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

The outreach team continued to track news and regulatory actions related to the Colstrip Facility this quarter. Regulatory actions taken at the Federal level this quarter that could indirectly or directly affect Colstrip include the signing of several Executive Orders by the Trump Administration: Executive Order on Energy Independence, the Executive Order on the Steam Protection Rule, and the Executive Order on the Waters of the U.S.

At the State level, Colstrip was the focus of numerous bills that were introduced during Montana's 2017 legislative session aimed at protecting coal-based economies and communities in Montana. Below is a summary of the bills that passed both the House and Senate. As of April 30th, most of the bills were waiting for the Governor's signature.

- SB 140 *"Allow low-interest coal tax trust fund loans to certain local governments"*
  This bill allows the Board of Investments to loan $10 million from the Coal Tax Trust Fund to Colstrip for infrastructure maintenance.

- SB 339 *"Establishing the coal-fired generating unit remediation act"*
  This bill would require the Colstrip owners to file a remediation plan with the Montana Department of Environmental Quality. The plan must outline all of the remediation obligations under existing law and provide information on how the owners intend to meet regulatory compliance requirements. The plan would be subject to public comment and DEQ oversight.

- HB 22 *"Appropriate money to assist/intervene/plan for closure of coal-fired generation"*
  This bill gives Montana's Attorney General $80,000 to discuss clean energy replacement and clean-up funding for Colstrip with Puget Sound Energy and the Washington State Utilities and Transportation Commission. This bill was signed by the Governor on April 14, 2017.

- HB 209 *"Extend funding to Coal Board"*
  This bill will double grant funding for coal communities from $1.6 to $3.2 million each year through 2019.

- HB 585 *"Provide for loans to an owner of a coal-fired generating unit"*
  HB 585 allows Talen Energy to borrow $10 million each year (up to $50 million) from the Coal Tax Trust Fund to operate Units 1 and 2 until their closure.

- HB 647 *"Generally revise education funding, implement general appropriations bill"*
  This bill maintains a $1.7 million annual grant for Colstrip schools.

Colstrip-related Senate and House bills that failed to pass this legislative session include:

- SB 37 *"Establish coal-fired generating unit decommissioning remediation act"*
- SB 38 *"Establish energy accountability act"*
- SB 338 *"Revise laws related to closure of certain coal-fired generation"*

- HB 60 *"Provide for communities affected by closure of coal-fired generation"*
- HB 624 *"Establishing a coal transition working group"*
- HB 625 *"Requiring an owner of a coal-fired generating unit to provide a bond"*

Additional information about each bill listed above can be found by searching the SB and HB bill number in the 2017 LAWS systems: http://laws.leg.mt.gov/legprd/law0203w$.startup?P_SESS=20171.

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 have been determined for bacterial and plant-based ureases. Thermal ureolysis kinetics were determined at increasing temperatures and increasing concentrations of urea and in the presence of calcium. Thermally induced precipitation has been promoted in sand filled batch reactors and the strength of the material was assessed. Montana State University and MET personnel have had discussions with several oil and gas companies and an oil field service provider to evaluate applications of mineralization-based subsurface technologies. Work continues with MET to design a mobile laboratory which will be used to implement these mineral precipitation technologies in the field.

Hirings

No additional hires were made this quarter. M.S. students, Arda Akyel, Vinny Morasko, Dicle Beser and Kyle DeVerna, and undergraduate student, Zach Frieling continue to work 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 proposal that represented a collaboration between MSU and Montana Emergent Technologies (MET) was submitted April 5, 2017, to the US Department of Energy (DOE) for $1M in additional funding. The objective of this proposal was to demonstrate the use of advanced mineral precipitation technologies in the field in a leaking oil and gas well and promote the commercial development of the sealing strategy.

Milestones

A. August 2015-June 2017: Perform laboratory bench experiments to extend the temperature range for mineral precipitation, and thief zone plugging for enhanced oil recovery (EOR)
    a. Ureolysis and enzyme inactivation kinetics: As reported previously, enzymatic urea hydrolysis kinetics from plant based sources (jack bean) of enzyme appear to be the fastest between 60 and 70° C. 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 six hours of exposure to 70° C, jack bean meal urease (JBM) urease demonstrated a > 97% decrease in activity. First order, series-parallel and series type mathematical models have been derived to predict the kinetics of deactivation of the JBM urease. A manuscript, titled "Plant-based ureolysis kinetics and urease inactivation at elevated temperatures for use in engineered mineralization applications" is in preparation presenting the results from the experimental and modeling results.

b. Thermally Induced Calcium Carbonate Precipitation: The kinetics of thermal urea hydrolysis (thermally induced calcite precipitation or TICP) were determined for temperatures between 30 and 130° C. Increasing ureolysis rates were observed with increasing temperatures (Figure 1). Data also suggested that in the presence of calcium, the ureolysis rate from thermal induction is slower than without calcium. This difference has been observed now at 100, 110, 120 and 130° C.

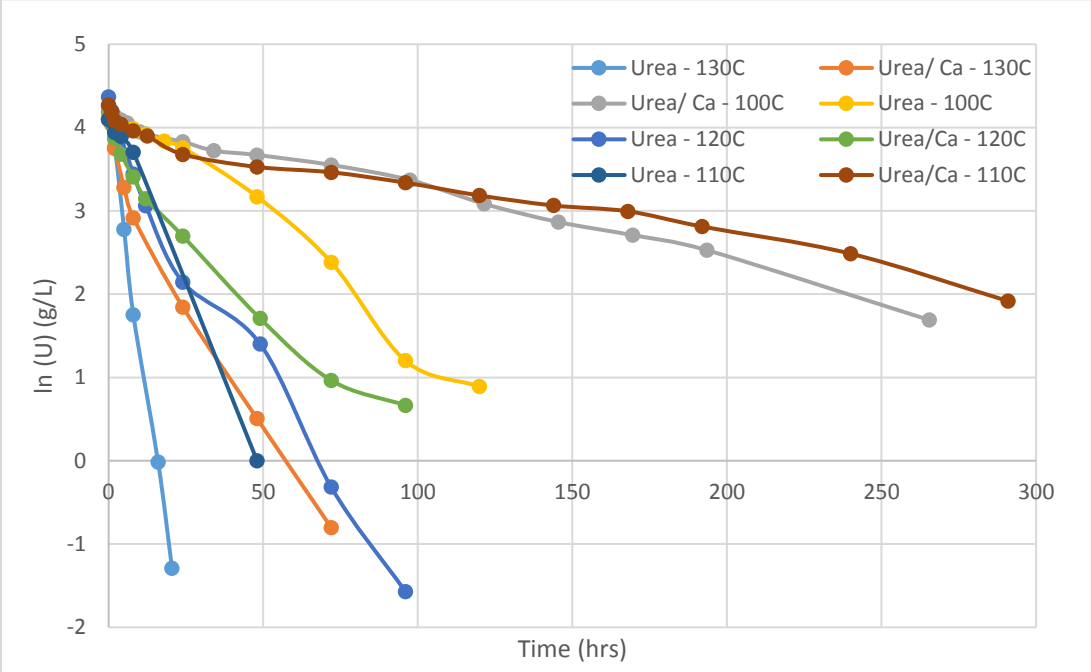

Figure 1. Thermally induced urea hydrolysis rates were observed to be influenced by the presence of calcium. In the presence of calcium, the rate of urea hydrolysis was observed to decrease as compared to the system without calcium.

Flow through reactor experiments were performed to assess the strength of the mineral materials promoted in a sand pack. Urea and calcium containing solutions were injected into columns (Figure 2) in an upflow configuration at 130° C. After 400 grams/L of urea were measured to have been hydrolyzed over the course of six days, the experiments were terminated and the sand packs were removed from inside the metal pipe. One of the sand packs where mineralization was promoted in water-based fluids was observed to crumble immediately after removal from the pipe. The other sand pack where mineralization was promoted in the presence

of a water-based fluids amended with an organic compound was cemented together (Figure 2) and tested for unconfined compressive strength.

Figure 2. 2"x4" reactor system (left), cemented sand column with the presence of organics (middle), crumbled sand column where precipitation was promoted without organics (right).

B. August 2015-June 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. MET 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 projects and advances the technology readiness level and commercialization potential of mineral-based sealing technologies.

Dissemination of Results

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.

Kirkland, C, Phillips, AJ, Codd, S. Low-field Borehole NMR as a Monitor for Subsurface Engineering Applications. SAGEEP 2017, March 19-23, 2017, Denver, Colorado.

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 (Poster)

Publications

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)

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, experiments were performed to assess the permeability reduction achievable in the fly ash materials provided by Southern Company. In addition, experiments were preformed to determine a method to spray the biomineralization solutions onto the surface of the ash materials to make a cap suitable to reduce dust formation. On March 23, 2017, Montana State University researchers and a representative from MET, a Montana small business in Butte, traveled to Colstrip to meet with Gordon Criswell. Samples were collected and ideas for upscaled laboratory experiments were discussed. A conference call with Ben Gallagher of Southern Company was held in April. He was updated on recent progress of biomineralization of CCRs, discussed the focus of the upcoming proposal, and put forth the idea of a site visit by MSU researchers to the Scherer plant in Georgia. Abby Thane presented "Remediation of Coal Combustion Residuals Using Microbially-Induced Calcite Precipitation" at the 2017 World of Coal Ash conference in Lexington, Kentucky. During the conference, discussions with Ben Gallagher of Southern Company were held and additional industry interaction was achieved.

Hirings

No hires were made this quarter. Steven Jones, a senior in Civil Engineering was hired in September 2016. Abby Thane and Steven Jones continued to research MICP (microbially induced calcite precipitation or biomineralization) in coal combustion residuals (CCRs) material.

Equipment Purchased

Minor equipment to simulate a wind tunnel was purchased during this reporting period.

Proposal *(leverage the overall MUS research enterprise)*

An additional funding opportunity with the Environmental Research and Education Foundation was identified and a pre-proposal is in preparation for a June 1, 2017, submittal. A $23,000 proposal was submitted in late April 2017 to Southern Company for upscaling work in ash materials to prepare for a field experiment.

Milestones

A. August 2015-June 2017: 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. Column Study: A column study (Figure 3) was performed to investigate changes in the permeability of fly ash treated with MICP. The columns contained fly ash

that was mixed with sand to compensate for the pressure limitations of the syringe pump. Two columns were treated with injections of *S. pasteurii* cells and calcium containing growth medium (biomineralization promoting solutions). Each time an injection was made, water was injected into a third column that was used as a control. Permeability data over the course of this experiment showed no permeability change in the control column. However, columns that were treated with MICP showed decreases in permeability of one and two orders of magnitude.

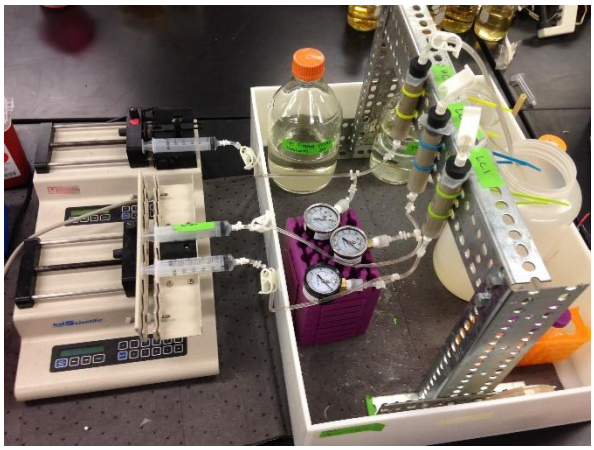

Figure 3. Ash materials mixed with sand were placed in a column and biomineralization solutions were injected over the course of 10 days. Three columns were prepared one with a higher concentration of ash (25%) and two with a 10% concentration of ash. One of the 10% concentration columns served as a control where water instead of biomineralization solutions was injected.

b. Capping Study: Ash materials were placed in a petri dish and biomineralization promoting solutions were added to the top of the materials. The resulting mineral precipitation was observed to form a cap on the ash that was bonded to the ash materials (Figure 4). Additional studies are planned to evaluate the manner with which the biomineralization promoting solutions are applied to scale this work up toward field application.

Figure 4. A whitish cap was observed to form on the surface of the ash material after treatment with biomineralization promoting solutions.

c. Upscaling Experiments: Based on the previous meeting with Gordon Criswell of Colstrip, plans have been made to conduct a meso-scale laboratory experiment. The experiment will be geared towards developing methods to create a partial cap between a closed fly ash pond and the baked shale layer above. The final reactor design will include layers of fly ash, baked shale, subsoil, and top soil samples taken directly from Colstrip. After MICP media is injected into the target area the reactor will be monitored to determine the spatial distribution of the resulting biocement.

B. August 2016-June 2017: Assess and plan field demonstration of MICP in CCR ponds (as appropriate). Work with MT company (MET) to implement the MICP technology in the field.
   a. Conversations with MET, Colstrip plant managers, and Southern Company continued on ideas for field deployment.
   b. A conference call was held in April 2017 with Ben Gallagher of Southern Company to discuss design of meso-scale experiments addressing the permeability reduction in ponded CCR materials. During the meeting and site visit to Colstrip with Gordon Criswell ideas were discussed for the upscaling of experiments with pond materials. Samples were collected in April during a site visit to provide enough material for the up-scaled experiments.

Dissemination of Results

Presentations

Thane, A, Phillips, AJ, Spangler, L, Cunningham, AB, Gallagher, B. Remediation of Coal Combustion Residuals Using Microbially-Induced Calcite Precipitation. World of Coal Ash Conference, May 8-12, 2017, Lexington, KY.

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 (Poster).

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).

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

Laboratory batch studies on mine influenced water were completed this quarter and research will progress towards flow studies using packed column reactors which more closely mimic potential remediation strategies. Enrichment studies with selenium reducing bacteria from the Colstrip

groundwater continued and the collected data will be utilized in a proposal to the NSF biogeosciences division for further studies of selenium fate in bioremediation scenarios. Laboratory studies of strontium co-precipitation in porous media flow reactors continued this quarter and have led to a manuscript in preparation.

Hirings

No additional hirings were made this quarter. Neerja Zambare, graduate student, continued work on the laboratory flow systems investigating strontium and barium remediation. Hannah Koepnick, graduate student, continued work on selenium reduction in Colstrip groundwater. Emily Stoick continued to work on the remediation of mine influenced water via microbial induced carbonate precipitation (MICP).

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. Laboratory studies completed this quarter had progressed beyond the use of model bacterial strains and synthetic wastewater and are detailed below. Contacts with the Montana Department of Environmental Quality (DEQ) abandoned mine lands program were made and potential application of MICP for water treatment of mine discharge near Belt, MT was discussed.

B. January 2017: Biomineralization studies in batch and flow reactors using real or synthetic waste water.
    a. Laboratory studies evaluating strontium precipitation with porous media flow systems continued this quarter. The findings from last quarter, including the revelation that calcium carbonate precipitation rate influences the extent of strontium removal, are currently being incorporated into a manuscript in preparation.

       Selenium bioremediation studies continued with batch reactors containing sampled microorganisms and groundwater from the Colstrip power plant site. After three months of cultivation, bottles supplemented with carbon sources (methanol, glycerol, molasses) began to show evidence of selenate reduction (Figure 5). Bottles amended with molasses as carbon source displayed the fastest reduction, although there may be logistical issues with employing molasses in a field remediation strategy due to the high viscosity and potential for freezing in winter temperatures. Further analysis is needed to determine whether selenium is reduced to its insoluble, elemental form and the ultimate stability of selenium in the solid phase within the aquifer.

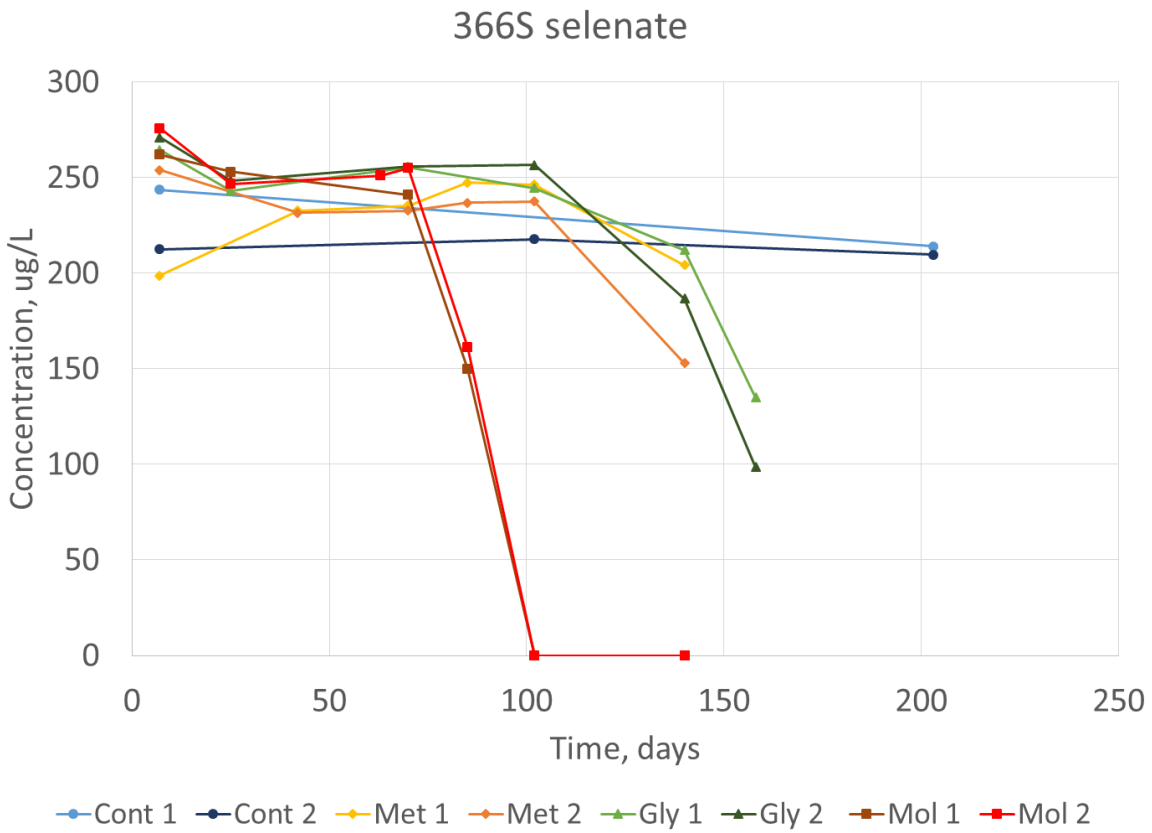

Figure 5. Selenate over time in samples from Colstrip well 366S, with additions of no carbon (Cont), methanol (Met), glycerol (Gly) and molasses (Mol).

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. This quarter, laboratory batch studies with mine influenced water from the Carpenter Snow Creek Mining District were completed using a bacterial strain isolated from sediment at the abandoned mine site. The organism is currently being identified via DNA sequencing. The experiments demonstrated successful ureolysis, carbonate precipitation and removal of some key heavy metal contaminants from the mine water. Batch tests conducted at 10° C indicated that ureolysis and precipitation of metals is possible at this lower temperature (Figure 6), close to that of the field. A lower range of urea concentrations were tested, due to the concern that production of ammonia (NH_3) would create nutrient problems in the streams and exceed total nitrogen and ammonia standards and aquatic toxicity levels for the receiving streams. To mitigate the nitrogen production, the possibility of a treatment wetland for nitrogen removal is being explored in collaboration with the treatment wetlands research group at MSU.

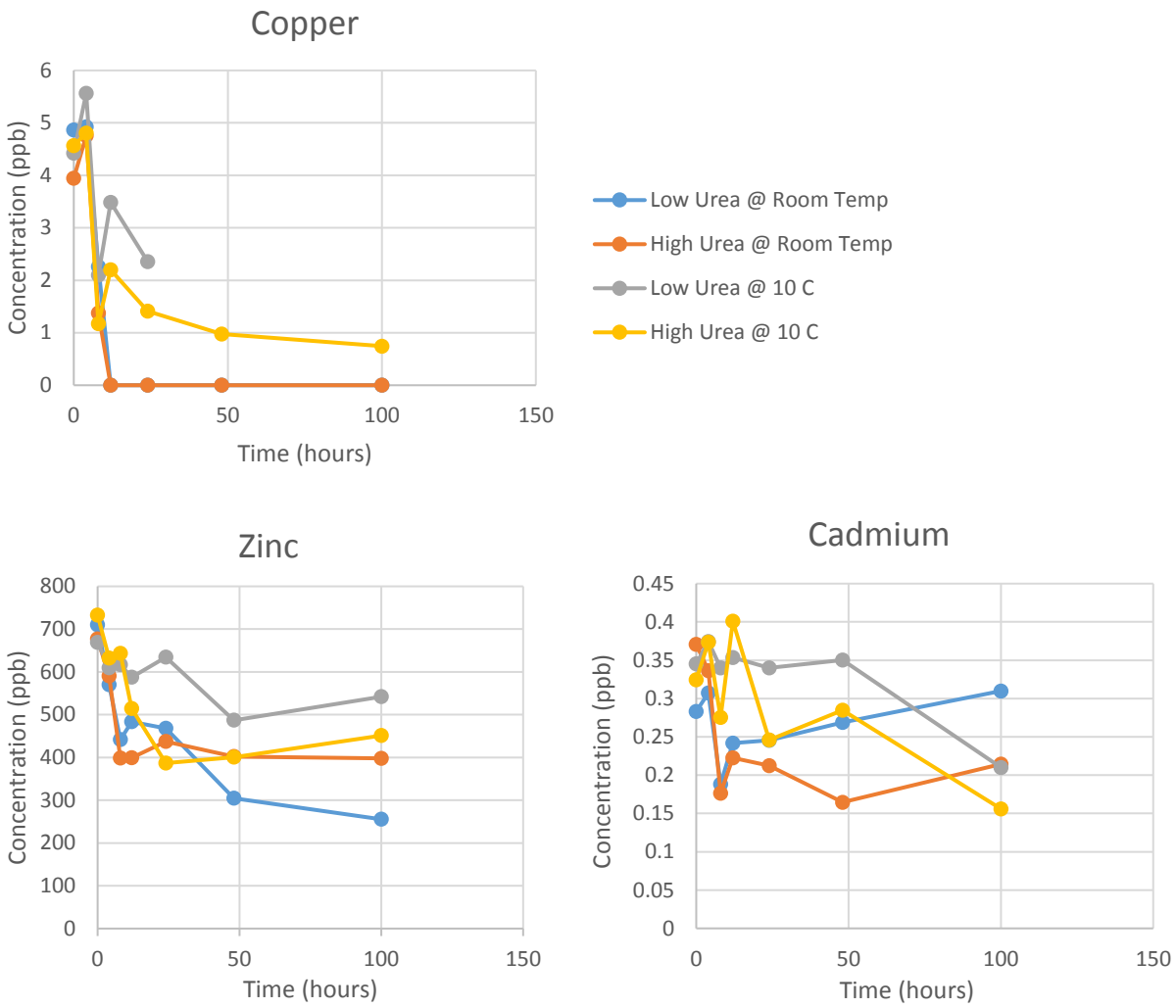

Figure 6. Concentrations of copper, zinc and cadmium in mine influenced water with native ureolytic bacterium.

Dissemination of Results

Presentation

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, MT.

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

Substantial progress was made on the CO_2-EOR regional capacity analysis near Colstrip this quarter. Data reviews and updates were completed for the CO_2-EOR field screening analysis, CO_2 capacity and EOR production estimates, emission reduction estimates and LCA model variables. Updates were incorporated into the Story Map graphics, interactive maps, and narrative text. In addition, work began on the draft sections of the final report including a white paper that provides a full narrative of the CO_2-EOR Suitability Assessment.

MSU completed a quality control review of the CO_2-EOR field reservoir screening analysis using field-specific information on oil field porosity, permeability, fracture pressure, residual oil saturation, formation volume factor, net pay thickness, oil gravity, temperature, and oil viscosity using a comprehensive literature review of published data. MSU reviewed well data from nearly 11,000 oil and gas wells within the regional vicinity of Colstrip and identified 471 producing field reservoir combinations (FRCs) with CO_2-EOR potential. MSU ran all 471 field reservoir combinations through an updated five step screening process illustrated in Figure 7 to assess suitability for CO_2-EOR. The screening process identified a total of 38 FRCs with CO_2-EOR potential that are within 80-130+ miles to the northwest, north east and southeast of Colstrip.

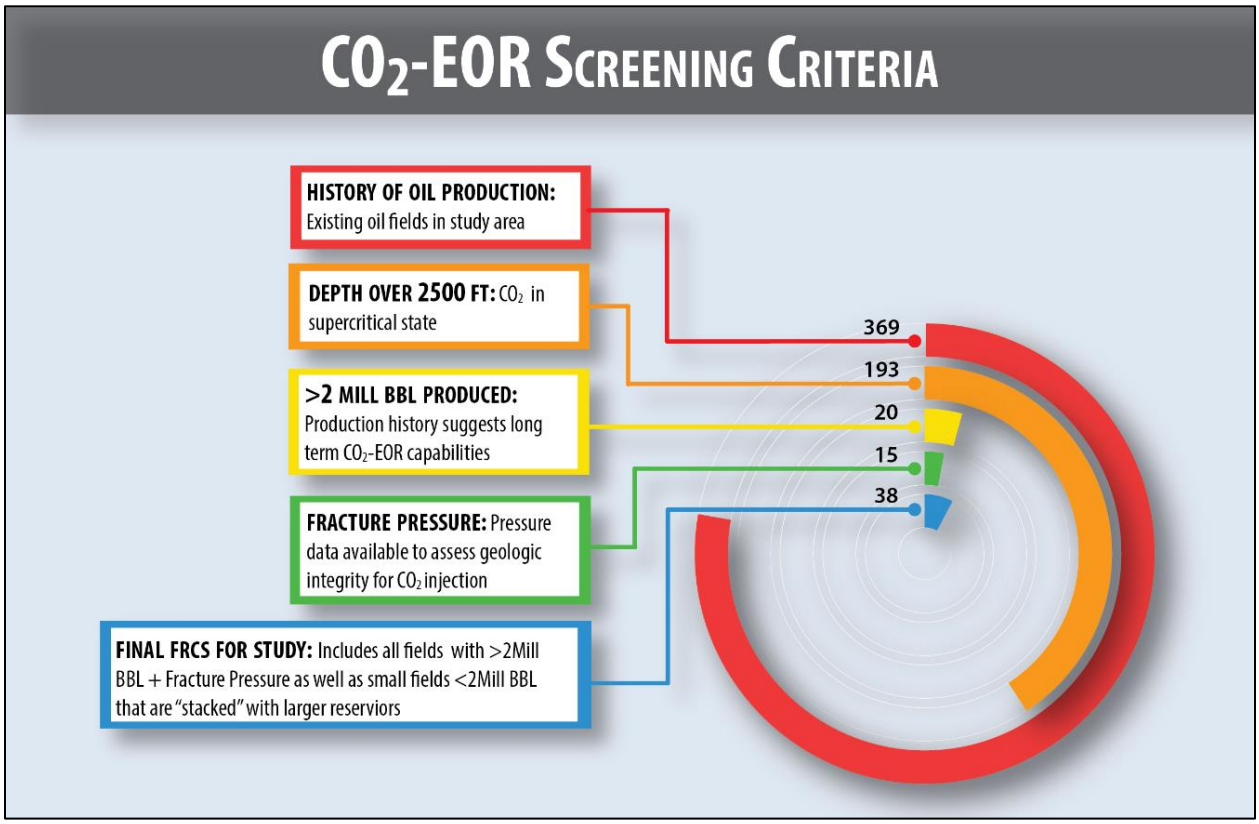

Figure 7. 5 step CO_2-EOR field reservoir screening criteria and results.

Once the final 38 FRCs were identified, MSU re-ran CO_2 storage capacity and oil production capacity calculations using the three different EOR production methods: *Conventional EOR+*,

Advanced EOR+, *Maximum Storage EOR+* (OECD/IEA 2015[1]). These estimates were then used to calculated potential CO_2 emissions reduction scenarios for the Colstrip facility.

MSU also completed a quality control review of the Least Cost Assessment (LCA) model input variables that were used to generate preliminary pipeline route alternatives between Colstrip and the 38 potential CO_2-EOR fields. Five variables were selected for the LCA model: land cover, slope, distance to roads, soil depth and land ownership. These variables represent key factors in early 'go' or 'no-go' pipeline routing decisions, touching on important environmental, engineering and regulatory factors with significant effects on project costs and overall success. A cost scale from 1 to 5 was applied to each variable, where scores in the 1 and 2 range represent pathways that minimize impacts and costs, and high scores in the 4 and 5 range represent pathways that would be difficult or costly to traverse. The scores were updated in the LCA model and new discreet cost maps were generated for each variable. From here, the discreet maps were combined to generate an aggregate cost map which was used to model the preliminary pipeline route alternatives between Colstrip and the potential CO_2-EOR fields. Figure 8 illustrates the LCA model process.

[1] OECD/IEA 2015. *Storing CO_2 through Enhanced Oil Recovery. Combining EOR with CO_2 storage (EOR+) for profit.* International Energy Agency, Insights Series 2015. 48pp.

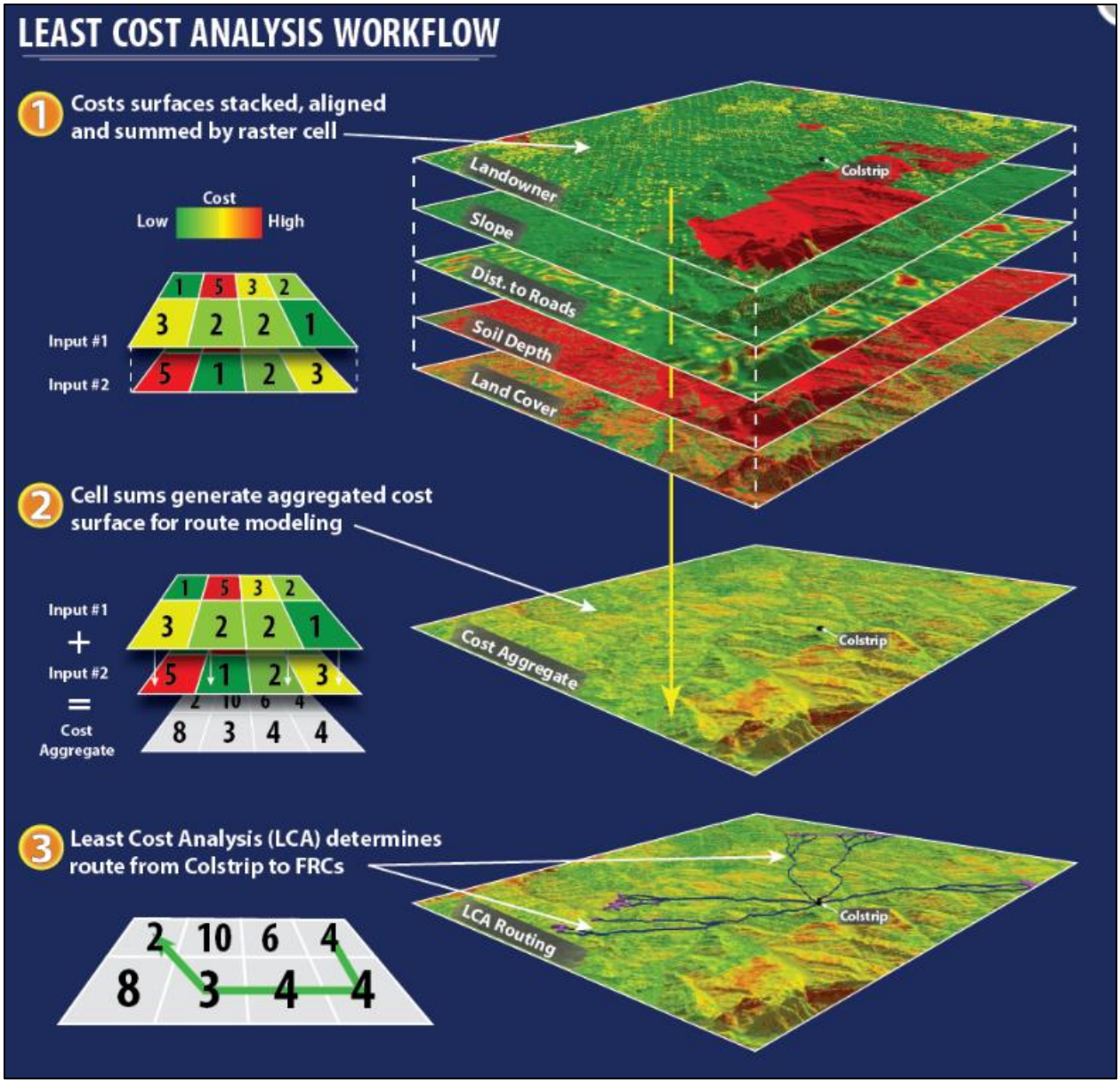

Figure 8. Least Cost Analysis Model work flow.

Substantial progress was also made on the Story Map presentation that is being developed through the GIS Portal application. MSU is using the Story Map as an online, interactive presentation of the CO_2-EOR Suitability Assessment that will be accessible to a broad stakeholder audience. The Story Map is predominately driven by graphics and maps with a supporting narrative to walk a user through the CO_2-EOR field screening process, CO_2 storage and oil production analysis, LCA model and pipeline route analysis, and Colstrip emissions reduction analysis. A significant portion of this quarter was dedicated to improving the content, continuity, graphics, and user-interface features of the Story Map.

Lastly, MSU began writing draft sections of the final report, including a white paper that will accompany the final deliverable package for this study. The white paper will provide a full

narrative of the CO_2-EOR Suitability Assessment and will include a more detailed description of the methodology, analysis, results, and discussion sections than what is provided in the Story Map.

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. A quality assurance and control review of the CO_2-EOR field screening evaluation was completed. Additional data sources were evaluated to bolster the datasets used to calculate the CO_2 storage and EOR production volumes. Estimated CO_2 storage and EOR production volumes were calculated using three different EOR production methods. The estimated CO_2 storage volumes were then used to calculate potential emission reduction scenarios for the Colstrip facility. A review of the LCA model input variables and their cost scores was completed. The LCA model was re-ran and revised pipeline route alternatives between Colstrip and the potential CO_2-EOR fields were generated.

B. December 2016: Completion of the interactive mapping application
    a. Progress continues on the new GIS Portal and Story Map application. Significant improvements were made to the Story Map content, continuity, graphics, and user-interface features.

C. June 30, 2017: Final Report and data package
    a. MSU began writing draft sections of the final report including a white paper that provides a full narrative of the CO_2-EOR Suitability Assessment.

Objective 5

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

Quarter activities and accomplishments

Culturing
From February through May 2017, we have continued to culture coal bed methane pond algal isolate PW-95, (*Neospongiococcum sp.*) and have collaborated with researchers at MSU on culturing techniques and to obtain new cultures as necessary. We currently have a thriving PW-95 culture in the Apple lab.

An additional culture of a coal bed methane pond algal isolate, *Nanochloropsis gaditana*, is now thriving, as is a culture of a non-toxic strain of the atmospheric nitrogen-fixing cyanobacteria, *Anabaena cylindrica*. While *A. cylindrica* was not isolated from the coal bed methane ponds, it is

integral to this research since it has great potential as a crop fertilizer that will not require the input of additional nitrogen.

Flocculation Experiment
To concentrate PW-95 with the goal of using it as a fertilizer for crops, graduate student Olakunle Ogunsakin has conducted an experiment in which he has added potassium hydroxide (KOH) across a spectrum of pH levels to determine the points at which flocculation (or in other words, formation of clumps of algae) is greatest. The clumps can then be collected and added to plants. The results of this experiment are currently being analyzed.

Biofilms Experiment
We have initiated an experiment to determine whether biofilms of the nitrogen-fixing cyanobactera, *A. cylindrica*, influence soil moisture, temperature, and electrical conductivity. Concentrated *A. cylindrica* cultures added to the surface of the soil of *Triticum aestivum*, (wheat), plants grown in potting soil in three-gallon pots is in the process of forming a biofilm. Smaller batches of biofilms are concurrently incubated under light in petri dishes containing *A. cylindrica*, potting soil, nutrients, and water. A 5TE soil moisture, temperature, and electrical conductivity sensor (Meter Instruments) is placed approximately 5cm (~2 inches) below the soil surface in each pot and attached to a datalogger with temperature recordings at five minute intervals for the duration of the experiment, which will be approximately one month. Aboveground measurements of biofilm formation are documented via photographs and sampling of surface soil to quantify *A. cylindrica* soils. Concurrently, the wheat plants are measured for growth, number of leaves, chlorophyll content, and other physiological and developmental parameters.

Coal Bed Methane
Analyses continue for the growth of algal consortia in non-sterile CBM production water and the changes in microbial community dynamics. Community analyses are underway to determine potential community dynamics for the selected algal consortium during growth in CBM production water.

Hirings

MSU
Hannah Goemann will start as a Ph.D. student in the Immunology and Microbiology Department, mentored by Dr. Brent Peyton at MSU. Hannah will be working on the development and field testing of cyanobacteria as a biofertilizer on the recently funded NSF EPSCoR project described below. This new project funding will also be used to support MSU student, James Valley, of the Crow Tribe, for summer research scaling up these systems to multiple 200L (~53 gallon) outdoor photobioreactors.

MT Tech
There were no new hires this quarter. Olakunle Ogunsakin continues to be employed by the MREDI project in his role as a graduate student in the Department of Environmental Engineering at Montana Tech and anticipates graduation in summer 2017.

Equipment Purchased

- Soil temperature, moisture, and electrical conductivity sensors and dataloggers
- Pipettor and pipettes
- Soil and pots
- Nutrient solutions
- Vortex mixer
- Camera with associated filters
- Statistical software
- Fluorescence and spectral analysis instrument for leaf physiology
- Portable greenhouse and larger scale algal cultivation ponds (200 liter) were ordered (supplemental funds from USGS)

Awarded Projects

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: Brent Peyton and Lee Spangler. Start Date: 08/01/2016; Award Amount: $6,000,000. Focused on sustainability in Montana's upper Missouri River Basin.

Proposal *(leverage the overall MUS research enterprise)*

SURF (Summer Undergraduate Research Fellowship) Funded: Joe Natale, an undergraduate student in Geophysical Engineering at Montana Tech, has received a SURF fellowship to work on the biofilm, wheat and biofilm project in the summer of 2017 with Xiaobing Zhou and Martha Apple as mentors.

NSF INFEWS/T3: EMBER-Engineered Multispecies Biofilms for Energy and Resources: Pending. Submitted to NSF Food, Energy, Water NEXUS, Matthew Fields, PI, Martha Apple, CO-PI ($140,000 for Apple part of proposal), Brent Peyton, Co-Investigator.

NSF Research Traineeship (NRT) Program: Pending. (Program Solicitation NSF 16-503) NRT: Engineering Biofilms for Energy and Resource Recycle. Matthew Fields, PI.

Milestones

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

    a. MT Tech: These are being scaled up from the 1-2 liter scale to the 19 liter carboy scale. Suitable conditions for outdoor growth are in the process of being assessed at MSU.

    MSU: The first attempt for outdoor growth was completed in September 2016. We are awaiting warmer weather in the spring to attempt further growth outdoors with natural sunlight.

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: Olakunle Ogunsakin was recruited in December 2015 for continuing work on this project and is culturing PW-95 (Neospongiococcum sp., microalgae isolated from the coal bed methane ponds) and the nitrogen-fixing cyanobacteria (Anabaena cylindrica) to test their macronutrient and micronutrient compositions.

b. MSU: 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 1 to 2 hours. Upon a second re-suspension (test 2), a small amount of additional biomass settled.

   Further cultivation is currently underway to produce more biomass and attempt collection via filtration with different filters (1 to 5 μm). The different fractions will then be used to test in Objective 6.

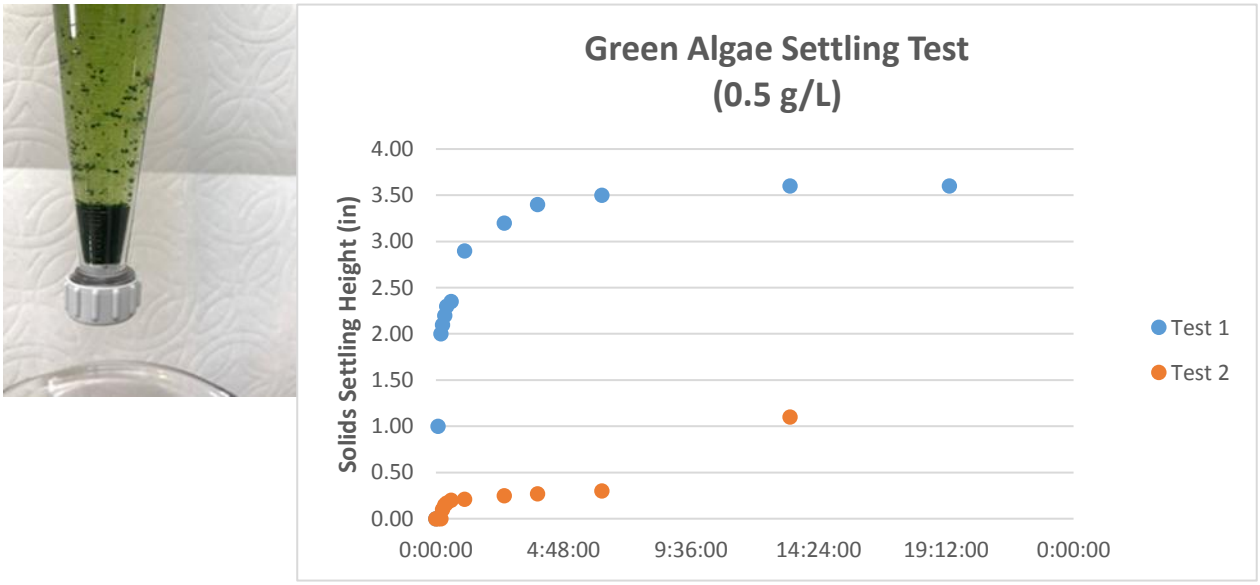

Figure 9. Green Algae Settling Test Comparison. Algal strain PW-95 was grown to a slurry concentration of 0.5 g/L. In Test 1 the green alga was ground, mixed in DI water for 20 minutes and shook for 1 minute. In Test 2 the algae slurry was stirred and mixed a second time approximately 12 hours later.

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

   a. MT Tech: Since wheat (*Triticum aestivum*) has shown the greatest response to algal fertilizer to date, further tests of the effectiveness of cyanobacteria will be conducted on wheat plants between now and July 2017.

Dissemination of Results
Presentation

Ogunsakin, O., Apple, M. Evaluation of the fertilization properties of PW-95 - a green alga isolate from the CBM ponds in Montana. National Society of Black Engineers Conference, Kansas City, Missouri, March 2017.

Objective 6

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

Quarter activities and accomplishments

Montana Tech finished a manuscript entitled "Extraction of surface water area of coal-bed methane water ponds from Google Earth images by a novel multi-component algorithm". The manuscript was developed from the algorithm development and CBM water extraction. The paper will be submitted in May 2017 to Remote Sensing Journal. This will be part of the final report of the project.

Montana Tech extracted water bodies from regular true-color images collected by a drone taken from a field trip in 2016 to the coalbed methane ponds (Figure 10). A drone was used to collect images of two ponds (Calamity Jane and Bronco Billy) in 2016. Additional images of more ponds will be collected in 2017 and pond areas extracted using the algorithm developed. Ground-control points will be set up by Montana Tech and direct sun light will be avoided when images are collected.

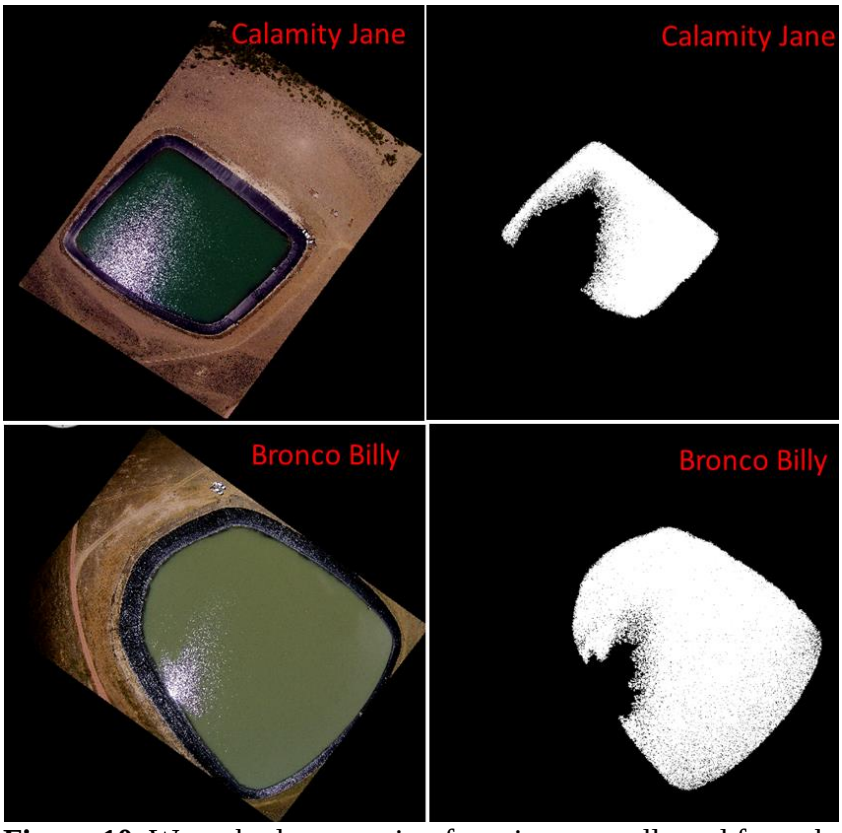

Figure 10. Water body extraction from images collected from the regular camera acquired from the Unmanned Aircraft System (UAS) platform. The left panels are the two true-color images and the right panels show the water bodies extracted from the two ponds. Direct sun light impact

can be alleviated by pointing the camera to the other direction. This will be improved in the field campaign in 2017.

Spectral data collection and analysis of the hyperspectral reflectance data of filamentous cyanobacteria (*Anabaena*), green algae (*PW-95*), and N. Gaditana contined. New microalgae indices were formed to investigate the best index - micro-algae cell concentration relationships.

Montana Tech continued Raman scattering spectra collection on filamentous cyanobacteria (Anabaena), green algae (Pw95), and N. Gaditana on the inVia Raman Microscope at Montana Tech. Raman Spectra of N. Gadotna will be collected at the end of May or early June 2017. Also, processing continued of the Raman spectra collected on Anabaena and PW-95. The Raman spectra of N. Gaditana will be processed once the spectra collection is finished.

Major activity this quarter include:

- Completion of the manuscript on the multi-component algorithm development for automatic water body extraction of CBM ponds
- Water body extraction from images collected from a drone in July 2016 during a field trip to the research site
- Microalgae indices development for effective derivation of microalgae concentration from hyperspectral data of microalgae
- Continuation of Raman spectra data collection, processing, and interpretation of cyanobacteria (Anabaena), green algae (PW-95), and N. Gaditana.

Hirings

No new hires were made this quarter. The graduate student continued image processing including image classification and hyperspectral data analysis. The student continued working towards his thesis entitled "Hyperspectral and Raman spectral Characterization of Various Microalgae from Coalbed Methane Ponds."

Equipment Purchased

No major equipment purchases were made this quarter.

Milestones

A. August 2015 – October 2016: Estimate areal coverage of CBM ponds using Hyperion or Landsat data
    a. For this objective, Montana Tech finished a manuscript on the multi-component algorithm to automatically extract water bodies from high-resolution Google Earth images. Water body extraction was performed from images collected from a drone flown at the research site.

B. July 2015 – December 2016: Evaluate time-course for methane production during consecutive stimulations
    a. Experiments were concluded that demonstrated repeated stimulation of coal-dependent methanogenesis using algal biomass in laboratory-scale enrichments. We are currently producing algal biomass (native PW-95 strain) grown in CBM

water and determining economical ways to collect (filtration) and process (high-shear mixing) biomass. The collected biomass will then be disrupted with high-shear mixing and collected via filtration. The fractions will be tested for the ability to stimulate coal-dependent methananogenesis.

C. July 2016 – June 2017: Monitor mesoscale growth of algae using spectral methods
   a. Montana Tech collected the hyperspectral and cell concentration data of cyanobacteria (Anabaena), green algae (PW-95), and N. Gaditana. Spectral data regression analyses with cell concentration are being performed using various methods: single bands, ratios, and microalgae indices. Raman scattering spectra of cyanobacteria (Anabaena), green algae (PW-95), and N. Gaditana continued to be collected. Raman spectra analysis is being performed to characterize the structure and chemical composition of the microalgae and to identify the implication for remote sensing from space.

Expenditures to Date

Quarterly Report 1/31/2017

	All Budgets	Spent to Date
Salaries & Benefits	717,237	549,216
Subcontract Payments		
Montana Tech	222,667	160,617
Montana Emergent Technologies	75,000	51,938
Operations	160,096	63,897
Equipment	25,000	28,000
Total Costs	**1,200,000**	**853,668**