# 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 Power Plant (Colstrip) this quarter. Two important updates on this front include the final merger of Talen Energy Corporation with Riverstone Holdings, LLC in December 2016, and the legislative activity related to Colstrip in Helena.

Legislative activity aimed at protecting coal-based economies and communities in Montana began last fall when the Montana Energy Telecommunications and Interim Committee (ETIC) drafted seven legislative proposals in response to the pending closures of Colstrip Units 1 and 2. Of the seven drafts, the committee amended three of the proposals and ultimately forwarded on a total of five bills for consideration in the 2017 Montana legislative session (see the Quarter 4 report for a description of the draft bills). Below is a summary and current status of each bill as of January 31, 2017. The current status of each bill can also be tracked in the 2017 LAWS systems: http://laws.leg.mt.gov/legprd/law0203w$.startup?P_SESS=20171.

- HB 0022 (previously reported on as LC COL1) is a bill to appropriate money to assist, intervene, and plan for future closures of coal-fired generating plants. The bill was introduced to the House on November 23, 2016, and is currently working its way through the Montana legislative process.

- SB 0037 (previously reported on as LC COL2) is a bill to establish the Coal-Fired Generating Unit Decommissioning Act which would vest in the Department of Environmental Quality the authority to review, approve, modify, or disapprove coal-fired generating unit decommissioning and remediation plans. This bill was introduced to the Senate on December 9, 2016, and as of January 31, continues to move through the legislative process.

- HB 0060 (previously reported on as LC COL3) is a bill to establish the Treasure State Restore and Rebuild Act which would provide support for communities affected by the closure of coal-fired generation plans. This bill was introduced to the House on December 9, 2016, and is working its way through the legislative review process.

- SB 0038 (previously reported on as LC COL5) is a bill to establish the Montana Energy Accountability Act which would require entities to notify the Public Service Commission, the Department of Revenue, and the Governor when they intend to retire a coal-fired generating unit. Entities would also be required to pay impact fees which would be used to support the communities, workers, and tax base that are affected by the closure. This bill was introduced to the Senate on December 9, 2016, and is currently in the legislative review process.

- HB 0021 (previously reported on as LC COL6) is a bill to establish a benefits and retirement security task force that would focus on the rights of employees affected by the exit or bankruptcy of natural resource-based businesses that operate in Montana. This bill was introduced to the House on November 11, 2016, and was tabled in committee on January 27, 2017.

## 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 including experiments performed by Montana Emergent Technologies, Inc. (MET) in a wellbore analog reactor at 80° C. 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 to begin to assess the strength of the materials. 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)*

No proposals have been submitted this quarter. A proposal to DOE for additional funding has been initiated with a slated submittal date for April, 2017.

**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: Enzymatic urea hydrolysis kinetics from plant based sources of enzyme 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 six hours of exposure to 70° C, JBM urease demonstrated a > 97% decrease in activity. A first order, series-parallel and series type mathematical model has been derived to predict the kinetics of deactivation of the JBM urease. A manuscript, entitled “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. Fracture Fixture Sealing at 80° C: Work continues to evaluate the urease enzyme from Sporosarcina pasteurii. Ureolytic activity can be observed up to approximately 80° C as demonstrated by sealing a 200 µm gap between cement and steel in a wellbore analog reactor. 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 80° C and injecting S. pasteurii organisms and allowing them to attach for 15 minutes. This was followed by a pulse of urea and calcium to initiate the mineralization process. As shown by the plot below, it took 26 pulses to reach a three order of magnitude permeability reduction, about twice as many as the 70° C tests (Figure 1). The photo (Figure 1) shows the calcite buildup at the gap "pinch point" where most of the sealing occurred.

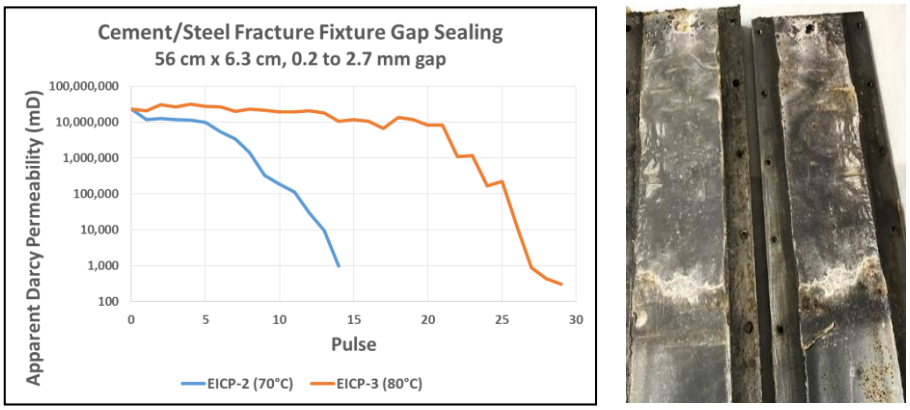



**Figure 1.** Left. Permeabilty decreased in the fracture fixture experiment as the number of calcium pulses increased. Right, a bridge of calcium carbonate was observed to form at the cement and steel interface where the gap size decreased from 500 µm to 200 µm (circled areas)

c. 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. Preliminary 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 at 100 and 130° C. Carbon steel pipes with caps, 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 2) and compressive strength testing of the specimens is planned. Additional experiments are planned to assess the mineral precipitation material properties with organic additives, calcite seeding, and different temperature treatment conditions.

**Figure 2.** Thermally induced calcium carbonate precipitation was observed to bind together particles of sand after removal from the steel pipe batch reactor.

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, Inc. (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

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, CA.

Publications

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

# 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, baghouse waste material of interest to Southern Company was investigated in the laboratory. This material was used to determine the potential of MICP to consolidate and bind the particles together. The experiments suggested that binding of the material was possible with MICP and the particle size was observed to increase which suggests the method can be used to reduce dust formation. On January 19, 2017, Gordon Criswell from Colstrip toured the laboratory, met with researchers, and provided input on the research being

performed and the proposed experiments. A conference call with Ben Gallagher of Southern Company was held in January. He was updated on recent progress of biomineralization of CCRs and put forth the idea of a site visit by MSU researchers to the Scherer plant in Georgia. An abstract entitled "Remediation of Coal Combustion Residuals Using Microbially-Induced Calcite Precipitation" was accepted for presentation at the World of Coal Ash conference to be held May 2017 in Kentucky.

**Hirings**

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

**Equipment Purchased**

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

**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. As previously reported samples of the Colstrip coal-fired power plant coal combustion residuals (paste) were collected in October 2015 and June 2016. Biomineralization experiments performed with the paste showed that noticeable binding of the materials was observed when mixed with increasing concentrations of paste with biomineralizing microbes and solutions. 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.

    Also described in the previous quarter, a method to enhance the ureolysis rate was determined. It was observed in previous experiments using fly ash that the pH is driven significantly higher than the optimal conditions for MICP. It was found that adding a phosphate buffer solution to the triplicate batch study flasks along with the microbes and mineralizing solutions resulted in complete ureolysis in high concentrations of ash materials. 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.

    In addition, biomineralized "caps" were successfully produced over Colstrip paste material (Figure 3). Producing a cap over the paste has the potential to do two things: (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.

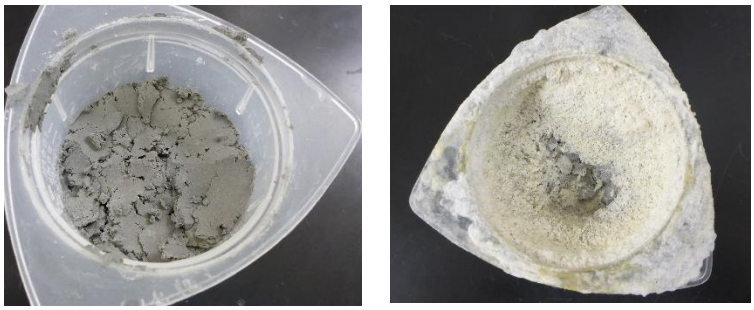


**Figure 3.** Left. Paste materials in a beaker. Right. Cap of biomineral formed on the top surface of the paste (whitish layer).

b. Experiments continue with the baghouse waste material supplied by Southern Company. The primary research question being addressed 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). During this period, several experiments were performed with baghouse waste material provided from Southern Company to investigate the minimum amount of liquid needed to achieve binding of particles to increase particle size and reduce dust formation. Using MICP, powdered activated carbon (PAC) ash was pressed into molds and hydrated at different percentages with microbes and water to determine the effect of initial water content on PAC ash binding. After a weeks' incubation time, a material sieve analysis test was performed to compare the particle binding achieved between each hydration percentage. The molds showed improved binding strength and higher material consolidation as the hydration percentage increased. Due to this success, work has begun developing a wind tunnel to analyze the resistance of MICP bound PAC ash to wind erosion.

B. August 2016-June 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. A conference call was held in January 2017 with Ben Gallagher of Southern Company to discuss design of meso-scale experiments addressing the consolidation of CCR materials of interest to Southern Company. During the meeting with Gordon Criswell of Colstrip, an idea was discussed for the upscaling of experiments with pond materials. Samples are planned to be collected in April 2017 during a site visit to provide enough material for the up-scaled experiments.

## 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**

The recent quarter included progress on cultivation of selenium reducing bacteria from the Colstrip groundwater. Addition of carbon sources molasses, glycerol, and methanol stimulated growth of bacteria obtained from groundwater wells. Selenium removal has been shown in the groundwater via the bacterial activity.

Experiments with barium and strontium removal from artificial groundwater in porous media flow reactors continued. The results have suggested that the spatial profile of strontium removal is correlated with reaction rate and thus can potentially be manipulated by control of the bacterial concentration and distribution.

Continued work with mine influenced water from the Carpenter Snow Creek District has been successful in catalyzing biomineralization of heavy metals from the water at temperatures similar to the environment. Native organisms capable of promoting biomineralization are being cultivated from sediment sampled at the mine sites.

**Hirings**

No new personnel were hired this quarter. Former undergraduate research assistant Kevin Burt graduated and was converted to a temporary research assistant II appointment to continue working on the laboratory biomineralization studies. Graduate student Neerja Zambare continued to work on barium and strontium precipitation in flow systems. Graduate student, Hannah Koepnick continued her work on cultivating bacteria extracted from wells at Colstrip to remove selenium from the groundwater. Graduate student Emily Stoick is continuing her work on biomineralization of metals in mine influenced water.

**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 flow reactors – strontium and barium remediation: Laboratory studies in porous media flow systems continued to investigate biomineralization driven removal of strontium and barium from artificial groundwater. Recent results indicate that the extent of strontium removal is dependent upon the reaction rate, which in turn depends upon the bacterial presence and activity. Thus, the method and extent of bacterial delivery and proliferation in an environment may be a key control factor in biomineralization driven remediation of contaminants such as strontium and barium.

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

   a. Colstrip Groundwater – Selenium reduction studies: The investigations toward utilizing native organisms to remove selenium from contaminated Colstrip groundwater continued this quarter. Microbes were sampled last summer from two wells in the area and grown in the laboratory to determine selenium reduction potential. Batch microcosms were cultivated with different carbon additions, methanol, glycerol, and molasses. Recently, after two months of cultivation, reductions in nitrate were observed with samples from both wells (Figure 4). In the 366S well, located in the shallow aquifer underlying the power plant, all carbon additions resulted in microbial activity. In the 633M well, located in the McKay coal seam, the molasses addition showed rapid nitrate reduction. The reduction of nitrate verifies microbial activity and nitrate removal typically occurs prior to selenium removal. Preliminary data on selenate indicate reduced selenate concentrations in the microcosms where nitrate has been depleted. Selenium data analysis is ongoing, but these initial results are promising.

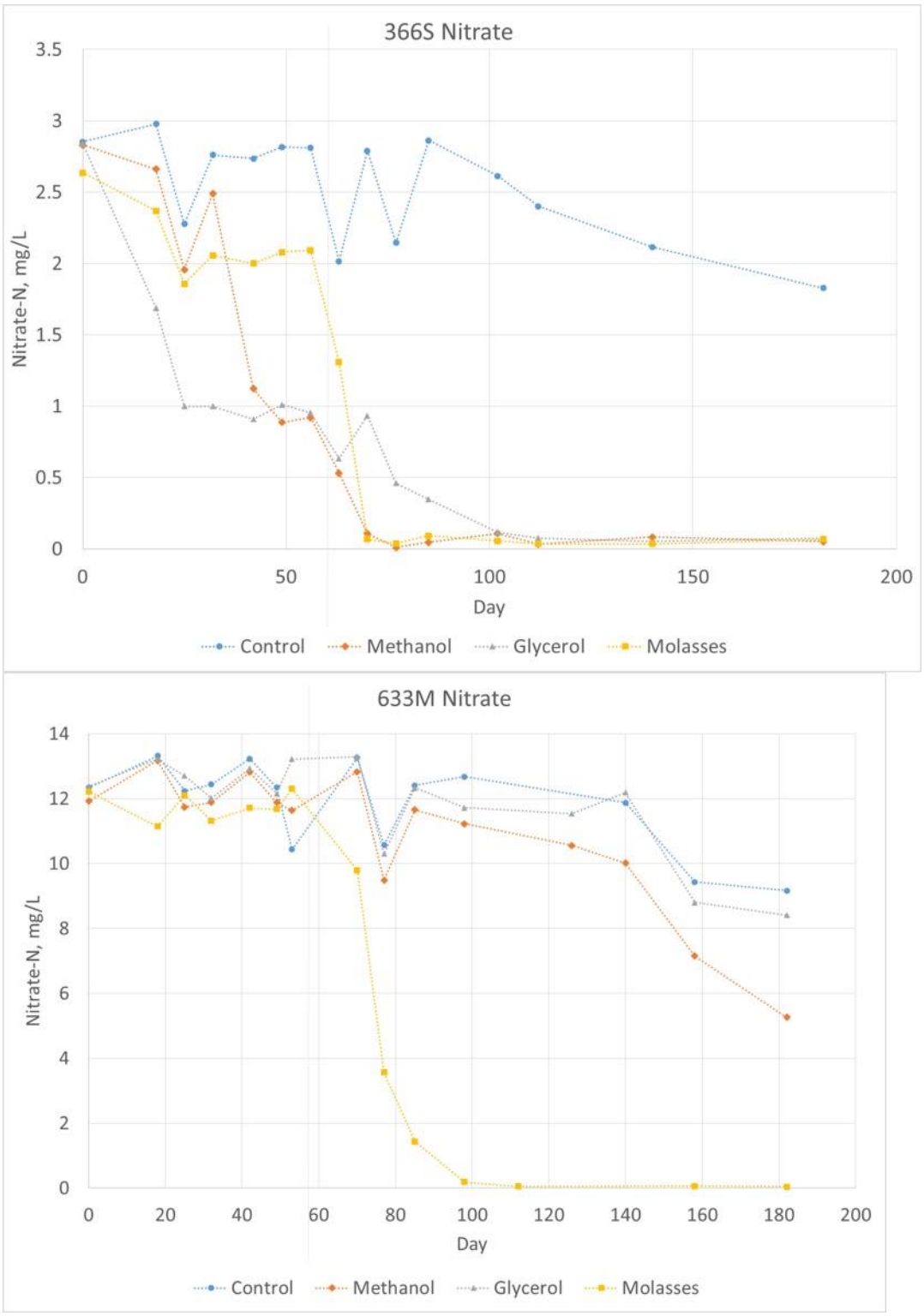



**Figure 4.** Nitrate reduction in microcosms with groundwater and bacteria cultivated from two wells at the Colstrip Power Plant.

b. Carpenter-Snow Creek (Neihart, MT) Mine Influenced Water – heavy metals removal: Laboratory tests continued on bacterially induced carbonate precipitation for heavy metals removal in mine influenced water from the Evening Star adit at the Carpenter-Snow Creek Mining District. Continued studies with the laboratory model bacterium, *S. pasteurii,* were conducted at room temperature and 10° C, which is close to the water temperature at the site. These studies indicated that the ureolysis reaction and carbonate precipitation resulting in reduction of heavy metals does occur at the lower temperature (Figure 5). However, growth of the laboratory strain did not occur at 10° C for the 24 hour duration of the test.

   Sediment samples collected from the site were used in batch incubations to cultivate native bacteria capable of promoting the process of ureolysis and metals removal via precipitation. In the sediment from the Evening Star adit, microbial growth occurred and a bacterial strain capable of ureolysis was isolated. Ongoing tests are underway to evaluate whether the organism is also capable of catalyzing heavy metal removal from the mine influenced water. The bacterium will also be identified via DNA sequencing in the near future.

   Emily Stoick, graduate student, received a 2017 Montana Water Center Fellowship to continue her studies on biomineralization of heavy metals in mine influenced water.

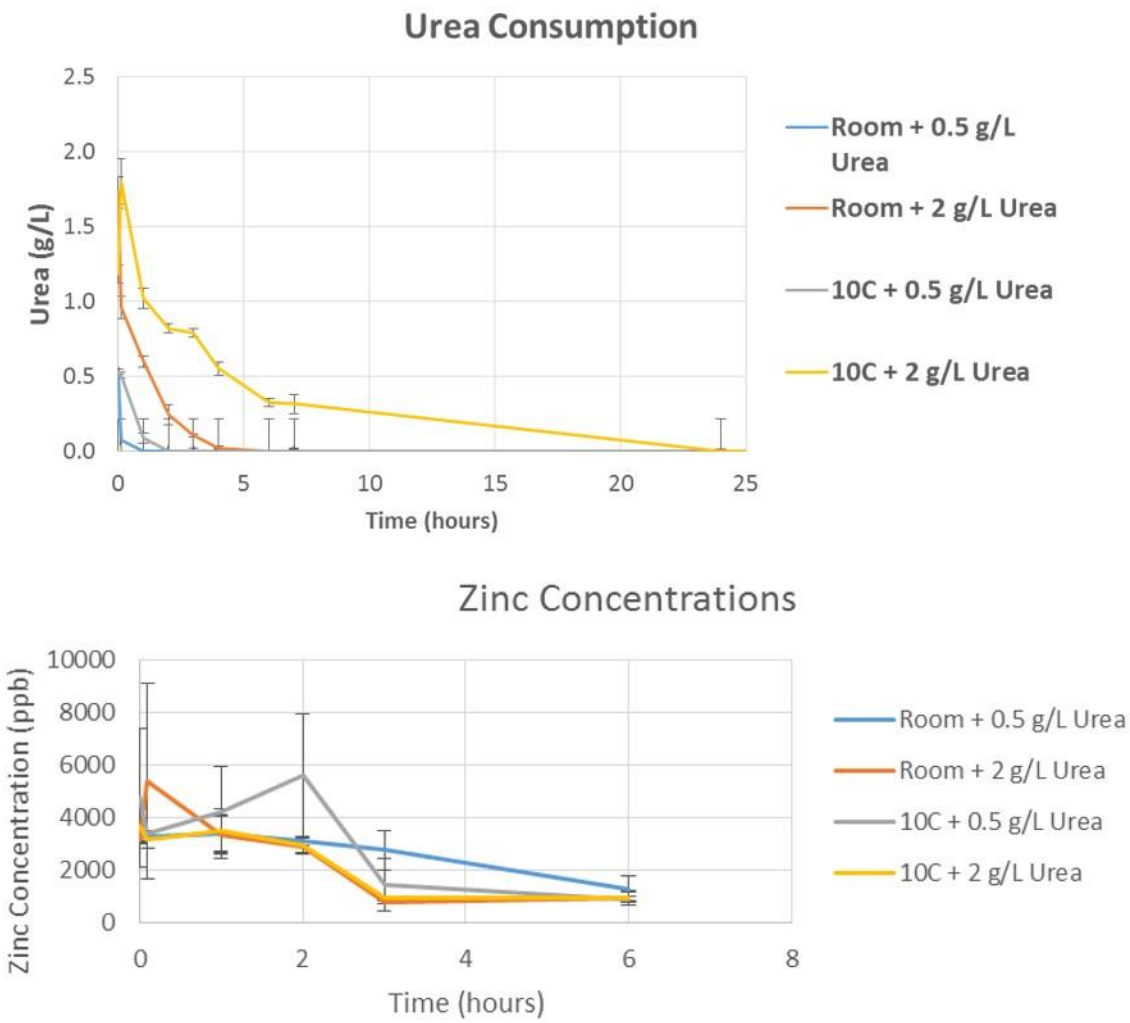



**Figure 5.** Urea and zinc concentrations in batch tests using S. pasteurii for ureolysis induced carbonate precipitation. Tests at 10° C, close to the water temperature at the site, were shown to be successful. Error bars are standard deviation of triplicate tests.

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. A meeting with Gordon Criswell of Talen Energy and the Colstrip Power Plant on January 19, 2017, resulted in a fruitful discussion of potential steps forward for the application of selenium reducing organisms to remediate contaminated groundwater at Colstrip. Plans are underway to return to Colstrip in Spring 2017 and sample for microbes in additional wells where selenium concentrations are lower than expected, indicating the possible presence of native selenium reducing bacteria.

## 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**

Work this past quarter largely focused on the $CO_2$ storage assessment and enhanced oil recovery (EOR) potential analysis near Colstrip. Substantial progress was also made on improving and expanding upon the capabilities of the GIS Portal that was implemented last quarter to house, organize, and share the results of the EOR assessment.

Work associated with the $CO_2$ storage assessment and EOR potential analysis included a detailed review of the $CO_2$-EOR field reservoir screening analysis for data quality assurance and quality control purposes. The review confirmed that the seven field-reservoir combinations (FRCs) described in the Quarter 3 report are the most suitable for $CO_2$-EOR in the Colstrip region. MSU also calculated the potential $CO_2$ storage and EOR production volumes for the seven FRCs using three different EOR production methods: *Conventional EOR+*, *Advanced EOR+*, *and Maximum Storage EOR+* (OECD/IEA 2015[1]). *Conventional EOR+* refers to the standard industry method of using $CO_2$ to maximize oil production. *Advanced EOR+* refers a method that uses even more $CO_2$ during the enhanced oil recovery process. The term *Maximum Storage EOR+* refers to an EOR method that focuses on using and storing as much $CO_2$ as possible during the recovery process. The analysis resulted in initial minimum and maximum $CO_2$-EOR estimates for each FRC.

Work also began on reviewing the model input variables that were used in the Least Cost Analysis model for the pipeline routes. The review is for quality assurance and quality control purposes to ensure the initial pipeline route analysis is complete and correct. The review will continue into the next reporting period.

In addition to the $CO_2$ storage and EOR production analysis work, MSU made substantial progress on the GIS Portal application that was described in the previous quarter's report. MSU's GIS leads resolved communication issues between the GIS system: the SQL databases, the ArcGIS for Server software, and the Portal for ArcGIS installation. Figure 6 illustrates how Portal interacts with desktop computers, data servers, and the internet. Portal is essentially a storefront for the REST services that the GIS server host. Many of these services are the same services in the interactive atlas mentioned last quarter. This allows all of the project information to be integrated from the same databases and maintain accuracy across all platforms. Portal was granted access to the project data as an operating system user on both the GIS server machine as well as the server that houses the SQL databases. After Portal was allowed to read/write on both of these servers, the data could be displayed in this new framework that MSU can reach via a web browser and removes the need to establish more ArcGIS Desktop users. This architectural change in the data structure creates a collaborative workspace within the GIS Portal that allows

[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.

project managers, technical leads, and web developers to work on a web application called a Story Map.

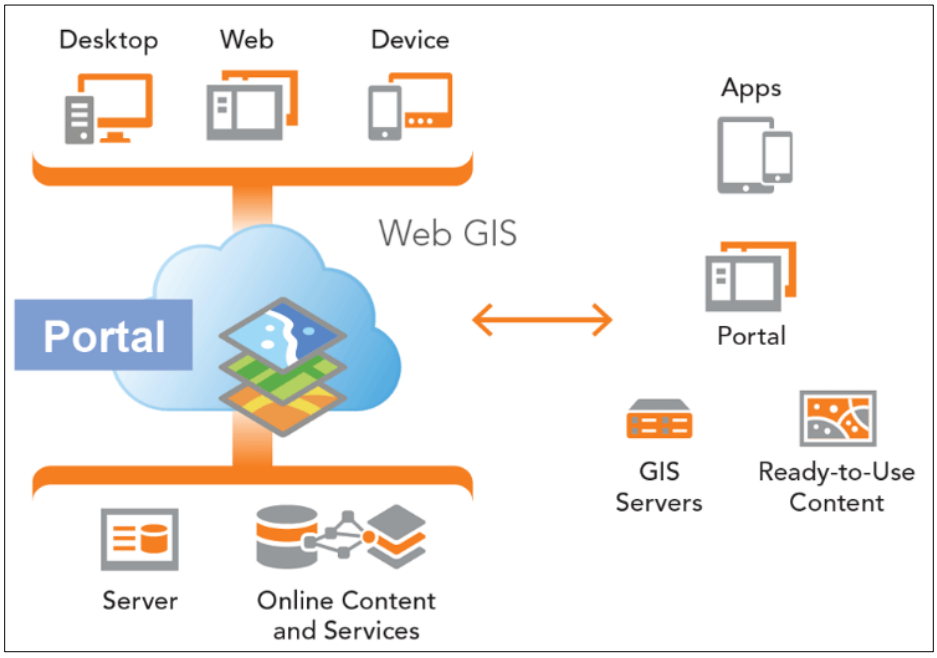



**Figure 6.** ESRI graphic showing the structure of the new web GIS system

A Story Map is an ESRI product that provides a data enriched reporting and/or presentation tool with access to live maps and real-time data displayed for interaction. There are several basic components that make up the graphical and narrative content of a Story Map including: data layers, web maps, and text. Data layers are used to build the web maps, and the web maps are the interactive maps or figures that are then embedded into the Story Map application. Text is also added to the Story Map to serve as a narrative that guides the user through the content. The Story Map content and the user's ability to interact with the content can be enriched by adding rules and actions to the web based graphic user interface (GUI) that is used to build the application. Links can also be added to the text to display additional information to enhance the Story Map application.

MSU continued developing a Story Map application for the Objective 4 study and made substantial progress on this effort this quarter. To begin the Story Map building process, MSU prepared a draft narrative and outline to organize the initial content of the Story Map into the following sections: (1) study introduction; (2) $CO_2$-EOR field screening analysis; (3) pipeline route analysis; (4) $CO_2$-EOR storage/production analysis; and (5) conclusions.

For each section of the Story Map, the MSU team prepared various data layers that were then used to create web maps. The web maps are the interactive maps and figures that were inserted into the Story Map application. Draft text was then added to the Story Map to provide a narrative for each section. Initial draft sections were completed for EOR field screening and $CO_2$-EOR production/storage sections. Figure 7 through Figure 11 show several screen shots from the $CO_2$-EOR field screen analysis section of the draft Story Map.

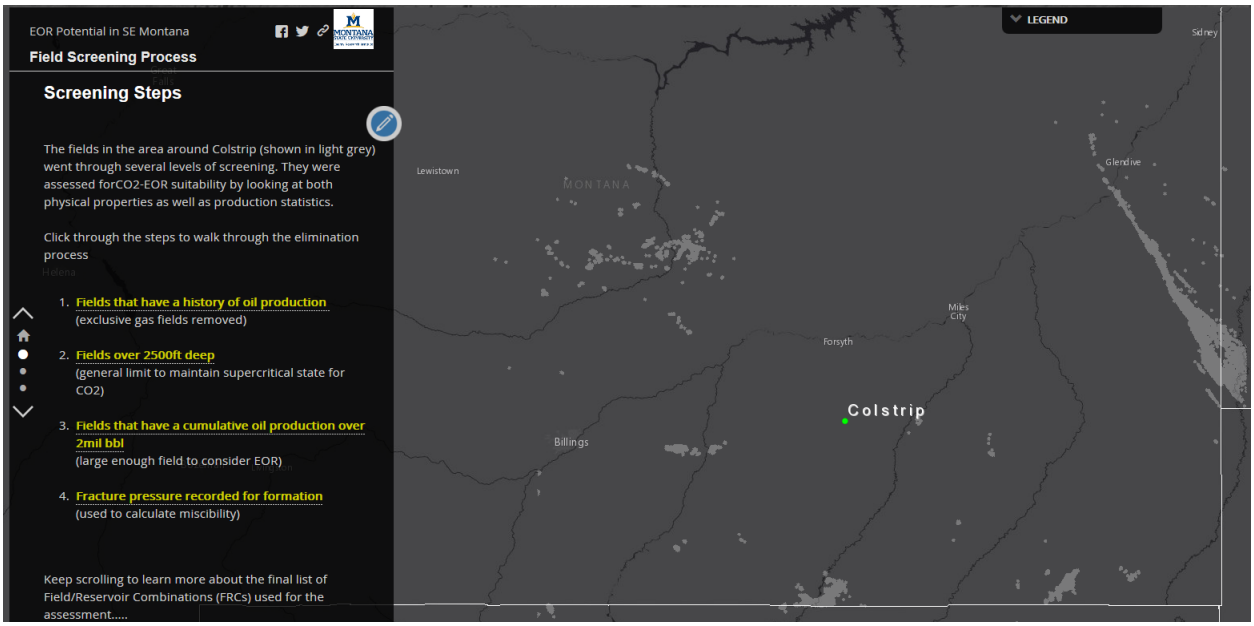



**Figure 7.** $CO_2$-EOR field screening analysis screenshot #1. The map on the right illustrates all of the oil and gas fields (in light grey) in the study area around Colstrip. Text on the left briefly describes the analysis and how to navigate the page.

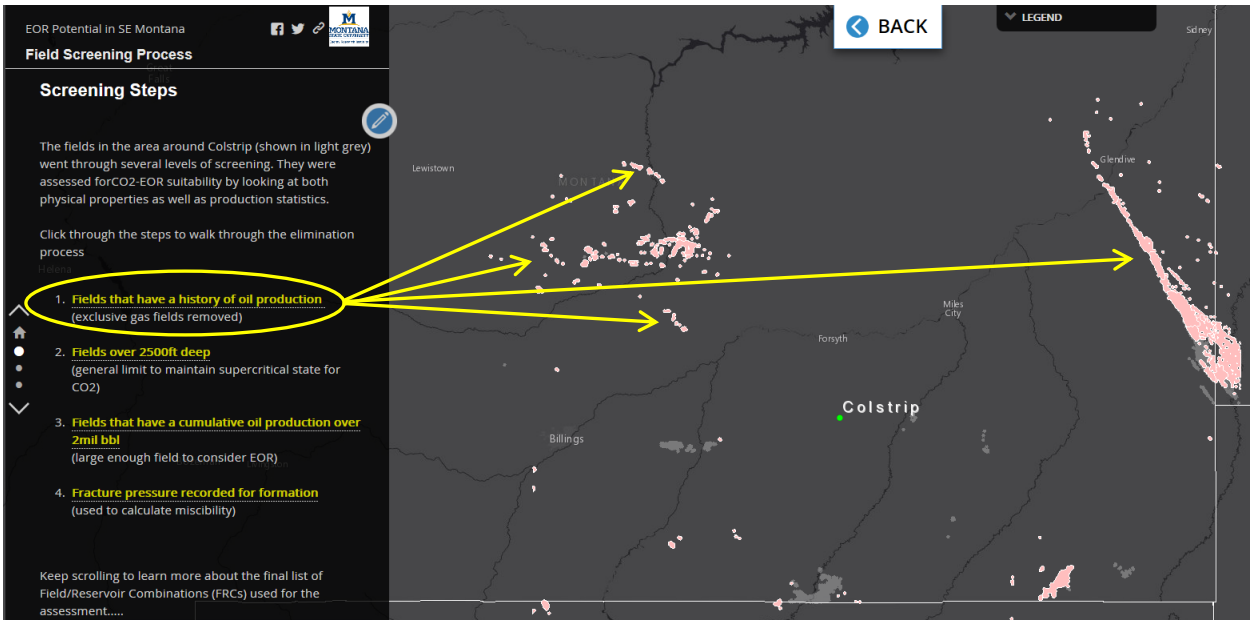



**Figure 8.** $CO_2$-EOR field screening analysis screenshot #2. When the user clicks on the yellow text circled above, the map on the right automatically updates to highlight the fields that have a history of oil production.

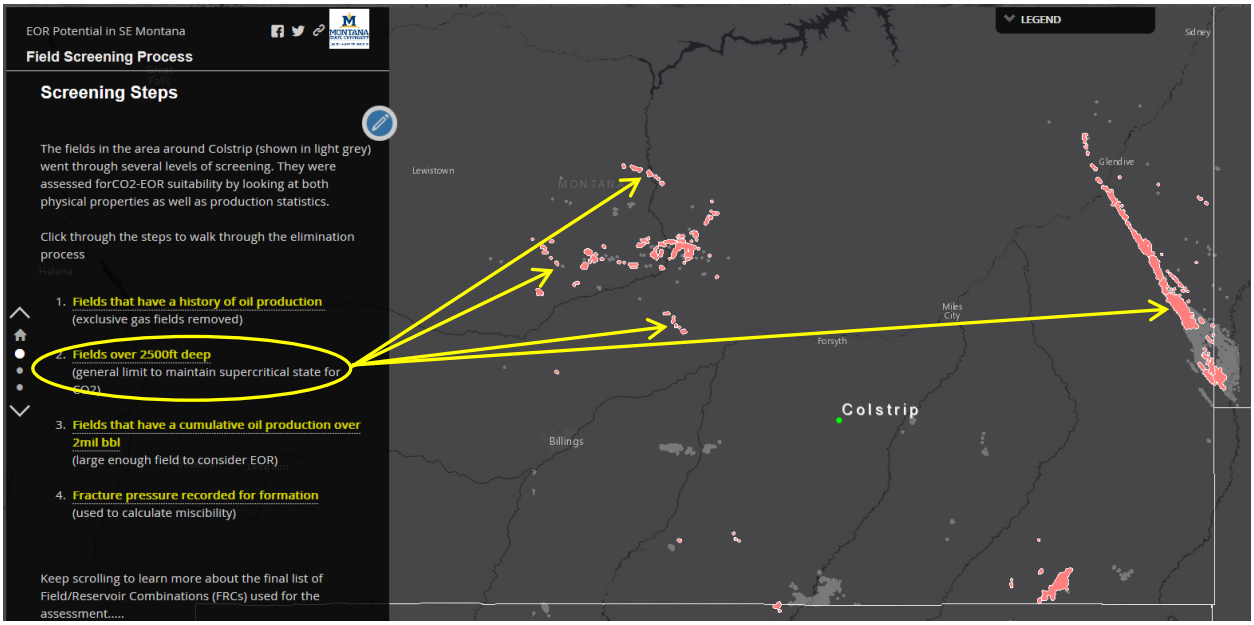



**Figure 9.** $CO_2$-EOR field screening analysis screenshot #3. When the user clicks on the next yellow text circled above, the map on the right automatically updates to highlight the fields that have a history of oil production and are also over 2,500 ft deep.

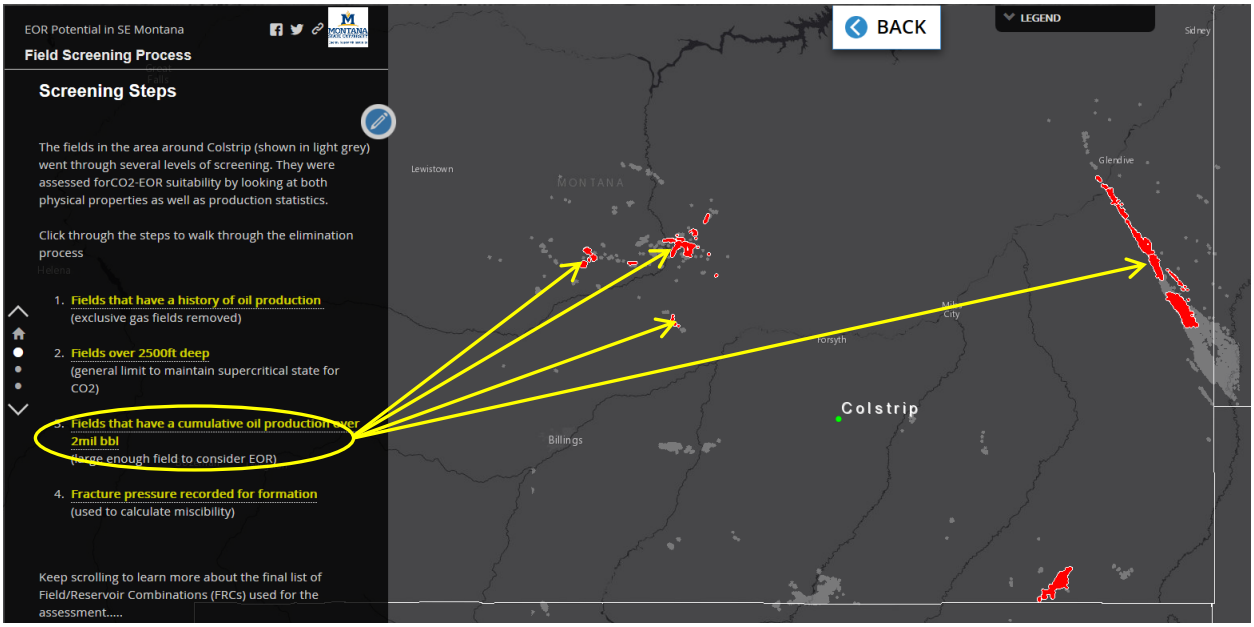



**Figure 10.** $CO_2$-EOR field screening analysis screenshot #4. When the user clicks on the next yellow text circled above, the map on the right automatically updates to highlight the fields that have a history of oil production, are also over 2,500 ft deep, and have a cumulative oil production >2mil bbl.

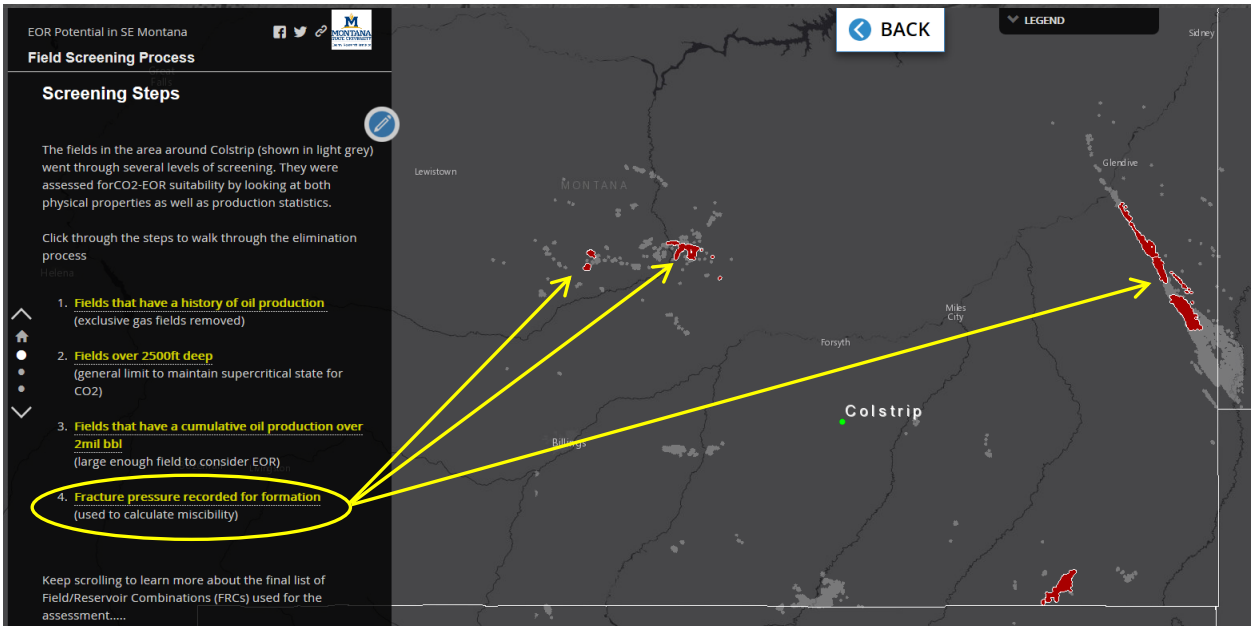



**Figure 11.** $CO_2$-EOR field screening analysis screenshot #5. When the user clicks on the last yellow text circled above, the map on the right automatically updates to highlight the fields that meet all of the screening criteria: fields that have a history of oil production, are over 2,500 ft deep, have a cumulative oil production >2mil bbl, and have suitable fracture pressure for miscibility.

Progress continues on the introduction, pipeline routing, and conclusion Story Map sections. MSU will also continue to refine the initial draft sections of the EOR field screening and $CO_2$-EOR production/storage sections.

Lastly, the team also participated in a project update meeting with Gordon Criswell of Talen Energy in January 2017 to present the current project status and discuss the next steps in the $CO_2$ storage and EOR potential analysis near Colstrip.

**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 CO2-EOR field screening evaluation was completed. Estimated $CO_2$ storage and EOR production volumes were calculated using three different EOR production methods. Additional data sources are being evaluated to bolster the datasets used to calculate the $CO_2$ storage and EOR production volumes. Verification of methods used to classify

data for pipeline routing analysis has begun, as well as discussions for consideration of changing weighting in model.

B. December 2016: Completion of the interactive mapping application
    a. Programming and development continues for the JavaScript-based interactive map as well as the new GIS Portal and Objective for Story 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**

During the short day lengths of November 2016 through February 2017, Montana Tech has concentrated on culturing coal bed methane algal isolate PW-95. PW-95 was previously thought to belong to the genus Chlorella, but has now been sequenced by other MREDI researchers and identified as (Neospongiococcum sp.). Other cultures include coal bed methane algal isolate Nanochloropsis gaditana, Chlorella vulgaris from the University of Texas culture collection, and the nitrogen-fixing cyanobacterial species, Anabaena cylindrica, which is cultured with continual aeration and stirring in 19 L flasks. A. cylindrica is potentially a very important fertilizer because it is autotrophic (does not require organic carbon), and especially because it fixes nitrogen and thus can use atmospheric nitrogen for its nitrogen requirements.

Cells of the coal bed methane algal isolate PW-95 cultures were settling out of solution and adhering to the inside of the culture vessels. This makes the alga fairly difficult to grow and collect so graduate student Olakunle Ogunsakin has collaborated with MREDI and biofilm researchers at MSU to obtain new cultures. These cultures, backup cultures, and cultures destined for experimental use are currently being grown in the Apple lab at Montana Tech. Mr. Ogunsakin is culturing coal bed methane algal isolate PW-95 (Neospongiococcum sp.) towards an experiment with flocculation as a way to concentrate the alga. He has also developed a means of using gravity and cone-shaped vessels to concentrate PW-95 into a slurry.

**Plants**

The experimental potato plants (Solanum tuberosum) which were treated with A. cylindrica, C. vulgaris, Hoagland's Nutrient Solution, or $H_2O$ were harvested in fall 2016, and data has been obtained on the dry weight of potato tubers and of potato plant biomass.

Flax plants (Linum usitassimum) continue to be cultivated in the Montana Tech greenhouse and have received slurries of A. cylindrica, C. vulgaris, Hoagland's Nutrient Solution, or $H_2O$ to compare their potential effectiveness as fertilizers.

Because the greatest positive responses to soil augmentation with PW-95 have occurred with wheat (Triticum aestivum), additional experiments are planned with wheat, algae, and cyanobacteria in the winter and spring of 2017. Hard red winter wheat seeds will be given PW-95, A. cylindrica, Hoagland's solution, or $H_2O$, after which they will receive water in a controlled regime. This will take place in the Apple lab to avoid variables introduced in greenhouse conditions. Biofilm formation will be monitored, and if practical, soil temperature and moisture sensors will be deployed to determine whether biofilm formation influences soil temperature and moisture.

**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*

The efforts of a postdoctoral research have been shifted to analyze the collected samples. Our sequencing instrument has been down for maintenance, and will hopefully, be running again soon. No further hires were made.

*MT Tech*

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. No further hires were made.

**Equipment Purchased**

*MSU*

No major equipment purchases were made this quarter.

*MT Tech*

Two 19-liter flasks and miscellaneous culturing supplies for growth of coal bed methane algal isolate PW-95 and *Anabaena cylindrica*.

**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. Further growth outdoors will be attempted with natural sunlight when the weather

is warmer in the spring. An additional larger-scale reactor and portable green-house is planned for purchase.

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.

    MSU: Elemental composition has been tested for indoor laboratory algal cultures. MSU will next test PW95 grown outdoors in spring 2017.

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 microalgae and cyanobacteria will be conducted on wheat plants between now and July 2017.

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

## Objective 6

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

**Quarter activities and accomplishments**

Montana Tech finished the algorithm development of water area extraction from satellite images and reported the new algorithm development and results at the 2016 American Geophysical Union fall meeting in San Francisco, December 12-16, 2017. A manuscript is being developed entitled "Automatic extraction of water area of coal bed methane water ponds from Google Earth images and regular photos from an unmanned aircraft system using a novel algorithm" which presents the algorithm development and CBM water extraction. A preliminary analysis was done of the hyperspectral reflectance data of filamentous cyanobacteria (*Anabaena*) and green algae (*Chlorella* sp.) and the relationships of hyperspectral reflectance with micro-algae cell concentration. Growing of N. Gaditana is in progress and when the cell concentration reaches high enough, the hyperspectral measurement of N. Gaditana will be made which is anticipated at the end of February 2017.

Major activity this quarter include:

- Finalizing the algorithm development for automatic water body extraction of CBM ponds.
- Validation of the water area extracted using developed algorithm and field measured area using differential GPS data in July 2016 during a field trip to the research site.
- Hyperspectral analysis of the spectral data of cyanobacteria (Anabaena) and green algae (Chlorella sp.) and identification of the most sensitive bands.

- Continuing regression analysis between the cell concentration and hyperspectral reflectance data at the most sensitive bands.
- Continuing analysis of Raman scattering spectrum of cyanobacteria (Anabaena) and green algae (Chlorella sp.)

**Hirings**

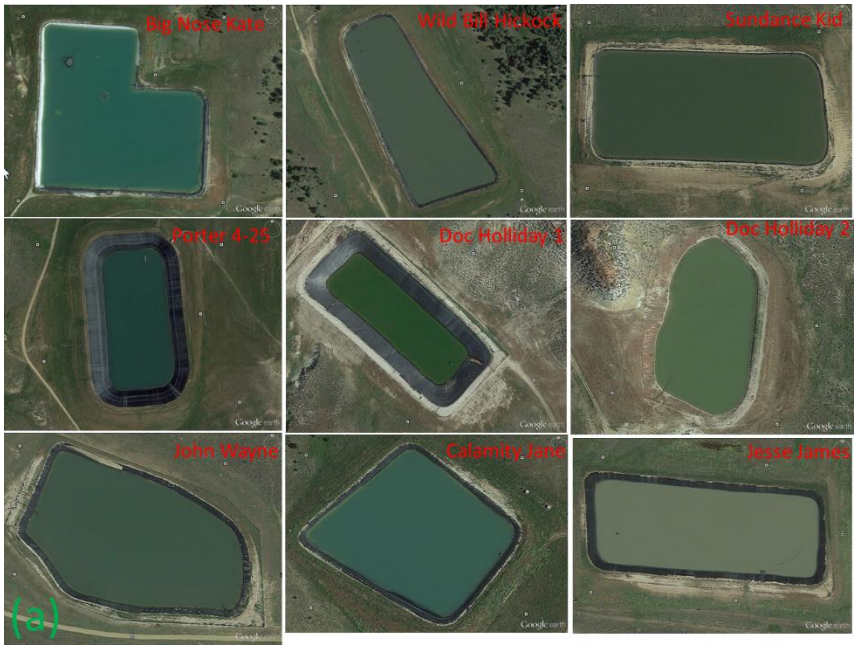
The graduate student continued image processing including image classification and hyperspectral data analysis and continues working towards his thesis. No new hires were made this quarter.

**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 finalized the algorithm to automatically extract water bodies from high-resolution Google Earth images and validation of the algorithm. The CBM ponds have great potential for micro-algae growth to generate biofuel and/or bio-fertilizers. The area of each CBM pond has been automatically extracted (Figure 12). Out of the ten ponds, a GPS survey was carried out along the edge of four of them. The area of the ponds from the GPS survey has been calculated using the triangulation method. The algorithm for water extraction was validated. The algorithm development and results were reported at the 2016 AGU fall meeting. A manuscript is being formed from these activities.



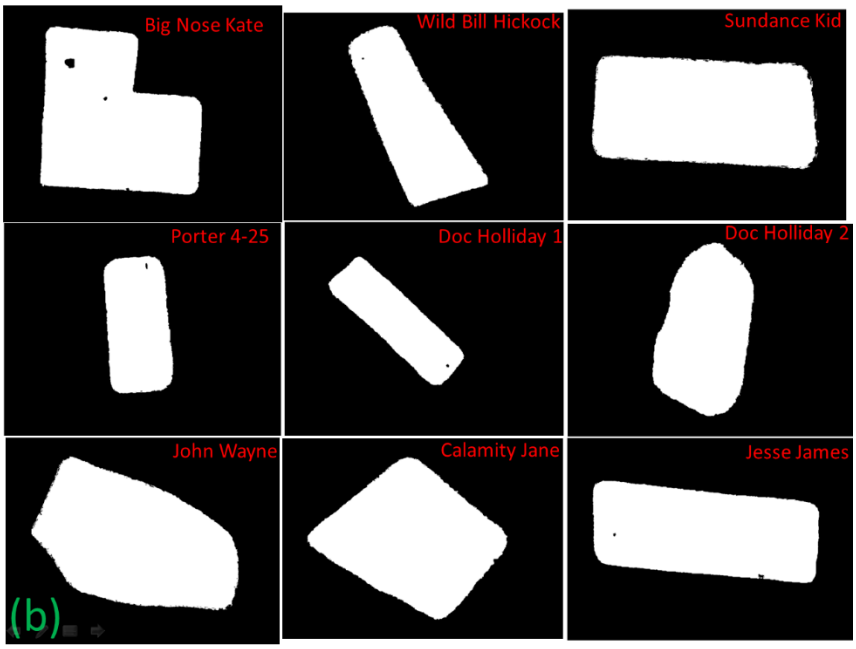



**Figure 12.** (a) Input Google Earth images each containing CBM pond; (b) Output water bodies (white area) automatically using the developed algorithm.

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

   a. Experiments are on-going for re-stimulation of coal-dependent methanogenesis. 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 (*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. 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.

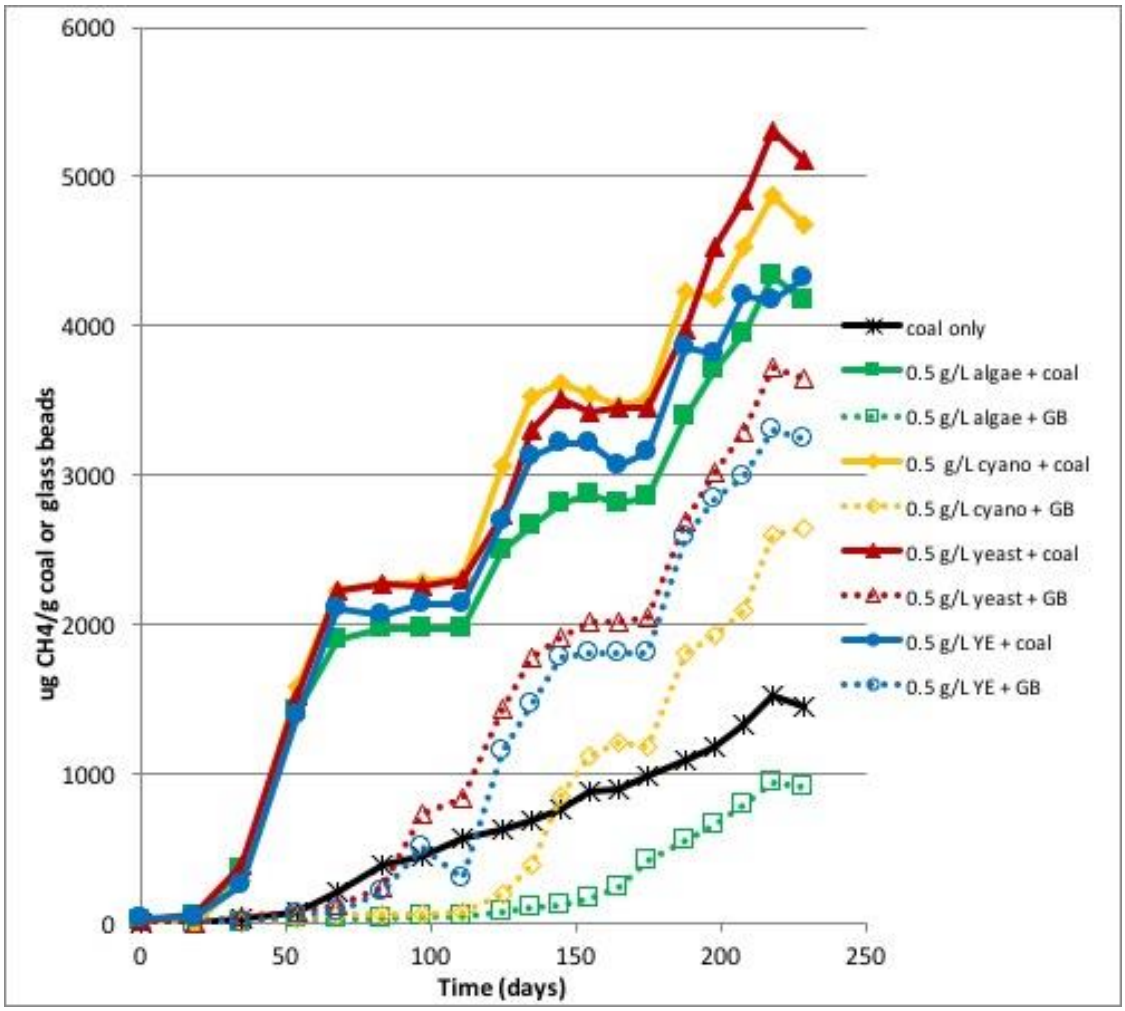



**Figure 13.** Four nutrient sources were tested for successive stimulations: algae, cyanobacteria, yeast, and yeast extract (all at 0.5 g/l). All nutrients were able to re-stimulate the same coal, but the amount of additional methane produced per amount of nutrient added decreased. In addition, there was a large difference between the ability of algae extract to stimulate successive coal-dependent methanogenesis over yeast or yeast extract.

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

a. Montana Tech has collected the hyperspectral and cell concentration data of cyanobacteria (Anabaena) and green algae (Chlorella sp.). The similar data collection for N. Gaditana will be completed by the end of February 2017. Several analysis are on-going: (1) a continuous analysis of the hyperspectral data for discovery of the most sensitive bands; (2) regression analysis between cell concentration and hyper-spectral reflectance; and (3) a continuous analysis of Raman scattering spectrum of cyanobacteria (Anabaena) and green algae (Chlorella sp.).

## Expenditures to Date

Quarterly Report 1/31/2017

| | All Budgets | Spent to Date |
|---|---|---|
| | | |
| **Salaries & Benefits** | 717,237 | 400,226 |
| **Subcontract Payments** | | |
| **Montana Tech** | 222,667 | 149,752 |
| **Montana Emergent Technologies** | 75,000 | 44,750 |
| **Operations** | 160,096 | 26,861 |
| **Equipment** | 25,000 | |
| **Total Costs** | **1,200,000** | **473,614** |