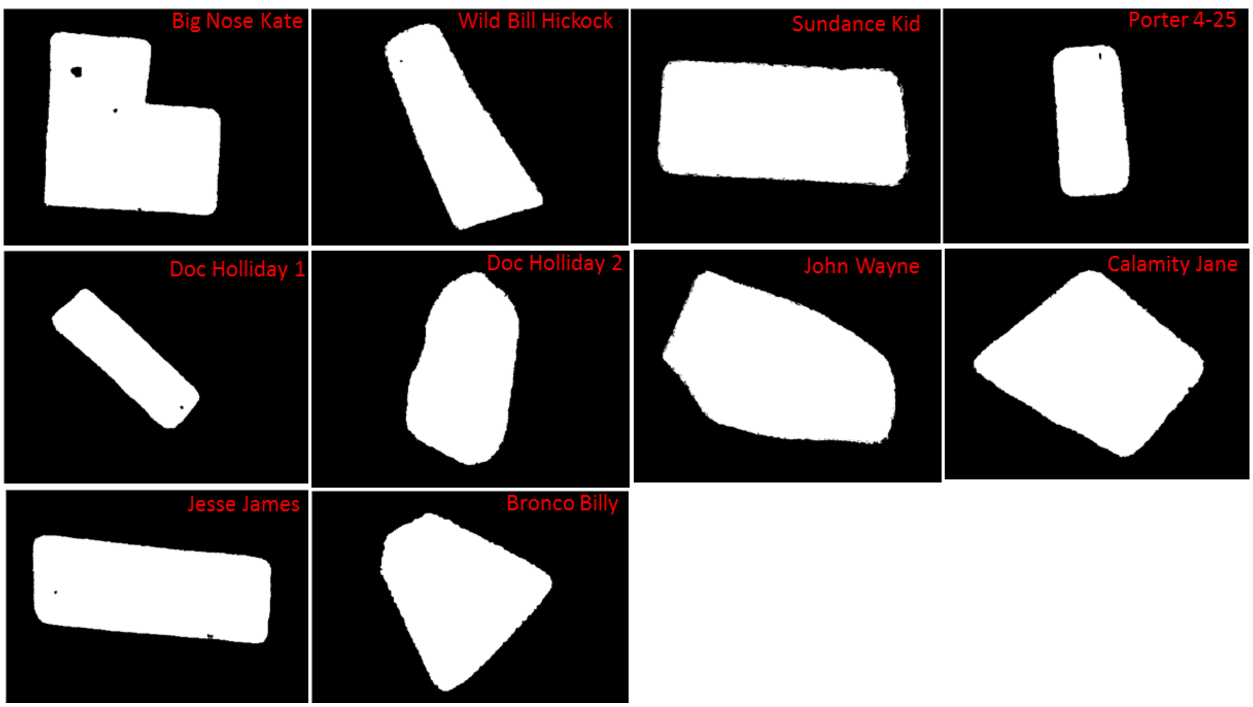

Figure 35. The water bodies extracted from the input images shown in Figure 34.

Table 4. Water area and exposed lined area extracted from Google Earth images and measured from GPS survey (NA = not available).

Pond Name	Image resolution (m)	Extracted water (m2)	Extracted lined (m2)	Measured (m2)	ARE Error
Big Nose Kate	0.34	50695.08	5600.41	50061.90	1.26%
Wild Bill Hickock	0.28	20297.18	2213.91	NA	NA
Sundance Kid	0.20	19508.60	978.79	NA	NA
Porter4-15	0.24	10301.76	8778.61	NA	NA
Doc Holiday 1	0.21	6213.16	6910.63	NA	NA
Doc Holiday 2	0.16	6943.20	NA	NA	NA
John Wayne	0.21	21699.61	3554.60	23538.59	0.26%
Calamity Jane	0.17	10902.39	2201.78	12928.21	1.36%
Jesse James	0.19	13903.27	3633.87	NA	NA
Bronco Billy	0.21	12343.09	4501.66	16368.60	2.91%

Hyperspectral spectra of CBM algae and the implication for hyperspectral remote sensing of microalgae in CBM ponds

Spectra-radiometers (350-1050nm) was used in Montana Tech's Remote Sensing Laboratory to measure the spectral reflectance at a total of 701 bands of each of the microalgae species (Anabaena cylindrical, N. gaditana, and PW-95). For each species, samples of different concentrations were prepared and the spectral reflectance was measured. Graduate student Zhaoming Zhou focused on the data collection, analysis, and modeling. This is for Zhaoming's thesis research and the work will be continued up until his graduate in December 2017. The objective of this task was to establish relationships between spectral signature and microalga concentration so that remote sensing of microalga and its concentration is possible.

Figure 36 shows the laboratory setting for the hyperspectral measurement of microalgae using the hyperspectral spectroradiomters. Figure 37 shows the spectral reflectance of Anabaena cylindrica, N. gaditana, and PW-95 from top to bottom panels isolated from CBM water and cultured in the laboratory. The spectral signatures are different for the different species especially when the concentration is relatively low, even though they have similar color by vision. For each specific species, the lower the concentrations, the higher the reflectance in the visible bands between 400 and 700 nm wavelength. The implication of the difference is that these species can be discriminated using hyperspectral images. A MATLAB program was developed to automatically select the band out of the 701 bands whose spectral reflectance is best linearly correlated with the concentration values in percentage. It was found that the red band of 704 nm for Anabaena cylindrica, the near-infrared band of 928nm for N. gaditana and the green band of 573 nm for PW-95 have the highest correlation coefficient and coefficient of determination with the corresponding microalgae concentration. Figure 38 (a) shows the linear relationships between spectral reflectance versus concentration of these bands. The implication of these relationships is that the reflectance at the optimized band for a specific microalgae species can be used for the estimation of the cell concentration once a field calibration is made.

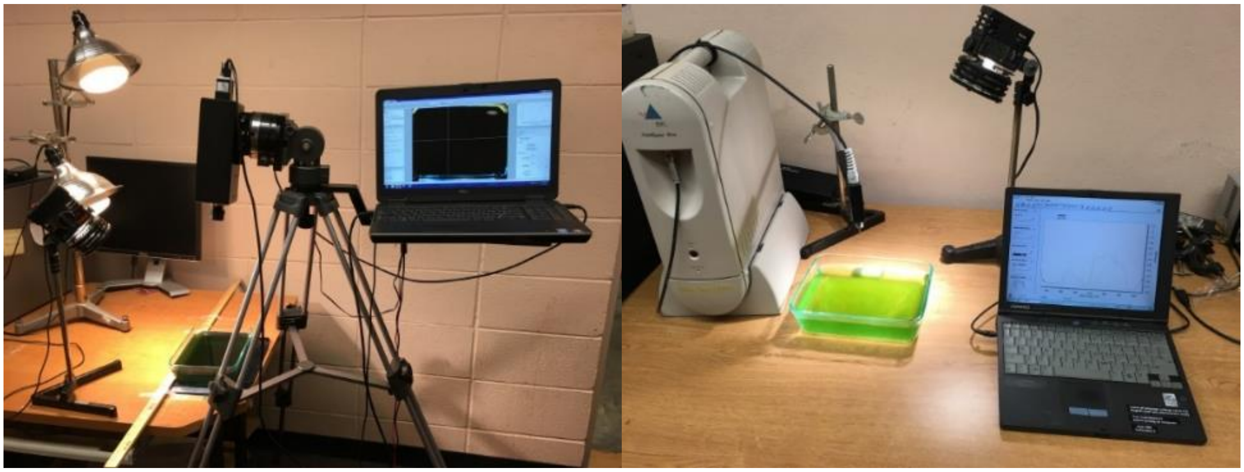

Figure 36. Hyperspectral systems used to spectral signature measurement of microalgae. Left-panel: measurement on filamentous cyanobacteria (Anabaena cylindrica) and right panel: spectral measurement on green algae (PW-95).

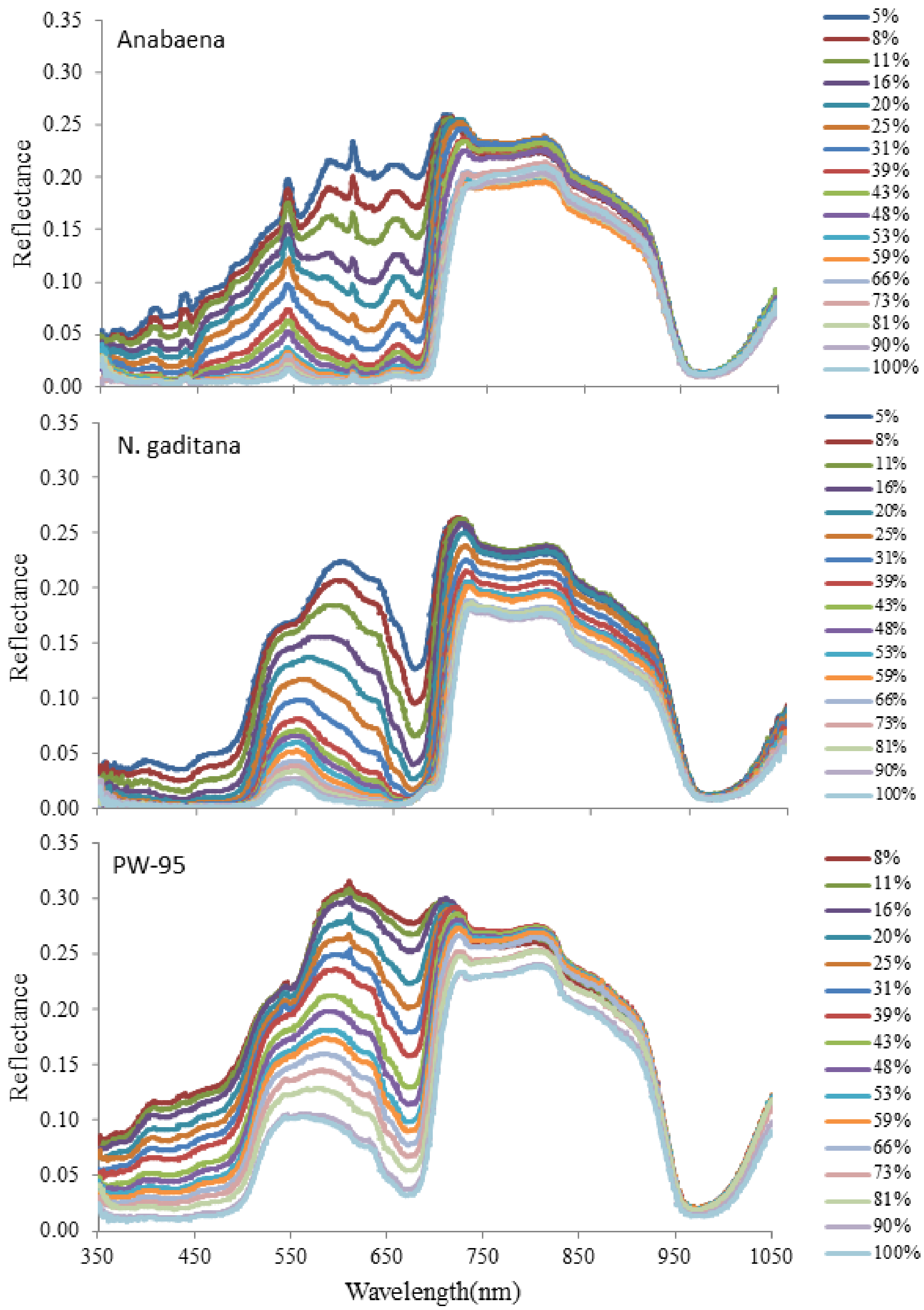

Figure 37. Hyperspectral reflectance of Anabaena cylindrica, N. gaditana, and PW-95 from the top to bottom panels, respectively at different concentrations. 100% concentration of Anabaena cylindrica, N. gaditana, and PW-95 means the cell concentration of 181,000 cells/ml, 26,507.25 cells/ml, 296,250 cells/ml.

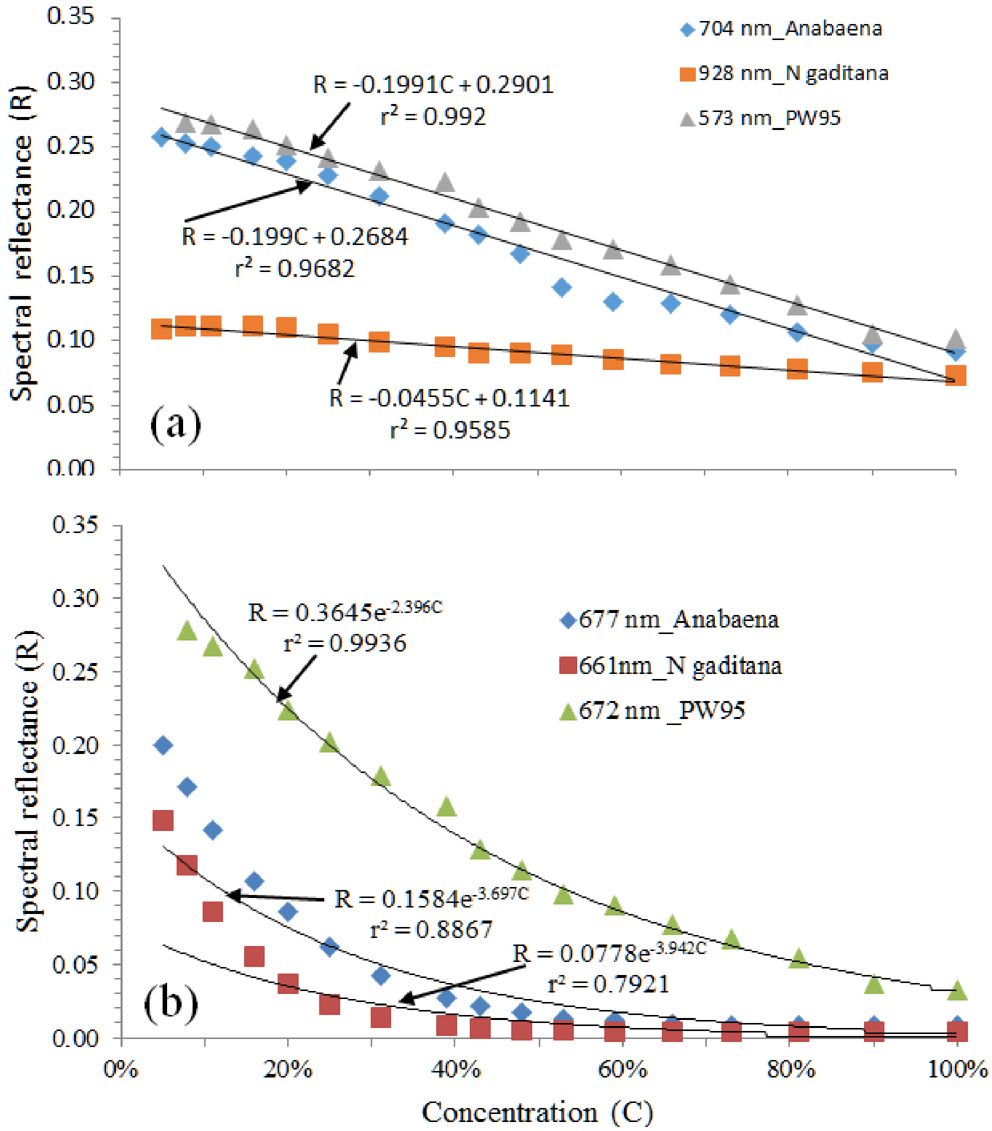

Figure 38. Optimized band reflectance versus cell concentration in percentage.

Another MATLAB program was developed to automatically select the absorption band (minimum spectral reflectance) within the spectral region (500nm ~ 700nm) for each concentration value. It was found that PW-95 has the most stable band (672 nm) when the concentration varies. N. gaditana's absorption red band downshifts from 672nm at lowest concentration (5%) to 648 nm at highest (100%) concentration as the concentration increases. The average value for the absorption band is 661m. The variability of the position of the absorption red band for Anabaena cylindrica when the cell concentration changes lies between the PW-95 and N. gaditana, with the average position being at 677 nm. Figure 38 (b) shows the exponentially decreasing relationships between the spectral reflectance at these bands and the cell concentration. The high coefficients of correlation and determination indicate that at these absorption bands, the absorption satisfies the Beer's law, especially for the PW-95 species. The significance is that the spectral reflectance at these absorption bands can also be used to estimate the concentration of the cell concentration of these species once field calibration is made. Both methods shown in Figure 38 provide useful tools for the algorithm development for remote sensing of microalgae concentrations once the microalgae species is discriminated using the spectral signatures shown in Figure 37.

Characterization of CBM Microalgae by Raman Spectroscopy

Laser Raman Spectroscopy (LRS) has proven to be a powerful and versatile characterization tool used for determining chemical composition of material systems or biological systems such as microalgae. Raman spectra of the CBM algae were collected to characterize the three species (Anabaena cylindrica, N. gaditana, and PW-95), The spectra were collected using the Raman scattering instrument recently purchased by the Montana Tech's Metallurgical & Materials Engineering Department and Center for Advanced Mineral and Metallurgical Processing. The objective was to look at the chemical compositions of microalgae and to better understand the hyperspectral signature of various microalgae species. The Raman spectroscope used is a high-end multi wavelength system. However the laser of 457 nm was found to produce the best Raman spectra for the CBM microalgae. The CBM microalgae were desiccated before the scanning. Figure 39 shows the Raman spectra of Anabaena cylindrica, N. gaditana, and PW-95 in top to bottom panels, respectively, after removing baselines and noise using the Savitsky-Golay filter size five and polynomial order of three. Peaks between 800-1600 cm-1 are mainly due to lipid molecules. It seems that Anabaena cylindrica and N. gaditana show more lipid molecules than PW-95. Anabaena cylindrica has the most. All three CBM microalgae show a strong peak at 1526 cm-1 (1521 cm-1 for Anabaena) that is caused by chlorophyll a. If chlorophyll b, there should be a second peak between 1600~1700 cm-1. There is a small peak between 1600~1700 cm-1 For N. gaditana, thus it is inferred that N. gaditana contain a small portion of chlorophyll b. The peak at 3048-3049 cm-1 may be due to double bond vibration in long chain unsaturated hydrocarbons within the lipid molecules. The peak at 2680 cm-1 (2677 cm-1 for Anabaena cylindrica) may be due to carotenoid. Further research on the analysis of the Raman spectra is needed and will continue.

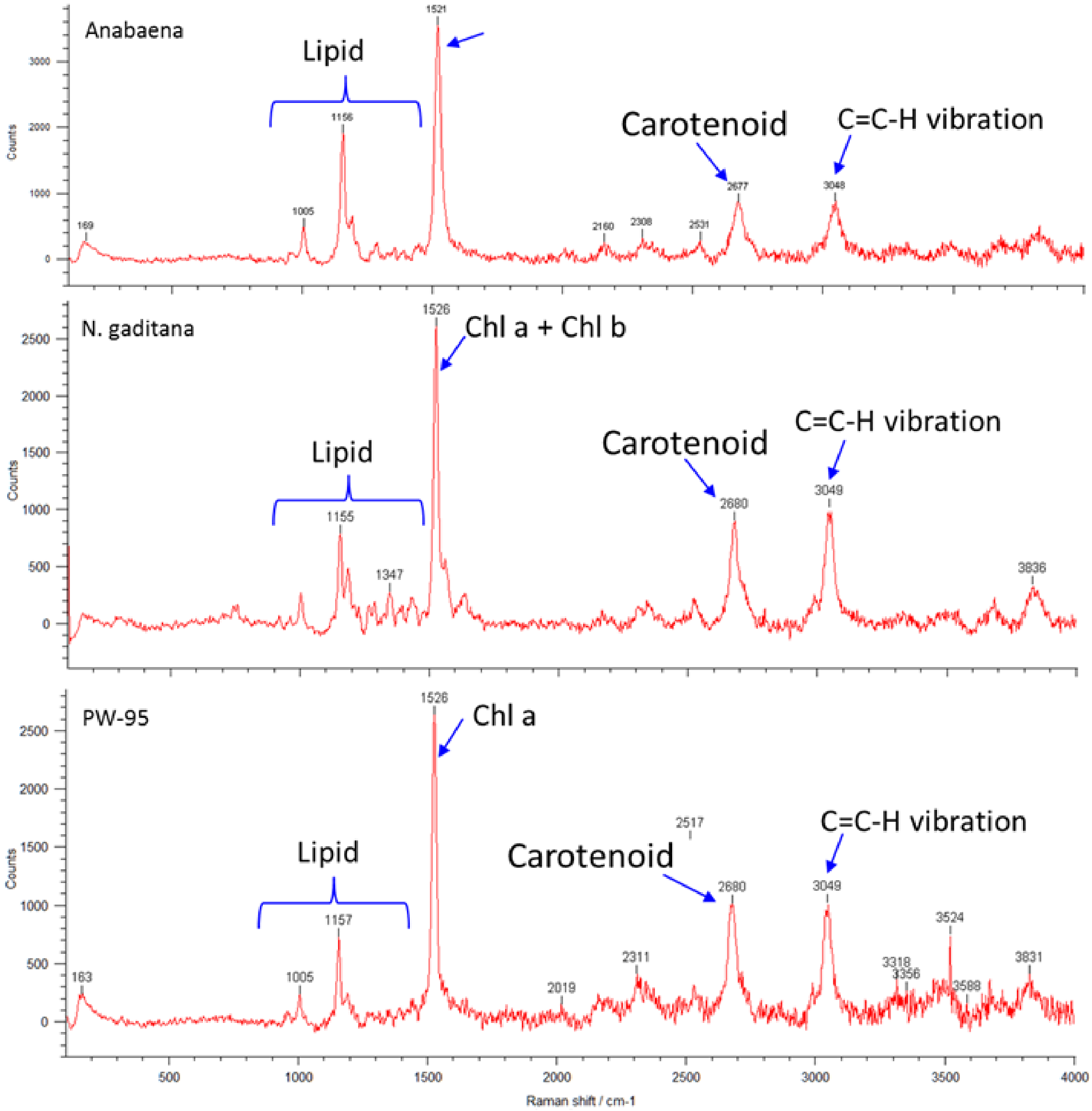

Figure 39. Raman spectra of the Anabaena cylindrica, N. gaditana, and PW-95 from top to bottom panels, respectively.

Results as compared to the original objectives

Responsible use of coal

The described work contributes to the utilization of Montana's vast coal reserves in an environmentally sound manner by investigating the use of locally-produced algal extracts in CBM production water to stimulate additional coal-dependent methane from existing well infrastructure. MSU demonstrated that algal extract could be used to stimulate and re-stimulate Powder River Basin coal. In addition, the growth of a unique algal species in CBM production water demonstrates usefulness because it provides a use for a low-quality waste water. Different methods to harvest the algal biomass were compared with respect to pre-processing for the stimulation of coal-dependent methanogenesis.

Raman Scattering Spectra

Almost all tasks as proposed in the original proposal were accomplished. Raman scattering spectra for the three species of the microalgae was additional research that was not originally included in the proposal. This work was a natural extension of the original objective, and the newly purchased Raman spectroscope at Montana Tech presented an opportunity to proceed.

Impact

Long-term Impact

The demonstrated results will be the basis to secure federal funds in partnership with MET, a Montana engineering business to demonstrate field-scale application. MSU has demonstrated alternative mechanisms to utilize existing infrastructure common in economically-depressed areas of Montana. The produced results will help inform techno-economic analysis for potential field-scale applications.

The algorithm for feature extraction from true color images can be used for feature extraction for surface water hydrology, water resources monitoring, and water surface area estimation such as water bodies from regular RGB images. The color images can easily be taken with a regular camera which has a higher resolution than near-infrared and infrared bands of imaging systems on board a specific satellite.

The acquisition of hyperspectral reflectance of the CBM microalgae and subsequent analysis will help to understand remote sensing and monitoring of CBM microalgae especially if they will be grown in the CBM ponds for their value-added products such as biofuel, biofertilizer, and biofilter for contaminated water processing.

LRS spectra and analysis to identify chemical compositions will help to identify the species that are candidates for oil production (high lipid content) or nutrients (high protein content), etc. They will also help us understand the chemical composition and hyperspectral remote sensing to monitor and quantify the concentration of microalgae in a CBM pond.

Future activities extending beyond the life of the original MREDI grant

The funds supported the continued partnership with the US Geological Survey (USGS) which has interests in securing additional funds to understand field-scale application of sustainable CBM production. The relationship with the USGS allows access to a unique field site near Birney, MT and provides expertise from the Helena, Denver, and Reston offices of the USGS.

Montana Tech's feature extraction algorithm will be applied for surface water hydrology. Dr. Zhou's group will write proposals on the application of the algorithm for surface water resources exploration and management based on images collected from UASs as the data acquiring platforms.

Spectral analysis and modeling studies of the hyperspectral data collected for the different CBM microalgae will continue. The possibility of growing CBM microalgae in the CBM ponds and subsequent monitoring from space- or air-borne platforms, especially using UASs will also be explored.

The Raman spectra analysis and identification of the compositions from such spectra and their relations with hyperspectra will continue. This will eventually help to understand remote sensing of CBM microalgae. At the same time, the Center for Advanced Mineral and Metallurgical Processing at Montana Tech will soon purchase a high resolution scanning electron microscope (SEM). The SEM will be used to investigate the lip content of these microalgae species and optimize which species is the best for biofuel production.

Metrics

Total additional grants received

Two additional grants were received

MSU in partnership with MET was awarded a DOE grant in the amount of $650,000. The project is titled Optimization, Scale-up, and Design of Coal-Dependent Methanogenesis in Preparation for *in situ* Field Demonstration.

MSU also received a Census of Deep Life Sequencing Program award titled valued at approximately $30,000. The project is titled Metagenomic Sequencing of Sulfate Transition Zones Located in Recalcitrant Carbon Rich Coal Seams.

Number of partnerships formed (private and public sector)

Four partnerships were formed: MET; Shell Global; Summit Gas Resources, Inc.; and USGS in: Helena, Montana; Denver, Colorado; and Reston, Virginia.

Commercial Products Produced

One computer program for feature extraction from true color images was produced.

Jobs created/supported:

- Research scientists/engineers: 1
- Technicians: 1
- Students: 3
- Post-docs: 2
- Visiting Scholar: 1

Publications

Hodgskiss, L.H., J. Nagy, E.P. Barnhart, A.B. Cunningham, and M.W. Fields. 2016. Cultivation of a native alga for biomass and biofuel accumulation in coal bed methane production water. Algal Res. 19:63-68.

Davis, K.J., S. Lu, E.P. Barnhart, A.E. Parker, M.W. Fields, and R. Gerlach. Type and amount of organic amendments affect enhanced biogenic methane production from coal and microbial community structure. Fuel (in review)

Davis, K.J., M.W. Fields, and R. Gerlach. Carbon conversion during enhanced microbial methane production from coal with repeated organic amendment. (in preparation)

Zhou, X., Z. Zhou, J. Miao, M. E Apple, and L. Spangler. Characterization of coal-bed methane water ponds using true-color images by developing a novel maximum uniformness/isotropy based multicomponent algorithm. ISPRS Journal of Photogrammetry and Remote Sensing, submitted, July 2017.

Presentations

Zhou, X., Z. Zhou, M. E Apple, and L. Spangler, Monitoring of coalbed water retention ponds in the Powder River Basin using Google Earth images and an Unmanned Aircraft System. NS42A-04 (poster

presentation), American Geophysical Union Fall meeting, December 12-16, 2016, San Francisco, California.

Ogunsakin, O. R., M. E. Apple, X. Zhou, B. Peyton, and L. Spangler, Green Algae from Coal Bed Methane Ponds as a Source of Fertilizer for Economically Important Plants of Montana. NS42A-04 (poster presentation), American Geophysical Union Fall meeting, December 12-16, 2016, San Francisco, California.

Zhou, Z., and X. Zhou, Accuracy comparison in mapping water bodies using Landsat images and Google Earth Images. NS42A-04 (poster presentation), American Geophysical Union Fall meeting, December 12-16, 2016, San Francisco, California.

Cumulative Metrics for All Objectives

Total additional grants received: 11, >$9,424,930

Total additional grants in progress: 13, >$1,690,000

Number of partnerships formed (private and public sector): 16

Number of new Montana businesses created: 1

Patents awarded or in progress: 1

Commercial products developed: 3

Jobs created/supported

- Private sector: 2
- Research scientists/engineers: 4
- Technicians: 4
- Students: 16
- Faculty: 8
- Post-docs: 4
- Visiting Scholar: 1

Publications: 13

Beser, D, West C, Daily, R, Cunningham, A, Gerlach, R, Fick, D, Spangler, L and Phillips, AJ. Assessment of ureolysis induced mineral precipitation material properties compared to oil and gas well cements. American Rock Mechanics Association 51st Annual Meeting Proceedings, June 25-28, 2017, San Francisco, CA. (Paper # 588) (Accepted).

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Davis, K.J., S. Lu, E.P. Barnhart, A.E. Parker, M.W. Fields, and R. Gerlach. Type and amount of organic amendments affect enhanced biogenic methane production from coal and microbial community structure. Fuel (in review)

Fairweather, Stacey; J. Blank, B. Winkelman and G. Nicholas. 2017. "A Story Map for Carbon Utilization and Storage Potential in Montana". Developed by Montana State University, Energy Research Institute and funded by the Montana Research and Economic Development Initiative.

Feder, M, Morasko, V, Gerlach, R, Phillips, AJ. Plant-based ureolysis kinetics and urease inactivation at elevated temperatures for use in engineered mineralization applications (in preparation).

Hodgskiss, L.H., J. Nagy, E.P. Barnhart, A.B. Cunningham, and M.W. Fields. 2016. Cultivation of a native alga for biomass and biofuel accumulation in coal bed methane production water. Algal Res. 19:63-68.

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Thane, A, Troyer, E, Gallagher, B, Phillips, AJ. Remediation of coal combustion residuals using microbially-induced calcite precipitation. World of Coal Ash Conference 2017 Proceedings (accepted)

Zhou, X., Z. Zhou, J. Miao, M. E Apple, and L. Spangler. Characterization of coal-bed methane water ponds using true-color images by developing a novel maximum uniformness/isotropy based multicomponent algorithm. ISPRS Journal of Photogrammetry and Remote Sensing, submitted, July 2017.

Zhou, X., Zhou, Z., Miao, J., Apple, M., and Spangler, L. 2017. Extraction of surface water area of coal-bed methane water ponds from Google Earth images by a novel multi-component algorithm. International Society for Photogrammetry and Remote Sensing (ISPRS) Journal of Photogrammetry and Remote Sensing. Submitted for review.

Presentations: 34

Feder, M, Phillips, AJ. Gerlach, R. "Advancing ureolysis driven mineral sealing strategies for environmental engineering applications." Platform Presentation. Goldschmidt Conference. June 26-July 01, 2016, Yokohama, Japan.

Filanoski, B, Phillips, AJ. "Microbial Induced Calcium Carbonate Precipitation of Coal Combustion Residuals". 31st Annual National Conference on Undergraduate Research, University of Memphis, April 6-8, 2017, Memphis, Tennessee.

Filanoski, B, Troyer, E, and Phillips AJ. "Microbial Induced Calcium Carbonate Precipitation of Coal Combustion Residuals". Montana State University Undergraduate Research Celebration, April 2016, Bozeman, Montana.

Filanoski, B, Troyer, E, and Phillips AJ. "Microbial Induced Calcium Carbonate Precipitation of Coal Combustion Residuals". Montana State University McNair Scholars Program Research Celebration, November 2015, Bozeman, Montana.

Frieling Z, Akyel A, Gerlach R, Phillips, AJ. "Urease Transport and Distribution to Better Understand its Subsurface Behavior". MSU Student Research Celebration, April 21, 2017, Bozeman, Montana.

Gerlach R, Phillips., AJ; Cunningham, AB, Spangler, L. "Biofilm-Mediated Mineral Precipitation Technology – From the Microscale to the Field-Scale." Platform Presentation. Goldschmidt Conference. June 26-July 01, 2016, Yokohama, Japan.

Gerlach, R, Phillips, AJ, Cunningham, AB, Spangler, L. "Controlling Fluid Flow in the Subsurface through Ureolysis-Controlled Mineral Precipitation", American Geophysical Union Fall Meeting, December 2016, San Francisco, California.

Gerlach, R. "Biocementation as an Advanced Well Remediation Technology –Technology Development from the Microscale to the Field-Scale." Invited Presentation. June 13, 2016, Los Alamos National Laboratory, New Mexicao.

Gerlach, R., Al B. Cunningham, Lee Spangler, Adrienne Phillips, Randy Hiebert, Jim Kirksey, Wayne Rowe, Richard Esposito, Bart Lomans, Andreas Busch (2016): Biocementation for Wellbore Integrity Restoration and Enhanced Resource Recovery. Platform Presentations at the RMF2016: 22nd Reservoir Microbiology Forum, November, 15/16, 2016, London, United Kingdom.

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Norton, D, Gerlach, R, Eldring, J, Thane, A, Hiebert, R, Cunningham, A, Spangler, L, Phillips, AJ "Visualizing and Quantifying Biomineralization in a Wellbore Analog Reactor". Geologic Society of America Annual Meeting, September 25-28, 2016, Denver, Colorado.

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Ogunsakin, O. R., M. E. Apple, X. Zhou, B. Peyton, and L. Spangler, Green Algae from Coal Bed Methane Ponds as a Source of Fertilizer for Economically Important Plants of Montana. NS42A-04 (poster presentation), American Geophysical Union Fall meeting, December 12-16, 2016, San Francisco, California.

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Phillips, AJ, Gerlach, R, Cunningham, AB, Spangler, L. “Wellbore Leakage Mitigation Using Advanced Mineral Precipitation Strategies” Department of Energy, Mastering the Subsurface through Technology Innovation & Collaboration: Carbon Storage & Oil & Natural Gas Technologies Review Meeting, August 17, 2016, Pittsburgh, Pennsylvania.

Phillips, AJ, Gerlach, R, Cunningham, AB, Troyer, E, Norton, D, Hiebert, R, Kirksey, J, Rowe, W, Esposito, R, and Spangler, L. “Biomineralization: A Strategy to Modify Permeability in the Subsurface”. Geologic Society of America Annual Meeting, September 25-28, 2016, Denver, Colorado.

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Stoick, E., and E. Lauchnor. Microbially Induced Calcium Carbonate Precipitation and Metal CO-Precipitation in Mine Influenced Water at the Carpenter-Snow Creek Mining District NPL Site. National Association of Abandoned Mine Lands Programs Annual Conference, September 25-28, 2016, Bozeman, Montana.

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Abbreviations

Ba	Barium
BECCS	Bio-Energy with Carbon Capture And Storage
$CaCO_3$	Calcium Carbonate
CBM	Coal Bed Methane
CCR	Coal Combustion Residuals
CO_2	Carbon Dioxid
CO_2-EOR	Carbon Dioxide Enhanced Oil Recovery
CSCMD	Carpenter-Snow Creek Mining District
DEQ	Montana Department of Environmental Quality
DNA	Deoxyribonucleic Acid
DOE	U.S. Department of Energy
EOR	Enhanced Oil Recovery
EPA	U.S. Environmental Protection Agency
EREF	Environmental Research and Educational Foundation
ERI	Energy Research Institute
GPS	Global Positioning System
ICP-MS	Inductively coupled plasma mass spectrometry
LCA	Least Cost Analysis
LRS	Laser Raman Spectroscopy
MET	Montana Emergent Technologies, Inc.
MICP	Microbially Induced Calcite Precipitation
MIW	Mine Influenced Water
MREDI	Montana Research & Economic Development Initiative
MSU	Montana State University
N	Nitrogen
NFC	Nitrogen Fixing Cyanobacteria
NPOC	Non-Purgable Organic Carbon
NSF	National Science Foundation
P	Phosphorus
PI	Principle Investigator
PW-95	*Neospongiococcum sp.*
RGB	Red, Green, Blue

SBIR	Small Business Innovation Research
Se	Selenium
SEM	Scanning Electron Microscope
SeO_3^{2-}	Selenite
SeO_4^{2-}	Selenate
Sr	Strontium
STTR	Small Business Technology Transfer
SURF	Summer Undergraduate Research Fellowship
UAS	Unmanned Aircraft System
USGS	US Geological Survey