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NCHRP IDEA Program

Rapid Rehabilitation of Highway Slopes Using Seeded Microbial Bio-Cement

Final Report for
NCHRP IDEA Project 200

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EXECUTIVE SUMMARY

Biological mineralization processes have been shown in laboratory studies to have promise to solve common soil engineering problems. In soil stabilization via biomineralization, a microbial process is used to produce mineral crystals between soil grains or clods that bind them together into aggregations or a solid mass. One example of biomineralization is microbial-induced calcite precipitation (MICP). MICP can have several forms spanning bioaugmentation and biostimulation of ureolytic microorganisms, sulphate reducing microorganisms, iron reducing microorganisms, denitrifying bacteria and others. The most common MICP treatments proposed is bioaugmentation with ureolytic *S. pasteurii*. In studies of this technology, soil crusts are developed with many treatments of large volumes of inoculate. However, for large areal applications along highways, single dosage treatments or a small number of treatments may be required logistically. If crusts develop too thick or strong, revegetation of slopes may be impossible. In this study laboratory and field experiments were performed to show effectiveness of single and small number of dosages MICP treatments to produce soil crusting that can mitigate soil erosion potential while maintaining the ability of revegetation.

When an intense wildfire occurs, the heat changes the chemical and biological makeup of the soil, impacting the microbial environment and increasing erosion risks that can propagate to devastating mudslides and debris flows. Both wind and water erosion of slopes poses problems to highways after fires. The fine particulate matter created from the burned organics easily becomes airborne which reduces air quality and visibility as wind erodes the surface. Unfortunately, there are no current large area technical or silvicultural measures known which could be put into place to make a forest fire area safe from erosion in an acceptable time and with a reasonable amount of effort. With wildfires becoming more pronounced in the wildland-urban interface, rapid watershed management actions to protect infrastructure, water quality, and ecosystem health are needed. Large amounts of burned soils can be eroded into streams and creeks, loading drainage and flood control infrastructure with large amounts of sediments while also removing roadway slope materials. In this research, the primary question being pursued is the effectiveness of low dosage surface MICP bio-stimulation biomineralization as a means to mitigate surface erosion in burned soils from wind and water; wildfires that damage roadways and transportation infrastructure. Likewise, in new construction, erosion risks along highway slopes are maximized until erosion mitigation techniques, most frequently silviculture in nature, can be installed.

This study broadens the knowledge of MICP treatments on various soil types, particularly soils subjected to wildfire and new construction through development of a more versatile technology herein referred to as BioCaN (biological soil treatments with calcium and nitrogen). In the study, different BioCaN soil applications were applied to burned, comparable unburned and clean sand soils in factorially designed laboratory experiments to test soil performance enhancement of six levels of treatment on key soil engineering properties. Naturally simulated conditions were created in the laboratory to test treated soils for wind erosion resistance, rainfall impact/sheet flow resistance, seed germination, vegetation reestablishment and surface strength crust development. The applications were also applied at three different field sites on sixteen plots to compare performance to existing technology including compost, newspaper pulp, seeding and fertilizer. In addition, an environmental study was completed to look at impacts of the microbial and chemical solutions to freshwater surface sources which determined that eutrophication is of concern as was originally suspected.

For Department of Transportation managers at all levels who have problems with erosion with or without fire or construction compounding factors, there are a variety of technologies that can be used under a variety of conditions to remediate erosion potential. The product developed in this research, BioCaN, is a valuable tool to add to the erosion control toolbox. Although not able to be used in every condition, the BioCaN treatment is effective after fires, on sandy soils, on clayey soils, on steep slopes and gentle slopes. It aids in revegetation, and in some cases may accelerate revegetation. The technology has some

environmental impacts, but these are similar to most of the environmental impacts associated with other technologies in the erosion control toolbox. When faced with difficult erosion and revegetation problems, we propose BioCaN as a new tool that DOT managers should evaluate and implement depending on the specifics of their situations.

INTRODUCTION AND PROBLEM STATEMENT

The field of geotechnical engineering has vastly expanded beyond the traditional hydro-mechanical bounds of soil behavior in recent decades to include the biological and chemical aspects to soils. Engineers are now researching methods to design soils and soils related infrastructure with natural processes, microbes and chemicals to help tackle difficult soils that cause problems to infrastructure such as: liquefiable sand, soil deposition, coastal and surface water erosion, structure instability from eroded soil foundations, airborne dust and so on. One of the primary new research fields is to understand biomineralization and to develop accelerated biomineralization methods to strengthen soils. With this new outlook on the field many other scientific areas must be understood and scientifically addressed to fully research and understand new technologies such as: botany, environmental health and science, chemistry, microbiology and so on.

Biomineralization via the precipitation of calcite initiated by microbes is a natural process that has inspired the creation of the biogeotechnology of microbial induced calcite precipitation (MICP) which is a geotechnical engineering special topic receiving an immense amount of interest throughout the world. MICP uses a urea-broth-calcium solution along with live ureolytic bacteria to create biocementation of soil particles through mineralization of primarily calcite, most prevalently experimented with on sand. In the late 1990s researchers successfully created over 30% by weight of calcite in clean sand using the ureolytic bacteria strain, *Sporosarcina pasteurii* [1]. Based on this original study and subsequent successes by researchers at the South Dakota School of Mines and Technology by Professors Sookie and Sangchul Bang [2-4] and others around the world [5-10], MICP is now being researched and experimented with all over the world. Much of the research has focused on making solid soil masses to serve more as a replacement for Portland cement. If successful, these studies may prove MICP to be an alternative to mixing cement in with soils for densification of sands to reduce impacts from earthquakes from liquefaction and stop contaminate transport. However, to create this solid soil mass of a thick erosion resistant crust, massive quantities of chemicals with many treatments (or continuous treatments over a time period of 4-10 days) are required. This may not be practical or feasible to implement for real-world large-scale soil surface erosion problems for hundreds to thousands of feet of highway slopes.

Though one might think that MICP could be a sustainable alternative to Portland cement (which is very energy intensive to create) there has been very little research into the environmental issues that MICP chemicals may cause or an environmental lifecycle analysis (LCA) on the transport and production of the chemicals and aggregates used in the MICP process. Other concerns with creating a solid "brick" of soil for an erosion resistant crust include seed germination and growth for revegetation of slopes. If erosion mitigation treatments are not conducive to revegetation over short or long time periods, this may cause more issues in addition to aesthetics. Another major concern with MICP biocementation is that calcite loses strength over time (especially when exposed to acidic water, i.e. dissolution of Karst) and therefore may not be a permanent solution such as if MICP "bricks" were to be used in constructing a building.

Little to no research has been completed using MICP chemicals and ureolytic bacteria on a more temporary basis in which a soil mass or "brick" is not being created and instead a much less robust crust is created on the surface of the soil. Thus, this research aims to fill the gap in knowledge of using much lighter MICP treatments and if this type of treatment has an engineering purpose. The researchers hypothesized for this project that to achieve a light crust only one or two MICP treatments would be required as opposed to the ten days of continuous medium treatment or 100+ treatments often needed to create a very solid mass of soil. The proposed use for light MICP treatments in this project is for the purpose of temporary erosion control of disturbed, problem and/or denuded soils until a vegetative fabric can replenish and take over as the primary source of erosion protection. This biomineralized and/or chemically charged light crust would still allow for vegetation germination and growth and may even provide for more plant available water (PAW) by reducing infiltration rates through the soil and holding more water particles in place. To determine if MICP treatments to create a light protective crust is a feasible and practical use for erosion control of disturbed soils a research plan was designed and developed to give soil performance data when only one treatment of

MICP solution treatments were applied to the soil.

The research and treatment types that inspired this research project are referred to as microbial induced calcite precipitation or, simply, MICP. Much of the research being actively pursued that use the "MICP" treatments is aiming to create a very solid and dense soil mass for the purpose of a very hard material that can be used as a building material, concrete replacement, liquefaction prevention or coastal dune permanent stabilization. However, this research project is not aiming to create a solid mass or thick, impervious soil crust, such as previous research has, and is instead intended to provide a light crust on the top of the soil surface which allows for a living and active system in which added bacteria and in-situ bacteria thrive; seeds and seedlings can have added protection from erosion and water loss; and in which no significant permanent changes will occur to the ecosystem or landscape due to the treatment. The treatment is intended to coexist and enhance the health of the environment and its inhabitants by providing cations, nitrogen-rich supplements and added "healthy" microbes. The purpose is not to create 30% calcite by mass or to completely densify all of the soil void space that exists within the soil matrix by adding large and numerous calcite crystals.

Throughout the research project the researchers discovered that their treatments worked much more like a 2-part process in which ionic bonds were likely just as responsible for the increase in strength gain that was achieved through the treatments on top of the calcification that is initiated by the bacteria [11]. With only a one-time treatment of the MICP solutions large crystals could not even be observed using X-ray Diffraction (XRD) or Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM/EDS) but the soil still gained significant strength. Due to the "lighter" treatments than normal, and the conclusions made in this research project that more factors are at play for the strength gain and soil stabilization than simply calcification and biocementation of soils it was decided that referring to the treatments as MICP treatments would be somewhat misleading due to calcite precipitation being in the name.

For the above stated reasons, the authors feel that referring to the treatments as MICP or even biomineralization are misleading because these aspects are likely only partially responsible for the erosion resistance incurred from the treatments [11]. Microbial induced calcite precipitation (MICP) does not accurately portray the treatments that were applied to the soils in this project and experimented with in this project. Therefore, the term **BioCaN** will be used to describe the treatments which more accurately portrays the chemical and biological treatments that were applied. The Bio- refers to the bacteria and the nutrient broth that was added with the soil treatment that will supplement augmented and in-situ soil bacteria. The -Ca- refers to added calcium chloride as well as the chemical processes that occur as a result of the urea that initiate and encourage the bonding of cations. The –N part refers to the nitrogen supplements that are added with the urea which are much like fertilizers that encourage and support both the growth of plants and the growth of mineral crystals such as urea, dolomite and calcite. ***BioCaN soil treatments help promote a healthy ecosystem which encourages microbial activity, plant growth, plant available water (PAW) storage, soil retention on site and cohesion with the existing ecosystem to help in recovering when negative natural or anthropogenic causes created problem disturbed soils.*** Some of the referenced events are wildfires (human or natural caused), construction, mining and so on. Though further research is needed to polish off and best accommodate certain situations of soil types and disturbance problems, which is explained later, this research project shows that BioCaN soil treatments can be a formidable solution in dust suppression and land rehabilitation under certain conditions and in certain situations. For the purpose of this document other research besides this product on the topic area will still be addressed as MICP research however the specific treatments used in this research project will be addressed as BioCaN which is specific to this project and only this project at this time.

CRITICAL TRANSPORTATION INFRASTRUCTURE PROBLEM AND PROPOSED SOLUTION

MICP is a proven method for stabilizing soils and for reducing permeability of soils. However, little research has been done on the erosion resistance of surface MICP stabilized soils particularly natural soils. MICP has been shown to lose strength over time. Thus, as the ideas were rolling for a research project, a temporary use for MICP stabilized soils seemed to be the most logical to avoid repeat treatments which would cut down on cost and potential environmental contamination. The Crow Peak fire in mid-summer of 2016 near the campus of the South Dakota School of Mines and Technology reminded that fires can cause soils to become very dry and vulnerable to both wind and water erosion and therefore if MICP solution works on burned soils similar to the MICP effectiveness on sandy soils it could be a solution for burned soil stabilization after a fire until revegetation. This is because plants burn and there are no longer the root systems to hold the soils in place. The loss of soil moisture can reduce the adherence of soil particles also making them more susceptible to erosion. A preliminary literary search on burned soils showed that calcium along with other nutrients increases in burned soils. This is likely due to the organic material decomposing through fire but may reduce the number of chemicals needed for MICP to successfully work by already have free Ca^{2+} ions available to bond with the carbonate. So, if MICP could temporarily stabilize the top portion of soils on sloped surfaces then two things could occur: (1) the loss of nutrients from the soils eroding would not occur because soil particles would be held in place and (2) there would not be mass loading into surface water sources from runoff depositing downstream in culverts and bodies of water.

The literature has many gaps in knowledge that do not allow for the question of if MICP could be used to hold burned soil particles in place. These gaps in knowledge included an expansive list but to name a few: the efficacy of MICP treatments on non-sand soils, the efficacy of MICP against rainfall erosion, the efficacy of MICP with only one or two treatments, the potential environmental impacts of putting MICP chemicals into the natural environment, and so on. Many parameters needed to be tested to answer the question, will MICP treatments work to stabilize burned soils and other disturbed soils? Figure 1 shows the immense deposition of soil particles after a fire to downstream infrastructure. It can also be seen how easily erodible the soil walls of the deposited burned soils are.

Figure 1. Burned soils from the Legion Lake Fire in Custer State Park, SD. Sediments have been washed and deposited downstream blocking roadway drainage structures. Photo taken from a highway looking upstream of a wash that runs through a blocked culvert.

When an intense wildfire occurs, the heat changes the chemical and biological makeup of the soil, impacting the microbial environment [12] and increasing erosion risks that can propagate to devastating mudslides and debris flows. Both wind and water erosion of slopes poses problems to highways after fires. The fine particulate matter created from the burned organics easily becomes airborne which reduces air quality and visibility as wind erodes the surface. Unfortunately, there are no current large areal technical or silvicultural measures known which could be put into place to make a forest fire area safe from erosion in an acceptable time and with a reasonable amount of effort [13]. With wildfires becoming more pronounced in the wildland-urban interface, rapid watershed management actions to protect sociological concerns, water quality, and ecosystem health are needed [14]. Large amounts of burned soils can be eroded in summer or winter rains into streams and creeks, loading drainage and flood control infrastructure with large amounts of sediments while also removing roadway slope materials. In this research, the primary question being pursued is the effectiveness of low dosage surface MICP bio-stimulation biomineralization as a means to mitigate surface erosion in burned soils from wind and water; rainfall and sheet flow across the burned soils as are common in the semi-arid American West in summers and the autumn after intense and widespread wildfires that damage roadways and transportation infrastructure. Likewise, in new construction, erosion risks along highway slopes are maximized until erosion mitigation techniques, most frequently silviculture in nature, can be installed.

The engineering use for MICP (i.e. BioCaN) soil treatments proposed in this research project focuses on using the same MICP treatment solutions and bacterial strain used in traditional "MICP" research for the stabilization of recently burned, disturbed or denuded soils but only applying the treatment one time instead of a continuous injection style or many (sometimes upwards of 100) treatments as has been commonly used in previous research studies. For the purpose of this paper "many" treatments will be defined as application of MICP treatment solutions to the same soil sample more than 10 times. This study compares the effectiveness of BioCaN soil treatments on disturbed soils to the effectiveness of the treatments on a clean sand and to the effectiveness of the treatments on a natural undisturbed soil. Burned soils are physically reduced to small particles and the vegetation root matrix are generally lost after a fire, which causes soils to become susceptible to erosion. If a fire is intense, it can sterilize the soils leaving behind no microbial communities which can further increase time until vegetation recovery. Previous studies as well as chemical analysis of burned soils completed for this project show that soils subject to burning typically contain an increased abundance of calcium, other cations and nutrients that exist in the soils due to burned organic matter. Erosion of burned soils become susceptible to erosion and cause sedimentation and contamination in surface waters which can lead to eutrophication and other environmental problems. Natural and prescribed burning near riparian zones causes concern because of the chemical changes to the soils and downstream surface water [15]. The loss of nutrients from soils further decreases the vegetation recovery time.

This research study aims to determine if adding ureolytic pure culture bacteria cells suspended in saline along with chemicals high in nitrogen and salts will improve land rehabilitation efforts by reducing erosion of soil particles and allowing for efficient vegetation regrowth. The study tests the effectiveness of varying types of chemical treatments with and without added bacteria to determine the best course of action to take when applying BioCaN soil treatments to three varying soil types: a manufactured sand, burned soil and unburned soil. The research investigates if the added cations, for the purpose of this research coming from calcium chloride, are needed for strength gain required to reduce erosion and how the addition of calcium chloride may impact vegetation regrowth. The study also investigates if the addition of ureolytic bacteria, strain *S. pasteurii*, is needed for strength gain and how this will impact vegetation growth. Finally, the study shows what the consequences would be if the chemical solutions were to be directly added to a surface water body, such as if a vehicle carrying the solutions were to spill into a lake. The study also gives some ideas on commercialization and how BioCaN soil treatments compare to existing and similar market products such as urea fertilizers and magnesium-chloride dust suppression solutions on a concentration and amount usage scale and will test out the technology on three field sites through the Black Hills of South Dakota area against commonly used erosion control methods.

Three different MICP or BioCaN chemical concentrations are tested: the regular or traditionally used concentrations (1x), half the original concentrations (0.5x) by mass of chemicals and double the original concentrations (2x). In addition, two variations on the originally formulated MICP technology are tested: (1) the 1x BioCaN soil treatment is tested without an calcium chloride and (2) without any added bacteria cells both of which will help to determine how these ingredients impact the efficacy of the treatment solutions. The three levels of concentrations along with the two variations and a control treatment of only distilled water in the same volume of liquid applications will all be tested with each of the three soil types. This is called a factorial designed experiment and tests 18 interactions that can occur based on the treatment type and soil type which basically means that each of the six treatment types are tested on each of the three soils types. This is done because due to the chemical, physical and biological complexities of soils it is likely that the treatment that works best on one soil type won't work best on another. The many physical, chemical and biological factors that come into play with soils when determining efficacy of a stabilization product include: soil particle size, infiltration rates, fines content, clay particle chemistry, soil compaction/density, chemical/crystalline constituents, biological makeup, void space, void connectivity, ionic bonds, capillary action, etc.

Soil erosion can cause eutrophication of downstream lakes and so can fertilizer runoff (non-point source pollution) both due to the nitrogen load in the watercourse. Since BioCaN soil treatments include urea-Nitrogen and ammoniacal-Nitrogen, eutrophication and water contamination are a concern from the chemical constituents of the solutions. BioCaN soil treatment solutions also contain a nutrient broth to supplement bacteria growth as well as a calcium source with the traditional supply coming from CaCl2. Previously, research studies have not been completed on MICP chemical solutions and the potential impacts to surface water sources. This research completes a preliminary laboratory scale study to show the potential of MICP to cause eutrophication. The study does find that BioCaN treatments, if catastrophically spilled in a water body, would likely cause first an overgrowth of bacteria and after one week an overgrowth of algae.

One of the most effective means of soil erosion prevention by wind and water is vegetation. This is why soil erosion becomes a problem on disturbed soils (fire and/or new construction) because the vegetation is lost. The purpose of this project is to determine if BioCaN soil treatment is effective to reduce soil erosion long enough for vegetation to recover with the hopes that by holding the soil in place, along with an abundance of nutrients, the vegetation will recover and take over the responsibility of holding the soil particles in place. This would result in the rehabilitate of the soil Horizon A which would result in no further erosion control treatments. The ideal situation would only require one BioCaN treatment (better economically and environmentally than many treatments) which would create a light charged carbonate crust that would help with plant growth due to increased water retention in the soils and would still allow plants to germinate and grow. The impacts to vegetation germination and growth were investigated for this project to determine if it is feasible to think that MICP solution treatments might work for the purpose of temporary erosion control until vegetation can recover.

SOIL MICROBIAL BIOMINERALIZATION

MICP via ureolytic bacteria occurs when an ureolytic bacterium releases an urease enzyme which, through a series of reactions, chemically converts urea into a carbonate. Adding calcium chloride with the inoculate solution allows the precipitation of calcium carbonate in the form of calcite, CaCO3(s) when live *Sporosarcina pasteurii* cells are present in the solution [1] or urease enzymes are added directly [16]. The phenomenon of bio-induced calcite precipitation has been discovered and observed in nature for many decades. However, the use of bio-initiated calcite for engineering purposes, known as MICP, got started in the late 1990s when researchers at the South Dakota School of Mines and Technology in Rapid City, SD successfully produced over 30% calcite by weight fraction in clean sand. Stocks-Fischer et al. [1] treated four samples continuously for 10 days, the first with live *S. pasteurii* cells in a urea-CaCl2 medium, the second with dead S. pasteurii cells in a urea-$CaCl_2$ medium, the third with a urea-$CaCl_2$ medium and the last with no bacteria or medium. The samples were subjected to an XRD quantitative analysis which only showed calcite in the first sample with live bacteria cells. The calcite made up 30.2% by weight fraction of sample 1 and there was no remaining urea detected in the samples. The sample with dead bacteria cells showed 8.3% gehlenite ($Ca_2Al[Al,Si]_2O_7$)) but it was unknown as to why this precipitation occurred.

Calcium carbonate exists in a variety of crystalline structures such as vaterite and aragonite, calcite has been found to be the most commonly created crystalline structure during MICP. Aragonite is another form of calcium carbonate but exists most commonly in corals. Even in coral formation biological components are of extreme importance in the size and shape of crystallization of calcium carbonate compounds [17] much like the bacteria and organic compounds in soils are thought to influence the crystals formed during MICP. Magnesium is also often precipitate with calcium and carbonates and forms crystalline carbonates such as dolomite which is 1:1 ratio of magnesium ions and calcium ions bonding with carbonates however it is not known if these other crystalline formations of biocementation can be engineered.

Marine peloidal deposits show various physical and chemical attributes that suggest that fine-grained precipitate of calcite was formed around bacterial cells and that the activity of the bacteria was vital in influencing the precipitation of the calcite [18]. The MICP process is known to occur naturally but it is accelerated with human intervention by adding the desired strains of ureolytic bacteria and the needed chemicals and nutrients for the bacteria to produce the urease enzyme. Urea hydrolysis has been found to be one of the most effective types of MICP however other types are known to occur [19]. The MICP bio-cementation process increases the stiffness and density of the soil which is well-documented in scientific literature. The technology has been extensively proven as a means that could increase soil strength, density and improve overall soil engineering properties [20-26]. Surface application of MICP can be performed by spraying the microbial solution combined with a nutrient broth and calcium solution on the surface of the soil. Volumetric mixing of the MICP solution with soils can be done if treatment is desired throughout a soil sample for laboratory experiments and has been a common technique used in MICP research [27]. Pressure injection can be used to apply below the surface if mixing is not feasible, as would likely be the case in a natural setting. However, this form of application does have challenges, the most difficult being the plugging of soils near the injection point disabling the flow of MICP solutions. Although bio-grouting has still been proposed as a means for ground improvement and effective methods have been simulated [28] and has been proven feasible in large-scale experiments [29].

Even though the effectiveness of MICP for soil biomineralization and resulting cementation has been proven in laboratory, bench scale, and small controlled field scale research the technology has yet to be implemented on a commercial platform. A rich literature exists on MICP with details on laboratory, bench scale and small field tests performed to date [2-10]. Using the ureolytic bacterium, *Sporosarcina pasteurii*, and the urea-broth-calcium solutions significant reductions of mass-loss from wind erosion have been observed in laboratory experiments through biomineralization of surficial soil grains and prevention of saltator mobilization [3, 20, 27]. The mechanistic and chemical details that occur during the MICP process can be dated back to mid-20th century by Warner and Cannan who observed the hydrolysis of urea [30].

Two types of engineered MICP are completed; either biostimulation or bioaugmentation. Biostimulation MICP, occurs when native populations of ureolytic bacteria are stimulated to produce biomineralized carbonates primarily as calcium carbonate in the crystalline form of calcite. In bioaugmentation, a typically pure culture of ureolytic bacteria are added to the soil to produce the biomineralized carbonates. It is important to note that in bioaugmentation of soils in the field that biostimulation also will likely occur. The native bacteria species will likely compete for nutrients with the supplemented bacteria species and it is plausible that the conditions will not be ideal for the supplemented bacteria and they will all die. The biomineralization precipitate reaction from the ureolytic bacterium *S. pasteurii* is well described in [1] and a conceptual overview is shown in Figure 2. Research testing engineered MICP using bioaugmentation has been implemented worldwide by various researchers [2-10, 20-26] while MICP biostimulation research is also being implemented but at a lesser capacity [31-33].

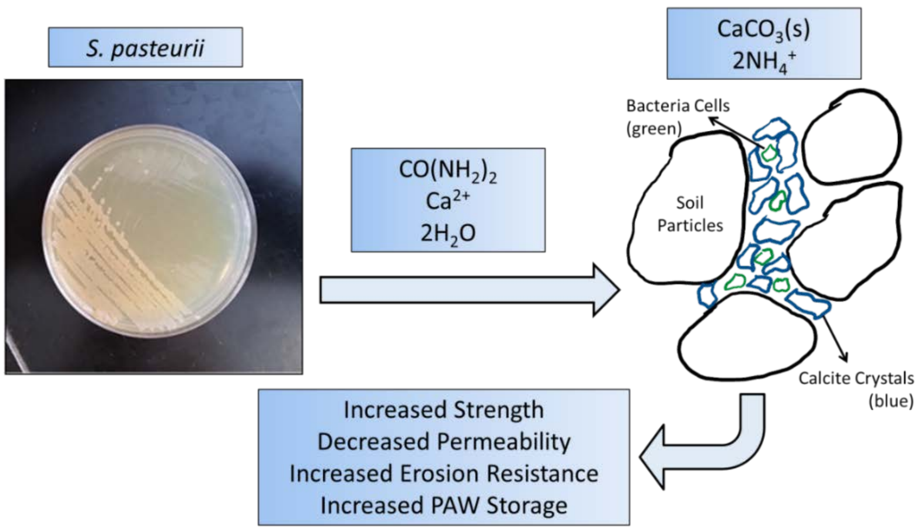


Figure 2. Conceptual overview of the MICP process.

In the MICP process, a bacterium produced enzyme catalyzes a series of chemical reactions which produce calcium carbonate ($CaCO_3$) in the crystalline formation known as calcite. Though in the document calcium carbonate and calcite are at times used interchangeably, they are technically different terms. Calcite is a crystalline formation of calcium carbonate. The theoretical hypothesis of the chemistry behind MICP is the initial reaction occurs when the bacteria releases a urease enzyme which initiates the conversion of urea and water into carbamate and ammonia. The carbamate molecule then degrades into carbonic acid and another ammonia molecule. Carbonic acid is not stable in the natural environment and in the presence of water it loses a hydrogen atom while ammonia gains a hydrogen atom. The ions now present in the aqueous solution are hydrogen, hydroxide, ammonium and bicarbonate. The ratio of hydroxide to hydrogen ions is 2:1 and so the pH increases. The increase in pH shifts the bicarbonate to form carbonate ions. In the presence of calcium, the carbonate and calcium form calcite. Typically, the calcium source used for MICP purposes is calcium chloride. The overall reaction for MICP is given in Equation 1.

Equation 1: The chemical reaction for MICP calcium carbonate precipitation.

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \leftrightarrow CaCO_{3(s)} + 2NH_4^+$$

The most commonly used bacteria that is effective at producing MICP is *S. pasteurii*, formerly known as *Bacillus pasteurii*. *S. pasteurii* is a Biological Safety Level (BSL) 1 microbe, which is the safest level and means that it poses no or low risk to humans. The rate of MICP correlates with bacteria cell growth and has been shown to be significantly faster than that of chemical precipitation of calcite alone without the biological component [1].

CURRENT MICROBIAL SOIL STABILIZATION METHODS

The biogeotechnology has been suggested for concrete manufacturing and healing for civil engineering structures in combining with fibers [2,4,5], for the improved durability and remediation of cemented materials [34-36] and as a building material which can heal itself [37] (De Muynck et al. 2010). The technology has even been suggested for the preservation of concrete art pieces such as statues for the healing properties the bacteria could provide [38]. The technology is widely being researched as a method to reduce the catastrophic impacts that result from soil liquefaction caused by earthquakes and to strengthen waterside embankments such as coastal dunes [10].

Due to the properties of reducing permeability, MICP technology has potential as a remediation tool to slow-down or prevent contaminate transport in groundwater through underground porous soil media. Thus, the dynamic and kinetic factors included in the process from the chemicals and bacteria have been investigated [39,40]. Due to the degradation effects of urea that bacteria are responsible for the technology could be used as a capture for ground water contaminants [41]. However, as bacteria are sometimes quite sensitive to toxicity, heavy metals research has indicated that another microbe, two species of fungi, could be used to precipitate heavy metals along with carbonate precipitation of minerals [42]. Even still, MICP has been suggested as a feasible tool in environmental remediation especially of heavy metals such as lead [43].

Various research studies have been completed to improve and optimize the MICP process. A study found that when doing injection application of MICP chemicals at a lower chemical concentration resulted in better distribution of calcite precipitation even if the cementation level was technically the same at high and low chemical injections [44]. The same team of researchers also found the following year that lower concentrations of urea-broth and calcium chloride solutions also produced stronger unconfined compressive strength in sand treated samples as well as resulted in a gradual and uniform decrease in soil permeability as compared to samples treated with high concentrations of chemicals [45]. These studies agree with results found in this study that indicate higher concentrations of chemicals does not always equal better results particularly when implemented at a field-scale on soils that need to establish vegetation and healthy microbial communities.

Some researchers have proposed that microbial induced calcium carbonate precipitation could result in increased durability in concrete structures when using non-ureolytic bacteria [46] and even enhance concrete crack remediation in a two-step process with calcium [47]. Another study suggested that the presence of both ureolytic (*S. pasteurii*) and non-ureolytic bacteria (*Bacillus subtilis*) lead to more efficient calcite precipitation due to the additional nucleation points of the non-ureolytic bacteria cells [48].

Studies on microbial remediation in concrete cracks have clearly identified that the use of a mixture of sand and microorganisms in concrete remediation increases the compressive strength. The chemical broths used to grow the bacteria are comprised of non-economical ingredients. A more economical solution is desirable that will still provide nutrients and the needed elemental components to the bacteria. Many industrial food processes create byproducts that still have abundant nutrients but are simply disposed of after the primary process, one of these researched solutions is lactose mother liquor (LML). When LML is used compressive strength of the bio-cement created during the MICP process was as great as the compressive strength of the bio-cement when the original nutrient broth for the MICP process is used. There was also no significant difference in the growth and urease production among the LML and original media which suggests that LML is an alternative source for standard media [49]. Eggshell mixed with vinegar as a calcium source was also found to produce the same amount of increased soil strength as when the traditionally used calcium chloride was the calcium source [50].

Research has been completed which has given other uses for MICP. One of these is the use of MICP to reduce wind erosion of soils. In one study, both washed and unwashed soils were treated with MICP solutions and S. pasteurii, both types of samples formed a crust-like layer on the surface and showed a significant reduction in mass loss. The original method of MICP includes the use of ureolytic bacteria which is combined with a urea broth solution and calcium chloride immediately prior to application to soils. Researchers are working on different methods to make the process more efficient and practical. One method that has been researched by various groups is to use the urease enzyme instead of the ureolytic bacteria in a process called enzyme-induced calcite precipitation (EICP) [16]. This would solve the issue of providing nutrients and ideal conditions for the bacteria to grow and produce the urease enzyme which is one of the most challenging parts of the procedure when aiming for commercialization. The urease enzyme is produced through agriculture, microbial or fungal procedures [16]. The enzyme is currently not economically feasible to use on large scale soil applications. However, research is investigating ways of producing the urease enzyme that would be economical. One method is looking at the obtaining urease from watermelon seeds [51].

WILDFIRE IMPACTS TO SOILS, MICROBES, AND VEGETATION ALONG ROADWAYS

Fires can have an intense effect on the environment including the terrestrial, hydrological and atmospheric components. In the terrestrial environment, the soils are both chemically, biologically and physically changed. These changes increase erosion of nutrients and contaminants to the hydrological system. Fire affects the physical and chemical characteristics of the soil, such as the structure, porosity, infiltration, nutrient content, and subsequent effects may also damage soil organisms [52]. The soil impacts from fire can occur from all of the types of fire which include prescribed, pile burning, and wildland (forest and grassland).

Fire disturbances can produce dramatic changes in hydrologic responses that can pose risks to human life, infrastructure, and the environment [53]. Forest fires account for significant amounts of nitrogen gases emitted to the atmosphere annually including: N_2 (pyro denitrification-removes fixed nitrogen from biosphere), N_2O (6%), NH_3 (15%), and NO_x (18%). There is also a significant release of CO_2 and CO during fires with smaller releases of CH_4, CH_3Br, CH_3Cl and SO_2 [54]. Elevated soil temperatures during burning contribute to the destruction of soil organic matter, degradation of soil structure and porosity, alteration of hydraulic properties, and changes in nutrient cycling and microbial processes [55].

Physical changes to soils from burning can greatly increase erosion of soil particles and can cause debris flows. This is because fire breaks down the soil particle size and burns the vegetation roots that hold the soil in place. Organic materials such as tree and bush stems, large forest floor organic litter, and accumulated leaves can mechanically support the creation of large volumes of cohesionless materials after a fire [56]. When this organic matter is destructed during a fire event the potential for wind and water erosion of sediments is greatly increased particularly during large storm events. The physical movement of sediments both into the air and water contribute to element cycling and downslope/stream sediment depositing while possibly creating poor air quality and destructive mudflows.

During a fire, soils are chemically changed in a way to which the water repellency of the soil is increased creating a hydrophobic soil layer underneath the ground surface. This hydrophobic layer is typically underneath the top layer which consists of small mineral soil particles and ash, and this is likely one of the contributors to increased erosion after fires. This layer decreases infiltration and because a large percentage of vegetation has been killed by the fire, there is little to no transpiration, these factors contribute to large runoff quantities taking the top layer of soil and ash with it, running off in a "tin roof" phenomenon of the hydrophobic lower layer. Studies show that the temperature gradient from the canopy burning (can reach over 1100°C), to the soil-litter interface (850°C), and down to 5 cm into the mineral soil (less than 150°C) creates optimal conditions to create a hydrophobic soil layer [57]. The heat produced by combustion of the litter layer on the soil surface vaporizes organic substances, which are then moved downward in the soil along the steep temperature gradients until they reach the cooler underlying soil layers, where they condense [57]. The hydrophobic layer varies in depth and thickness but is typically a few centimeters below the surface and parallel to the mineral soil surface which allows everything above it to be highly susceptible to erosion.

A management program using controlled burns on oak savannah and oak forest was implemented in 1964 in a historic area of east-central Minnesota and summarized the effects of burns on aspects of the soil and vegetation [58]. This study found that in general there was an increase in pH of soils after a burn which supports other studies that have found similar evidence in areas annually burned [59]. One potential reason for the increase in pH is that as the organic rich layer of the soil is exposed to extreme heat there is an increased conversion of nitrates into ammonia and could give higher levels of usable nitrogen [59]. Based on this theory, Fowells and Stephenson [60] suggested that the effects of a higher pH and ammonia levels enhance nitrogen fixation and nitrification increasing total nitrogen. This agrees with another study that found higher levels of inorganic nitrogen and available phosphorus in soils after burning [61].

Fire can have varying impacts on nutrient cycling depending on the specific fire characteristics and where the fire occurs such as: a grassland, forest or savanna. The intensity of the fire, as well as, the frequency of fire all contributes to the overall impact the fire has on the soils and nutrient cycling. An experiment was carried out in Florida scrubby flatwoods in which the biogeochemical properties of soil were measured before and after a burn and analyzed while monitoring the duration and temperature of the fire [61]. Soil samples taken immediately preceding and two weeks after the burn were analyzed and data showed that inorganic nitrogen, available phosphorous and potassium, nitrogen mineralization rates, and potential nitrification rates significantly increased after fire. Nitrogen and phosphorus level increases were positively correlated with both fire intensity and vegetation. Nitrogen mineralization strongly correlated with available phosphorus changes which suggested phosphorous stimulated nitrogen mineralization [61]. While annual fires have the positive effect of increased utilization of such nutrients as phosphorus, when soil is too frequently burned it could result in a net loss of nitrogen to the system [58]. The frequency when a decrease in nitrogen in the soils was observed was at a fire frequency greater than eight burns in 20 years. The litter and biomass above ground decrease with fire temporarily while the soil carbon increases after a burn. Studies completed in Illinois, Mississippi and Kansas all showed changes in organic matter cycling below ground and increases in organic carbon [62]. However, if soil is frequently burned some studies have shown a decrease in carbon storage.

Cations such as calcium, potassium and magnesium are important nutrients for plants and also for MICP. In various studies cations, along with pH, have been shown to increase in burned soils which have been attributed to the combustion during fire from inorganic elements in the plant material [62]. As these elements combust the char created, which is carbon rich, also contains basic salts which hydrolyze in the presence of water, producing alkalinity, thereby raising the pH and releasing cations into the solutions [63]. Thus, vegetation burning can create a liming effect. Both a rise in pH as well as the abundance of cations after burning could indicate that MICP could potentially work in burned soils and have some of the key constituents already in place.

Vegetation diversity has been observed to increase with fire up to a point. An experiment in central-Minnesota showed that vegetation diversity increased with a burn frequency up to eight to nine fires over a 20-year period but any more fires and species diversity decreases [58]. In Iowa, it was determined that naturally occurring fires prevented the takeover of vast tall-grass prairie by expanding oak forests[59]. As civilization has moved into the natural environment people have started suppressing fires and this has shifted the vegetation balance in many environments. Where there was once grassland it has been taken over by forest since there is not fire to decrease woody plant growth. On the other side of the coin, increased numbers of fires from climate change and other human activities can allow invasive species to take over areas that were previously covered with native species. Many invasive plant populations are enhanced by fires and can help to establishment of other invasive species [64]. Seeding after fire for land rehabilitation is of utmost importance, both to stop unwanted invasive species and due to the reduction in seed germination and seedling establishment and growth because the seeds are killed in the top 2 cm of the soil, however heating from fire can increase seed germination [59].

Microbes are essential to soil health. Microbes produce nutrients that aid in plant life and growth and are the building blocks of living earth systems. Soil microbes are very important for the maintenance of soil health and soil nutrient systems [52]. When a fire occurs, the heat can impact the microbes changing the biological makeup of the soil. The changes in chemistry of the soil, such as pH and elemental composition and availability, also commonly changes which greatly impacts the soil microbes. Soil damage from fire could have negative impacts on how quickly organisms, such as plants and animals, can recover after a burn which will also impact microbes. Microbial survival after a fire depends on a few factors, one of the most important being the burn intensity, both duration and temperature. In some cases of severe burn, the soil may become sterile. The severity of burn to the soils depends on the water content prior to the fire. Soils with a higher water content actually burn worse because the water conducts the heat whereas dry soils are poor heat conductors. Studies indicate that fungi have a much harder time surviving and thriving after fire than bacteria, likely due to the heating and drying of soils during the fire and the increased water repellency of the soils. Bacteria can even thrive after a fire because of the increase in usable elements such as carbon and nitrogen. Fire creates more soluble carbon and mineralizes organic nitrogen into inorganic nitrogen in the forms of nitrates and ammonia. Bacteria can thrive after a fire until the easily used resources are gone, then there is much less organic matter and the bacteria may struggle overtime. Although some basic theories on how microbes are affected by fire have been proposed there is still very little knowledge on how fire directly and indirectly affects the microbial community.

There are no technical or silvicultural solutions to protect soils in an area impacted by fire that are feasible or time conducive known at this time. As wildfires becoming more pronounced in the wildland-urban interface due to increases in fires and expansion of human developed areas, rapid watershed management actions to protect sociological concerns, water quality, and ecosystem health are needed. Since there is currently no rapid response method to decrease soil loss an erosion control method needs to be developed to help with reduced erosion along severely burned roadways to reduce cost and loss of property which is why MICP is being investigated as a solution. Figure 3 shows newly scorched earth and sediment erosion from the slopes and migration of sediments toward a highway.

Research on changes in nutrient cycling and soil response to fire have been conducted in a very limited number of environments such as oak savanna, prairie, and scrubby flatwoods and so more diverse information is needed to further analyze soil constituents response to fire. Fire impacts have been well studied in terms of plant community responses, but the effects of fire on soil characteristics and post-fire plant-microbial interactions in these systems are mostly unknown. The importance of microbial communities decomposition and nutrient cycling is known but little is known about how microbe temporal and spatial diversity and plant litter impacts this. With limited knowledge of the diversity and cycling of microbial organisms it is difficult to understand how fire influences the microbial communities that have such a great impact on the restoration of an area after fire. This also makes it difficult to manage lands with the best management practice if the influence of fire is not well known on microbial specimens.

Soils are changed by fire physically, chemically and biologically. Fire reduces soil particle size, increases pH, creates water repellent soils and changes carbon, nitrogen, phosphorus and cation cycling. Vegetation can be either positively or negatively impacted by fire depending on the type of plants and the environment in which the fire occurred. An application that has not yet been researched for MICP is the immediate reduction of erosion of recently burned soils until vegetation can recover. MICP used on burned soils is a potentially viable option that will help to keep the nutrient rich upper layer of the burned soil intact so that vegetation can use the nutrients to recover the land. As was shown earlier, physical changes to soils from burning can greatly increase erosion of soil particles and can cause debris flows. Burned soils are dry and broken down into small and easily eroded particles. Wind and water can easily carry these particles away and result in environmental problems such as eutrophication of lakes. Among other nutrients, fire increases the presence of free cations in soils including calcium. Basic salts contained in vegetation and soils hydrolyze in the presence of water, producing alkalinity, thereby raising the pH and releasing cations into the solutions. MICP could work well with burned soils because for MICP to occur there must be an alkaline pH around 9.0 and calcium must be present. Currently, $CaCl_2$ is the chemical used to provide the calcium for calcite precipitation. However, tests results have shown a reduction in pH when high concentrations of $CaCl_2$ were used in the biomineralization solution and have shown the bacteria to be less effective [31]. Therefore, when used on burned soils there could still be efficient calcite production by MICP because a reduced amount of $CaCl_2$ could be used due to the increased presence of calcium ions in the soils.

Figure 3. Soils burned in the Legion Lake Fire of South Dakota in December 2017. The large sediment load in the stream is heading for a downstream culvert, which clogged.

IDEA PRODUCT, CONCEPT AND INNOVATION

The IDEA product of this report is to use seeded low dosage MICP, called herein as BioCaN, soil treatments using the same MICP treatment solutions and bacterial strain used in traditional MICP for the stabilization of recently burned, disturbed or denuded soils but only applying the treatment one time instead of a continuous injection style or many (sometimes upwards of 100) treatments as has been commonly used in previous research studies. For the purpose of this paper "many" treatments will be defined as application of MICP treatment solutions to the same soil sample more than 10 times. This study compares the effectiveness of BioCaN soil treatments on disturbed soils to the effectiveness of the treatments on a clean sand and to the effectiveness of the treatments on a natural undisturbed soil. Burned soils are physically reduced to small particles and the vegetation root matrix, as well as the chemical bonds that held the soils in place, are mostly lost after a fire which causes soils to become very susceptible to erosion. If a fire is intense it can sterilize the soils leaving behind no or very few living microbial communities which can further increase time until vegetation recovery. Previous studies as well as chemical analysis of burned soils completed for this project show that soils subject to burning typically contain an increased abundance of calcium, other cations and nutrients that exist in the soils due to burned organic matter. New construction and burned soils become susceptible to erosion and cause sedimentation and contamination in surface waters which can lead to eutrophication and other environmental problems. Natural and prescribed burning near riparian zones causes concern because of the chemical changes to the soils and downstream surface water. The loss of nutrients from soils further decreases the vegetation recovery time.

This study aimed to determine if adding ureolytic pure culture bacteria cells suspended in saline along with chemicals high in nitrogen and salts will improve land rehabilitation efforts by reducing erosion of soil particles and allowing for efficient vegetation regrowth. The study tests the effectiveness of varying types of chemical treatments with and without added bacteria to determine the best course of action to take when applying BioCaN soil treatments to three varying soil types: a manufactured sand, burned soil and unburned soil. The research investigates if the added cations, for the purpose of this research coming from calcium chloride, are needed for strength gain required to reduce erosion and how the addition of calcium chloride may impact vegetation regrowth. The study also investigates if the addition of ureolytic bacteria, strain *S. pasteurii*, is needed for strength gain and how this will impact vegetation growth. Finally, the study shows what the consequences would be if the chemical solutions were to be directly added to a surface water body, such as if a vehicle carrying the solutions were to spill into a lake. The study also gives some ideas on commercialization and how BioCaN soil treatments compare to existing and similar market products such as urea fertilizers and mag-chloride dust suppression solutions on a concentration and amount usage scale and will test out the technology on three field sites through the Black Hills of South Dakota area against commonly used erosion control methods.

Soil erosion can cause eutrophication of downstream lakes and so can fertilizer runoff (non-point source pollution) both due to the nitrogen load in the watercourse. Since BioCaN soil treatments include urea-Nitrogen and ammoniacal-Nitrogen, eutrophication and water contamination are a concern from the chemical constituents of the solutions. BioCaN soil treatment solutions also contain a nutrient broth to supplement bacteria growth as well as a calcium source with the traditional supply coming from $CaCl_2$. Previously, research studies have not been completed on MICP chemical solutions and the potential impacts to surface water sources. This research completes a preliminary laboratory scale study to show the potential of MICP to cause eutrophication. The study does find that BioCaN treatments, if catastrophically spilled in a water body, would likely cause first an overgrowth of bacteria and after one week an overgrowth of algae.

This project includes various hypotheses/research questions which it will address that are:

1. Will a one-time treatment of BioCaN soil treatments be effective at reducing erosive forces generated from wind? Rainfall?
2. What types of soil hazards will the one-time treatment successfully reduce? Burned? Construction? Acidic? Highly compacted fines? Sloped?
3. Will vegetation be allowed to germinate and grow when a light crust is formed due to BioCaN soil treatments? How do the treatments impact germination rates on a timescale and quantity-scale? Could BioCaN treatments increase drought resistance in vegetation?
4. How do BioCaN soil treatments compare to other soil erosion reduction techniques on real-world problem sites in three field trials? How do the BioCaN treatments impact soil pH, shear strength, unconfined

compressive strength, vegetation recovery and qualitative erosion observations?
5. How would BioCaN soil treatment solutions impact surface water quality during a one-time contamination event with and without microbes present in the solution?

One of the most effective means of soil erosion prevention by wind and water is vegetation. This is why soil erosion becomes a problem on disturbed soils because the vegetation is lost. One purpose of this project is to determine if BioCaN soil treatment is effective to reduce soil erosion long enough for vegetation to recover with the hopes that by holding the soil in place, along with an abundance of nutrients, the vegetation will recover and take over the responsibility of holding the soil particles in place. This would result in the rehabilitate of the soil Horizon A which would result in no further erosion control treatments. The ideal situation would only require one BioCaN treatment (better economically and environmentally than many treatments) which would create a light charged carbonate crust that would help with plant growth due to increased water retention in the soils and would still allow plants to germinate and grow. The impacts to vegetation germination and growth were investigated for this project to determine if it is feasible to think that MICP solution treatments might work for the purpose of temporary erosion control until vegetation can recover.

An essential part of the innovation of this IDEA is that BioCaN is assessed and evaluated for the specific circumstances of creating a vegetated erosion resistant crust along highway slopes that have been denuded by new construction or fire. In development of this innovation, it is essential to differentiate between the conventional MICP treatments being proposed by others and our proposed BioCaN. In BioCaN, several other mechanisms for erosion resistant crust are included in the theoretical model (and verified by the experimental program). Table 1 lists the comparison between conventional MICP and BioCaN by highlighting the several sources of crusting and crust strength identified in BioCaN. Table 1 shows that BioCaN is a hybrid of bio-augmentation MICP and bio-stimulation MICP plus strengthening of clay particles. Of particular importance in Table 1 is inclusion of non-calcite crystals along with microbial byproducts from both the introduced S. pasteurii and the native microbes. Most notably the effects of native bacteria and fungi are expressed by biopolymers and polysaccharides, which are known to be active participants in biological soil crusting [65-74].

Table 1. Sources of crusting and crust strength in BioCaN.

Source of Strength	Mechanism	Critical Nuances	Differences to the Classic Understanding of MICP
Bio-stimulation of biomineralizing microorganisms	Biomineralization, generally calcium carbonate precipitation.	Ureolytic bacteria in the native microbiome can induce carbonate precipitation rather than the added *S. pasteurii* if the native microbes out-compete or cannibalize the introduced bacteria.	In bio-stimulation, the crusting may be in-part from non-ureolytic bacteria biomineralizing, and or/from other biogeochemical processes. The engineer has no control over this.
Bio-augmentation of biomineralizing *S. pasteurii* microorganisms	Biomineralization, generally calcium carbonate precipitation.	As there are many ions in soils along roadways, particularly from de-icing and dust suppression, there may be many polymorphs of calcium carbonate.	In addition to calcite, there may be development of vaterite and aragonite depending on magnesium ion concentrations in the soil.
Amorphous chlorine and other amorphous crystals	The complex biogeochemistry of BioCaN can result in amorphous crystals binding soil grains together.	In bio-augmentation MICP and BioCaN treatments, large amounts of chlorine and urea are added to the soil that form a variety of amorphous compounds.	In conventional MICP treatments, the effects of amorphous chemical compounds are generally neglected. Some of these compounds are water soluble.
Crystalline chlorine and other chemical compounds	The complex biogeochemistry of BioCaN can result in non-mineral crystals binding soil grains	Chlorine oxide and urea crystals are just two examples of potential crystalline products from liquid MICP and BioCaN treatments.	In conventional MICP treatments, the effects of non-calcium carbonate crystals are generally neglected. Some of these crystals are water soluble.

Source of Strength	Mechanism	Critical Nuances	Differences to the Classic Understanding of MICP
	together.		
Flaky unused salts	Unused salts act as a deliquescent	In dry applications, this may be an important part of BioCaN fugitive dust control but dissipates as saturation levels increase. This effect lasts for more than a single inundation.	In conventional MICP treatments, it is assumed that the unused salts play no role in fugitive dust control or that they are completely removed by transient water inundation.
Electrochemical changes to clay particles	Ion substitution in clay particles and changes to surface polarity changes their agglomeration and their adhesion.	During intense rainfall, sheet flow across the slope, and flooding the effects will lessen as saturation of the soil increases.	Conventional MICP is performed on clean sands with negligible silt and clay content. General use of BioCaN along roadway slopes will be in soils with a small to large amounts of fines.
Biopolymers and polysaccharides	Produced by the metabolism and other biological functions of microorganisms, these organic compounds can add significant tensile strength to soils and bind particles together.	Development of biopolymers and polysaccharides depends on a variety of complex factors including native microbial colonies, water content during treatments, development of biofilms, and stimulating fungi.	In conventional MICP treatments, the effects of biopolymers and polysaccharides are generally neglected, or is performed in soils with no fungi or other microbes.
Matric suction	Enabled by the presence of silt and clay sized particles under unsaturated conditions.	During intense rainfall, sheet flow across the slope, and flooding, the matric suction will dissipate as saturation of the soil increases.	Conventional MICP is performed on clean sands with negligible silt and clay content. General use of BioCaN along roadway slopes will be in soils with a small to large amounts of fines.

WHAT NEED DID THE PROJECT ADDRESS?

This project addresses 1) stabilization of burned soils and loose soils after highway slope construction, 2) erosion potential mitigation of said slopes, 3) accelerated revegetation, and 4) fugitive dust control from low volume roads and highway slopes during/after construction prior to vegetation or after fires.

RESEARCH ACCOMPLISHED

Three different MICP or BioCaN chemical concentrations are tested: the regular or traditionally used concentrations (1x), half the original concentrations (0.5x) by mass of chemicals and double the original concentrations (2x). In addition, two variations on the originally formulated MICP technology are tested: (1) the 1x BioCaN soil treatment is tested without an calcium chloride and (2) without any added bacteria cells both of which will help to determine how these ingredients impact the efficacy of the treatment solutions. The three levels of concentrations along with the two variations and a control treatment of only distilled water in the same volume of liquid applications will all be tested with each of the three soil types. This is called a factorial designed experiment and tests 18 interactions that can occur based on the treatment type and soil type which basically means that each of the six treatment types are tested on each of the three soils types. This is done because due to the chemical, physical and biological complexities of soils it is likely that the treatment that works best on one soil type won't work best on another. The many physical, chemical and biological factors that come into play with soils when determining efficacy of a stabilization product include: soil particle size, infiltration rates, fines content, clay particle chemistry, soil compaction/density, chemical/crystalline constituents, biological makeup, void space, void connectivity, ionic bonds, capillary action, etc.

BENEFITS TO STATE DEPARTMENTS OF TRANSPORTATION

State Departments of Transportation have persistent problems nationwide during and after construction of highway slopes with erosion and fugitive dust. Although there are many means available for fugitive dust control and erosion potential mitigation, these alternatives may be at odds with one another, and may interfere with vegetation of the slopes to maintain long-term erosion resistance. This technology provides short-term erosion resistance and fugitive dust control all while accelerating or not interfering with revegetation. In states with wildfire hazards, this technology allows State Departments of Transportation to rapidly remediate wind and water erosion of the burned soils along the highway and remediate massive sediment loads into drainage infrastructure. The proposed innovation has similar environmental impacts to currently used technologies for erosion resistance and fugitive dust control. This technology adds a critical new \tool to the Department's toolbox of technologies for erosion potential remediation and control of fugitive dust and can be applied to multiple cases and after extreme events (most especially wildfire).

ALTERNATIVES TO MICROBIAL APPLICATIONS

A number of alternative technologies exist in the marketplace for erosion potential remediation. These technologies include vegetated compost blankets, seeded straw mats, hydroseeding, geosynthetic products, mulches, and multi-stage revegetation efforts that include hydroseeding and mulching. Existing revegetation protocols may take several years to be fully effective [75]. In the FHWA roadside revegetation with native plants approach [75], a seeded MICP method can augment or take the place of hydroseeding, fertilizing, Mycorrhizae procurement and augmentation, top soiling, and/or planting. Likewise, there are a plethora of alternative technologies that can be used for fugitive dust control and reductions in wind erosion of soils along highways. These technologies can be applied to unpaved roads, slopes during or after construction, or slopes in desert areas. These technologies include water absorbing products such as calcium chloride or magnesium chloride flakes, sulfonated oils, organic and synthetic polymers, bentonite powders, and organic nonpetroleum products such as lignosulfonates and polysaccharides from vegetable matter [76]. A number of enzyme products are available on the market which are used to stabilize soils. These are often proprietary enzyme products that produce electrochemical changes to soils, often require a certain percentage of clay fines in the soil to work and modify the absorbed water characteristics of the soil [76]. All of the products listed in Table 2 have key similarities with the MICP and BioCaN technologies of this study. Table 1 lists several of the key similarities and differences. Table 2 is a synthesis of references [1] to [76], personal communications with State Department of Transportation erosion and dust control experts and maintenance crews, and the professional experiences of the authors.

Table 2. Comparison of BioCaN with alternative technologies.

Technology	Purpose of Technology	Similarities to BioCaN	Differences from BioCaN
Bio-stimulation MICP	Soil stabilization	Ureolytic bacteria are used to produce urease enzymes, which in turn catalyze calcium carbonate precipitation.	Native microbes are stimulated to produce calcium carbonate. Vegetation not considered.
Bio-Augmentation MICP (others)	Soil stabilization	Ureolytic bacteria are used to produce urease enzymes, which in turn catalyze calcium carbonate precipitation.	Many treatments are used (as opposed to single treatments in BioCaN). Vegetation not considered.
EICP	Soil stabilization	Urease enzymes are used to catalyze calcium carbonate precipitation.	Urease enzymes are added directly to the soil. Vegetation not considered.
Proprietary enzyme augmentation	Soil stabilization	Enzymes are added to the soil to strengthen the soil, increase stiffness and prevent erosion/dust.	Electrochemical stabilization rather than generation of binding crystals between soil grains.
Slope paving or armoring	Erosion potential mitigation	A "crust" is placed atop the soil to prevent erosion.	No vegetation possible

Technology	Purpose of Technology	Similarities to BioCaN	Differences from BioCaN
Hydroseeding	Erosion potential mitigation	A tackifier is used to prevent seeds from eroding short-term, while seeds are applied to long-term.	Tackifier does not deeply penetrate soil and provides only a weak mitigation of erosion potential compared to other technologies. May or may not include fertilizer in initial application.
Vegetated Compost Blankets	Erosion potential mitigation	Compost is used to provide seeds and seedlings nutrients and to prevent against soil erosion while plants take root.	Thick compost blankets required. Compost is spread mechanically. Compost availability and quality varies by locality.
Geosynthetics	Erosion potential mitigation	Vegetation is protected during initial seeding or planting from erosion. Plant available water is maximized.	Geosynthetics must be spread my manual labor or mechanically. Aesthetics during vegetation. Difficult to place on some steep slopes.
Straw and straw mats	Erosion potential mitigation	Straw is used to provide seeds and seedlings nutrients and to prevent against soil erosion while plants take root.	Straw mats and mulches must be spread my manual labor or mechanically. May or may not include fertilizer in initial application.
Native plant revegetation	Erosion potential mitigation	Native plants are used to vegetate slopes and prevent erosion.	Can take 3 to 6 years to implement. Little short-term erosion protection.
Calcium Chloride flakes or solutions	Fugitive dust control as a deliquescent	Calcium chloride is the primary source of calcium in BioCaN. Effective for several storms.	Only applied as a water absorber. Any microbial calcification is an unintended byproduct. Harmful to vegetation.
Magnesium Chloride	Fugitive dust control as a deliquescent	A spray of an ionic solution is applied to the soil surface, which strengthens the soil temporarily and prevents fugitive dust.	Only applied as a water absorber. Any microbial biomineralization is an unintended byproduct. Harmful to vegetation.
Organic petroleum products (dust oils)	Fugitive dust control	Liquid surface application. Binds soil particles together.	Petroleum byproducts. Can waterproof the road.
Asphalt cutbacks and emulsions	Fugitive dust control	Liquid surface application. Binds soil particles together.	Petroleum byproducts rather than microbial biocementation. Can waterproof the road.
Lignin derivatives (lignosulfonates)	Fugitive dust control	Liquid surface application. Binds soil particles together.	Susceptible to being destroyed in heavy rains. Can be used under higher moisture conditions. No cementation.
Molasses and cane/beet sugar extracts	Fugitive dust control	Liquid surface application. Binds soil particles together. Much of the binding is from polysaccharides.	Susceptible to being destroyed in heavy rains. Can be used under higher moisture conditions. No cementation.
Tall-oil derivatives	Fugitive dust control	Liquid surface application. Binds soil particles together. Works best under dry conditions.	Susceptible to being destroyed in heavy rains. No cementation.
Vegetable oils	Fugitive dust control	Liquid surface application.	Binding agents can oxidize

Technology	Purpose of Technology	Similarities to BioCaN	Differences from BioCaN
		Binds soil particles together.	rapidly and become brittle. No cementation.
Electrochemical ionic treatments	Fugitive dust control	Performance is sensitive to clay mineralogy and soil chemistry.	Changes characteristics of the clay size particles rather than binds soil grains together.
Sulfonated oils	Fugitive dust control	Performance is sensitive to clay mineralogy and soil chemistry.	Changes characteristics of the clay size particles rather than binds soil grains together.
Synthetic polymers (acetates and acrylics)	Fugitive dust control	Liquid surface application. Binds soil particles together. Much of the binding is from biopolymers.	Rather than cementation or biomineralization, the particles are bound by polymer adhesive forces.
Clay additives	Fugitive dust control	Increases dry strength by agglomerating fine particles together.	Creates "slippery" surfaces when wet. No cementation.

INVESTIGATIVE APPROACH

This study was designed to investigate if the proposed BioCaN innovation is effective for remediation of erosion and fugitive dust for highway slopes that have been denuded. Experiments included soil baseline testing, bacteria culturing optimizations, pilot studies on MICP of clean sand, laboratory erosion testing, laboratory vegetation experiments, field erosion and vegetation experiments, and an environmental water quality impacts experiment. This experimental design was developed to show the span of BioCaN treatment applicability and effectiveness for multiple aims: short-term surface erosion protection, long-term surface erosion protection, compatibility with revegetation, and environmental impacts. Experiments were not performed for erosion resistance in active water courses or channels (i.e. scour erosion was not studied). Work was performed in the biogeotechnical engineering laboratory at the South Dakota School of Mines and Technology.

BACTERIA CULTURING AND TREATMENT SOLUTIONS

Cultures of bacterium *S. pasteurii*, and all chemical nutrient broths were prepared in the biogeotechnical laboratory at SDSMT. All solutions were sterilized prior to application to soils by autoclave or filter sterilization. Sterile disposable pipets were used exclusively including for surface-drip application to the soil samples. Water used to make the broths for all experiments and field trials was distilled or deionized.

The procedures for the chemical and bacteria nutrient broths that were used to culture bacteria used in the erosion, vegetation and environmental experiments are presented in this section. In the preparation of *S. pasteurii* and the BioCaN soil application formulas, three bacteria growth media were used: ATCC 1376, ATCC 1832 and urea-broth solution [1]. The first growth media, ATCC 1376, was used to rehydrate and preserve the original ordered stock culture of freeze-dried *S. pasteurii*. ATCC 1376 can be prepared as a liquid growth media or agar plate growth medium or half and half which is what was completed for this process resulting in 500 mL of liquid growth media and 20 agar plates. The plates used in the growth of bacteria used for all the experiments in this study started from colonies plated on ATCC 1376 agar plates or directly from prepared freezer stocks. ATCC 1832 was the growth media used to grow S. pasteurii into the desired growth phase and cell population density/quantity culture. Once to quantity culture, the bacteria cells were then washed and quantified to add to the soil solutions in the desired cell concentration. The third growth media used was the urea-broth which was used to supply the nutrition and proper chemical elements to the bacteria after applied to soils to produce the biomineralization or hardening of the soils. The desired number of *S. pasteurii* cells were added to the urea-broth immediately before soil application along with the calcium chloride solution if used in the BioCaN treatment. The 1x calcium chloride solution is 0.25 M.

The urea-broth and the calcium chloride solutions are added to one another immediately prior to application for erosion control treatments. If using bioaugmentation, a saline solution with washed S. pasteurii cells in the desired concentration is also added. When urea-broth and calcium chloride solutions are combined together the total liquid volume becomes 0.33 M urea and 0.25 M Ca2+ being applied to the soils. Throughout this document those concentrations are referred to as either regular concentration or 1x concentration of urea-broth or calcium chloride solutions. Also, tested in this research is a half or 0.5x concentration which contained half the mass of the dry chemicals in the same amount of liquid or double or 2x concentration which consists of double the mass of chemicals in the same amount of liquid. If large volumes of urea-broth or calcium chloride was desired the recipes were just increased by volume of liquid and mass of ingredients proportionally. An 0.85% sterile saline solution is used for bacteria cell washing and storage. Saline solution is used for bacteria storage because it maintains microbial integrity by protecting the cells while also not allowing growth. Therefore, the cells can stay in the intended growth phase and correct population density.

To prepare the regular concentration MICP or BioCaN treatment solution, i.e. the 1x solution of urea-broth the following ingredients are combined:

- powdered nutrient broth,
- crystalline urea,
- crystalline ammonium chloride, and
- distilled water.

The pH is adjusted to 6.0 using 4N sodium hydroxide or 1M hydrogen chloride. The urea-broth is autoclaved and stored aseptically until needed.

When growing pure culture bacteria, aseptic conditions are required to maintain a pure culture of bacteria. Therefore, a properly sterilized benchtop and workspace is essential for each step. Maintaining these sterile conditions throughout all procedures up until the bacteria/chemical application to soils is likewise essential. Soils are not sterile unless treated to be so. However, sterility treatments of soils change their microbiomes, chemistry, structure, and hydro-mechanical properties. Therefore, sterilizing soils is not an option. Likewise, in this work it is essential preserve the existing microbiota so that interactions between applied chemicals, applied pure culture and existing soil conditions can be investigated. This is because there would never be a situation in the real world in which sterile soils are used at a feasible level.

Cultivated *S. pasteurii* for BioCaN treatments are obtained from ATCC. ATCC maintains a library of pure microbial cultures, and freeze-dried seed stock can be ordered for *S. Pasteurii* and thousands of other strains. Once the bacterial seed stock has arrived it must be rehydrated. Rehydration activates the dormant microorganisms so that cultures and/or freezer stocks can be prepared. Freezer stocks are prepared so that if something happens to bacterial colonies in the petri dish colonies, more bacteria specimens can be easily obtained without rebuying the freeze-dried seed stock. Also, it is best when culturing new bacteria to always return to the freezer stock to maintain pure culture so that the cultures being used in ongoing experiments are not evolving or otherwise modifying their DNA makeup.

Bacteria culture of *Sporosarcina pasteurii*, ATCC # 11859, was ordered from American Type Culture Collection (ATCC) in Bethesda, Maryland. The bacteria strain was originally isolated from soils, is known to produce urease and is rated as biosafety level 1. The bacteria arrived in a small vial with freezer-dried stock cultures and therefore does not need refrigeration upon arrival. The sample was stored until ready to rehydrate in a cool dark place. The initial step to rehydrate the sample is to heat the tip of the tube in a Bunsen burner, dropping water on the tip until it cracks and then hitting the tip with a file. Sterile ATCC 1376 broth was pipetted into the bacteria tube and mixed until the flaky freeze-dried stock cultures are dissolved. The solution was then put in a sterile testing tube and placed in an incubator on a drum roller which was checked after 24 hours for growth. The bacteria *S. pasteurii* grows at 30°C in an aerobic atmosphere in the incubator. The bacteria were then plated using agar plates made from ATCC # 1376 media. Gram staining was completed on the bacteria to make sure that the bacteria came back as bacillus (rod-shaped) and gram positive (purple stained) to ensure a pure culture of the correct bacteria strain. Two freezer stocks were made from the plate cultures for long term storage. The freezer glycerin stocks were made by micro pipetting 50% DI water/50% glycerin solution (mixed previously) and *S. pasteurii* solution into sterile tubes after 48 hours of incubation. All materials used and steps taken to make the freezer stocks were aseptic. These freezers stocks were used to plate and grow all the bacteria used in this project. Best practice is to take the bacteria from an original and verified source as it assures that the correct bacterium is being grown and used and limits evolution of the bacterial strain over time.

To incubate the bacterial culture, ATCC 1832 is added aseptically to centrifuge tubes. Each tube is inoculated with a separate *S. pasteurii* bacteria colony from an agar plate. The centrifuge tubes with bacterial solution are placed into a tube rotator placed inside an incubator. The bacteria are incubated for 8 hours at 30°C and 30 RPM. ATCC 1832 is then added each to the flasks and placed in a hood to bring the fluids to ambient room temperature for 8 hours while bacteria incubate. Alternatively, these can also be placed inside the incubator. After the 8 hours of incubation, the bacteria solutions are removed. Lastly, the flasks are placed securely onto a shaker plate and placed into an incubator for 16 hours at 30°C and 150 RPM.

The bacteria solution is removed from the incubator and is then washed and centrifuged to produce pure pellets. Once finished in the centrifuge, there should be a pellet of bacteria in the bottom with the supernatant liquid. Pour off the supernatant from all tubes into a flask labeled waste with the chemical constituents listed. Add 0.85% saline solution to each of the tubes with bacteria and tighten caps. Use a vortex mixer to thoroughly disperse bacteria into saline solution. Centrifuge for the 2nd time for 10 mins at 4,000 RPM. Remove the tubes from centrifuge, pour off liquid, add 5 mL of saline to each tube, vortex mix and centrifuge for the 3rd time for 10 minutes at 4,000 RPM. Repeat washing one more time by pouring off supernatant, add saline to each tube, vortex and mix. For the 4th and final time centrifuge for 10 minutes at 4,000 RPM. Pour off the 10 mL of saline from bacteria cells and add 5 mL of saline back into tube. Vortex mix and store refrigerated until soil experiments.

The concentration determinations of the bacterial specimen after cell washing is determined using a microplate reader at a wavelength of 600 nm which gives the optical density at said wavelength for the growth curve equations. Appropriate growth curve equations were used because the bacteria growth procedures primarily incubation times were similar and for the same strain. A 96-well plate was used to determine triplicate 200 µL samples from each of the tubes prepared as explained in the previous section. The average of the three measurements was used to calculate the population density of *S. pasteurii cells* in each tube. The cell concentration in each tube was then used to determine the amount of the solution that must be combined with the urea-broth and calcium chloride for the BioCaN soil treatments.

The bacteria-urea-calcium solution can be applied to the soils by volumetric mixing, injection or surface application. To prepare typical sand soil samples for experimental testing by volumetric the application rates previously used of MICP solution approximately 2 mL solutions is used per 100 grams of sand. After volumetric mixing with soil the soil mixture can be compacted into sample cups to the desired density. Most of the BioCaN soil treatments in this research project were surface applied by spray or drip method at a rate of 1 mL of solution liquid per square inch of soil surface area exposed.

SOIL SAMPLE COLLECTION AND PREPARATION

To properly analyze the impacts that BioCaN soil treatments have on varying types of soils, each of the soil types chosen for this research program must have various geotechnical index testing completed to classify and understand the soil behaviors. If possible, the tests were first completed on clean sand due to the fact that the properties of clean silica sand are well known, and this helps ensure a higher quality of testing by ensuring the procedures and equipment were in proper working order and giving reliable results. The soils classification phase of this project gave a better understand of the constituents and chemical makeup of the various soil types used for experimentation which helps to better understand the reasons the soils behaved in the manner they did during treatments and testing. This phase of the research project also gives insight into the chemical and physical changes that occur to soils during fire and other disturbance events.

Geotechnical index testing was completed to physically characterize the soils via the following indices: liquid limit and plasticity index, particle size distribution, drained shear strength via direct simple shear, constant head permeability, etc. Scanning electron microscopy along with energy dispersive x-ray spectroscopy (SEM/EDS) was completed to give images of the soil particles and a generalized idea of the elemental differences that may be caused by fire. X-ray diffraction (XRD) was completed to analyze the crystalline composition of the soils which can also be compared to the SEM/EDS results. It must be noted that XRD does not quantify the percent of the soils that are amorphous, nor does it give information on crystals that are nano-sized but does give quantification of the micro-sized crystalline compositions. Elemental and crystalline compositions obtained from EDS and XRD testing are a rough estimate based on a very small sample window. The tests can be used to estimate the overall composition of soils included treated soils but are really better for identifying only small areas of a sample and care must be taken to not overtly classify soils based only on these test results. Most tests were completed in triplicate and an average of all measurements taken and used as the results. Outliers were identified, if any, in the process of testing, and retest performed as needed to rectify outliers if sufficient material quantities were available for retests.

During fire sampling the depth of the top layer of burned soils was measured. The top layer was determined based on the layer of ash and char which was darker colored and degraded. The layers were sampled during the Crow Peak Fire of July 2015 at 15 different locations within the fire perimeter. Most of these sites were in the naturally burned area while a few were in an area where back burning was performed as a fire fighting tool. Areas that were back burned typically resulted in a less severe burn whereas areas that were burned where the fire spread to naturally were less controlled and resulted in a medium to high severity burn. The top layer of burn typically resulted in about one-inch (25-mm) depth of char and ash both when calculated by modal and mean.

To obtain samples of burned soils scorched in intense wildfires, the research team worked with the USDA Forest Service, South Dakota Fire, Custer State Park, and the South Dakota Division of Forestry to collect samples most typically within 1 week of fire suppression in the Black Hills of South Dakota. All soil samples came from the same formation, a lean silty sand with gravels common to the region in the hills. Fines content is approximately 1-20%, and gravel content 5% to 15%. Soils burned in the Crow Peak and Legion Lake Fires constitute the bulk of materials obtained for this phase of testing, with additional samples obtained after three other smaller fires. Samples were obtained prior to rainfall of 2-mm or greater or winds of 15mph or higher. Figure 4 shows sampling of burned soil after the Legion Lake fire of December 2017, obtained from Custer State Park, with the ground still warm from the fire and the fire actively still burning nearby. Care was used to not disturb the hydrophobic layer in the soil developed during burning. Ash was preserved as best as possible in the samples and preserved throughout sample transport, handling, and preparation in the laboratory for BioCaN solution treatments. Unburned soils from each site were also sampled for laboratory testing.

Figure 4. Field sampling of burned soils after a wildland fire, assisted by the USDA-FS and Custer State Park fire officials.

The specimen cups for laboratory testing were disk shaped with a diameter of 4.25 in (108 mm) and a depth of 1.75 inches (45 mm) which gives a top surface area of 14.2 in2 (91.6 cm2) and a volume of 24.8 in3 (406.9 cm3). Soil specimens were prepared flush to the top of the container. Once the desired density by soil type was determined based on in situ or practice preparation with common compaction methods the cups were prepared in binary layers with different densities. The burned soils and unburned soil test specimens used different soils for layer 1 and 2 from the 1st and 2nd layers obtained in the field. In the sand cups the same soil was used for both layers. Layer 1 for the burned soils and the unburned soils consisted of the top layer collected from the field and subsequently layer 2 consisted of soils collected from the 2nd layer down in the field. Refer to Section 3.2 for more information on how these samples were collected from the field, layering was determined, and soils were mixed. The soil cup preparation and compaction procedures for the 3x6 factorial design wind erosion and rainfall erosion were identical. The 3x6 factorial designed vegetation experiment was identical except for the 4th lift (top lift) and the variation will be explained at the end of the procedure. Refer to Table 3 1 for the specifications of density, lift weights and hits per lift by soil type. After lifts 1 and 2 were completed the cup was measured to ensure 1" (25-mm) deep remained open on top and if it was not 1" (25-mm) than an additional 100 tamps were done. After the soil for lift 4 was placed into the cup than the majority of hits were completed but if soil fell off during the process it was placed back on top of the cup and the hits continued. For the vegetation cups 20% of the top lift soil was left off, the seeds were planted and lightly raked. The remaining soil was placed on top of the soil, packed by hand and lightly raked. The cups were then treated, planted or whatever else was needed for the specific experiment.

Seeded BioCaN biomineralization solution was applied in three different levels: 0.5x, 1x and 2x concentration. All specimens were prepared and testing in triplicate so that statistical variation could be constrained, and outliers identified. Solutions were applied at a rate of 1 mL/in2 of surface area of the soil specimen. The 1x BioCaN solution resulted in concentrations of 333 mM urea (CH_4N_2O) concentrations and 252 mM Ca2+. In addition to three levels of BioCaN treatments (0.5x, 1x, and 2x), soils were treated with only the bacteria inoculated urea-broth solution with no supplemented cations (BioN, i.e. no additional calcium), and with the cation solution and no inoculate (CaN, i.e. no bacteria were added so that bio-stimulation was encouraged rather than bio-augmentation). These two additional treatments were for comparative purposes to show the interactions of the independent variables and to show the relative merits of bio-augmentation versus bio-stimulation and also of leveraging existing cations in the soil for biomineralization. Soil was not sterilized or autoclaved to preserve existing microbiota. All solutions were sterilized prior to application to soils and sterile pipets used for surface-drip application to the soil samples. Soil was not sterilized or autoclaved to preserve existing microbiota. Solutions were applied most typically by sterile drip pipet method to ensure and even treatment application across the entire surface of the soil so equal infiltration and depth of treatment could be expected throughout.

LABORATORY STUDY

Treated and untreated soils were tested for chemical composition and had imaging performed for chemistry controls in the laboratory experiments. Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy for Imaging and Elemental Content Spot and Spectrum Identification were used for this purpose. The SEM examination was completed using the Zeiss Supra40VP variable-pressure field-emission SEM. X-ray microanalysis was performed with an Oxford Instruments X-Max 80mm2 SDD detector and the Aztec microanalysis system. Soil samples were prepared using double sided carbon tape. The soil particles were sprinkled over the tape and then loose particles were taped or pushed off to try to obtain an even surface with minimal loose debris. Some samples were not coated with carbon to try to reduce the charge buildup while others were carbon coated. The secondary electron detector (SE2) was used to take images with an acceleration voltage of 1.00 kV. Pictures were taken with either a line interval of N=256 scan speed of 3 or with the frame integration of N=10 and a scanning speed of 5. The EDS detector was used on the samples with an acceleration voltage of 15.00 kV and a 30.00 µm aperture. SEM use electron beams to detect objects. Great magnification can be used with an SEM. If the SEM is equipped with an EDS detector it can give a detailed report on the elemental makeup of a sample. Since the detection is completed with an electron beam the sample must have a conductive layer for the electrons to go into. This is why nonmetallic and biological materials are commonly prepared for SEM analysis with a thin (a few nanometers thick) layer of carbon or gold coating. Since the surface is the detection point flatter surfaces are better.

X-Ray Diffraction (XRD) was also used for mineralogic analysis. Soil samples to be tested in an XRD analysis were first oven dried overnight. The soils then must be pulverized by pestle and mortar (pictured in Figure 5) or sample shaker/mixing mill with beads. A small sample is evenly spread onto a glass slide and then placed inside the Rigaku X-Ray Diffractometer Ultimate Plus which is pictured in Figure 3 6 and located in the mining industries building on the SDSMT campus. The x-ray diffraction scans were run with a copper x-ray tube and a scintillation detector performed from 3 to 6 degrees and run at 2° per minute at a 2 s step-time. The instrumentation works being measuring the diffraction angles off of the sample as it is rotated 180°. Bragg's law is then used as a function of the diffraction angle and the wavelength of radiation to determine the spacing where the peaks are observed in the output scans. At least three diffraction peaks are needed to identify a phase of material. The output scans were analyzed used Jade 6 XRD software analysis software and compared against large databases of known crystalline structure scans to determine the likely crystalline composition. Quantities of each crystalline structure contained in the materials are estimated from the scans although this does not account for amorphous material or nano-sized particles.

Figure 5. The X-Ray diffractometer used to identify crystalline structures in burned, unburned and sand untreated and treated soils for this study. The picture on the left is a pulverized sample ready for testing, the middle picture is the outside of the instrument and the picture on the right is the internal components while a scan is being run.

The specimens used in the testing with the scanning electron microscope, the energy dispersive x-ray spectroscopy (EDS) and the x-ray diffraction (XRD) were sampled from various fires. A note going forward: all SEM data must be read and interpreted with caution due to the electrically dipolar soil particles moving and being charged under electron bombardment, and the very small area of analysis and the error that is inherently involved. SEM works better with inert, solid and flat objects and therefore analysis of loose soils is difficult. Soils were taped and/or glued with carbon but results still may not be as reliable as would be expected if completing the tests on a polished rock or a metal.

A preliminary investigation was completed on burned soils which were sampled from within the fire perimeter area a couple weeks after the Crow Peak Fire was contained. The 2,733-acre fire, which was 4 miles west of Spearfish SD, burned U.S. Forest Service, SD State and private land in summer of 2016. The fire primarily burned in a Ponderosa Pine forest with areas of mortality from pine beetle infection and large amounts of downed and decaying tree matter. A visual inspection of the burned area showed that the upper layer (top 1 to 3 inches) of ground had broken down into very fine ash particles that became airborne with any disturbance. After a light rainfall event the individual rain droplet were observed in the soil and made a cone-shaped depression. The soil matrix structure was almost completely lost in the upper 3 to 6 inches, depending on the area. The burned soil samples were collected and further analyzed with an SEM/EDS. Figure 6 (burned soil) and Figure 7 (unburned soil) show SEM images of the soils. The breakdown of soil particles from the fire event can be seen on a microscale. The unburned samples were taken on the outside of the fire line in the same area and close elevation as the burned samples. The burned sample was taken from an area that appeared to have an intense burn.

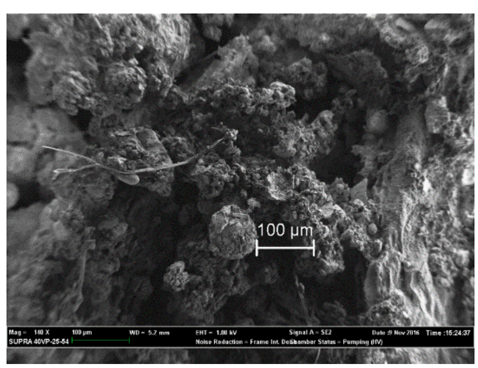


Figure 6. Image taken of burned soil from the top layer of soil in sampling location CPF 8. The magnification is at 140X, the working distance 5.2 mm, acceleration voltage of 1.00 kV and the detector systems used was the

SE2.

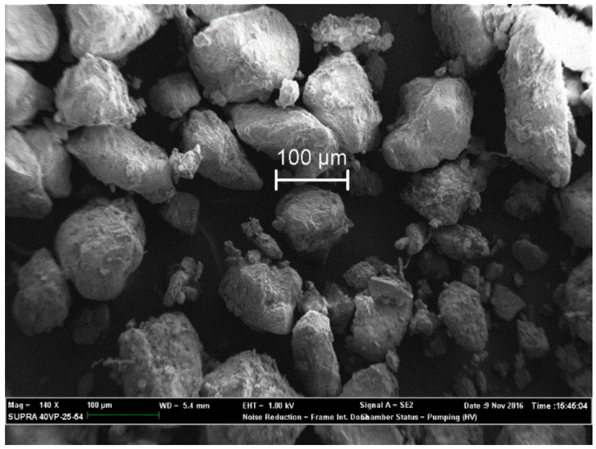


Figure 7. Figure 4 2 Image taken of unburned soil retrieved from the dozer line in the sample location CPF 7. The magnification is at 140X, the working distance 5.4 mm, acceleration voltage of 1.00 kV and the detector systems used was the SE2.

An Energy Dispersive X-Ray Spectroscopy (EDS) was used to analyze the unburned and burned soil samples. Results from this study are found in Figure 8 and Figure 9. The working distance used in the SEM/EDS is at 8.8 mm, the sample is at 90X magnification, the acceleration voltage is 15.00 kV, the aperture is 30.00 microns and the current are normal not high. A map spectrum was taken in areas of both samples. This is a very limited study of a very small area of the sample and therefore more testing is needed to make a final conclusion on element composition of the soils. However, this small-scale study agreed with studies that have shown an increase in calcium content in burned soils. The unburned soils showed 0.7 % calcium and the burned soils showed 6.7 %. Other samples observed with the EDS also showed a higher calcium content in burned than unburned. When the voltage was turned up to 15.00 kV as is recommended for the EDS detector the soil particles on the carbon tape started to move and shift around. This behavior could have somewhat skewed the results from the EDS analysis. Figure 9 gives a calcium composition of 6.7% in the burned soils whereas Figure 8 only shows a 0.7% composition of calcium. The elemental analysis is completed over such a small area that it cannot be taken as conclusive evidence that the calcium is increased so substantially by fires. However, this data does support previous research on burned soils.

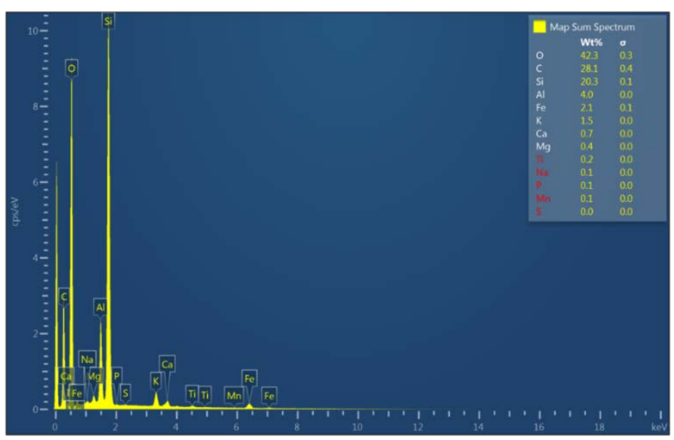


Figure 8. Mapped spectrum of soil sample CPF7 from the dozer line, this is unburned soil.

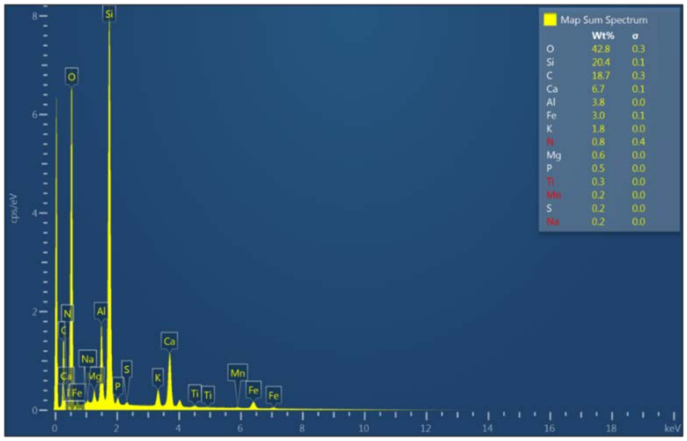


Figure 9. Mapped elemental spectrum of soil sample CPF6 from the top layer of burned soil in a group torch burned area.

The initial findings and the literature both show that burned soils exhibit both a broken-down soil matrix highly susceptible to erosion as well as an increase in calcium content needed for BioCaN soil treatments. Evidence supports that severely burned soils lose the physical and chemical properties that allow erosion resistance and BioCaN increases erosion resistance in soils.

X-ray diffraction scan were run on various burned and unburned soils. All soils showed the highest composition of quartz which is to be expected from soils in this area. Samples were treated with the same six treatment types as were used in various experiments throughout this study of which they are: (1) None, (2) No Bacteria only 1x Urea-Broth and $CaCl_2$, (3) 1x BioCaN, (4) No $CaCl_2$ only 1x Urea-Broth and S. pasteurii cells, (5) 0.5x BioCaN and (6) 2x BioCaN. These samples were then tested by penetrometer for surface strength (crust development) and then the sand samples and untreated burned and unburned were analyzed by XRD. Samples were one square inch and half an inch deep of soil. The soil samples were submerged in solution and allowed to cure under 16 hours per day of broad-spectrum lighting in aluminum tins. Very high strength readings were achieved to the extent that sand with treatments 2, 3, and 6 could not give readings and could not be broken apart using metal utensils. Table 3 shows the penetrometer readings of the samples. Note that the second letter in the sample names gives the soil type, s=sand, u=unburned and b=burned and the number in the sample name represents the treatments given earlier in this paragraph. Figure 10 shows the scans of the silica sand treated and untreated samples. All of the samples, even though displaying very different strengths, showed the same crystalline composition on the XRD scan. Note that XRD analyses cannot detect nano-scale crystal structures, only micro and macro scale. In single treatment BioCaN, this indicates that calcite and other calcium carbonate precipitates have not grown to sufficient size to be detected in XRD.

Table 3. Surface strength after treatment with BioCaN solutions

Sample	Strength (tsf)	Sample	Strength (tsf)	Sample	Strength (tsf)
X-S1	0	X-U1	0.3	X-B1	0.75
X-S2	MAX (>4.75)	X-U2	0.75	X-B2	1.0
X-S3	MAX (>4.75)	X-U3	0.75	X-B3	1.25
X-S4	3.25	X-U4	0.35	X-B4	1.5
X-S5	2.5	X-U5	0.5	X-B5	1.5
X-S6	MAX (>4.75)	X-U6	0.45	X-B6	1.5

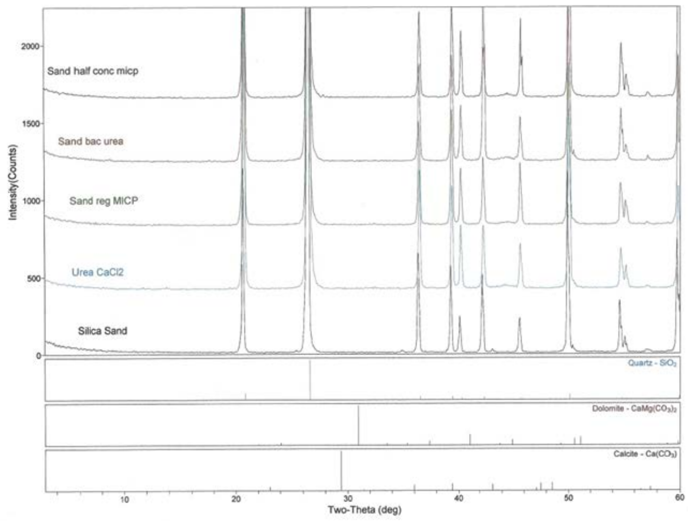


Figure 10. XRD scan of various BioCaN treatments to silica sand. Note that the treated samples showed far greater strength than the untreated, yet crystalline structure is the same by XRD testing.

Strength and Soil Index Testing

Typical geotechnical index testing to classify the soils for comparison methods included: particle size distribution by sieve shaking for 10 minutes (ASTM D-422), particle size distribution using hydrometer for the fines passing the #200 sieve (ASTM D-422), engineering classification by American Association of State Highway and Transportation Officials (AASHTO), engineering classification by Unified Soil Classification System (USCS), constant-head permeability testing (ASTM D-2434) and field unit weight of compaction using the sand cone method (ASTM D-1556). Strength testing included field shear vane testing, pocket penetrometer testing and direct simple shear testing (ASTM D-3080). Penetrometer testing was used for the surface strength determinations which are frequently presented with the erosion and vegetation data throughout this document. Figure 11 shows the pocket penetrometer being used to check crust strength and thickness.

Figure 11. Penetrometer measurements of samples prepared for XRD analysis. On the left is clean sand treated with 0.5x BioCaN treatments and reads a strength of about 2.5 tsf (240 kPa). On the right is a clean sand treated with 2x BioCaN treatments and maxed out the instrument to produce a strength greater than 4.5 tsf (430 kPa). Note the soil crust is not broken in the sample cup on the right as the strength exceeded the capacity of the pocket penetrometer. Untreated clean sand produces 0 tsf (0 kPa) strength.

The soils tested in this research program are organized into 3 categories: manufactured, burned and unburned. The only manufactured soil that was tested is a uniform graded Ottawa clean silica sand. This soil was used as a baseline/control test due to the fact that MICP and silica sand interactions have been studied before and this way soil treatment efficacy can be more easily monitored. The material is chemically inert, poorly graded, and has uniform particle shape. Table 4 shows results of geotechnical index tests on all soil materials used in laboratory and field experiments.

Table 4. geotechnical indexing parameters for the primary soils used in the laboratory experiments and the soils from the three field sites.

	Laboratory Soils					**Field Soils**		
Test Parameter	**Burned**		**Unburned**		**Clean Sand**	**RC Landfill**	**Custer State Park**	**Rush. X I-90**
	Top Layer	**2^{nd} Layer**	**Top Layer**	**2^{nd} Layer**				
Moisture Content	5-6 %	6 %	10 %	11 %	0.1 %	30 %	25 %	12 %
Liquid Limit	43	35	NP	24	NP	89-118	65	32
Plastic Limit	40	31	28	21	NP	44	62	21
Plasticity Index	3	4	NP	3	NP	45-75	3	11
Fines	18 %	9 %	<1 %	<1%	0.2 %	15-30%	11%	20%
Sand	74 %	80 %	92 %	89 %	99.8%	70-85%	50%	74%
Gravel	8 %	11 %	7 %	11 %	0 %	0-5%	39%	6%
Friction Angle (DSS)	29°		29°		19°		3°	
Cohesion (DSS)	215 psf		285 psf		240 psf		200 psf	
Density When Compacted	60 pcf	80 pcf	80 pcf	100 pcf	110/ 120 pcf	-	-	-

Field site 1 was at the Rapid City Landfill, on the slopes of an excavation to be used as a waste cell after 2024. Site soils are weathered shale with sand, cobbles, and boulders frequent in the formation and a fines content of 15 to 30%. The soil fines are predominantly fat clay and silt with a plasticity index ranging from 45 to 75% and a liquid limit from 90 to 120 %. The fines content is somewhat misleading as it was determined after the delicate formation was broken down whereas in situ the fines content would be much lower as the fines are bound into clods and peds which would not behave as fines. The soils are a dark slate grey in color.

Field site 2 is a slash pile burn area on a gentle slope in Custer State Park near Lame Johnny Creek that was burned in the December 2017 Legion Lake fire and again in early 2018 during a slash pile burn. The Custer site soils were composed of 50% sand and 40% gravel with a fines content of about 10% with a thin one-inch ash layer over top of a two inch layer of soil with vitrified clay and hydrophobic layer from the fire. The soil has a LL around 65 but low plasticity with a PI of only 3 thus consistent with the laboratory burned soils. The average water content for the Custer State Park burned soils on treatment day was 25%. The location of the test site was a pile of mechanically placed forest debris and logs, making the burn hot, intense, and long, which made a consistent site with no vegetated cover and an open, loose structure to the soil. Previously completed XRD and SEM/EDS tests performed on burned soils have shown high levels of calcium in the soil, freed by the fire and available for plant or microbial use. The soils were dark grey to black in color except for where erosion had occurred at which they were brown. Field site 3 was on the side of Interstate 90 in Rapid City next to Rushmore Crossing shopping center.

Site 3 soils were a highly compacted sandy lean clay embankment fill. Fines content of the soil was 20% with only 5% gravel. Site 3 had a 2.5H:1V slope and was a location where SDDOT has had a difficult time establishing vegetation and preventing erosion. Site 3 soils very much behaved as a “tin roof” in which very little water is able to penetrate and everything washes off. The soils appear to be very hydrophobic in nature rather than over-compacted. When wet the soils cannot even be driven on because the vehicle will have no control and slide off. At the same point the soils are unique because they exhibit high levels of erosion due to wind from passing trucks as well as sheet erosion from rainfall and runoff. The moisture content at the time of BioCaN treatments was 12%. The soils have a relatively low liquid limit of 32 and a plastic limit of 21. The soils are brown with a red tint.

Pilot Study of MICP on Clean Ottawa Sand

Pilot studies were completed to demonstrate that procedures developed by [1-5] could successfully be reproduced the same protocols and materials as those foundational studies that initiated the study of MICP. This pilot study MICP treatments had bacterial concentrations in the 10^6-10^7 cells/mL ranges. The treatments were volumetrically mixed with clean Ottawa Sand at a rate of 1 mL MICP treatments with 50 grams of sand, cured in a 39°C dark oven and tested every few days with penetrometer measurements. Over ten days the lower bacterial concentration treated samples developed crusts with pocket penetrometer resistances of up to 3.5 tsf (335 kPa). However, the 2x higher bacterial concentration samples developing less than half the strength as the 1x.

After 10 days the treated samples (and a control sample of untreated sand) were saturated with distilled water and monitored for surface strength for an additional 9 days. The purpose of this was to see how MICP treatments would maintain functionality after extended saturation. All three samples had a strength reading of zero within a few days after saturation! This raises the question to if after a single treatment, sufficiently large calcite crystals can develop as shown in the previous work by [1-5] wherein dozens of treatment cycles were used. In typical MICP studies, specimens are rarely saturated in unconfined conditions for 10 days to monitor strength loss as a function of time, or the materials have been treated dozens of times, or with massive volumes of inoculate. In some studies, sands are treated immersed in reactors for several days to weeks. The pilot study highlights that in single treatment scenarios, the method developed in this innovation, BioCaN differs from MICP in that calcite crystals do develop, at nanoscale, in single treatments, yet significant strengths can still be obtained! The different mechanisms listed in Tables 1 and 2 thus become more critical than calcite formation in single treatment BioCaN scenarios.

After 9 days of allowing evaporation in a controlled setting at room temperature (20°C) the control sample returned to a strength of 0 psi while the treated samples with MICP treatments increased in strength to pocket penetrometer resistances of 1.4 tsf (134 kPa) and 2.1 tsf (200 kPa)! 200 kPa strength in treated clean Ottawa sand after a cycle of wetting and drying. Thus, the BioCaN crusts developed and maintained in a moist condition, weakened under saturation, and then increased in strength again once dried. This behavior is quite unusual compared to results in the literature. However, in the literature, many treatments are made, and an extended inundation phase is typically not presented in the literature. These observations and measurement agree with data obtained in later rainfall erosion experiments. The research team performed replication experiments to confirm these behaviors of the pilot study.

The MICP treatments also appeared to reduced moisture loss through evaporation with the MICP treated samples with higher and lower bacterial concentrations maintaining a water content by a factor of 3 and 2.75 greater than that of the untreated sand control. This pilot study supports literature that states a light mineralized carbonate crust in soils will help maintain moisture which will help with plant growth. It is still unknown the extent to which the increased moisture content is due to the carbonate crust, hygroscopic nature of salts or something else. Further studies should be completed in which a study similar to this is repeated with a large sample size and more treatments some of which include no addition of salt. This pilot study also supports the hypothesis that higher concentrations of *S. pasteurii* treatments does not always mean better soil improvement. After this study, other pilot studies and the bacterial growth/concentration optimizations some adjustments were developed to provide for better testing of the biogeotechnology for soil stabilization treatment testing to use in the laboratory experiments and field trials. These developed procedures are given previously herein.

Through other testing and analysis, it was determined for this study that the name BioCaN was a better representation of the treatments than MICP since other stabilization drivers are occurring than simply the precipitation of calcite (See Tables 1 and 2). This is because the name represents the solution treatment constituents being put into the soil instead of only one of the processes at work of which the others are still being understood. One of the developed aspects that the researcher believes had the most impact on greatly increased strength development observed in the erosion studies as compared to that seen in this pilot study is the curing of the samples under broad spectrum lighting for 16 hours a day. This was theorized to work due to the intense dependence of many living creatures on light, which includes soil microbes that are used to being exposed to light every day. Testing in conditions more similar to real-world conditions was also thought to be more realistic for the proposed application of BioCaN treatments to improve soil erosion resistance and allow vegetation growth of which the treatments would be exposed to light for approximately 16 hours a day during the growth season and thus it is considered best to cure all of the future experiments with BioCaN treatments under broad spectrum lighting simulating real-world conditions.

Wind Erosion

The results for all of the wind erosion experiments and procedures that are experiment-specific are given in Chapter 5 of Hodges (2019)[11]. This section gives the overall wind testing procedures. A wind tunnel was used for wind erosion testing of specimens, seen in Figure 12. This push-type wind tunnel has variable wind velocity control and has fully turbulent air flow that was developed, calibrated and validated for laboratory or field soil erosion testing. The wind tunnel was calibrated with an anemometer prior to any wind testing to determine the required settings needed to obtain wind speeds of 10 mph (16 km/hr.), 20 mph (32 km/hr.) and 30 mph (48 km/hr.). The wind tunnel was checked on the testing day with the anemometer every time a new experiment was to be conducted to ensure calibration accuracy was still in place. Figure 12 shows the wind tunnel used for wind erosion experiments.

Figure 12. Wind tunnel for soil erosion experiments at SDSMT in the laboratory.

After BioCaN treatments were applied to the soil specimen cups, the soil surface strength was measured every 24 hours for four days using pocket penetrometer prior to wind testing. The control and treated soil specimen were allowed to cure at room temperature, 21°C for four days under broad spectrum fluorescent lighting for 16 hours a day to simulate natural conditions. The disturbance of the crust caused by the penetrometer measurements created a rough surface, which is preferable for wind erosion testing over a perfectly smooth surface as it allows saltators to break free and impact more realistically. After four days of curing, the soil specimens were placed in groups of three identical cups placed in a row perpendicular to wind current to reduce deposition from one cup into another. The initial weight of each cup was recorded prior to wind testing. Each group (3 identical cups) was subjected to turbulent wind flow for 2 minutes at 10 mph and then weighed to determine mass loss. This was repeated at 20 mph and 30 mph to end up with the mass loss after being subjected to each of the three wind speeds for 2 minutes. The final weight gave the overall mass loss of each soil specimen during the 6 total minutes of wind erosion testing.

Two of the primary and most effective causes of physical soil erosion are from Aeolian and hydraulic forces. Aeolian transport of soil particles comes from a results of three primary mechanisms: suspension of finer particles, saltation in which particles move along the ground into other soil particles releasing them and causing more erosion and lastly surface creep in which large particles move along the ground and are often broken into smaller particles becoming susceptible to suspension and saltation erosion. It is was not measured as to which of the three Aeolian forces were most at play in the wind erosion testing completed for this study due to the difficulties that type of testing would add that would have made testing in a factorial design type of experiment impractical. All three types of erosion that occurred would be accounted for in the results from the erosion testing due to mass loss. In a 3x6 factorial design experiment with triplicate samples the experiments resulted in the testing of 18 varieties of specimen with a total of

54 specimen tested per experimental run. Three runs were performed. One without seeding completed 4 days after treatment and one with seeding, a second BioCaN treatment and daily water for 2 Months Prior to Wind Erosion Testing. The first sample set was designed only to test for crust development by penetrometer and wind erosion resistance of non-vegetated, bare soils and therefore no seeds were planted with the BioCaN treatments. The second set used in the BioCaN round 2 vegetation experiments which was seeded immediately prior to BioCaN treatments and watered daily for two months prior to wind erosion testing. The sample specimens were also subjected to "tilling" to break apart the intense compaction that geotechnical structures are often subjected to as well as treated a second time with BioCaN treatments.

Measurements were taken by penetrometer after treatment but prior to wind erosion testing on the unseeded BioCaN treated sample cups. A surface strength reading measuring unconfined compressive strength on the top crust of the soil as measured by a penetrometer was taken prior to BioCaN soil treatments for each sample as an initial soil sample strength. The samples were then treated and allowed to cure six inches under broad spectrum fluorescent lighting for 16 hours a day for four days prior to wind erosion testing. The room temperature was maintained between 20°C and 21°C. The temperature at six inches under the curing lights at the elevation of the soil sample surface was typically between 22°C and 27°C depending on the time of day. Each sample had four penetrometer readings taken after treatment and before wind erosion testing. The treated samples exhibited a brittle crust which broke apart, the worst being in the sand samples, during penetrometer reading. Once the crust fractured the penetrometer readings typically read lower. The broken crust allowed for a more realistic condition by allowing saltators to break free during the wind erosion testing. Natural conditions would rarely exhibit a smooth crust such as the one prior to penetrometer testing.

Penetrometer data was averaged for the three identical samples and then normalized to the control cup by soil type. The data was normalized by reducing strength changes experienced by treated samples by the amount of strength changes observed in the control sample. This was done to eliminate the changes that likely occurred to the specimen as a result of the distilled water included with the sample as well as environmental conditions because it was assumed that changes that were experienced by the control sample were experienced by all samples of the same soil type and not due to the treatment solutions. Standard deviation was calculated for the samples by soil type for strength changes of all samples of that soil type for this experiment. The average of the maximum strength increases of three identical soil cups that the samples experienced can be found in Figure 13.

Clean sand soil samples showed a much larger increase in soil surface strength then samples of burned and unburned soils. Of importance is burned and unburned soils had a pre-treatment strength whereas the clean sand did not. The untreated clean sand measured no strength for all of the measurements. All six of the burned soil samples had a pre-treatment strength of 0.25 tsf and five of the unburned soil samples had a pre-treatment strength of 0.67 tsf with combination NU4 (unburned 1x no $CaCl_2$) having an average strength of 0.58 tsf. Both the control sample data for burned and unburned had slight surface strength changes through the three days of penetrometer testing and therefore the other data was normalized with these changes. The normalized surface strength readings that were taken in the three days following BioCaN soil treatments are graphed in Figure 13. Standard deviations of the population based on n=3 for the triplicate sample cups were calculated but are not include on the following results figures due to the confusion too many lines and points would add to the graphs. The trends are much simpler to analyze without the standard deviations included.

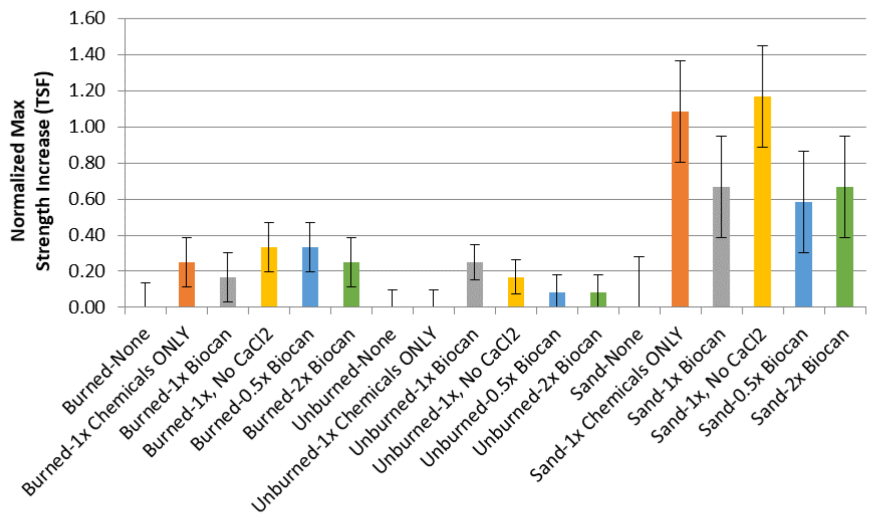


Figure 13. The normalized maximum strength increase exhibited by each sample within the first 3 days after treatment. The error bars show the standard deviation as calculated by soil type on all strength change measurements.

The maximum strength readings by soil type and treatment type are shown next to the initial strength readings (black bar on left) with the number of hours until the maximum strength (top number) in Figure 14. The measurements are an average of triplicate sample readings. The initial strength readings (black bar) are not present on the sand samples because untreated sand exhibits no surface strength. These samples were not normalized to the initial strength reading since it is also displayed on the chart.

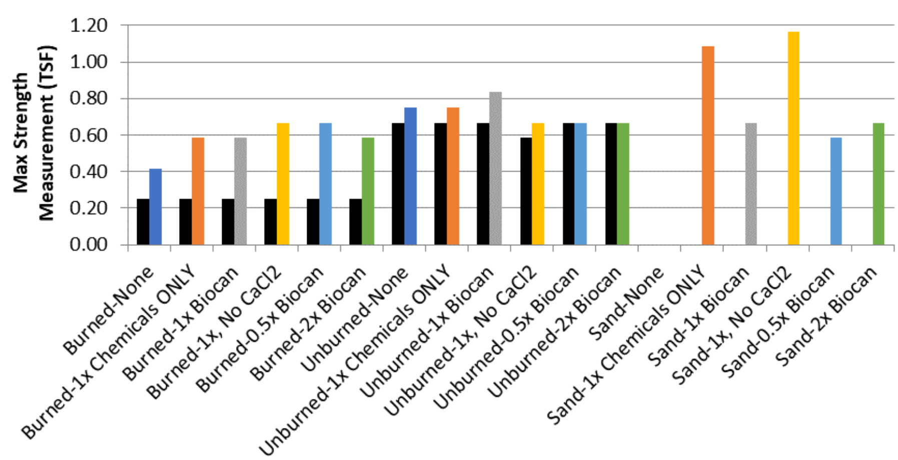


Figure 14. The non-normalized maximum surface strength measurement for each soil/treatment type. The black bar on the left is the initial strength.

Figures 13 and 14 shows that the sand samples treated with BioCaN solutions far away showed the greatest strength gain with the burned soils showing the second greatest strength gain and the unburned samples across all treatment types showing very little strength gain from the initial. In fact, the control sample showed more strength gain than some of the treated unburned samples with the 0.5x and 2x BioCaN treatments showing no strength gain at any point during the surface strength testing. However, as the next section presents, though strength gain was less in the unburned as compared to the burned samples the erosion resistance provided from BioCaN treated samples was greater in the unburned than the burned samples. This indicates that even though unburned samples treated with BioCaN did not experience an increase in surface strength they did still experience increased erosion resistance which means that surface strength testing, while an indicator crust development is not necessarily a good indicator of provided erosion resistance but can give some indication on surface tensile strength gain.

Figure 15a and b show that the most effective treatment in unburned soil was the 2x BioCaN treatment in terms of preventing mass loss. Though it was only marginally more effective. The two least effective treatments in burned soils, were treatment 2 and treatment 4, which were among the more effective treatments in unburned soils. The most effective treatment in unburned soils was the 2x, or treatment 6. The erosion resistance for burned soils from treatments as compared to the control was a three times reduction in mass loss at 20 mph. The treatments failed to reduce erosion in burned soils at 30 mph. One large factor in the wind erosion resistance is the breakup of the BioCaN crust into large pieces in the destructive testing of the penetrometer. However, in a real-world situation it would not be feasible to expect that a BioCaN formed crust would remain intact without disturbances (i.e. footsteps of humans or animals, impact of hailstones, ATV or motorcycles, etc.) and therefore this would likely be a representation of performance in the field.

Figure 15b shows that very little erosion at 20 mph for all tested materials including control. However, at wind speeds of 30 mph all treatment types proved to be effective at reducing wind erosion as compared to the control, which experienced nearly double the mass loss as any of the treatments. At 30 mph, the 0.5x BioCaN providing the least erosion resistance. All treatments proved to be effective at reducing mass loss from wind erosion up to 30 mph in clean sand as can be seen in Figure 15 c and d. Figure 15 d gives an up close look at the treatments without the control for comparison, however, it must be noted that even at 30 mph even the least effective treatment on sand, 0.5x BioCaN, still proved to prevent less than 4% mass lass as compared to the nearly 75% mass loss experienced in the control cup.

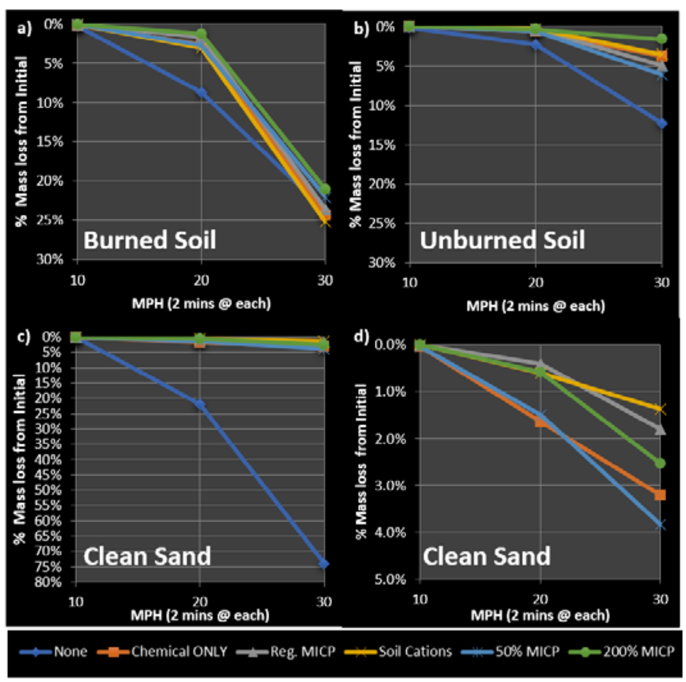
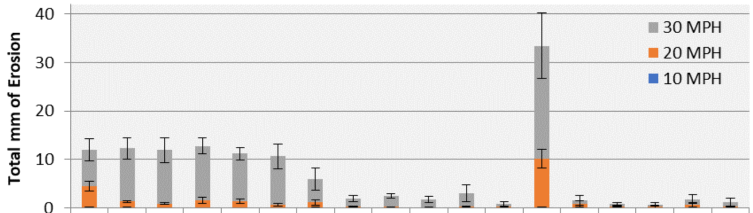


Figure 15. Cumulative mass loss during each speed of the wind testing.

The depth of erosion in sample cups is important to examine, i.e. volume loss rather than mass loss. This is especially true when working on roadside ditches along highways. When the volume is reduced in the soil it can create stability issues along roadways which will be seen later in this document in the field site 3 along I-90. Figure 16 shows the incremental height lost from the surface of the soil (extrapolated from the mass loss data) by soil type, treatment type and wind speed. The error bars represent the standard deviation by soil/treatment interaction type. At 10 mph there was either no height lost or only 0.1 mm at the most which is so small it is negligible. By far the most height lost was from the untreated sand control specimen which lost a much larger 3.3 cm from surface of the soil after only be subject to 2 mins at each of the three wind speeds. Surprisingly, the burned soil control actually lost the least amount of height at 30 mph. This could indicate that the crust formed by the treatments actually allowed wind vectors to get into the top of the crust and since very light it could erode away in large cemented clods at a time. The inlets may have been produced by the destructive penetrometer testing.



	Burn (1) No BioCaN	Burn (2) CaN (Biostimulation)	Burn (3) 1x BioCaN	Burn (4) 1x BioN (No CaCl2)	Burn (5) 0.5x BioCaN	Burn (6) 2x BioCaN	Unb (1) No BioCaN	Unb (2) CaN (Biostimulation)	Unb (3) 1x BioCaN	Unb (4) 1x BioN (No CaCl2)	Unb (5) 0.5x BioCaN	Unb (6) 2x BioCaN	Sand (1) No BioCaN	Sand (2) CaN (Biostimulation)	Sand (3) 1x BioCaN	Sand (4) 1x BioN (No CaCl2)	Sand (5) 0.5x BioCaN	Sand (6) 2x BioCaN
30 MPH	7.5	11.0	11.1	11.2	9.8	10.0	4.9	1.6	2.3	1.6	2.7	0.6	23.3	0.7	0.6	0.4	1.1	0.9
20 MPH	4.3	1.2	0.8	1.5	1.4	0.6	1.1	0.3	0.2	0.2	0.3	0.2	10.1	0.7	0.2	0.3	0.7	0.3
10 MPH	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Average Incremental Height of Soil Lost During Testing (mm) - 2 Mins at each Wind Speed

Figure 16. Incremental height lost at each wind speed during erosion testing.

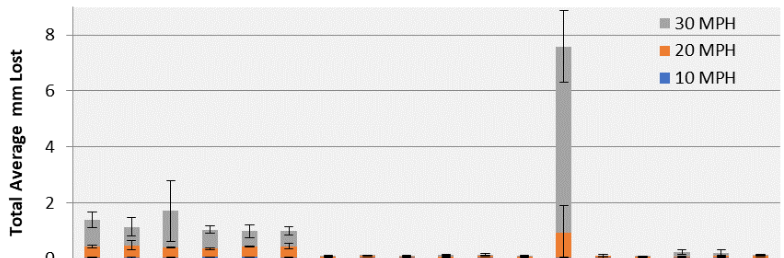
During 20 mph wind erosion testing the BioCaN treatments did reduced mass loss from wind erosion by a minimum of 65% from that of the control in which case the treatment with no $CaCl_2$ proved to be the least effective at 20 mph for burned soils. The treated unburned soils experienced very little height loss at all wind speeds. At 30 mph the unburned soils did start to experience wind erosion in which the control experienced nearly two times the loss of soil height as the least effective treatment which was the 0.5x BioCaN. The most effective treatment at wind erosion resistance for unburned soils was the 2x BioCaN in which the control specimen had over eight times the erosion from this type of treatment. Figure 17 shows the samples prior to wind erosion testing. Each type of soil and treatment type combo is pictured but does not show the other two identical samples because they all looked similar at the start of erosion testing. Note on the burned and the sand treatment type 1 (control) without any BioCaN solutions has noticeably less of a crust than the other 5 treatment types. The unburned control sample appears to have a less crumbly crust on the surface but for the most part looks similar.

Figure 17 shows the sand specimen prior to any wind erosion testing on the top image and after the 30-mph wind testing in the bottom image. The untreated sand proved to be completely susceptible to 30 mph wind speeds in which one cup even lost all of the sand and the cup blew to the end of the wind tunnel. Aside from a few clumps of biocemented sand missing (example: middle cup in far-left column) and erosion of the sand around the treatment area (example: back or top cup in 2nd column from the right) the treated sand cups looked nearly identical in the before and after photos.

Figure 17. Sand specimen subjected to wind testing, the top image shows the samples prior to wind testing, the bottom image shows the samples after the wind erosion testing at 30 mph. The treatment type is shown on the labels and is the 3rd digit or the 1st number. Note that the control is on the right of the picture.

Wind erosion testing of the vegetation cups after 3 months of watering, plant growth (though very little), drilling and a second treatment (still over 2 months prior to wind testing showed very different wind erosion results than the cups which were tested just 4 days after treatment. Significantly less mass loss was observed across all soil types and treatment types after two months than in the first set of wind erosion testing. Figure 18 shows the untreated sand lost less than 1 cm whereas in the previous experiment it lost 3.3 cm after all three rounds of testing. No other specimen cups lost more than 2 mm of height. None of the unburned samples lost enough soil to even register.

Figure 18 shows the cumulative percent mass loss as an average of three by soil type and treatment type. Burned soils and unburned soils showed no statistical differences from the control and therefore after the watering, disturbances and seeding the treatments were no longer effective after 2 months of having a BioCaN treatment applied. Surprisingly after all of the disturbances to the specimen cups the untreated sand cup still showed significantly more mass loss from wind erosion testing than any of the other treatments. After 30 mph wind erosion testing the untreated sand still lost 30% of the top layer whereas none of the BioCaN treated sands even lost 1% of the top layer by mass. Thus, BioCaN treatments continued to work months after application in clean sand in a controlled laboratory setting that simulated real-world conditions of rain and soil disturbances.



	Burn (1) No BioCaN	Burn (2) CaN (Biostimulation)	Burn (3) 1x BioCaN	Burn (4) 1x BioN (No CaCl2)	Burn (5) 0.5x BioCaN	Burn (6) 2x BioCaN	Unb (1) No BioCaN	Unb (2) CaN (Biostimulation)	Unb (3) 1x BioCaN	Unb (4) 1x BioN (No CaCl2)	Unb (5) 0.5x BioCaN	Unb (6) 2x BioCaN	Sand (1) No BioCaN	Sand (2) CaN (Biostimulation)	Sand (3) 1x BioCaN	Sand (4) 1x BioN (No CaCl2)	Sand (5) 0.5x BioCaN	Sand (6) 2x BioCaN
30 MPH	1.0	0.7	1.3	0.7	0.5	0.5	0.0	0.0	0.0	0.0	0.1	0.0	6.7	0.0	0.0	0.1	0.1	0.0
20 MPH	0.4	0.4	0.4	0.3	0.4	0.4	0.1	0.1	0.1	0.1	0.1	0.1	0.9	0.1	0.1	0.1	0.1	0.1
10 MPH	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0

Average Incremental Height of Soil Lost During Testing for 2 Mins at each Speed

Figure 18. Incremental height lost during wind erosion testing of samples used in vegetation BioCaN experiments.

Water Erosion

BioCaN treated sample specimen were subjected to wind erosion testing in the previous sections and the positive impact BioCaN treatments can have on reducing mass loss from soil erosion was proven. This section explores what an intense 45-minute rainfall can do to the same three soil types with the same six levels of treatment that were investigated in the 54-cup wind erosion experiment with no vegetation. The hydraulic erosion process used for this experiment was from rainfall erosion which results in four primary types of erosion mechanisms: splash erosion from the initial impact of the raindrop, sheet erosion as the water runs off of the soil taking soil particles with it, rill erosion as the sheet runoff develops into channels and finally gully erosion in which the eroded particles are carried through the channels formed from the rill erosion on a larger scale. The rainfall erosion testing completed for this research only accounted for splash erosion and sheet erosion whereas larger specimen samples would need to be tested to account for gully and rill erosion. In the field trials there was some observance of erosion resistance of BioCaN soil treatments to rill erosion and gully erosion. . In a 3x6 factorial design experiment with triplicate samples the experiments resulted in the testing of 18 varieties of specimen with a total of 54 specimen tested per experimental run. The water erosion testing resulted in 162 experimental runs for each sample set.

A rainfall simulator was constructed that stood 14 feet tall (4.3 m) and created a 9’ by 9’ (2.9 m by 2.9 m) square of relatively equal distribution of rain droplets. The nozzle used is specially designed to simulate natural raindrops that fall evenly over the 81 square foot pattern. The experiment was carried out inside under a controlled environment in which all cups were tested at the same time so as to rule out any factors that could have impacted the cups if carried out at different times. The sample cups which did not have drained holes were placed in aluminum containers with drainage and a 0.2-micron filter lining the entire tray to catch the runoff. The samples cups within the filter lined trays were placed on boards angled at a 33% gradient to simulate a roadside embankment. There were two sample cup holder boards with three rows and nine columns, so each board held 27 samples. The identical sample cups were placed in a single row together so a sample of each type would be at the highest, middle and lowest elevation and thus closer and/or further from the raindrop source. The rainfall simulator, sample cup holder and filter trays with soil cups can be seen in Figure 19.

The sample cups were prepared in triplicate in binary density layers. The densities of the sample cups by soil type are as follows in which the top layer for each cup is one inch deep and the bottom layer is ¾ inch deep (19 mm): sand top 110 pcf (17.28 kN/m^3), sand bottom 120 pcf (18.85 kN/m^3), burned top 60 pcf (9.43 kN/m^3), burned bottom 80 pcf (12.57 kN/m^3), unburned top 80 pcf (12.57 kN/m^3) and unburned bottom 100 pcf (15.71 kN/m^3). The same treatment levels were used for the rainfall erosion testing as were used for the 54-cup wind erosion test and are: 1-no treatment; 2-1x BioCaN chemicals with no bacteria augmentation; 3-1x BioCaN chemicals and *S. pasteurii* at concentration of 4.1x105 cells/mL; 4-1x urea-broth, S. pasteurii at concentration of 4.1x105 cells/mL with no calcium chloride; 5-0.5x BioCaN chemicals and S. pasteurii at concentration of 4.1x105 cells/mL; and 6-2x BioCaN chemicals and *S. pasteurii* at concentration of 4.1x105 cells/mL. The sample cups were treated and allowed to cure in a room at 20°C under 16 hours of broad-spectrum fluorescent lighting for 3 days. The cups with soil were weighted prior to testing and the trays, securing materials and filters were also weighed prior to testing. The rainfall simulator was run for 30 minutes at 1 inch/hour (25.4 mm/hr.) intensity. A 35-minute break was taken so as to allow the trays to drain due to the speed of filtration out of trays, if this break was not taken many of the trays may have overtopped and eroded sediments would have been lost. The boards holding the samples were rotated at this time in case there were slight differences in rainfall intensity/coverage so as to give more fair and balanced test results. The rainfall simulator was run for another 15 minutes however it was observed that not much more erosion probably took place at this time and most of the erosion occurred during the first 30 minutes of rainfall. Figure 20 show the sample cups after experimentation during the break when the filter trays were allowed time to drain.

Figure 19. The image shows the rainfall simulator apparatus at the beginning of the BioCaN rainfall erosion test on 3 different soil types with 6 treatment levels conducted in triplicate.

Figure 20. Sample cups after 30 minutes of rainfall were allowed to drain so that overtopping did not occur. During the experiment the samples were carefully monitored to make sure no overtopping was occurring.

After the rainfall experiment was completed the soil cups, trays and filters were allowed to sit for 72 hours at which time they were weighed, however, they still contained significant moisture at this time and so the samples were allowed to air dry completely in a protected environment, so as not to be tampered or disturbed, for 30 days. After 30 days the soil cups and tray, securing materials and filters were weighed separately. Figure 21 shows the mass loss results after the 30-minute rainfall testing. Significant mass loss was observed in all specimens, no matter the treatment under 1-inch per hour rainfall intensity. Single application BioCaN appears to be ineffective under intense rainfall prior to vegetation.

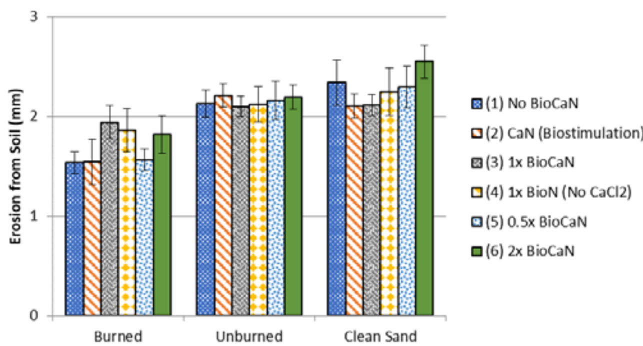


Figure 21. The height of erosion from the top of soil cup from rainfall erosion experiment. Data shown is an average of the triplicate cups.

Vegetation

Ecosystem health heavily depends on vegetation growth and preservation. When an area is severely disturbed, such as from a wildfire or construction activity, it is of utmost importance to rehabilitate the land in such a manner so that native vegetation can reestablish and live successfully. As was reviewed previously, plants must have certain nutrients, water supply and soil conditions to successfully grow. Soil conditions such as elemental constituents, nutrient availability, compaction, void space, void connectivity, suction and water content (PAW), mineralogy, pH and so on can all greatly impact the plants ability to germinate and grow. If compaction is great delicate young roots may not have the strength to penetrate through the soil and even if they can there may not be enough water or suction capabilities for the plant to get the water supply needed to grow strong. Soil treatments of any kind can impact these crucial soil characteristics sometimes in a positive way but also in a negative way. Some nitrogen supplement may be good for plant growth, too much will prove deadly. A light carbonate crust in the topsoil horizon can prove to be beneficial to plants by providing protection to seeds and PAW however, too dense of crust could hinder plant growth. Thus, it is very important when a technology is to be introduced into the natural environment that the impacts to vegetation are understood particularly when it is changing the soil biology, chemistry and physical properties such as occurs when BioCaN soil treatments are applied to soils.

When surface applied BioCaN treatments have been proven to reduce wind erosion potential, however, the slope will also need to be revegetated for long-term slope stabilization and aesthetics. An interaction occurs between the induced microbiological reactions and the native grasses and plants being seeded or planted on and near the slope.

This section demonstrates a laboratory study into the interactions between BioCaN treatments and seedling germination, sprouting, and growth. This study shows there as an environmental effect of BioCaN treatments on germination and growth of grass species. The study was completed as a 4 by 4 factorial design experiment and therefore 16 soil and treatment interactions were tested. Due to material and laboratory constraints at the time of this experiment, triplicate samples were not done. The samples were all prepared consistently, and good quality assurance and control followed. Future vegetation experiments were carried out in triplicate and a third vegetation experiment will be carried out.

Figure 22b) shows seedling rates for all treatment types as a function of soil. In terms of germination, the two types of burned soils produced similar rates until 17 days at which point the burned soil with the ash layer indicted jumped nearly 25% ahead of the burned soils without the 1" ash layer. The unburned soils produced the highest germination rates until 17 days at which point it leveled out and fell behind the burned soils with ash to a similar rate as the burned soils without ash layer intact. Clean sand, as was hypothesized, resulted in the worst rates over the 25-day period. For the clean sands, few seeds sprouted after 17 days, and those that had sprouted before 17 days struggled to survive. Around 30 days, there was a three day "drought" period in which the plants that were growing in the sand nearly all died. Once watering resumed, they never were able to recover. After 30 days the experiment continued to be qualitatively observed but not quantitatively measured. From Figure 22b) it is clear that clean sand is the worst soil for revegetation, as is consistent with the existing literature. Analysis of the BioCaN treatments and the reasoning for the lower performance in revegetation of the full BioCaN treatment must analyze the treatment types and the performance in the individual soils. Figure 22a-h shows the various interactions by treatment and soil types to analyze in the factorial designed method.

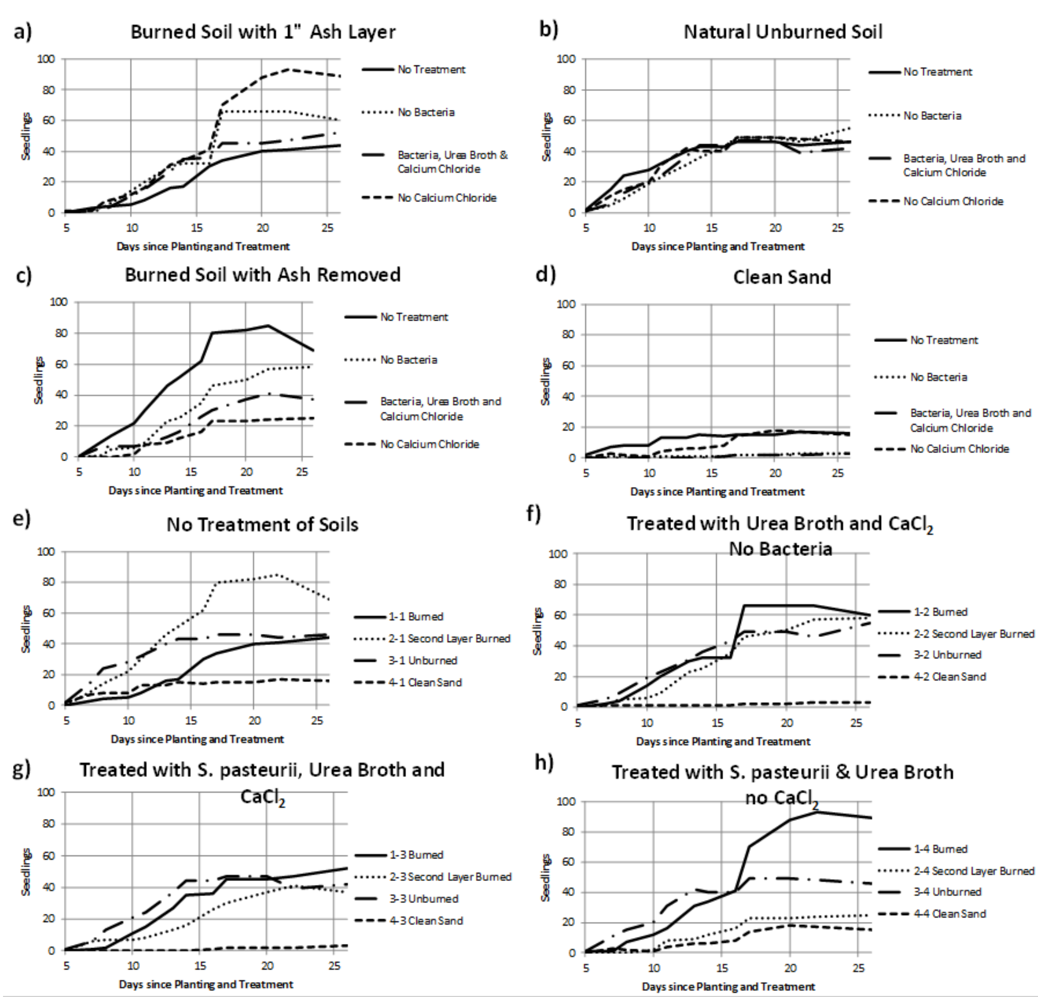


Figure 22. Detailed results of the seed germination experiment as a function of soil type and treatment type for all tested species.

FIELD RESEARCH

During summer and fall of 2018 three field trials were conducted in western South Dakota. The first site at the Rapid City Solid Waste Facility consisted of highly acidic soils (pH~3.0) in which vegetation is difficult to grow and soil erosion is noticeable by the deep gullies. The second site at Custer State Park was in an area burned by the Legion Lake Fire in December 2017 and again during a slash pile burn in early spring 2018 which resulted in severely burned soils. The third site sits along I-90 as it passes through Rapid City, SD by Rushmore Crossing. This site consisted of soils that were eroded by wind, water and passing traffic and the SDDOT has tried various methods for rehabilitation with little to no vegetation able to grow. All three of these soils are easily eroded by wind and water and very little vegetation persists in the area (with the exception of the Custer State Park site). Fifteen plots treated with control, existing technology and BioCaN treatment variations were tested for unconfined compressive strength, shear strength, pH, vegetation growth and observational erosion resistance. Results varied by site and were difficult to analyze due to the conditions and challenges of field experimentation, however, some erosion resistance was observed on plots with half, regular and double concentration BioCaN treatments. Strength measurements showed limited consistency amongst sites and plots. Vegetation was difficult to grow at sites 1 and 3 but grew well in all plots that were seeded on site 2, burned soils.

The three field sites were selected because they all represent a real-world situation in which a solution is needed to help in land rehabilitation or recovery. Previous studies looking at MICP have applied as a combination of a lab grown bacteria such as S. pasteurii, a urea-broth and a calcium supplement such as calcium chloride which are basically the same treatments (slightly different concentrations in some cases) as applied in this study. However, since the authors believe there are more complex chemical and biological constituents providing for soil stabilization than just the bacterial release of the urease enzyme initiating the formation of calcite the author prefers the term BioCaN soil treatments. Many fertilizers contain urea nitrogen as one of the primary ingredients and therefore the BioCaN solution would also likely work as a fertilizer to enhance plant growth assuming too much nitrogen is not added. In addition to the soil stabilization and the fertilizer the bacteria in the presence of urea and calcium release the urease enzyme which leads to the formation of calcium carbonate. Previous studies have shown a calcium carbonate formation on the soil horizon A to provide plant available water (PAW) which will further enhance the land and soil rehabilitation process. Therefore, the hypothesis is that the BioCaN technology, when applied to the surface of highly disturbed soils, would hold the soil particles in place, reducing wind and water erosion for long enough that the calcite and ionic chemical stabilization will form and then the combination of the nitrogen source and the increased PAW will help in the faster recovery of plants. However, previous research and studies completed and presented in this document have shown that calcium chloride is a hindrance to both microbial efficiency in producing calcium carbonate as well as a hindrance to plant growth particularly in sandy soils.

Three field test sites were chosen in the Black Hills of South Dakota near to Rapid City. These three sites present a range of conditions for installation, performance, soil conditions, and environmental conditions. Figure 23 shows the locations of the three sites along with a closeup picture of the erosion of soils on each site. The soils classification, testing results and characteristics of each site can be found in Chapter 4 of Hodges (2019)[11]. Site 1 exhibits erosion gullies that reached depths of over 4 feet. Site 2 exhibits rills flowing off the severely burned site where fine ash and broken-down soil particles have been transferred downhill by sheet flow. Site 3 exhibits soil that

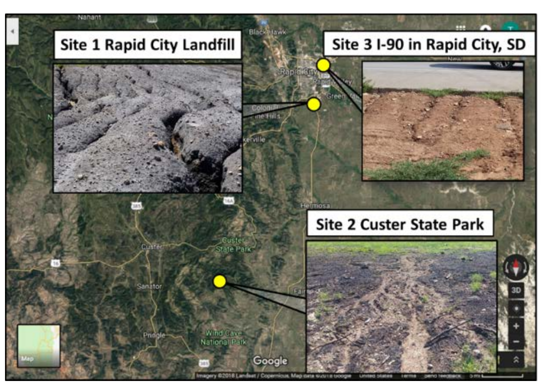


Figure 23. Locations of three field test sites in the Black Hills of South Dakota. Background imagery courtesy

of Google Maps™.

Figure 24 shows the layout of the field testing, with plots laid out in a row along the slopes. Each plot is 4 feet wide and 8 feet long. A silt fence was placed upslope and downslope of all plots to prevent sediments from depositing on the test plots from upslope runoff. Seeded BioCaN solution was applied in three different levels: 0.5x, 1x and 2x concentration, with 1x or regular concentration of treatment solution consisting of: 100 mL urea-broth solution with 0.3 g nutrient broth, 2 g urea , 1 g ammonium chloride, 2.8 g $CaCl_2$. The solution with no calcium chloride adds consisted of the same amount of nutrient broth, urea and ammonium chloride but in only 90 mL of distilled water. The bacteria were applied at a concentration of $4.1x10^5$ cells/mL at all treatment levels that include a bacterial component. Nutrient broth was included in the soil treatments that were not bioaugmented to encourage biostimulation. Application rates of liquid solutions consisted of 1 mL/in^2 which resulted in 2.2 liters of solution or distilled water per plot. This translates to $9.02x10^8$ cells of *S pasteurii* applied per plot.

The fifteen 32 SF rectangular test plots where laid out so that the longer dimension ran parallel to the slope side by side so as to reduce cross-contamination. An area of 3 feet by 5 feet was treated, allowing at least 1 ft between treatment areas to reduce the chance of contamination between plots. Plots with comparative technologies (newspaper pulp, fertilizer, seeded compost blankets, and conventional seeding) were placed along with plots in which BioCaN treatments were applied with and without calcium chloride, bacteria, or seeds. Also applied were seeded BioCaN treatments using commercial fertilizers as well as lower quality chemicals instead of laboratory grade chemicals. BioCaN was applied using surface spraying via backpack sprayers used in landscaping to apply liquid fertilizer and BioCaN treatments. Bacteria cells were cultured, washed and counted off site on the campus of SDSMT in the biogeotechnical engineering laboratory and transported in chilled conditions to the site. All solutions and their containers were autoclaved prior to use in backpack sprayers. Sprayers were sterilized with a bleach solution between each use. Water used in experiments distilled. Seeds were commonly used mixes by SDDOT and are the same species used in highway reseeding in the Black Hills, with excellent records of germination, growth and longevity as native grasses and flowers.

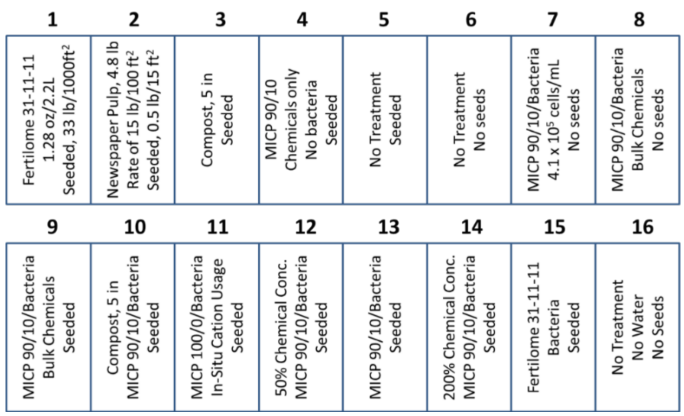


Figure 24. Layout of field test plots. Plots with bacteria listed means they were augmented with S. pasteurii at a concentration of 4.1 x 105 cells/mL with all treatment solutions applied at a rate of 1 mL/in2. Note that the plots were laid out in a single row.

Soil strength was measured every two to three days for three weeks using pocket penetrometer and field inspection shear vane. Randomized locations were chosen in each plot each day and the average of 3 measurements were taken as representative for the day for each plot. Erosion rates were measured from accumulated sediment on the downslope silt fence, while vegetation rates were observed using a 1 SF (0.093 m2) box for percent cover estimation. Samples were taken approximately 1 time per week from each plot and taken to the lab for pH and other testing. Erosion and vegetation were monitored for three months after treatment and recheck after 1 year.

Figure 25 shows strength data from the three field sites over the first two weeks after treatment. Plots 5 and 6 are the control plots and used to temper the data so that changes in soil moisture content (which directly influences soil strength) can be seen independent of any treatment. Ironically, it is the site with the most clay in the soil that saw the most improvement from treatment with BioCaN, counter to the conventional wisdom that biomineralization will be most difficult in clayey soil. The sandiest soil in test site 3 had the worst performance, and overall, loss of strength was seen at site 3. Site 3 had the most rainfall, including large storms in consecutive nights after treatment. Thus, it is likely that none of the treatment technologies at site 3 had a chance to “set up” and achieve a stable result before being damaged by rain. Overall, BioCaN fared better than other technologies in strengthening the soil. At the Landfill and at Custer, good strength gain was seen in all plots despite the buffer zones between plots with BioCaN treatments. The plots were grouped in a manner to reduce cross contamination between plots as much as feasible in a field study. Plots 1-6 consisted of treatments that did not have bacteria applied and plots 7-15 contained bacteria thus to reduce bacterial contamination to none bioaugmented plots. As far as BioCaN treatments on burned soils, the 2x or double concentration treatment was qualitatively shown to be most effective, although bio-stimulation proved successful, as did use of commercial fertilizer grade urea and CaCl2 rather than laboratory grade.

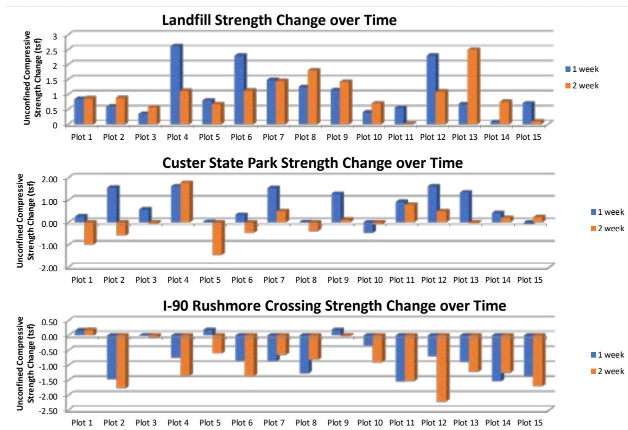


Figure 25. Strength change over time at the three test sites for each plot.

In addition to the quantitative strength gain data, more qualitative data in terms of vegetation grown, erosion amounts (not precise enough to be “quantitative”) and erosion patterns were observed. Erosion patterns are the most telling qualitative result of the testing. Figure 26 shows an example of how erosion rill patterns can be used to demonstrate effectiveness of an erosion mitigation technology. In Figure 26, the erosion rills coming downslope from “top of the page” deflect around the 1x BioCaN treatment and the 0.5x or half concentration treatment and moved in between the two treatment boundaries. However, the erosion rills stopped completely at the treatment boundary of the 2x BioCaN treatment and showed no evidence of erosion across or around the plot whatsoever. In terms of vegetation, compost blankets performed very well compared to other technologies except in Custer State Park at Site #2 on burned soils where compost performance was mixed. Most test sites had good erosion resistance, but not all had good vegetation outcomes.

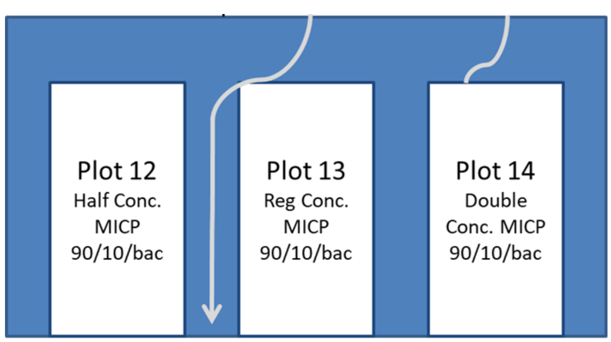


Figure 26. Runoff erosion rill pattern between and around three plots of BioCaN treatment areas. Erosion rills divert around plot 13 and between plot 12 and 13. Erosion rill completely stopped at Plot 14.

Upon a 1-year revisit to the site plots 3 and 10 (compost plots) showed wonderful rehabilitation with plentiful vegetation growth and no erosion. Site 2 at Custer State Park had plots 2, 4, 7, and 12 show the greatest 1-week strength increase with plot 4 showing the greatest 2-week strength increase. The site was trampled by buffalo and so the strength and vegetation data were significantly impacted. All plots that were seeded had at least some vegetation growth. There was observed erosion resistance on BioCaN treated plots. Site 3 along I-90 in Rapid City, SD gave mostly unreliable strength data due to the perpendicular flow conditions across the site. Lots of erosion of seeds, soil, and newspaper pulp was observed however, very little compost erosion was observed. Compost had the most vegetation growth, and nearly all plots had vegetation growth along the silt fence where the seeds had eroded to.

IMPLEMENTATION PLANS

In order to implement BioCaN, additional environmental impact studies need to be performed. BioCaN surface application rates used in the study were in the range of 5.25 to 21 lb. N/1000 ft^2 which compare to 2.5 to 5 lb. N/1000 ft^2 as would be used for typical fertilizer application rates. However, since bacteria and/or urease is added with the urea much of the urea would be processed and therefore more is needed than would be needed for plant nutrition alone. BioCaN as classified by the industry standard for fertilizers would be labeled as a 36-0-0 fertilizer (28% urea-N and 8% ammoniacal-N). When compared to existing calcium dust suppression technology, BioCaN application rates for this study use only 6-12 % of the calcium chloride and provide comparable or better dust suspension prevention. There are wide concerns about eutrophication in waterways from these levels of surface fertilizer applications and of salt burn from chlorides, and this needs to be further evaluated for permits to be received from USDA and other agencies for widespread applications.

Throughout this research various treated soils were monitored for pH changes after BioCaN treatments. The pH of a soil is known to have major impacts on plant growth and microbial activity and therefore the impacts to soil pH that incur from a soil treatment is important to identify. The pH is also a driver of many chemical reactions including microbial induced calcite precipitation (MICP) because as the bacteria releases urease and converts urea to carbamates the pH rises and the series of subsequent reaction rates to create calcite are increased. The pH of soils is difficult to obtain and for this study the slurry method was used to determine soil pH. Soil pH changes during treatments to round 1 vegetation cups were measured to determine if this could have affected the success of vegetation growth by treatment type based on the soil type. PH was measured in the sample cups by soil type and treatment type after treatment and vegetation growth occurred for 2 months. Note that distilled water that was used for BioCaN treatment solutions was measured to have and average pH of slightly less than 7.0, while tap water which was used to water seedlings had an average pH of 6.24. Testing and tracking pH changes are important so that the effects of the salts in the BioCaN treatment solutions can be separated from changes in pH. However, separate testing of highly saline versus highly alkaline conditions were not performed in this research and thus this is an area of research that still needs to be performed so that salinity and acidity can be formally separated for the impacts to revegetation.

In the time that the additional environmental related research recommended above is being performed, state Departments of Transportation can use Table 2 of this report to enable erosion potential mitigation technology selection. If a local area is desirous to implement BioCaN treatments, a small pilot study would be required to tailor the treatment to local conditions (such as soil pH and salts), along with a site-specific water quality test for local waterways. These small site-specific tests would take approximately 3-months for the research team of this report to perform. Other laboratories could implement these pilot studies, assuming that the necessary laboratory equipment is available for both bacteria culturing and water quality testing. Field testing results in this report showed marginal improvements compared to alternative technologies in the pilot scale testing performed herein. Before a strong recommendation for BioCaN over other technologies can be made, additional field testing and optimization is needed. Therefore, next steps in implementation need to occur.

Next steps on implementation are to do the follow-up testing described above using the mechanisms described here:

- State by State campaign – States that are prone to wildfire need to be made aware of the proposed solution so that <u>small scale trials can be made tailored to the unique soil conditions of each state</u> for use in developing a knowledge base for widespread implementation. The goal is to perform a suite of field tests, drawing on the experiences of this study, that test a wide range of field conditions. This state by state campaign will involve a virtual tour of each state DOT geotechnical and erosion control departments using contacts through TRB committees, contacts in existing pooled fund study efforts and academic collaborator network. One of the primary goals of the state by state campaign is to find a partner DOT for the NCHRP Implementation Program. This effort is not to develop a pooled fund study at this time. This effort is a long-term implementation plan as there are many states, and not all states have funding immediately available if they are interested in the technology.

- NCHRP Implementation Program – Key to the NCHRP Implementation Program is to identify and partner with a state DOT to make the application to the program. The P.I. cannot make that application, although the P.I. will be doing the work proposed by the state. This Implementation Program project will focus on pH issues and compatibility with plant pH tolerance.
- USDA Forest Service Collaboration – as discussed above, the environmental impacts need to be evaluated. For this the project team is seeking partnerships with the USDA-FS for projects examining eutrophication potential in waterways and watercourses from BioCaN spills or over-applications. These partnerships are in context of wildfire and post-wildfire remediation.
- West Dakota Water Development District Study – A key issue to water quality agencies is salt load in waterways. Overland water flow tends to accumulate and transport salts from the soil and bring them into waterways. This is well known to impact water quality. As a result, local water quality agencies such as the West Dakota Water Development District are interested in research that can reduce the salt applications for de-icing and for dust control. A salt-reduction focused study for BioCaN targeted at calcium chloride reductions is being discussed.

SUMMARY AND CONCLUSIONS

The effects of MICP treatment to clean sand has previously been thoroughly studied. Previous to this study the effectiveness of MICP/BioCaN treatments on non-sandy soils has not been thoroughly studied and therefore the differences in effectiveness of the biogeotechnology when applied to clean sand versus natural soils is not well understood. In this research the efficacy of the treatment is determined by the crust formation (determined by surface strength changes), the resistance to wind erosion, the strength gain after rainfall inundation and the vegetation germination and growth as all of these parameters are important when deciding if the soil treatment “worked” on a particular soil type. Conclusions to this report are as follows:

- All BioCaN and MICP treatments are effective at creating an erosion resistant crust in clean sand, but in clean sands all of the treatments have negative effects on vegetation, some extremely negative.
- The most effective treatments in the clean silica sand that balanced a crust and vegetation were treatment 2 (the regular chemical concentrations BioCaN treatment with no bacteria) and treatment 4 (the regular concentration urea-broth with no calcium chloride).
- Follow up research should investigate if a sand is treated with only urea-broth to determine if the strength gain is indeed coming from the supplemented bacteria or perhaps from the chemicals or existing microbes on the clean sand.
- A robust crust was observed in burned soils through BioCaN treatments. Though the crust strength gained in burned soils was not as great as that experienced in unburned soils it still nearly doubles the crust strength in even the least effective treatment as that seen in the control.
- The least effective BioCaN treatment in the burned soils from a surface strength gain perspective was the 1x regular concentration treatment.
- The two most effective treatments from a crust forming perspective in the burned soils was treatment 4 (1x urea-broth and bacteria cells) and treatment 5 (0.5x BioCaN treatments). Thus, the two treatments with the least calcium chloride were proven to be the most effective.
- The vegetation germination in burned soils treated with BioCaN varied based on if the ash layer was still intact or not. If the ash was removed, then no treatment provided the best growth with treatment 2 (no bacteria) being the second most effective. However, if the ash layer was still intact then all BioCaN treatments allowed for more vegetation germination than the control specimen. The most effective at allowing germination growth was the BioCaN treatment 4 with bacteria, urea-broth and no calcium chloride.
- Since the 0.5x chemical concentration also proved to be quite effective on burned soils this treatment should be tested for vegetation growth in burned soils as well as without the calcium chloride and only using 50% of the chemicals in the urea-broth.
- One of the most important outcomes to results from this research is the extreme differences that chemical additives have to soils based on what constituents make up the soils. This complex interaction between chemicals, biological components and soil particles dictate the functionality of land rehabilitation efforts and must be adequately accounted for when stabilizing soils and promoting plant growth.

- The BioCaN solutions applied to the soils created a quickly developed strength gain most abundant in clean sand while also providing a robust crust in burned soils. This crust provided soil erosion resistance nearly immediately. Over time the strengthening and erosion resistance continued through the ionic bond strengthening as well as the likely development of small carbonate-based crystallizations.
- In an environment where the ureolytic bacteria can continue to thrive, or at the very least live, additional urea could potentially be added to continue a biomineralization from urea to carbamate to carbonates to calcite which was indicated by the strength gain after rainfall inundation.

REFERENCES

1. Stocks-Fischer, S., Galinat, J. K., and Bang, S. S. (1999). "Microbiological precipitation of $CaCO_3$." *Soil Biology and Biochemistry*, 31(11), 1563-1571.
2. Bang, S. C., Bang, S. S., Choi, S. R., Lee, S. J., Lee, J. H., and Kim, J. S. (2015). "Method of manufacturing soil reinforced by microbe-based bio-binders and soil produced by the method." *Google Patents*, US Patent # 9074134 B2.
3. Bang, S. C., Min, S. H., and Bang, S. S. (2011). "KGS Awards Lectures: application of microbiologically induced soil stabilization technique for dust suppression." *International Journal of Geo-Engineering*, 3(2), 27-37.
4. Bang, S. S., Galinat, J. K., and Ramakrishnan, V. (2001). "Calcite precipitation induced by polyurethane-immobilized Bacillus pasteurii." *Enzyme and Microbial Technology*, 28(4), 404-409.
5. Bang, S. S., Lippert, J. J., Yerra, U., Mulukutla, S., and Ramakrishnan, V. (2010). "Microbial calcite, a bio-based smart nanomaterial in concrete remediation." *International Journal of Smart and Nano Materials*, 1(1), 28-39.
6. Anderson, J., Bang, S., Bang, S. S., Lee, S. J., Choi, S. R., and Dho, N. Y. "Reduction of Wind Erosion Potential Using Microbial Calcite and Soil Fibers." *Proc., Geo-Congress 2014: Geo-characterization and Modeling for Sustainability*, 1664-1673.
7. DeJong, J. T., Fritzges, M. B., and Nüsslein, K. (2006). "Microbially induced cementation to control sand response to undrained shear." *Journal of Geotechnical and Geoenvironmental Engineering*, 132(11), 1381-1392.
8. DeJong, J. T., Mortensen, B. M., Martinez, B. C., and Nelson, D. C. (2010). "Bio-mediated soil improvement." *Ecological Engineering*, 36(2), 197-210.
9. DeJong, J. T. M. A., Nelson, D. C., and Proto, C. J. (2016). "Biomediated Permeability Reduction of Saturated Sands." *Journal of Geotechnical and Geoenvironmental Engineering*.
10. Shanahan, C., and Montoya, B. "Strengthening coastal sand dunes using microbial-induced calcite precipitation." *Proc., Geo-Congress 2014: Geo-characterization and Modeling for Sustainability*, 1683-1692.
11. Hodges, T.M. (2019). *Microbial and chemical soil applications to stabilize burned and other erosion susceptible soils and rehabilitate vegetation.* Dissertation. South Dakota School of Mines and Technology.
12. Mataix-Solera, J., Guerrero, C., García-Orenes, F., Bárcenas, G. M., Torres, M. P., and Barcenas, M. (2009). "Forest fire effects on soil microbiology." *Fire effects on soils and restoration strategies*, 5, 133-175.
13. Conedera, M. (2005). "Erosion and surface drainage after forest fires." *Information for Forest Management. Retrieved August 19, 2016:* http://www.waldwissen.net/waldwirtschaft/schaden/brand/wsl_erosion_nach_waldbrand/index_EN.
14. Yochum, S. E., and Norman, J. (2015). "Wildfire-induced flooding and erosion-potential modeling: examples from Colorado, 2012 and 2013." *Proceedings of the 3rd joint federal interagency conference on sedimentation and hydrologic modeling*, 953-964.

15. Hubbert, K. R., Busse, M., Overby, S., Shestak, C., and Gerrard, R. (2015). "Pile Burning Effects on Soil Water Repellency, Infiltration, and Downslope Water Chemistry in the Lake Tahoe Basin, USA." *Fire Ecology*, 11(2), 100-118.
16. Almajed, A., Khodadadi Tirkolaei, H., and Kavazanjian Jr, E. (2018). "Baseline Investigation on Enzyme-Induced Calcium Carbonate Precipitation." *Journal of Geotechnical and Geoenvironmental Engineering*, 144(11), 04018081.
17. Meibom, A., Cuif, J. P., Hillion, F., Constantz, B. R., Juillet-Leclerc, A., Dauphin, Y., Watanabe, T., and Dunbar, R. B. (2004). "Distribution of magnesium in coral skeleton." *Geophysical Research Letters*, 31(23).
18. Chafetz, H. S. (1986). "Marine peloids; a product of bacterially induced precipitation of calcite." *Journal of Sedimentary Research*, 56(6), 812-817.
19. Dhami, N. K., Reddy, M. S., and Mukherjee, A. (2013). "Biomineralization of calcium carbonates and their engineered applications: a review." *Frontiers in microbiology*, 4, 314.
20. Anderson, J., Bang, S., Bang, S. S., Lee, S. J., Choi, S. R., and Dho, N. Y. (2014). "Reduction of Wind Erosion Potential Using Microbial Calcite and Soil Fibers." *Geotechnical special publication.*, 2(234), 1664-1673.
21. Cheng, L., Cord-Ruwisch, R., and Shahin, M. A. (2013). "Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation." *Canadian Geotechnical Journal*, 50(1), 81-90.
22. Chu, J., Stabnikov, V., and Ivanov, V. (2012). "Microbially induced calcium carbonate precipitation on surface or in the bulk of soil." *Geomicrobiology Journal*, 29(6), 544-549.
23. De Muynck, W., Debrouwer, D., De Belie, N., and Verstraete, W. (2008). "Bacterial carbonate precipitation improves the durability of cementitious materials." *Cement and Concrete Research*, 38(7), 1005-1014.
24. Lee, M. L., Ng, W. S., and Tanaka, Y. (2013). "Stress-deformation and compressibility responses of bio-mediated residual soils." *Ecological engineering*, 60, 142-149.
25. Lin, H., Suleiman, M. T., Brown, D. G., and Kavazanjian Jr, E. (2016). "Mechanical behavior of sands treated by microbially induced carbonate precipitation." *Journal of Geotechnical and Geoenvironmental Engineering*, 142(2), 04015066.
26. Whiffin, V. S., van Paassen, L. A., and Harkes, M. P. (2007). "Microbial carbonate precipitation as a soil improvement technique." *Geomicrobiology Journal*, 24(5), 417-423.
27. Meyer, F. D., Bang, S., Min, S., Stetler, L. D., Bang, S. S., Conference Conference Dallas, T. X., and Mar (2011). "Microbiologically-Induced Soil Stabilization: Application of Sporosarcina pasteurii for Fugitive Dust Control." Reston, Va.; American Society of Civil Engineers; c2011, p. 4002-4011.
28. Van Wijngaarden, W., Vermolen, F., Van Meurs, G., and Vuik, C. (2011). "Modelling biogrout: a new ground improvement method based on microbial-induced carbonate precipitation." *Transport in porous media*, 87(2), 397-420.
29. van Paassen, L. A., Ghose, R., van der Linden, T. J., van der Star, W. R., and van Loosdrecht, M. C. (2010). "Quantifying biomediated ground improvement by ureolysis: large-scale biogrout experiment." *Journal of geotechnical and geoenvironmental engineering*, 136(12), 1721-1728.
30. Warren, L. A., Maurice, P. A., Parmar, N., and Ferris, F. G. (2001). "Microbially mediated calcium carbonate precipitation: implications for interpreting calcite precipitation and for solid-phase capture of inorganic contaminants." *Geomicrobiology Journal*, 18(1), 93-115.
31. Burbank, M. B., Weaver, T. J., Green, T. L., Williams, B. C., and Crawford, R. L. (2011). "Precipitation of Calcite by Indigenous Microorganisms to Strengthen Liquefiable Soils." *Geomicrobiology Journal*, 28(4), 301-312.
32. Crawford, R. L., Burbank, M. B., Weaver, T. J., and Williams, B. C. (2013). "In situ precipitation of calcium carbonate (CaCO3) by indigenous microorganisms to improve mechanical properties of a geomaterial." Patent # US 8420362 B2.

33. Bachmeier, K. L., Williams, A. E., Warmington, J. R., and Bang, S. S. (2002). "Urease activity in microbiologically-induced calcite precipitation." *Journal of Biotechnology*, 93(2), 171-181.
34. Abo-El-Enein, S., Ali, A., Talkhan, F. N., and Abdel-Gawwad, H. (2012). "Utilization of microbial induced calcite precipitation for sand consolidation and mortar crack remediation." *HBRC Journal*, 8(3), 185-192.
35. De Muynck, W., De Belie, N., and Verstraete, W. (2010). "Microbial carbonate precipitation in construction materials: a review." *Ecological Engineering*, 36(2), 118-136.
36. Ramachandran, S. K., Ramakrishnan, V., and Bang, S. S. (2001). "Remediation of concrete using micro-organisms." *ACI Materials Journal-American Concrete Institute*, 98(1), 3-9.
37. Bang, S. S., and Ramakrishnan, V. "Microbiologically-enhanced crack remediation (MECR)." *Proc., proceedings of the international symposium on industrial application of microbial genomes*, Citeseer, 3-13.
38. Al-Salloum, Y., Hadi, S., Abbas, H., Almusallam, T., and Moslem, M. (2017). "Bio-induction and bioremediation of cementitious composites using microbial mineral precipitation–A review." *Construction and Building Materials*, 154, 857-876.
39. Ferris, F., Phoenix, V., Fujita, Y., and Smith, R. (2004). "Kinetics of calcite precipitation induced by ureolytic bacteria at 10 to 20 C in artificial groundwater." *Geochimica et Cosmochimica Acta*, 68(8), 1701-1710.
40. Glover, P., Zadjali, I., and Frew, K. (2006). "Permeability prediction from MICP and NMR data using an electrokinetic approach." *Geophysics*, 71(4), F49-F60.
41. Montoya, B., DeJong, J., and Boulanger, R. (2013). "Dynamic response of liquefiable sand improved by microbial-induced calcite precipitation." *Géotechnique*, 63(4), 302.
42. Dhami, N. K., Quirin, M. E. C., and Mukherjee, A. (2017). "Carbonate biomineralization and heavy metal remediation by calcifying fungi isolated from karstic caves." *Ecological engineering*, 103, 106-117.
43. Achal, V., Pan, X., Zhang, D., and Fu, Q. (2012). "Bioremediation of Pb-contaminated soil based on microbially induced calcite precipitation." *J Microbiol Biotechnol*, 22(2), 244-247.
44. Al Qabany, A., and Soga, K. (2013). "Effect of chemical treatment used in MICP on engineering properties of cemented soils." *Géotechnique*, 63(4), 331.
45. Al Qabany, A., Soga, K., and Santamarina, C. (2012). "Factors affecting efficiency of microbially induced calcite precipitation." *Journal of Geotechnical and Geoenvironmental Engineering*, 138(8), 992-1001.
46. Xu, J., and Yao, W. (2014). "Multiscale mechanical quantification of self-healing concrete incorporating non-ureolytic bacteria-based healing agent." *Cement and concrete research*, 64, 1-10.
47. Xu, J., Yao, W., and Jiang, Z. (2013). "Non-ureolytic bacterial carbonate precipitation as a surface treatment strategy on cementitious materials." *Journal of Materials in Civil Engineering*, 26(5), 983-991.
48. Gat, D., Tsesarsky, M., and Shamir, D. (2011). "Ureolytic calcium carbonate precipitation in the presence of non-ureolytic competing bacteria." *Geo-Frontiers 2011: Advances in Geotechnical Engineering*, 3966-3974.
49. Achal, V., Mukherjee, A., Basu, P. C., and Reddy, M. S. (2009). "Lactose mother liquor as an alternative nutrient source for microbial concrete production by Sporosarcina pasteurii." *Journal of Industrial Microbiology & Biotechnology*, 36(3), 433-438.
50. Choi, S.-G., Wu, S., and Chu, J. (2016). "Biocementation for sand using an eggshell as calcium source." *Journal of Geotechnical and Geoenvironmental Engineering*, 142(10), 06016010.
51. Javadi, N., Khodadadi, H., Hamdan, N., and Kavazanjian Jr, E. (2018). "EICP treatment of soil by using urease enzyme extracted from watermelon seeds." *IFCEE 2018*, 115-124.
52. Song, H.-G., Kim, O.-S., Yoo, J.-J., Jeon, S.-O., Hong, S.-H., Lee, D.-H., and Ahn, T.-S. (2004). "Monitoring of soil bacterial community and some inoculated bacteria after prescribed fire in microcosm." *The Journal of Microbiology*, 42(4), 285-291.

53. Beeson, P. C., Martens, S. N., and Breshears, D. D. (2001). "Simulating overland flow following wildfire: mapping vulnerability to landscape disturbance." *Hydrological Processes*, 15(15), 2917-2930.
54. Schlesinger, W. H., and Bernhardt, E. S. (2013). *Biogeochemistry: an analysis of global change*, Academic press.
55. Hubbert, K. R., Wohlgemuth, P. M., Beyers, J. L., Narog, M. G., and Gerrard, R. (2012). "Post-fire soil water repellency, hydrologic response, and sediment yield compared between grass-converted and chaparral watersheds." *Fire Ecology*, 8(2), 143-162.
56. Spittler, T. E. (1995). "Fire and the debris flow potential of winter storms." *Brushfires in California Wildlands: Ecology and Resource Management.'(Eds JE Keeley, T Scott) pp*, 113-120.
57. DeBano, L. F. (2000). "The role of fire and soil heating on water repellency in wildland environments: a review." *Journal of hydrology*, 231, 195-206.
58. Tester, J. R. (1989). "Effects of fire frequency on oak savanna in east-central Minnesota." *Bulletin of the Torrey Botanical Club*, 134-144.
59. Mitros, C., McIntyre, S., and Moscato-Goodpaster, B. (2002). "Annual burning affects soil pH and total nitrogen content in the CERA oak woodlands." *Tillers*, 3, 29-32.
60. Fowells, H., and Stephenson, R. (1934). "Effect of burning on forest soils." *Soil Science*, 38(3), 175-182.
61. Dean, S., Farrer, E. C., and Menges, E. S. (2015). "Fire effects on soil biogeochemistry in Florida scrubby flatwoods." *The American Midland Naturalist*, 174(1), 49-64.
62. Sherman, L. A., and Brye, K. R. (2009). "Sequential burning effects on the soil chemistry of a grassland restoration in the Mid-Atlantic coastal plain of the United States." *Ecological Restoration*, 27(4), 428-438.
63. Daubenmire, R. F. (1968). *Ecology of fire in grasslands*, Academic Press, London ;.
64. Pyke, D., McArthur, T., Harrison, K., and Pellant, M. (2003). "COORDINATED INTERMOUNTAIN RESTORATION PROJECT œ FIRE, DECOMPOSITION AND RESTORATION."
65. Duniway, M. C., Herrick, J. E., and Monger, H. C. (2010). "Spatial and temporal variability of plant-available water in calcium carbonate-cemented soils and consequences for arid ecosystem resilience." *Oecologia*, 163(1), 215-226.
66. Jangid, K., Williams, M. A., Franzluebbers, A. J., Schmidt, T. M., Coleman, D. C., and Whitman, W. B. (2011). "Land-use history has a stronger impact on soil microbial community composition than above ground vegetation and soil properties." *Soil Biology and Biochemistry*, 43(10), 2184-2193.
67. Le Bissonnais, Y. l. (1996). "Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology." *European Journal of soil science*, 47(4), 425-437.
68. Madsen, M. D., Kostka, S. J., Inouye, A. L., and Zvirzdin, D. L. (2012). "Postfire restoration of soil hydrology and wildland vegetation using surfactant seed coating technology." *Rangeland Ecology & Management*, 65(3), 253-259.
69. Miller, M. E., Bowker, M. A., Reynolds, R. L., and Goldstein, H. L. (2012). "Post-fire land treatments and wind erosion–lessons from the Milford Flat Fire, UT, USA." *Aeolian Research*, 7, 29-44.
70. Owen, D., Davis, A. P., and Aydilek, A. H. (2019). "Use of Compost for Permanent Vegetation Establishment and Erosion Control." Maryland. Dept. of Transportation.
71. Pascual, J., Garcia, C., Hernandez, T., Moreno, J., and Ros, M. (2000). "Soil microbial activity as a biomarker of degradation and remediation processes." *Soil Biology and Biochemistry*, 32(13), 1877-1883.
72. Passioura, J. (1991). "Soil structure and plant growth." *Soil Research*, 29(6), 717-728.
73. Robichaud, P., Jennewein, J., Sharratt, B., Lewis, S., and Brown, R. (2017). "Evaluating the effectiveness of agricultural mulches for reducing post-wildfire wind erosion." *Aeolian research*, 27, 13-21.
74. Wertz, S., Degrange, V., Prosser, J. I., Poly, F., Commeaux, C., Freitag, T., Guillaumaud, N., and Roux, X. L. (2006). "Maintenance of soil functioning following erosion of microbial diversity." *Environmental microbiology*, 8(12), 2162-2169.

75. Steinfeld, D. E., Riley, S. A., Wilkinson, K. M., Landis, T. D., and Riley, L. E. (2007). "Roadside revegetation: an integrated approach to establishing native plants."
76. Bolander, P., and Yamada, A. (1999). *Dust palliative selection and application guide*. United States Department of Agriculture Forest Service Technology Development Program Report.

APPENDIX

INVESTIGATOR'S PROFILES

Dr. Tasha Hodges was born in Wyoming in the 80s moving to South Dakota at a young age. Tasha graduate from Spearfish High School in 2001 as a recipient of the Regents' Scholar Diploma and a member of the National Honor Society. Tasha attended Black Hills State University and graduated in May 2005 with a Bachelor of Science degree in environmental physical science with triple minors in mathematics, physics and earth sciences. In December of 2012, Tasha graduated from South Dakota School of Mines and Technology with a Master of Science degree in civil and environmental engineering as well as a PhD earned in 2019. Tasha is an engineer-in-training in the state of South Dakota. Tasha has over a decade of professional experience with private and governmental agencies in management, land/construction surveying, environmental/engineering planning and design. She interned with Scull Construction, HDR Engineering and the Black Hills National Forest. Tasha's management experience started as the head lifeguard at a YMCA during college. Tasha was the LEED project specialist on the first LEED silver-certified school in South Dakota and survey team lead on engineering design projects in South Dakota and Louisiana. Tasha was a project manager/specialist for Project Solutions and worked as a hydrologist for the US Forest Service. During her doctoral graduate school career, Tasha received a research assistantship and served as president of the Graduate Student Society, vice president of Graduate Women in Science and has participated in various outreach programs for youth in STEM.

Dr. Bret Lingwall was born in Salt Lake City Utah and grew up in southern Utah hiking and mountain biking on the red sands and slickrock. He specializes in geotechnical, bio-geotechnical, and earthquake engineering. Dr. Lingwall has a BS, and MS, and a PhD from the University of Utah, all in Civil and Environmental Engineering. He studied under Dr. Steven Bartlett. His active research portfolio includes static and dynamic numerical modeling of geologic materials, dams and levees, liquefied soils, seismic hazards analysis, surface fault rupture mitigation, very soft soils, retaining walls, geosynthetics, paving materials, laterally loaded foundations, and ground improvement. Recently, Dr. Lingwall has begun working in biogeotechnical, soil ecology and soil biological soil crusting, and biomimetic research areas. Dr. Lingwall also researches EPS Geofoam, and its use to prevent damage to buried structures from faulting, landslides, and blasting. Dr. Lingwall has expertise with accelerated construction management, quality control and quality assurance from his days as a practicing engineer. Dr. Lingwall has 14 years of global design and construction experience on billion-dollar infrastructure, transportation, energy, and DoD projects, and has served as a technical advisor for a variety of projects in Canada, South Korea, Guam, and across the US. As an engineering consultant, Dr. Lingwall managed site investigations and assessments for erosion, slope stability, and environmental/water quality. He continues to serve the profession as a soils and earthquake engineering consultant and peer reviewer.

Research Results

RAPID REHABILITATION OF HIGHWAY SLOPES USING SEEDED MICROBIAL BIO-CEMENT

Development of a microbial biomineralization technique for remediating erosion potential that also accelerates revegetation

NCHRP IDEA Program Committee

FEBRUARY 2020

Project Title:
Rapid Rehabilitation of Highway Slopes Using Seeded Microbial Bio-Cement

Project Number: 200

Start Date: May 1, 2017

Completion Date: August 30, 2019

Product Category: Highway Rehabilitation and Safety

Principal Investigator:
Bret Lingwall
Assistant Professor
South Dakota School of Mines and Tech
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WHAT WAS THE NEED?

Erosion losses of highway slopes is a persistent issue across the United States. These erosion losses are more persistent and pernicious in new construction and after wildfire, cases wherein soils have been denuded of vegetation and their microbiome or in which vegetation and a microbiome have not been established. These cases are particularly difficult in that across most states new construction and wildfire both occur in warm weather months, in which high-intensity thunderstorms are most likely to occur unexpectedly, which can wash away slope materials before conventional erosion protection and/or revegetation treatments can be applied.

In the case of new construction or wildfire, a new technology that can be rapidly deployed needed to be developed that can supplement or augment existing technologies, or act as a stand-alone technology. For this new technology, it is imperative that both erosion potential is rapidly remediated, but also that revegetation is not impaired. To meet these twin objectives, a seeded microbial biomineralization technique was proposed.

WHAT WAS OUR GOAL?

The goal was to transition the successful Microbially Induced Calcite Precipitation (MICP) laboratory-based technology to the field to develop a rapid erosion control method that also accelerated revegetation as compared to existing technologies.

Burned soils after a fire as viewed from the highway, erosion losses evident

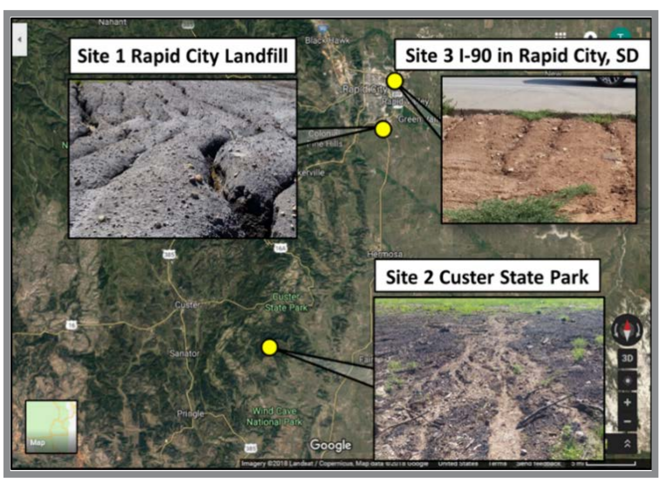
Research Results

WHAT DID WE DO?

The project was divided into two phases: A laboratory study and a field study. In the laboratory study, soil samples of burned soils, soils from construction projects, and native soils from the Black Hills area were collected and brought into the laboratory for a suite of MICP treatments tests under multi-variate controls and subsequent wind erosion testing and rainfall erosion testing plus seed germination studies. Wind erosion testing was performed with a wind tunnel. Rainfall erosion testing was performed with a rainfall simulator. Field testing was performed at three sites local to Rapid City, SD. Field testing included all of the multi-variate treatments that were tested in the laboratory. Erosion rates and vegetation rates were monitored in the field under natural conditions.

WHAT WAS THE OUTCOME?

Laboratory testing showed that the most robust crust is developed in clean sands, but that an erosion resistant crust still develops on a range of soil types including clayey, provided that the clayey soil is not highly compacted. Field testing showed that the technology takes at least 24 hours to set-up before it can resist intense thunderstorms but is viable in most loose soils and after wildfire.

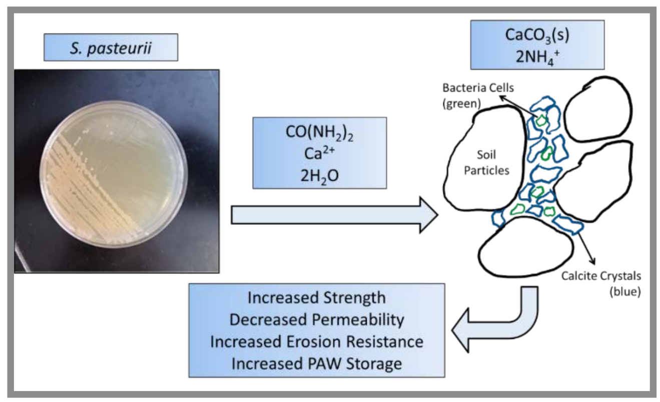


WHAT IS THE BENEFIT?

The hybrid seeded-MICP approach is able to rapidly develop an erosion resistant crust at a myriad of sites and soil conditions for after construction, maintenance, or wildfire while at the same time accelerating revegetation provided the local DOT native grass and plant seed mixture contains a variety of pH and salt tolerant species. The technology reduces wind and rain erosion rates significantly for wind speeds under 40 miles per hour and rainfall intensities of 1-inch/hour or less.

LEARN MORE

To view the complete report: email *bret.lingwall@sdsmt.edu*



Conceptual overview of the MICP process and locations of

Research Results