Quarterly Report

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

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Enhancing Montana's Energy Resources

During the reporting period, the outreach team focused on tracking news and regulatory actions related to the Colstrip Power Plant. The Colstrip Power Plant is located in the town of Colstrip, Montana east of Billings. Colstrip has four generating units which are capable of producing 2,094 megawatts of electricity. Units 1 and 2 came online in 1975 and 1976 and Units 3 and 4 came online in 1984 and 1986. It is the nation's 8th-largest producer of greenhouse gases, emitting 13.5 million metric tons annually, according to the EPA. Units 1 and 2 are its oldest and biggest polluters. The ownership of the plant is divided among five companies: Talen Energy LLC, Puget Sound Energy Inc. (PSE), Portland General Electric Company (PGE), Avista Corporation, PacifiCorp, and NorthWestern Energy. Talen Energy is the operator of the plant. Over the past year, there have been several pieces of legislation in other states that will affect the future of the plant. The paragraphs below summarize the legislation and the impacts it may have on Colstrip.

Washington has passed legislation, Senate Bill 6248, to create a fund to pay for the eventual decommissioning and remediation costs of Units 1 and 2 which PSE owns 50% of, if they're closed after 2023. PSE has said shutting down the plants and cleaning them up would cost between $130 million and $200 million. The bill does not require the units to be closed nor does it set a timeline for shutdown. Separately, PSE must tell Washington state utilities regulators in early January its plan for the Colstrip units, including "a narrow window of dates" for the planned retirement of Units 1 and 2 as well as the costs. Closing the two Colstrip units would remove 614 megawatts of coal-generated electricity from the grid, equal to between 5 and 6 million tons of carbon-dioxide emissions annually.

Oregon passed the "Clean Electricity and Coal Transition" plan, Oregon Senate Bill 1546B. Electricity provided to customers of Pacific Power and Portland General Electric would be coal-free by 2030, with the exception of a small amount from PGE's ownership of Colstrip, which would be cut out of the Oregon mix no later than 2035. The bill also increases the Renewable Portfolio Standard (RPS) in Oregon to 50% in 2040. This increase is staged at 27% at 2025, 35% at 2030, 45% at 2035 and 50% at 2040. Consumer protections in the existing RPS law are maintained. There is also a safety valve that allows the Oregon Public Utility Commission (PUC) to temporarily suspend the requirement for a utility if meeting the RPS would conflict with grid reliability. The bill doesn't mention Colstrip specifically, but targets Pacific Power and Portland General Electric, both of which share ownership of Colstrip Units 3 and 4.

Both in Washington and Oregon, the drive to get out of Colstrip comes from consumers and environmentalists who want to decrease the dependence on coal-fired power because of worries about climate change and public health.

Talen Energy has put in notice to relinquish being the operator in two years or less. Another development is that an investment firm Riverstone LLC is going to buy Talen Energy – this won't change operation plans.

In June, a Missoula District Judge ruled that a lawsuit related to leaking groundwater at the Colstrip Power Plant pond can proceed. Three environmental groups are suing the state of Montana for failing to take action to remedy the water leakage problem that has existed for 30

years. The plant is leaking 300 gallons per minute totaling 200 gallons per year into groundwater in the Colstrip area. The environmental groups say that the water is laden with heavy metals and other contaminants from the air pollution controls at the plant. The contaminants are entering the groundwater from the leaking ponds.

On July 11, 2016, a settlement was reached for a separate lawsuit against the plant for Clean Air Act violations. The Sierra Club and Montana Environmental Information Center sued the Colstrip Power Plant owners for sulfur dioxide and nitrogen oxide emissions violations. The settlement calls for the closure of Units 1 and 2 by 2022 and for a plan to reduce emissions until the shutdown. A timeline for the shutdown has not been developed yet.

On July 14-15, 2016, Lindsey Tollefson attended the Energy Telecommunications Interim Committee (ETIC) meeting. The meeting agenda may be found here http://leg.mt.gov/content/Committees/Interim/2015-2016/Energy-and-Telecommunications/Meetings/July-2016/final-agenda-july.pdf . Representatives from Talen Energy and Pudget Sound Energy presented on the Future of Colstrip Units 1 and 2. The video and audio meeting minutes may be found here http://leg.mt.gov/css/Video-and-Audio/archives/av.asp. The legislators expressed great concern for the community of Colstrip and the future of energy production and transmission in the state of Montana. In response to the shutdown news for Units 1 and 2, the ETIC committee has drafted seven bills intended to protect Montana residents and businesses from the impact of the closure. The ETIC has requested public comments on the draft bills and they will discuss them further when they meet at their next meeting scheduled for September 8-9. As part of the stakeholder work, Lindsey Tollefson is reviewing the bills and will summarize them for the MREDI team members at their next quarterly meeting.

During the reporting period, Lindsey Tollefson also followed up with Gordon Criswell of Colstrip to plan to present at the next owners meeting in September. Mr. Criswell informed Lindsey that the agenda for the September meeting was full given the recent events and that we could try for their next meeting scheduled for November.

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 ERI biomineralization technology.

Quarter activities and accomplishments

Research continued to extend the temperature range for in situ mineral precipitation. Ureolysis kinetics as well as inactivation kinetics for a number of bacterial and plant-based ureases were determined. Thermal ureolysis kinetics were determined at increasing temperatures. A reactor system was designed and constructed to assess thermally induced calcium carbonate precipitation. Montana State University (MSU) and Montana Emergent Technologies (MET) personnel have had discussions with several oil and gas companies to evaluate possible applications as well as field deployment strategies in support of the development of biomineralization-based technologies. Work has begun in collaboration with MET on the design of a mobile laboratory that could be used to implement these mineral precipitation technologies in the field.

Hirings
One additional undergraduate student was hired this quarter, Zach Frieling, a senior pursuing a degree in Chemical and Biological Engineering at MSU. In addition, a graduate student, Kyle DeVerna, was recruited to work on the project. Postdoctoral researcher Dr. Marnie Feder, M.S. students Arda Akyel and Kyle DeVerna, and undergraduate students Zach Frieling and Vinny Morasko are working on the development of advanced mineral precipitation strategies and are studying the differences in material properties between abiotic, enzymatic and bacterially precipitated calcium carbonates.

Equipment Purchased
No equipment has been purchased to date.

Proposal *(leverage the overall MUS research enterprise)*
A Budget Amendment Request was submitted for DOE Project DE-FE0024296 "Methods to enhance wellbore cement integrity with microbially-induced calcite precipitation (MICP)". This budget amendment request was approved by DOE to add an additional $140,000 to the project for design and construction of a mobile laboratory. This mobile laboratory will be used in upcoming field demonstrations.

Milestones
A. September 2015-September 2016: Perform laboratory bench experiments to extend the temperature range for mineral precipitation, and thief zone plugging for enhanced oil recovery (EOR)
    a. The team has continued to assess the temperature range and upper temperature limits of jack bean meal urease (JBM). Cottonseed (CS), pigeon pea (PP) and soy bean (SB) were identified as additional possible sources of urease and have been tested (Figure 1). Activity is observed in the soybean, pigeon pea, and cottonseed meal studies although the rates of ureolysis were less than those observed with JBM. Work continues to try to evaluate the soy and cottonseed sources of enzyme as they show greater economic promise for use in field application.

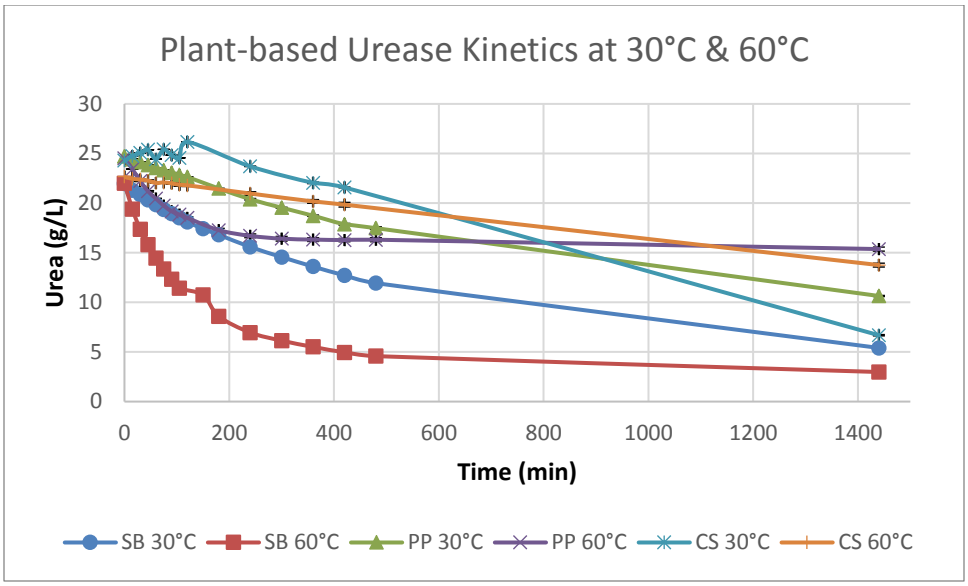

Figure 1. Urea concentrations during 24 hour batch experiments at 30°C and 60°C for soybean (SB), pigeonpea (PP) and cottonseed (CS) . While urea hydrolysis occcured, moreso at 60°C than 30°C, urea hydrolysis rates were less than those demonstrated across the 20-80°C temperature range used with jack bean urease.

Enzymatic urea hydrolysis kinetics appear to be the fastest between 60 and 70 °C; and inactivation of the jack bean urease was observed as a function of time and temperature. At 80 °C the urease was observed to be inactivated after 45 minutes. After 6 hours exposure to 70 °C, JBM urease demonstrated a > 97% decrease in activity. A mathematical model has been derived to predict the kinetics of deactivation of the JBM urease.

Work continues to evaluate the urease enzyme from *Sporosarcina pasteurii*. Ureolytic activity can be observed up to approximately 80 °C but growth of *S. pasteurii* ceases at approximately 40 °C. Methods are being developed to extract the enzyme from the cell and compare the bacterial urease from *S. pasteurii* to the enzyme derived from plant based sources.

b. The kinetics of thermal urea hydrolysis (thermally induced calcite precipitation or TICP) have been determined for temperatures between 30 and 130 °C. Increasing ureolysis rates were observed at higher temperatures. Additional experiments are being performed to determine the type of mineral and amount of mineral that can form during TICP. A flow-through reactor system (Figure 2) has been designed to assess mineral precipitation efficiency at 130 °C.

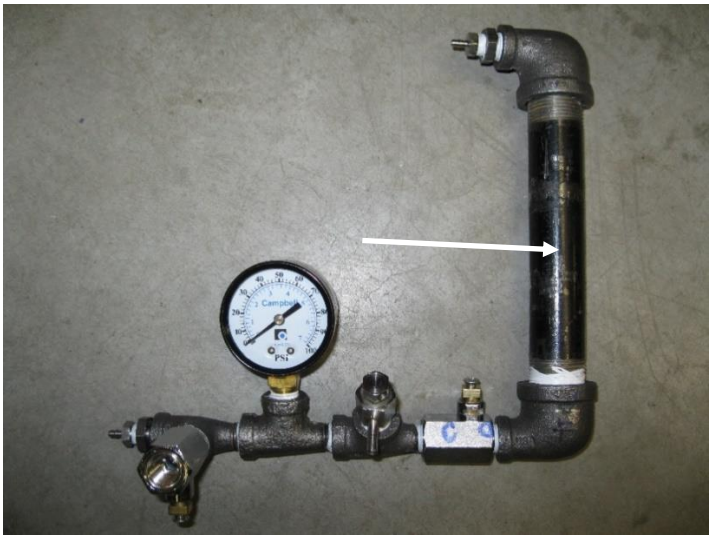

Figure 2. Steel reactor for analysis of TICP under flow conditions. Sand is packed in the straight 6" pipe portion of the reactor (arrow). Flow will be established in upflow direction and a pulsed injection strategy will be utilized initally, in which urea- and calcium-containing fluids are injected and then allowed to remain stagnant for ~24 hours.

Thermal gravimetric analysis of the precipitates will be conducted next quarter to assess the relative content of organics versus calcium carbonate in the precipitates.

B. September 2015-September 2017: Leverage federal funds and partner with a Montana company to initiate and plan a mineral precipitation well sealing field test. Identify interested stake holders, share relevant results and field plan.
    a. Montana Emergent Technologies (Butte, Montana) and MSU are continuing to pursue the development of biomineralization-based technologies. Conversations with several oil and gas companies have occurred. Wells and field deployment strategies have been discussed. Montana Emergent Technologies is a collaborator on the design and construction of the mobile laboratory which was funded by DOE through a budget amendment request. The mobile laboratory will be used for upcoming field project and advances the technology readiness level and commercialization potential of mineral-based sealing technologies.

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).

Quarter activities and accomplishments

During this reporting period, Cody Cole and Steve Christian of Talen Energy provided a tour of the Colstrip coal-fired power plant and discussed ways that MSU research could contribute to their operations. MSU collected samples of ash/scrubber paste material during the visit on June

15, 2016. Southern Company sent new baghouse waste material of interest to possibly determine alternate disposal methods. This material of interest is the primary subject of experiments to be performed during the 2016 funding period. A manuscript summarizing the previous results is in development to be submitted to a peer reviewed journal and/or the World of Coal Ash conference proceedings. A poster presentation, "Remediation of Coal Combustion Residuals Using Microbially-Induced Calcite Precipitation" was made at the July Montana Biofilm Meeting in Bozeman, Montana.

Hirings
No additional researchers were hired this reporting period. Eric Troyer and Abby Thane continued to research MICP (microbially induced calcite precipitation or biomineralization) in coal combustion residuals (CCRs) material.

Equipment Purchased
No equipment was purchased this reporting period.

Proposal *(leverage the overall MUS research enterprise)*
The following proposal was submitted: Laboratory testing of the effects of biomineralization on coal combustion residuals. PI: A. Phillips. Collaborators: A. Cunningham (MSU), Ben Gallagher (Southern Company). Total: $40,000. 08/16-12/16.

Milestones

A. September 2015- September 2016: Collect samples of bottom ash, fly ash and pond water at the Colstrip plant ponds. Perform laboratory studies to assess the feasibility of MICP CCR pond remediation.
    a. Samples of the Colstrip coal-fired power plant coal combustion residuals (paste) were collected in June 2016. These paste samples were similar to those collected in October 2015. Biomineralization experiments performed with the paste collected in October 2015 showed that noticeable binding of the materials was observed when we mixed increasing concentrations of paste with biomineralizing microbes and solutions. Increasing concentration (by mass %) of the paste mixed with biomineralizing solutions was noted to decrease the ureolysis rate. Though the rate was reduced, ureolysis and enhanced material binding was observed to still occur even in 50% paste and 50% mineralizing solution mixtures. The promise of these findings is the potential to enhance binding to minimize fugitive dust emissions from paste materials. This could allow for less water to be used to store the materials. The advantage to using less water is reduced risk of leaching of contaminants from the paste into the water which could impact groundwater sources. Biomineralization of paste particles also points to the potential for safely storing these materials dry where little or no water could be used.

       In this quarter, a method to enhance the ureolysis rate was determined. It was observed in previous experiments using fly ash. The pH is driven significantly higher than the optimal conditions for microbially induced calcite precipitation (MICP). Therefore, in order to control the pH, phosphate buffer solution was added to the triplicate batch study flasks along with the microbes and mineralizing

solutions. The flasks were filled with 25% fly ash by volume, a phosphate buffer solution, the mineralization promoting reagents, and microbes (or no microbes as the negative control). A positive control was also analyzed where microbes, buffer, and mineralization reagents were added without the ash material. It was observed that by controlling the pH with the buffer system improved, the microbially induced ureolysis rate in high concentrations of ash materials (Figure 3).

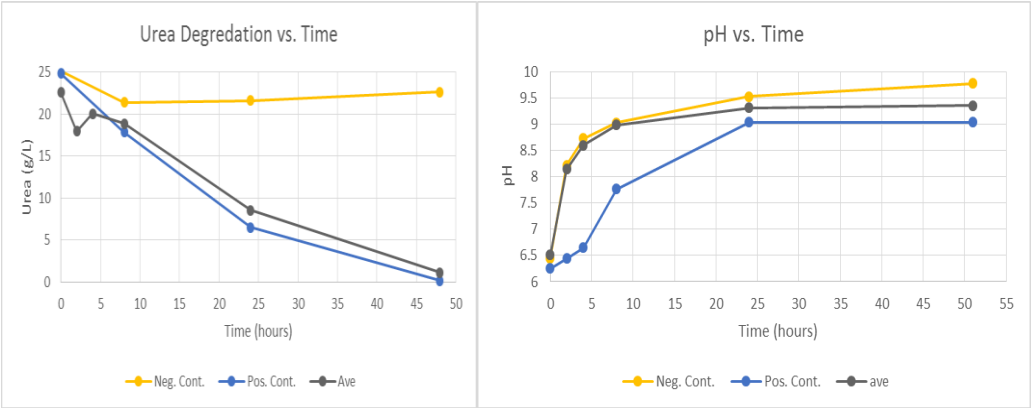

Figure 3. Ureolysis rates (left) in samples with 25% ash material (dark grey line) were observed to have ureolysis rates comparable to the positive control (blue line), where all the urea is hydrolyzed within 48 hours. The negative control (yellow line) showed no decrease in the urea concentration as expected. The pH (right) was observed to remain below 10, which is a pH condition favorable for MICP to occur.

These results show promise for biomineralization to bind together CCR materials at higher concentrations to reduce the amount of water needed to store these materials. Additional work to understand how to control the pH in samples with higher concentrations of fly ash is underway. Leachability studies are still in the planning stage but are scheduled to begin in the next quarter.

b. Additional experiments have begun with the baghouse waste material supplied by Southern Company. The primary research question we address is: Can baghouse waste cohesiveness be improved with biomineralization?

   An initial screening study was performed to assess the capacity of MICP to bind together the baghouse waste materials. It was observed that urea hydrolysis occurred over 48 hours in samples with up to 50% baghouse waste and ureolytic microbes or jack bean meal (another source of the urease enzyme). Additional screening studies are underway.

B. September 2016-September 2017: Assess and plan field demonstration of MICP in CCR ponds (as appropriate). Work with MT company (Montana Emergent Technologies, MET) to implement the MICP technology in the field.

   a. Conversations with MET continued on ideas for field deployment.

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

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.

Quarter activities and accomplishments

This quarter, efforts of biomineralization expanded from coal combustion wastewater to include studies of strontium and barium, found in produced water, and heavy metals found in mine adit discharge. Porous media flow reactors are being utilized to evaluate co-precipitation of strontium and barium under flow and mixing of fluids in porous media. Water samples taken from several mine adits in the Carpenter-Snow Creek mining district are being analyzed for removal of heavy metals via biomineralization of carbonate minerals. Investigations are underway in collaboration with the Colstrip Power Plant and Talen Energy to evaluate the potential for selenium bioremediation in contaminated groundwater due to a leaking evaporation pond at the power plant. Thus far, microbial samplers have been used to extract potential microorganisms capable of reducing selenium in the contaminated groundwater.

Hirings

Emily Stoick, recent Civil Engineering MSU graduate was hired for the summer months to work on removal of heavy metals from mining adit runoff. Lydia Amann resigned to pursue a summer internship in industry. Ph.D. student Neerja Zambare and undergraduate Kevin Burt continue to work on biomineralization studies in laboratory flow systems.

Equipment Purchased

No equipment was purchased this quarter.

Milestones

A. May 2016: Laboratory studies in synthetic mining wastewater with key heavy metal contaminants using model bacterial strains. Contact site(s) of interest to obtain water samples. Discuss potential and strategies for implementation of the technology with local Montana companies (e.g. Montana Emergent Technologies and Enviromin).

    a. Water samples taken from several mine adits in the Carpenter-Snow Creek mining district near Neihart, Montana, are being analyzed for removal of heavy metals via biomineralization of carbonate minerals. The mining district is a superfund site and contains a number of adits with discharge water containing heavy metal contamination. Two sampling trips have been conducted with the help of Tetra Tech and the EPA superfund site manager. Water samples were collected from three mine adits, the Compromise, Moulton and Evening Star adits. Primarily, the water samples contain high concentrations of zinc (0.5-3 mg/L), manganese (1.5-10 mg/L) trace concentrations of lead, cobalt and nickel.

Liquid batch tests have been conducted on the water samples to evaluate remediation potential via addition of ureolytic bacteria (*Sporosarcina pasteurii*) capable of inducing calcium carbonate precipitation. Thus far, microbial activity in Evening Star water samples has been shown to induce pH increase and precipitation of calcium carbonate. Reductions in dissolved manganese and zinc have been measured in preliminary tests and analyses are ongoing.

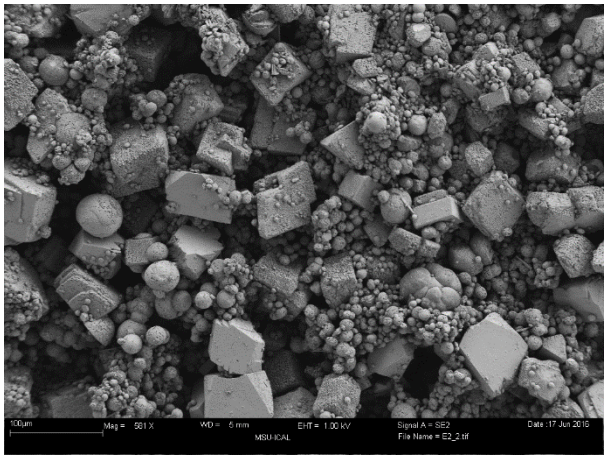

Figure 4. Scanning electron micrograph of biomineral precipitate from Evening Star mine influenced water. Elemental analysis identified calcium as a major constituent of the precipitate.

B. January 2017: Biomineralization studies in batch and flow reactors using real or synthetic waste water.

   a. Two flow systems have been constructed for laboratory studies, one with two parallel inlets to investigate the influence of fluid mixing on biologically driven mineral formation. The two inlets represent biostimulation, or addition of a nutrient fluid to a contaminated subsurface environment and mixing with the native fluid. One inlet contains nutrient medium with urea and the other inlet contains artificial groundwater with strontium and barium. Efficient removal of dissolved barium and strontium during biologically driven carbonate precipitation has been demonstrated in the system under flow conditions. The system with parallel inlets is shown in Figure 5, where the precipitation, shown as a white band, occurs primarily in the flow path of the artificial groundwater (lower inlet).

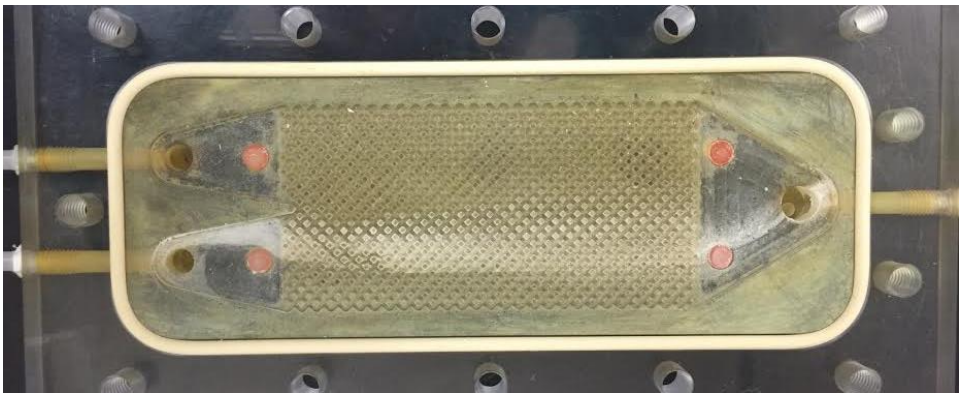

Figure 5. Dual-inlet flow cell after biomineralization experiment, showing white precipitation where calcium, barium and strontium were deposited. Inlets are located on the left side.

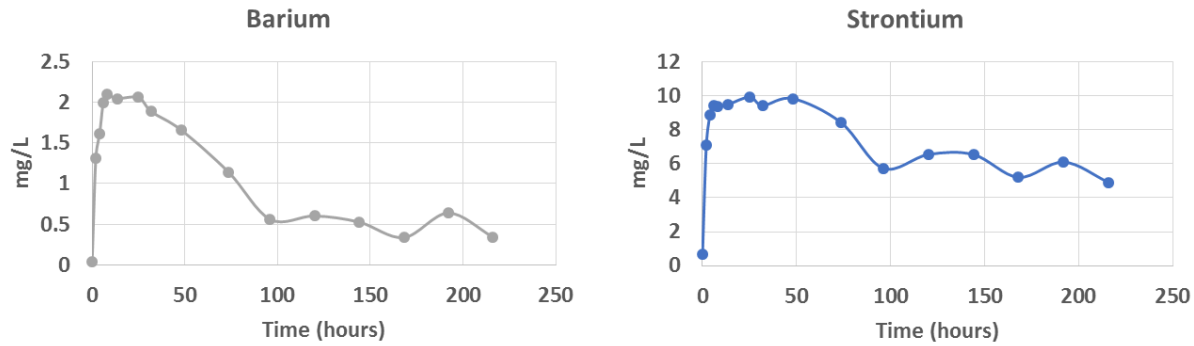

Figure 6. Measured concentrations of dissolved barium (left) and strontium (right) in the reactor effluent during operation.

b. A second system with spatial sampling locations allows for analysis of reaction kinetics along the flow path in the reactor. Strontium coprecipitation has been observed in the system and resulting precipitates are currently being analyzed to determine spatial distribution of strontium within the biologically induced calcium carbonate precipitate.

C. June 2017: Completion of laboratory investigations on technology scale-up and final assessment of potential for bioremediation of coal- and enhanced oil recovery-generated industrial wastewater.

a. Discussions with Talen Energy engineers at the Colstrip power plant were initiated to evaluate the potential for remediation of selenium groundwater contamination due to a leaking evaporation pond. Microbial samplers were installed down-well in two selenium contaminated wells at the power plant to screen for bacterial strains capable of selenium reduction. The samplers were

removed after 5 weeks and are undergoing laboratory enrichments to evaluate the potential for selenium bioremediation of the groundwater using native bacteria. Microbial activity has been measured in the enrichments as oxygen consumption and efforts are underway to determine if selenium reduction is occurring. DNA based analyses will also be performed to assess the microbial community present in the subsurface.

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.

Quarter activities and accomplishments

This quarter the screening results from the previous quarter were used to assess potential pipeline routes using geospatial analysis. The method used was a Least Cost Analysis (LCA), giving weighted values to various aspects of the landscape between Colstrip and each of the miscible field-reservoir combinations identified last quarter. This LCA routing model is concerned with avoiding geological, social, and environmental hazards; these include slope, urban developments, land cover, etc. (Huseynli, 2015). The LCA method determines the most "cost" effective route between a source and a destination on a cell by cell basis to create a raster surface. Each of the landscape attributes considered gets valued in different ways depending on their sensitivity or ability to hinder pipeline construction. The processing tools go through the eight neighboring cells around each location to find the next lowest-cost cell to add to the path. This then creates a pathway of the smallest sum of raster cell values to get from point A to point B.

The data used in this analysis included:

- Slope surface from 30m Digital Elevation Model (DEM)
- Land Cover (Northwest ReGAP classifications)
- Transportation Framework (Roads, State MT)
- Soils Data (SSURGO)
- Landownership

The elevation and slope data were used to assess the terrain for potential pipeline path, using lower slopes as the preferred path and assigning higher cost to steeper slopes where construction difficulties would be increased. Land cover was adapted from the Northwest ReGAP project classifications, which used 30 meter satellite imagery to define classes for vegetation and land cover (Comer et. al.2003). For this study vegetation types were grouped by vegetative physiognomy, aquatic and alpine cover classes, as well as human land uses. Land that has been recently disturbed or deemed for human use were desirable, wetlands and other sensitive areas were weighted with more cost. The roads from within the Montana Transportation Framework dataset were used as a way to find more suitable routing as well, within 5 kilometers of a road was most suitable. The Soil data that was incorporated into the analysis was the Soil Survey Geographic (SSURGO) database and used as a way to establish a minimum depth to bedrock surface. This factor plays into placement for a buried pipeline set below the frost line. Places over 45 to 50 inches of soil depth were desirable. Lastly, landownership was used as a metric in

the analysis. National forests, state parks, national monuments and parks, as well as recreation areas and tribal lands, were weighted as less desirable in the routing decisions.

Each dataset was weighted with equal importance, meaning each of the map layers held 20% of the importance for the decision making. Using a raster calculator, each of the classified surfaces was multiplied by 0.20 to impose the weight on the class values. From there, using the raster calculator allows the surfaces to be summed up and divided by the sum of the weights to create the cost surface input for the path analysis. Using the cost surface as input into a Cost Distance tool in ArcGIS, we were able to create pathways that represent the lowest cost based on the input to get from Colstrip out to the miscible fields identified during the screening.

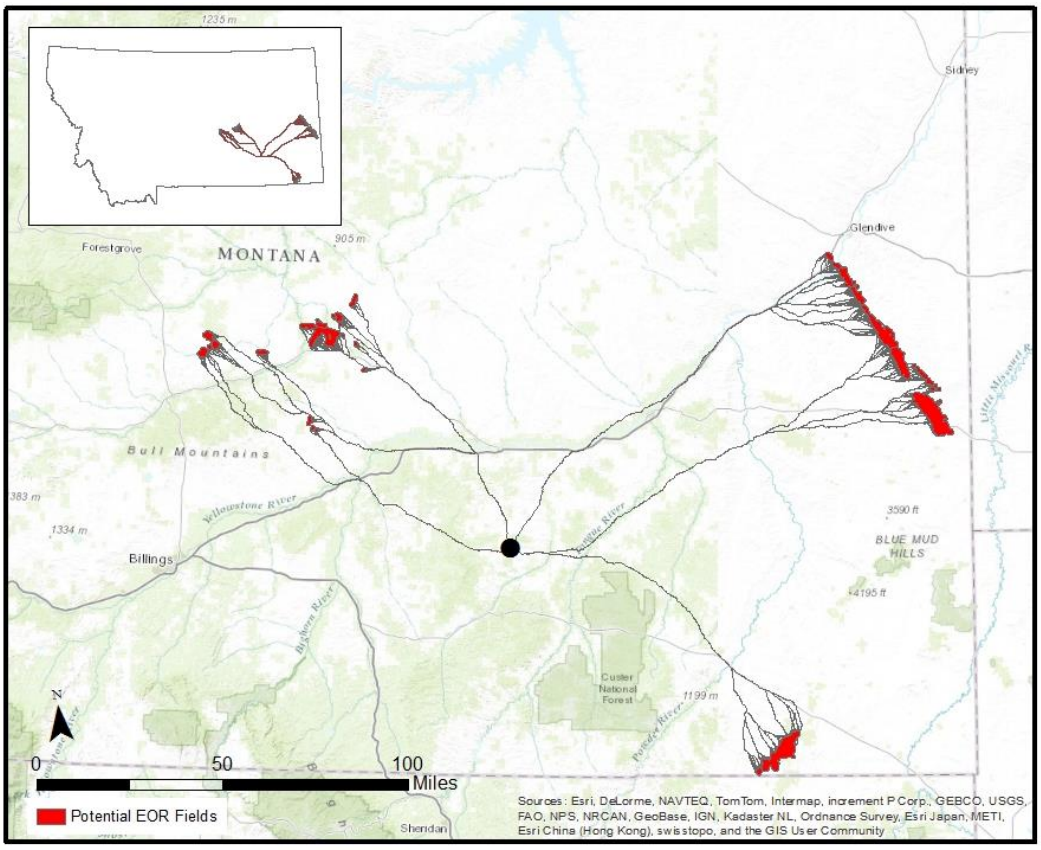

Figure 7. Map of LCA routes between Colstrip and high-potential miscible field-reservoir combinations.

Development has also continued on the interactive mapping application. Rest services that contain these decision making datasets have been established and coded into the programming testing environment that was created last quarter.

Hirings

There were no new hires this quarter.

Equipment Purchased
No equipment has been purchased to date.

Milestones

A. July 2015 – July 2016: Assessment of carbon storage and EOR potential
    a. Oil and gas data for all producing horizons in the study area underwent three levels of screening based on reservoir characteristics including oil gravity, depth, thickness, fracture pressure, temperature, and historical production. Seven regional field-reservoir combinations were found to have complete data and meet all screening criteria. Network analysis has been conducted on the high-potential target fields, and routes to each were determined using a Least Cost Analysis method.

B. December 2016: Completion of the interactive mapping application
    a. Screening and network analysis results were added to the project geodatabase, which will ultimately house the complete dataset and be tuned for use with the interactive mapping application software. Setup and programming continues for the JavaScript-based interactive map.

C. June 30, 2017: Final Report and data package
    a. No activity to report this period.

Objective 5

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

Quarter activities and accomplishments

Culturing
Montana Tech cultured unicellular green algae from the Coal Bed Methane Ponds of Southeastern Montana. This culture is known as CBM-W, which stands for Coal Bed Methane Water and grows in freshwater with the addition of Bold's Basic Nutrient Medium. After the algal cultures were bright green (signifying a high number of cells), we further concentrated the algae letting it sink through the water column of a funnel and decanting the algae with a valve. The cell density was then determined via microscopy and a hemacytometer (a gridded microscope slide for counting cells).

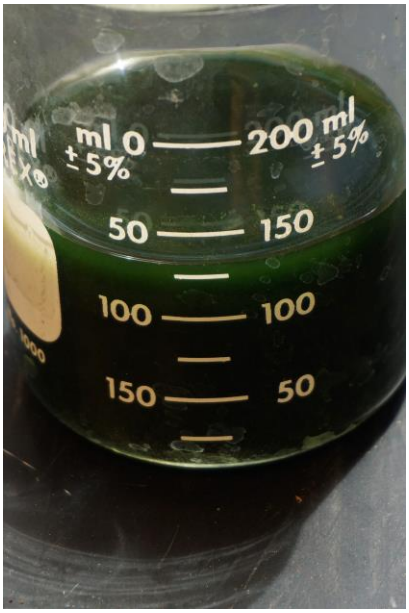

Figure 8. Beaker of concentrated CBM-W algae.

MSU continued analyses for the growth of the algal isolate in non-sterile CBM production water and the changes in microbial community dynamics. Experiments have been initiated to grow mixed algal cultures (strain PW95, strain WC-1, and strain X) together in defined medium and to test for outdoor growth (natural light and temperature fluctuations) along with biomass and lipid accumulation.

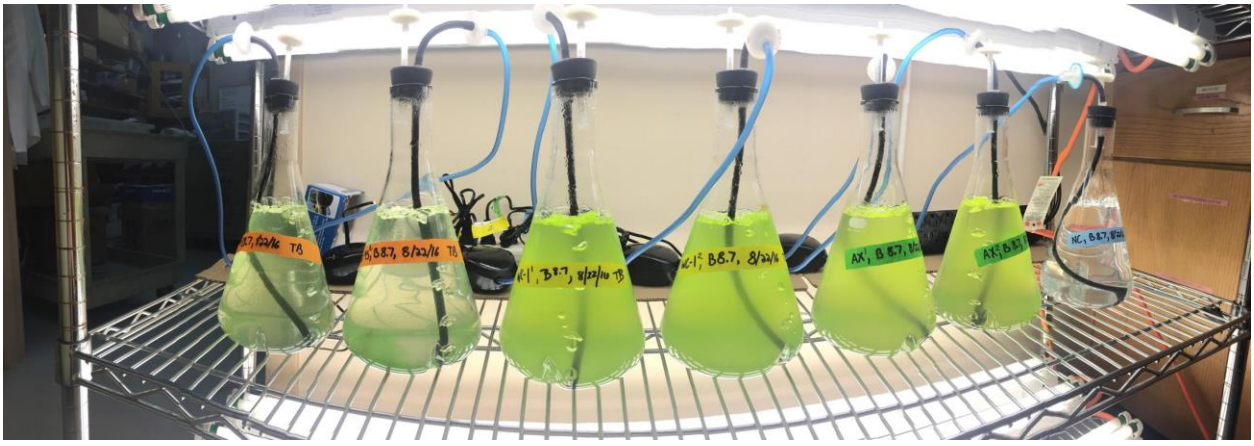
Figure 9. Cultivation of individual algal strains in preparation for scale-up of inoculum. Cultures are being slowly acclimated to outdoor conditions.

Plants

Wheat, Triticum aestivum, is an important crop in Montana. In May 2016, Montana Tech planted hard red spring wheat in pots in the greenhouse at Montana Tech and added CBM-W algae to the soil. When compared to other wheat plants given only water or water with commercial fertilizer, the wheat plants that were given water plus the algae had more chlorophyll per leaf area, produced more tillers per plant (tillers are additional stems), and more inflorescences (flower

clusters) per stem. The wheat plants are scheduled to be harvested in August 2016, when root:shoot ratios and grain production will be determined. Wheat seed was purchased from Northern Seed Company, LLC.

Potatoes, Solanum tuberosum, are also an important crop in Montana. In June 2016, we planted Russet Ranger seed potatoes in soil and supplied them with just water, water and Hoagland's nutrient solution, or water and CBM-W algae. As the potato plants develop, time of flowering, height of stems, chlorophyll content, and other parameters will be measured. During the late summer harvest, production of tubers (potatoes) will be quantified in the different treatments. The seed potatoes were supplied by Dr. Nina Zidack of the MSU Potato Lab.

Figure 10. Experimental wheat and potato plants at the MT Tech greenhouse.

Nitrogen fixing cyanobactera (NFC)

Nitrogen fixing cyanobactera (NFC) populations have potential for 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 N 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, 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*.

Strategies to overcome the current limitations of using cyanobacteria as a large scale biofertilizer are being developed by researchers at Montana State University. MSU grew *Anabaena cylindrica* strain 16 in liquid medium without added nitrogen. This culture is highly resistant to contamination, and in the past, small scale tests with wheat has served as an excellent fertilizer for enhancing plant growth.

MSU cultured *Anabaena cylindrica* strain 16 in liquid medium without added nitrogen at multiple scales including 12L and 200L raceway ponds. The cell density was determined via dry weight for determination if enough biofertilizer had been produced. The 200L raceway test unfortunately failed due to very high temperatures and solar irradiance during the initial growth period.

Cultures were restarted in three identical 12L ractors and soon developed enough biomass to transfer to Dr. Marth Apple (MT Tech) for recently started growth tests on potatoes. Dr. Peyton and student James Crawford met with Dr. Martha Apple and her group at the quarterly meeting at MSU to develop collaborations on the use of cyanobacteria as a means of fertilizing economically important crop plants of Montana. Cyanobacteria has the advantage of being able to fix atmospheric nitrogen, so additional nitrogen is not required before it is added to the plants.

Coal Bed Methane Ponds

In July, Montana Tech traveled to the coal bed methane ponds of Montana's Tongue River Valley to collect water samples (Apple, Ogunsakin) and to study geophysical parameters of the ponds (Zhou and Xhou). The water samples contained some microalgae, which we are now beginning to culture.

In addition, Martha Apple conducted a brief survey of the flora and fauna of the coal bed methane ponds.

Figure 11. Posterized view of the John Wayne CBM Pond.

Hirings

MSU
James Crawford was hired (half time) as a student researcher in Chemical and Biological Engineering to grow and characterize the cyanobacterial biomass.

Efforts to recruit a Ph.D. student have been shifted to conduct experiments with mixed algal cultures and the cultivation outdoors.

MT Tech
Mr. Olakunle (Kunle) Ogunsakin continues with his research on this project as a Master's student in Environmental Engineering.

Equipment Purchased

MSU
No major equipment purchase were made this quarter.

MT Tech
We have purchased culturing equipment that includes an aquarium, light sources, and nutrient media to culture the algae, as well as pots and soil for growing the wheat and potatoes.

Milestones

A. December 2016: Growth characteristics under outdoor conditions (temperature and sunlight) in meso-scale ponds will be determined

   a. MT Tech: Growth characteristics under outdoor conditions (temperature and sunlight) in meso-scale ponds will be determined. We are currently scaling up to this by increasing the volume of the CBM-W cultures, which are grown in natural lighting. We have also visited the raceways for volume growth of cyanobacteria at MSU.

      MSU: Cultivation equipment continues to be setup for outdoor use once cultures are acclimated to outdoor conditions.

B. July 2016: Obtain and test algal byproducts for macronutrient and micronutrient composition. Recruit a graduate student to work on this project.

   a. MT Tech: Algal byproducts have been tested for macronutrient and micronutrient composition and we have recruited and hired Olakunle Ogunsakin as a graduate student for this project.

      MSU: In August, *Anabaena cylindrica* strain 16 was cultured in liquid medium without added nitrogen. Enough biomass was developed to transfer to Dr. Martha Apple (Montana Tech) for recently started growth tests on potatoes.

      Methods are being developed for the growth of algal biofilms to aid in biomass collection and elemental composition.

C. July 2017: Tests will be targeted towards those plants that showed responses to the algal fertilizer.

   a. MT Tech: Tests will be targeted towards those plants that showed responses to the algal fertilizer. We are currently testing the responses of wheat and potato plants to the algal fertilizer and to date, the wheat plants exhibit a strongly favorable growth response when given algae as a fertilizer.

      MSU: Tests will be targeted at scaling up to the 200L outdoor raceway systems including better control of light and temperature during culture initiation. Additional biomass will be provided for tests run in spring and summer 2017.

Objective 6

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

Quarter activities and accomplishments

During the fourth quarter, we continued the algorithm development of water area extraction from satellite images, field data collection from the coal bed methane ponds within the Montana Powder River Basin (PRB), and setting up of the hyperspectral system for algae monitoring and quantization. The main activities included setting-up of the hyperspectral imaging system for algae image collection, algorithm development, Google earth satellite data processing, water area extraction, continuation of literature review, and field data collection.

Major accomplishments this quarter include:

- Image collection of algae using the hyperspectral imaging system and spectral data collection using a spectro-radiometer.
- Development of an algorithm for water area extraction of the Google high-resolution satellite images covering the ten coal bed methane ponds within the Montana Powder River Basin.
- Original documents on the ten coal bed methane ponds within the Montana Powder River Basin were collected.
- Field data including GPS data along the edges of the ponds and images of the ponds using a drone (Unmanned Aerial System)was collected at four of the coal bed methane ponds

Hirings

No new hires were made this quarter. The graduate student continued image processing including image classification and learning how to set up and use the hyperspectral imaging system for algae image collection.

Equipment Purchased

The purchase request including three quotes for a hyperspectroradiometer was submitted to the business department. The equipment is expected to be ordered next quarter.

Milestones

A. July 2015 – July 2016: Estimate areal coverage of CBM ponds using Hyperion or Landsat data

   a. For this objective, we developed an algorithm to extract water area from the Google image of very high-resolution (0.20-0.30 m) covering the ten CBM ponds within PRB. The algorithm can successfully extract water area of some ponds but not all. Montana Tech continues to improve the algorithm and test new ones.

   b. Original documents were collected were collected from Summit Gas Resources, Inc. on the ten ponds that will help to characterize the ponds and validate the area extraction algorithms.

   c. Field data including GPS data and UAS images was collected at four of the ten ponds.

   d. The GPS data will be used to calculate the area of the ponds. The area calculated will be used as ground truth for algorithm validation.

   e. The imaging system was successfully set up and is being used to collect hyperspectral data on algae grown by Marth Apple's research group.

   f. Literature review continued on the algorithm development. An abstract was submitted to American Geophysical Union (AGU) fall meeting to be held in December of 2016 in San Francisco.

B. July 2015 – December 2016: Evaluate time-course for methane production during consecutive stimulations

   a. Algal extract is being tested and compared to yeast extract and cyanobacteria extract. Preliminary results suggest that coal-dependent methanogenesis can be sequentially

stimulated; however, the coal-dependent nature of the methane production appears to subside. Algal and cyanobacterial extract appears to perform better in terms of subsequent stimulations for coal-dependent methanogenesis. Community dynamics for the initial stimulation are being determined, and both the bacterial and archaeal populations are different for both the type and amount of nutrient amendment. Analyses are on-going.

C. July 2016 – July 2017: Monitor mesoscale growth of algae using spectral methods

   a. The hyperspectral imaging system was acquired and set up for acquisition of hyperspectral images of algae.

   b. Some preliminary measurement were taken on the microalgae grown in Marth Apple's lab. Hyperspectral images on the algae were collected. Spectral data was also collected using a spectro-radiometer.

   c. Literature review continued for the algae monitoring from remote sensing point of view.

Expenditures to Date

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	All Budgets	Spent to Date
Salaries & Benefits	717,237	130,297
Subcontract Payments		
Montana Tech	222,667	11,564
Montana Emergent Technologies	75,000	29,500
Operations	160,096	36,993
Equipment	25,000	
Total Costs	**1,200,000**	**208,354**

References

Associated Press, Johnson, C. (2012, January 11). EPA: Power plant main global warming culprits. Billings Gazette. Retrieved from http://billingsgazette.com/news/state-and-regional/montana/epa-power-plants-main-global-warming-culprits/article_03ebdd92-3c90-11e1-9e01-001871e3ce6c.html.

Huseynli, S. (2015) *Determination of the Most Suitable Oil Pipeline Route using GIS Least Cost Path Analysis* (Unpublished master's thesis). Nova University of Lisbon, Lisbon, Portugal.

Comer, P., Faber-Langendoen, D., Evans, R., Gawler, S., Josse, C., Kittel, G., Menard, S., Pyne, M., Reid, M., Schulz, K. and Snow, K. (2003) Ecological systems of the United States: a working classification of US terrestrial systems. *NatureServe, Arlington, VA.*