Remediation of Coal Combustion Residuals Using Microbially-Induced Calcite Precipitation

Abby A. Thane[1], Eric Troyer[1], Al Cunningham[1], Lee Spangeler[2], Ben Gallagher[2], Adrienne J. Phillips[1]

[1]Center for Biofilm Engineering, Montana State University, Bozeman, MT 59717, USA
[2]Southern Company Services Inc., Atlanta, Georgia 30308, USA

CONFERENCE: 2017 World of Coal Ash – (www.worldofcoalash.org)

KEYWORDS: Coal Combustion Residuals, Fly ash, Calcite Precipitation, *Sporosarcina pasteurii*, Particulate material, Coal-fired Power Plant

ABSTRACT

The final rule recently published by the Environmental Protection Agency places new standards on existing and future CCR storage facilities to reduce potential environmental impacts.[1] Closure of existing facilities and corrective actions may be necessary if environmental impacts are identified. In-situ stabilization (ISS) is a common corrective action used in environmental remediation. ISS is accomplished by mixing Portland cement or similar binder with in-situ materials. However, this method may be challenging or impossible to implement in large CCR facilities.

An alternative to ISS may be the use of microbially-induced calcite precipitation (MICP). In MICP, the urease enzyme produced by *Sporosarcina pasteurii* promotes the hydrolysis of urea (ureolysis). If Ca^{2+} is present it will react with CO_3^{2-} formed during ureolysis to produce $CaCO_3$ which can be used to bind particulate materials together. Batch tests and a flow through column experiment were performed to investigate the production of $CaCO_3$ in the presence of CCRs. Samples taken at different time points were analyzed for decreasing urea concentration and increasing pH, both of which are indicative of ureolysis. Data collected from batch testing and presented here shows MICP successfully producing $CaCO_3$ in the presence of fly ash, scrubber waste water, and flue gas desulfurization material (gypsum). It was observed that the resulting $CaCO_3$ formed around the CCR particles, binding them together. This method of binding CCRs and forming an effective “biocement” has potential for applications in corrective action for closing facilities. It may also be useful in controlling fugitive dust emissions for operating facilities.

INTRODUCTION

For decades coal-fired power plants have been one of the most economical and widespread solutions to the world’s energy demands.[2] As a result, waste from coal-fired power plants, also known as coal combustion residual (CCR), has become a growing

problem. Second only to household trash, CCRs are one of the largest waste products in the United States. Recent estimates suggest that although ~50% of coal ash is recycled, 1.5 billion tons are currently stored in landfills or surface impoundments.[3] Despite the national shift towards renewable energy sources, CCR production is expected to rise. The American Coal Ash Association predicts a 2.6% increase in CCR production through 2033.[4]

In a typical plant, CCRs are produced when pulverized coal is burned in a boiler. Immediately following combustion, heavier ash particles sink to the bottom of the chamber (bottom ash), while lighter particles (fly ash) travel with the hot flue gases and are filtered out using a bag house, an electronic precipitator, or both. After filtering, flue gasses may undergo additional treatment in a flue-gas desulfurization unit (FGD) to capture and remove sulfur dioxide. Typically, FGD systems react an alkaline sorbent like limestone with flue gas to form a solid byproduct such as gypsum. Many CCRs, including fly ash and gypsum, can be recycled into building products such as concrete and wallboard.[3] Recycling not only minimizes the amount of land needed for disposal, it can also improve building materials, reduce the need for mining, and decrease atmospheric CO_2 emissions.[4] However, recycling is not always feasible. If these materials are improperly disposed, they may pose a threat to environmental and human health through releases to groundwater or fugitive dust emissions.[3, 5]

The Environmental Protection Agency (EPA) recently published a final rule to regulate the disposal of CCRs and reduce potential risks posed by certain CCR management units. The rule sets minimum criteria for existing and new CCR landfills and surface impoundments by establishing administrative requirements, location restrictions, design and operating criteria, closure requirements, and post closure care.[1]

Coal-fired power plants face a challenge and considerable expense to comply with the new regulations.[6] One novel method that holds promise for various applications in CCR treatment is to bind these materials together using a process known as microbially-induced calcite precipitation (MICP). This process, also known as biomineralizeation, uses microorganisms and porous media to produce biocement. The incorporation of biocement in CCR material has the potential to fill pore space between particles, improve strength and reduce the flow of water through ponded ash. In addition, biocement in landfilled CCR may agglomerate particles thereby reducing the possibility of them entering the atmosphere and contributing to fugitive dust emissions. This could improve dry disposal of CCRs.[7] It may also be possible to remove contaminants from wastewater streams for their safe disposal or discharge through co-precipitation of minerals and metals.[8, 9]

MICROBIALLY-INDUCED CALCITE PRECIPITATION

Microbes are capable of altering the chemistry of their surrounding environments. Certain chemical reactions promoted by microbially produced enzymes can result in supersaturation and precipitation of minerals. In MICP, *Sporosarcina pasteurii* (*S. pasteurii)* produces the urease enzyme which promotes the hydrolysis of urea (ureolysis). This reaction shown in equations 1-5 influences the chemical saturation

state by increasing the dissolved inorganic carbon concentration and the pH value. This results in increased carbonate alkalinity which will react with free calcium (if present) to form calcium carbonate ($CaCO_3$) precipitate (Equation 6). [10-12]

$$CO(NH_2)_2 + H_2O \xrightarrow{Urease} NH_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \xrightarrow{Spontaneous} NH_3 + H_2CO_3 \quad (2)$$

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^- \quad (3)$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad (4)$$

$$HCO_3^- + H^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2H_2O \quad (5)$$

With Calcium:

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3 \quad (6)$$

Precipitation of $CaCO_3$ depends also on the presence of nucleation sites. These can be provided by the microbes (including those attached to surface in the biofilm phenotype) and other particles in the environment.[10] The primary advantage of MICP technology is the use of low viscosity aqueous solutions to promote precipitation and significantly reduce permeability in porous materials. These solutions can be applied in small pore spaces or fractures that are otherwise difficult to penetrate with higher viscosity fluids such as cement. In porous media $CaCO_3$ will form around individual particles, binding them together to create biocement.[13, 14] MICP can also deposit calcite in preferential flow paths or can be applied to the surface of porous materials for dust suppression.[15, 16]

MICP has already been proven successful a wide range of engineered applications including: environmental remediation, amending or improving construction materials and cementing porous media.[17-22] Previous subsurface investigations show that it has the potential to reduce leakage of geologically sequestered carbon dioxide and methane.[12, 15, 23, 24] It may also improve the containment of nuclear waste.[25] Due to its previous success, it was hypothesized that MICP can also be applied to CCRs to remediate current management units, improve the handling of new materials, and to comply with the new EPA requirements.

In this study, we examined the use of MICP to bind together CCR materials. The objective of this study was to determine (1) if biomineralization (MICP) could occur in the presence of CCRs, (2) to discover any inhibitory factors that may exist and (3) if biomineralization could successfully bind CCR particles together. Batch testing and a flow-through column study was used to assess the impacts of MICP on different types of CCRs. These tests focused on the use of fly ash, gypsum, and flue gas desulfurization (FGD) waste water from a power plant fueled with western sub-bituminous coals.

MATERIALS AND METHODS

Growth Media

Calcite Mineralizing Medium (CMM-) was comprised of 3 g/L nutrient broth (Fisher Scientific), 10 g/L ammonium chloride (Amresco), and 20 g/L urea (Fisher Scientific). This served as the solution to promote growth of the microorganisms and could be amended with 49 g/L CaCl (CMM+) to promote precipitation when necessary. Media were adjusted to pH 6.0-6.3 with 2 M Hydrochloric Acid (Fisher Scientific). For high concentration fly ash studies, 400 mM N-(2-Hydroxyethyl)piperazine-N-2-ethanesulfonic Acid (HEPES) buffer (Fisher Scientific) was added to the media to mediate and control high pH levels. Media were filter sterilized using Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with PES membrane (Fisher Scientific). Microbial inoculum was prepared by centrifuging an overnight culture grown from thawed frozen stock of *Sporsarcina pasteurii* in 37g/L Brain Heart Infusion (Becton Dickinson) amended with 2% urea (Fisher Scientific). The pellet of microbes was re-suspended in CMM- to an optical density measured at 600 nm (OD_{600}) of 2.0 prior to addition to the screening study, batch studies, or flow-through column study described below. The optical density was measured by aliquoting 200 µL of resuspended culture in triplicate to the wells of a polycarbonate 96 well plate (VWR). The absorbance of 600 nm light (light path length 0.26 cm) was measured by a BioTek Instruments (Winooski, VT, USA) Synergy HT Multi-Mode Microplate Reader and the data were analyzed using Gen5 software.

Sample Analysis

Samples of the batch or column study fluids were collected for analysis of their pH and urea concentrations. The pH of each sample was taken with a Fisher Scientific™ accumet™ pH combination electrode (Fisher Scientific) and Symphony SB70P multimeter (VWR). The urea concentration was analyzed using a colorimetric assay modified from the published Jung assay method.[26, 27]

Calcium Concentrations

Samples with 50% weight/volume (w/v) fly ash and gypsum were prepared by mixing 50 g of the CCR with 100 ml of deionized water. The concentration of Ca^{2+} in fly ash, gypsum, and FGD waste water were analyzed by ion chromatography with a Metrosep C4 150 mm x 4 mm cation column (Metrohm USA, Inc.) equipped with a Metrohm 732 conductivity detector. 20 mM dipicolinic acid at pH 2.7 served as eluent. Prior to IC analysis, samples were diluted 1:200 in deionized water. The sample's peak areas were compared with those of known concentration standards to determine concentration.

Screening Study

A screening study was performed in a polycarbonate 48 well plate (Fisher Scientific) to examine the relationship between CCR concentration and ureolysis. Wells contained either fly ash, gypsum, or a fly ash/gypsum mixture immersed in 1 ml CMM-. Fly ash and gypsum were tested at concentrations of 5%, 10%, 25%, 35%, and 50% w/v. Mixtures were tested in triplicate at ratios of 25:75, 50:50, and 75:25 fly ash:gypsum with a total concentration of 25% w/v in the well. Wells were inoculated with 10 µL *S.*

pasteurii at time zero, sampled for initial urea concentration, and left on a shaking table (IKA® MTS 2/4 digital) at 300 rpm. Final samples were taken at 24 hours.

Batch Studies

Batch studies were run for each type of CCR treatment in Erlenmeyer flasks. Treatments containing media, CCR, and *S. pasteurii* were prepared in triplicate (Table 1) along with positive and negative controls (Table 2). Positive controls contained medium and *S. pasteurii* while negative controls contained medium and CCR material. Fly ash and gypsum were tested at 50% w/v concentration (for example if 50 g of the CCR was added to the flask, 100 ml of the fluid would be added as well). Treatments containing FGD waste water were prepared by adding CMM- chemicals directly to the waste water.

Table 1. Batch Study Material Matrix (components used in a given treatment are indicated by a check mark.)

Treatment	CMM-	CMM+	HEPES Buffer	*S. pasteurii*	FGD Waste Water	Gypsum	Fly Ash
1	✓			✓	✓		
2	✓			✓		✓	
3	✓			✓			✓
4	✓		✓	✓			✓
5		✓	✓	✓			✓

Table 2. Positive and Negative Controls for the Batch Experiments (Components used in a given treatment are indicated by a check mark.

	Media	*S. pasteurii*	CCR
Positive Control	✓	✓	
Negative Control	✓		✓

Medium was added to each reactor immediately before inoculation in a biosafety cabinet (Sterilgard Hood, Class II Type A/B3, The Baker Company Inc.). At time zero, reactors were inoculated with 1 ml *S. pasteurii*. Reactors were placed on a shaking table (Innova 2300 Platform Shaker, New Brunswick Scientific) at 150 rpm for 48 hours. Samples were collected in 1 ml aliquots at 0, 4, 8, 24, and 48 hours. Samples were analyzed using the methods described above.

Flow-through Reactor Study

A column study was performed by mixing fly ash and sand (to achieve initial permeability to accommodate the syringe pump's (KD Scientific) limited pressure setting) before adding the materials to a 30 ml syringe (Becton Dickenson). The syringe was plumbed together with silicone tubing attached to a syringe pump (Figure 1). Flow rates were controlled by a KD Scientific syringe pump; the flow rate was 5 ml/min to begin and were lowered as pressure increased.

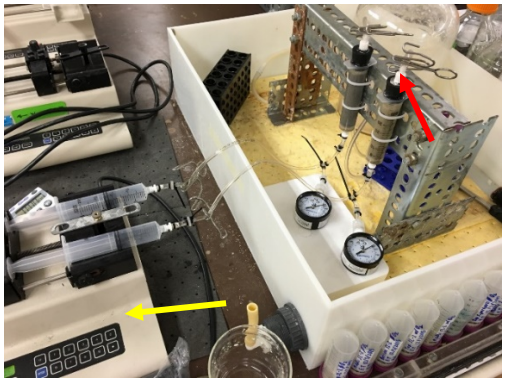

Figure 1. Flow through reactor system designed to assess the reduction in permeability from MICP in CCR materials, specifically, fly ash. A syringe pump (yellow arrow) was used to inject fluids and samples were collected from the effluent of the column (red arrow).

The column was inoculated by injecting a 24h culture of *S. pasteurii* grown in YE- medium. YE- consisted of 24 g/L urea, 1 g/L ammonium chloride, and 1 g/L yeast extract (Fisher Scientific). Medium was pH adjusted and filter sterilized as described above. The culture was allowed to sit for 30 minutes prior to the injection of calcium growth medium which was allowed to react for 3 hours. Three calcium pulses were injected per day prior to re-inoculation of the column with *S. pasteurii* which was allowed to sit in the column overnight. The injection strategy was repeated daily until a total of 20 calcium pulses were performed. Pressure (gauges produced by Omega Engineering) and flow relationships were monitored to determine permeability. Permeability was assessed prior to injection of the microbes, during, and at termination of the experiment. Samples were collected from the effluent of the column to assess urea conversion and calcium precipitation per pulse. At the termination of the experiment, the column was cut open with a Dremel tool and the resulting cemented fly ash column was observed.

Imaging

Samples of the biocemented CCRs were assessed at the particle scale using the Zeiss Supra 55VP Field Emission Microscopy (FEM) in the Imaging and Chemical Analysis Laboratory and Montana State University. Samples were air dried, attached to a pin mount, and sputter coated with iridium for 30 sec. Images were taken at 1.0 kV at a working distance of 4.0 mm of fly ash and gypsum before and after MICP. Elemental analysis was performed with energy-dispersive X-ray spectroscopy (EDX) at 20 kV and a working distance of 15 mm.

RESULTS AND DISCUSSION

Calcium Concentrations

IC analysis showed that the Ca^{2+} concentrations naturally existing in fluids incubated in the presences of fly ash and gypsum were 0.06 g/L and 0.99 g/L respectively. The FGD wastewater was determined to have 30.1 g/L as Ca^{2+}. Based on this, it was hypothesized that calcium addition to the medium was not necessary when treating gypsum, but was required for both eastern bituminous and western sub-bituminous fly

ash treatments. One test condition studied was to amend CMM- with FGD waste water since Ca^{2+} was observed in concentrations favorable for MICP treatment.

Screening Study

Results from the screening study indicate a correlation between inhibition decrease in the urea hydrolyzed over 24 hours and an increasing concentration of fly ash. As shown in figure 2, at the lowest concentration (5% w/v) of fly ash 93% of urea was hydrolyzed and virtually no inhibition was observed. However, as fly ash concentrations were increased, the amount of urea hydrolyzed decreased. The highest concentration (50% w/v) of fly ash had only 6% of its urea hydrolyzed over 24 hours. This was compared to tests using gypsum where 86-95% of urea was hydrolyzed in all wells regardless of the concentration of gypsum added to the well. A trend was also observed in mixtures of fly ash and gypsum where increasing concentrations of fly ash created conditions that inhibited ureolysis. Wells containing 75:25 fly ash:gypsum ratios had only 10% of their urea hydrolyzed whereas 25:75 fly ash:gypsum wells had 67% urea hydrolyzed.

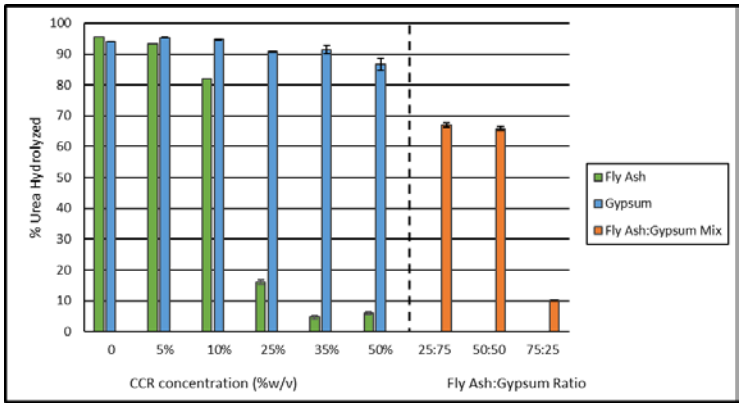

Figure 2. Graph showing the percent of urea hydrolyzed after 24 hours for different concentrations of fly ash, gypsum, and fly ash:gypsum mixture. A decrease in the percentage of urea hydrolyzed suggested some sort of inhibition of the reaction from the increasing concentration of fly ash.

For this technology to be applicable in the field, higher weight of CCR to liquid ratios would be advantageous to reduce the water necessary for storage. Thus, batch tests were designed to examine CCRs at concentrations of 50% w/v. Samples were taken at various time points for the duration of the tests to further investigate the link between fly ash concentration and inhibition of ureolysis.

Batch Tests

Figure 3 shows batch tests utilizing FGD waste water and gypsum (Treatments 1 and 2 respectively) undergoing complete ureolysis (from 20 to 0 g/L) in 48 hours similarly trending to the positive controls. The pH values for FGD waste water, gypsum, and the positive control ranged from 7.7 to 9.3.

In preliminary tests, with 50% w/v fly ash (Treatment 3), no ureolysis was observed. Ciurli et al. demonstrated that 80% of *S. pasteurii* urease activity was lost at pH levels above 9.0 and below 6.0. As a result, it was hypothesized that the high pH levels

observed in fly ash batch tests (pH as high as 10.5) were inhibitory to the urease enzyme.[28, 29] To overcome the pH inhibition, the fluid was buffered with 400mM HEPES to prevent a significant pH increase. Studies with 50% fly ash then showed 100% of urea consumed within 48 hours and pH ranging from 7.7 to 9.6 with CMM- (Treatment 4) and 90% consumed and pH ranging from 7.6 to 9.1 with CMM+ (Treatment 5). One method to overcome the inhibition to ureolysis from high pH was to control the pH of the solutions using buffer. In addition, mixing the different types of waste materials together might also serve as a method to control high or low pH conditions.

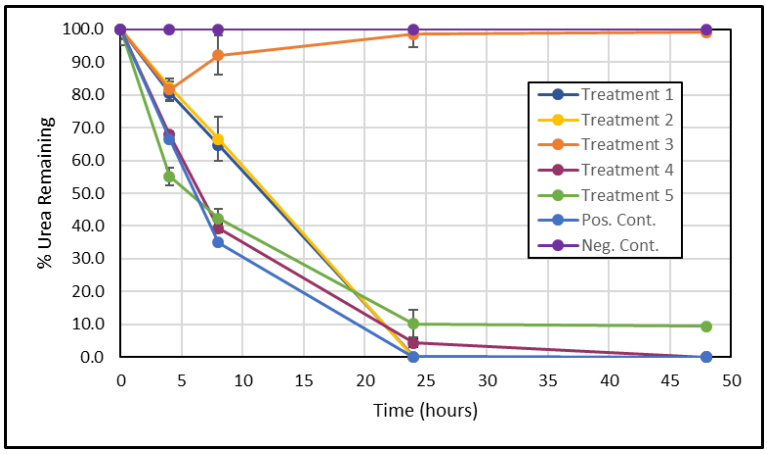

Figure 3. Percent urea remaining in batch test reactors at 0, 4, 8, 24, and 48 hours. The composition of each treatment is shown in Tables 1 and 2. Complete ureolysis was observed in treatments 1, 2, 4 and the positive control. In treatment 5, it was observed that 10% of the original urea concentration remained after 48 hours which is similar to results observed in the positive controls containing calcium. Treatment 3 which contained fly ash without HEPES buffer showed no ureolysis occurring, similar to the negative control.

Flow-Through Column Study

It was observed in the flow through reactor study that ureolysis occurred in every calcium pulse (data not shown), significant permeability reduction was achieved, and biocement was produced throughout the column binding the fly ash particles together into a solid column (Figure 4). Although a control column was not run in this experiment, in batch studies with fly ash material that were not inoculated with *S. pasteurii* it was observed that binding of the ash material was not observed. Therefore, the binding and permeability reduction observed here was attributed to the MICP. During the experiment the initial flow rate was 5 ml/min with a differential pressure of 7.5 psi and at termination the flow rate was decreased to 2 ml/min with a differential pressure of 51 psi. The flow rate was reduced over the course of the experiment to remain below system pressure during the injection period. The successful binding and permeability reduction in the fly ash material suggested this technology could be used for *in situ* stabilization of ponded materials.

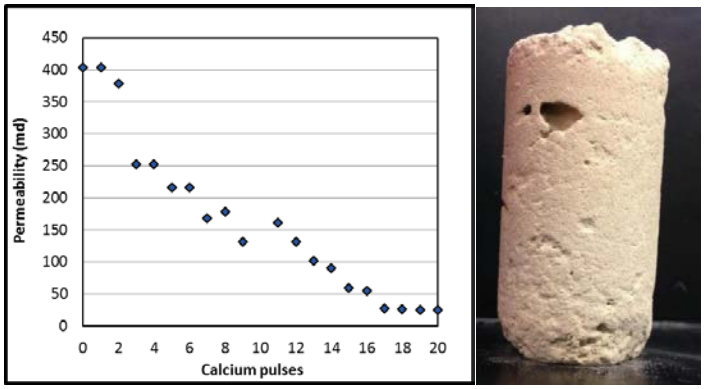

Figure 4. *Left:* Permeability was reduced 94% from approximately 404 to approximately 25 millidarcy. *Right:* Biomineralized fly ash column, the biomineral was observed to bridge the particles and cement the fly ash together.

Image

Images of CCRs before and after batch testing were taken with a field emission microscope (FEM). Post-batch test images of fly ash and gypsum (Figure 5) show mineral crystal formation (most likely $CaCO_3$) surrounding individual particles. The particles appear to be bound together by the mineral. EDX spot analysis of these minerals show that they contain calcium (data not shown), supporting the theory that these are $CaCO_3$ crystals formed by MICP.

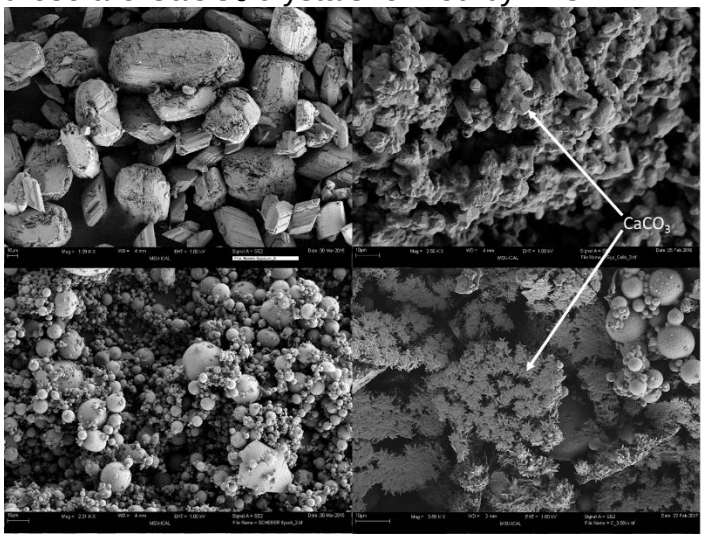

Figure 5. FEM images taken after batch testing of gypsum (top right) and fly ash bottom right) show increased binding of particles as compared to raw gypsum (top left) and raw fly ash (bottom left). In addition, post-batch test images show minerals similar to $CaCO_3$ that were not found in the raw materials.

CONCLUSION

Experiments described here have shown that MICP can occur and produce calcium carbonate in the presence of fly ash, gypsum, and FGD waste water. This calcite forms between individual particles, successfully binding them together and creating a novel form of biocement. Additionally, a flow-through reactor study shows decreased

permeability in fly ash treated with MICP. This study showed that (1) biomineralization (MICP) occurred in the presence of CCRs, (2) high pH caused inhibition of ureolysis but that could be overcome by buffering the solution and (3) biomineralization successfully bound CCR particles together, reducing permeability in a column study. This research provides support for the idea that MICP can be applied to CCR management units to suppress fugitive dust, reduce permeability, and strengthen surface impoundments to comply with new EPA regulations.

FUTURE WORK

Further research will be conducted in an effort to scale up this technology and develop methods for field study applications. Specifically, investigations will address potential applications of a biocement agglomeration of landfilled CCR for fugitive dust suppression. In addition, procedures and methods will be developed for injection into ponded ash to develop biocement within pore spaces with the goals of increasing strength and reducing the ability of water to flow through the materials.

REFERENCES

[1] Agency, U. S. E. P. Final Rule: Disposal of Coal Combustion Residuals from Electric Utilities. https://www.epa.gov/coalash/coal-ash-rule (April 27, 2016)

[2] Breeze, P. Coal-fired Power Plants. In: *Power Generation Technologies*, 2 ed.; Elsevier Ltd., United Kingdom, 2014, pp. 29-65.

[3] Ritter, S. K. A New Life For Coal Ash. *Chemical & Engineering News***,** 2016, 94, (7), pp. 10-14.

[4] A Sustainable Future. https://www.acaa-usa.org/About-Coal-Ash/A-Sustainable-Future (7/26/2016)

[5] Mueller, S. F., Mallard, J. W., Mao, Q., Shaw, S. L. Fugitive particulate emission factors for dry fly ash disposal. *Journal of the Air & Waste Management Association***,** 2013, *63*, (7), pp. 806-818.

[6] Ellison, K. Industry perspective on integrating ELG and CCR compliance through wastewater encapsulation: Challenges, opportunities, and technology readiness. In: *World of Coal Ash Conference*, Nashville, TN, USA, 2015.

[7] Baldrey, K., Sjostrom, S., Ellison, K. Options to stabilize and utilize tomorrow's fly ash: Approaches and initial results. In: *World of Coal Ash Conference*, Nashville, TN USA, 20155.

[8] Yeboah, N. N., Ellison, K., Minkara, R., Rhodes, D. Treatment and disposal alternatives for flue gas desulfurization wastewater. In: *World of Coal Ash Conference*, Nashville TN, USA, 2015.

[9] Lauchnor, E. G., Schultz, L. N., Bugni, S., Mitchell, A. C., Cunningham, A. B., Gerlach, R. Bacterially induced calcium carbonate precipitation and strontium coprecipitation in a porous media flow system. *Environ Sci Technol***,** 2013 *47*, (3), pp. 1557-64.

[10] Phillips, A. J., Gerlach, R., Lauchnor, E., Mitchell, A. C., Cunningham, A. B., Spangler, L. Engineered applications of ureolytic biomineralization: a review. *Biofouling***,** 2013, *29*, (6), pp. 715-33.

[11] Hammes, F., Verstraete*, W. Key roles of pH and calcium metabolism in microbial carbonate precipitation. *Reviews in Environmental Science and Biotechnology, 2002, 1*, (1), pp. 3-7.

[12] Mitchell, A., Dideriksen, K., Spangler, L., Cunningham, A., Gerlach, R. Microbially enhanced carbon capture and storage by mineral-trapping and solubility-trapping. *Environmental Science & Technology,* 2010, pp. 5270-5276.

[13] Phillips, A. J., Lauchnor, E., Eldring, J. J., Esposito, R., Mitchell, A. C., Gerlach, R., Cunningham, A. B., Spangler, L. H. Potential CO2 leakage reduction through biofilm-induced calcium carbonate precipitation. *Environ Sci Technol,* 2013, *47*, (1), pp. 142-9.

[14] Ebigbo, A., Phillips, A., Gerlach, R., Helmig, R., Cunningham, A. B., Class, H., Spangler, L. H. Darcy-scale modeling of microbially induced carbonate mineral precipitation in sand columns. *Water Resources Research,* 2012, *48*, (7), n/a-n/a.

[15] Phillips, A. J., Lauchnor, E., Eldring, J., Esposito, R., Mitchell, A. C., Gerlach, R., Cunningham, A. B., Spangler, L. H. Potential CO2 Leakage Reduction through Biofilm-Induced Calcium Carbonate Precipitation. *Environmental Science & Technology,* 2013 *47*, (1), pp. 142-149.

[16] Bang, S., Min, S.-H., Bang, S. S. Application of microbially induced soil stabilization technique for dust suppression. *International Journal of Geo-Engineering*, 2011, Vol. 3, pp. 27-37.

[17] van Paassen, L., Ghose, R., van der Linden, T., van der Star, W., van Loosdrecht, M. Quantifying biomediated ground improvement by ureolysis: large-scale biogrout experiment. *Journal of Geotechnical and Geoenvironmental Engineering,* 2010, *136*, (12), pp. 1721-1728.

[18] DeJong, J. T., Mortensen, B. M., Martinez, B. C., Nelson, D. C. Bio-mediated soil improvement. *Ecological Engineering,* 2010, *36*, (2), pp. 197-210.

[19] Tobler, D., Maclachlan, E., Phoenix, V. Microbially mediated plugging of porous media and the impact of differing injection strategies. *Ecological Engineering,* 2012, *42*, pp. 270-278.

[20] Fujita, Y., Taylor, J., Gresham, T., Delwiche, M., Colwell, F., McLing, T., Petzke, L., Smith, R. Stimulation of microbial urea hydrolysis in groundwater to enhance calcite precipitation. *Environmental Science & Technology,* 2008, *42*, pp. 3025-3032.

[21] De Muynck, W., De Belie, N., Verstraete, W. Microbial carbonate precipitation in construction materials: a review. *Ecological Engineering,* 2010, *36*, (2), pp. 118-136.

[22] De Belie, N., Verstraete, W. Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete. *Journal of Industrial Microbiology & Biotechnology,* 2012 *39*, (4), pp. 567-577.

[23] Cunningham, A. B., Phillips, A. J., Troyer, E., Lauchnor, E., Hiebert, R., Gerlach, R., Spangler, L. Wellbore leakage mitigation using engineered biomineralization. *Energy Procedia,* 2014 *63*, (0), pp. 4612-4619.

[24] Dupraz, S., Menez, B., Gouze, P., Leprovost, R., Benezeth, P., Pokrovsky, O., Guyot, F. Experimental approach of CO_2 biomineralization in deep saline aquifers. *Chemical Geology,* 2009 *265*, pp. 54-62.

[25] Cuthbert, M. O., McMillan, L. A., Handley-Sidhu, S., Riley, M. S., Tobler, D. J., Phoenix, V. R. A Field and Modeling Study of Fractured Rock Permeability Reduction Using Microbially Induced Calcite Precipitation. *Environmental Science & Technology,* 2013, *47*, (23), pp. 13637-13643.

[26] Jung, D., Biggs, H., Erikson, J., Ledyard, P. U. New Colorimetric Reaction for End-Point, Continuous-Flow, and Kinetic Measurement of Urea. *Clinical Chemistry,* 1975, *21*, (8), 4.

[27] Phillips, A. J. Biofilm-Induced Calcium Carbonate Precipitation: Application in the Subsurface. Montana State University, Bozeman, MT, 2013.

[28] Ciurli, S., Marzadori, C., Benini, S., Deiana, S., Gessa, C. Urease from the soil bacterium Bacillus pasteurii: immobilization on Ca-polygalacturonate. *Soil Biology and Biochemistry,* 1996, *28*, (6), pp. 811-817.

[29] Lauchnor, E. G., Topp, D. M., Parker, A. E., Gerlach, R. Whole cell kinetics of ureolysis by Sporosarcina pasteurii. *Journal of Applied Microbiology,* 2015, *118*, (6), pp. 1321-32.