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Microbiologically-Induced Soil Stabilization: Application of *Sporosarcina pasteurii* for Fugitive Dust Control

F.D. Meyer[1], S. Bang[2], S. Min[3], L.D. Stetler[4], and S.S. Bang[*]

[1]Department of Chemical and Biological Engineering, South Dakota School of Mines and Technology, 501 E. St. Joseph St., Rapid City, SD, 57701-3901; PH (605) 394-2421; FAX (605) 394-1232; e-mail: fletcher.meyer@mines.sdsmt.edu

[2] Department of Civil and Environmental Engineering, South Dakota School of Mines and Technology, 501 E. St. Joseph St., Rapid City, SD, 57701-3901; PH (605) 394-2440; FAX (605) 394-5171; e-mail: sangchul.bang@sdsmt.edu

[3]Institute of Construction Technology, Samsung C&T Corporation Co., Ltd., Seoul, Korea; PH 82-2-2145-6518; email: soohong.min@samsung.com

[4]Department of Geology and Geological Engineering, South Dakota School of Mines and Technology, 501 E. St. Joseph St., Rapid City, SD, 57701-3901; PH (605) 394-2464; e-mail: larry.stetler@sdsmt.edu

[*](Corresponding Author), Department of Chemical and Biological Engineering, South Dakota School of Mines and Technology, 501 E. St. Joseph St., Rapid City, SD, 57701-3901; PH (605) 394-2426; FAX (605) 394-1232; e-mail: sookie.bang@sdsmt.edu

ABSTRACT

In this study, we have introduced a biological dust control technique utilizing a naturally occurring soil microorganism, *Sporosarcina pasteurii*, which is capable of inducing calcium carbonate precipitation in the environment. To evaluate the dust suppressive potential of this microbial calcite, *S. pasteurii* was suspended in medium and applied to locally available sand. The treated soil samples were tested via a wind tunnel at intervals and mass losses were measured. In order to identify the optimum conditions of microbial dust suppression, we examined the effects of: a) the concentration of *S. pasteurii*, b) the temperature and humidity, and c) the soil preparation method (washed or unwashed). Both types of the soil samples treated with *S. pasteurii* formed a crust-like layer on the surface and showed a significant reduction in mass loss. Our study demonstrated the potential of this microbially-mediated process as an effective, environmentally friendly means of airborne fugitive dust control.

INTRODUCTION

Airborne dust and debris from building materials (concrete, sand, etc.) often not only damage construction equipment but also present a major health hazard. The traditional dust suppression methods including spraying water, salts, chemicals, and petroleum products onto sources of airborne dust particles are well studied (Cowherd

et al. 1988; Borlander and Yamada 1999). Calcium chloride ($CaCl_2$) is commonly applied as a 38% brine solution to unpaved roads, as the hygroscopic nature of this inorganic salt slows the evaporative loss, thereby increasing the dust suppression effort (Lohnes and Coree 2002). However, at such high concentrations, calcium chloride is extremely corrosive to metals and concrete. It also poses a potential environmental hazard, as it can leach into soil and aquifers (Rushing et al. 2006).

Calcium carbonate ($CaCO_3$) precipitation induced by *Sporosarcina pasteurii* is a potentially long-lasting, environmentally innocuous process that can be used to suppress dust from landfills, open pit mines, unpaved roads, and construction sites. *S. pasteurii* produces the urease enzyme (Benini et al. 1996; Braissant et al. 2003), which hydrolyzes urea to produce both ammonium and carbonate ions (Reaction 1 below). This reaction raises the pH of the surrounding environment (Stocks-Fischer et al. 1999; Warren et al. 2001), ultimately precipitating calcite from carbonate and calcium ions (Reaction 2 below). The reactions are summarized as follows.

$$H_2NCONH_2 \text{ (urea)} + 2H_2O \xrightarrow{urease} 2NH_4^+ + CO_3^{2-} \quad (1)$$

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

Microbial calcite induced by *S. pasteurii* has been well-documented as an agent used for mineral plugging (Gollapudi et al. 1995), concrete crack remediation (Bang et al. 2001), and improved cement mortar strength and longevity (Ghosh et al. 2005; DeMuyck et al. 2008). This microbially-induced cementation process also slows contaminant leaching in soils (Fujita et al. 2000; Mitchell and Ferris 2006) and has been shown to stabilize loose sand structures (DeJong et al. 2006). In particular, *S. pasteurii* has recently been explored as a potential dust palliative when applied to the surfaces of different soils including poorly-graded commercial sandblasting sand, silt, and clay soils (Bang et al. 2008; Bang et al. 2009).

In this study, we modified several factors to optimize the *S. pasteurii* proliferation on the surface of nearly well-graded sand (SW), thereby maximizing the calcite precipitation and the dust suppression. The cell concentration, volume of dust suppressant applied, temperature, and humidity were varied to determine the effectiveness of *S. pasteurii* as a dust suppressant.

MATERIALS AND METHODS

Soil Preparation and Analysis

The soil was obtained from a local construction contractor, Pete Lien and Sons, Inc. (Rapid City, SD). Percentages of soil components of various sizes were determined through sieve analysis. Particulate matter is nearly well graded, with a uniformity of coefficient of 4.80 – 5.68 and a coefficient of gradation of 0.80 – 0.95. This size distribution is very close to the soil designation of 'SW' (well-graded sand) based on the Unified Soil Classification System (ASTM D 2487). In this study, unwashed and washed soils were used, where the washed soil was prepared using the procedure described by Bowles (1992). Both washed and unwashed soils were dried at 85^{o}C for 48 hours prior to being loaded into soil cups.

Soil particles were examined using a Z16 APO Macroscope (Leica, Inc., Wetzlar, Germany). There were visual differences between unwashed and washed soils (Figure 1), showing a noticeable reduction in the amount of small particles among larger particles after washing. Sample cups (75 mm in height, 65 mm in diameter, and 3.32×10^3 mm^2 of total surface area) were filled with approximately 310 g of the soil to the rim and left at room temperature overnight before receiving bacterial applications the following day.

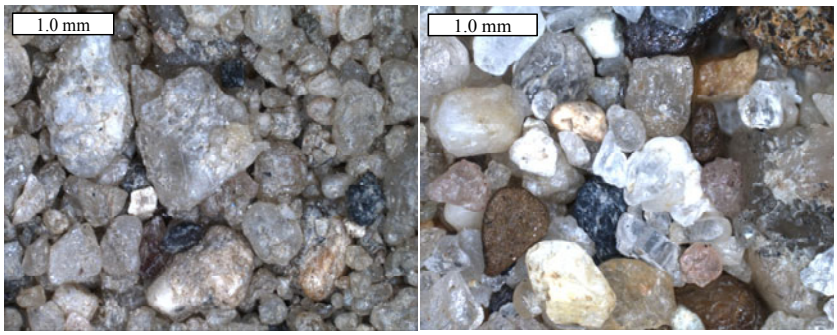

Figure 1. Unwashed (left) and washed (right) soil, bar, 1.0 mm.

Chemical components of the unwashed and washed soils were analyzed using an Energy-Dispersive X-ray Spectrometer (EDS) (Princeton Gamma-Tech, Princeton, NJ). Both soils comprised relatively high silica (SiO_2) content, with lesser but significant levels of iron oxide (Fe_2O_3), calcium oxide (CaO), and aluminum oxide (Al_2O_3) (Table 1).

Table 1. Energy Dispersive X-ray Spectroscopy (EDS) analysis of soil

Compound	Unwashed (%)	Washed (%)
SiO_2	71.31	72.64
Fe_2O_3	8.97	7.60
CaO	7.06	6.82
Al_2O_3	6.52	6.66
K_2O	2.83	3.52
P_2O_5	2.02	0.59
Na_2O	1.07	1.19
TiO_2	0.10	0.26
MgO	0.08	0.55
MnO	0.05	0.17
	100%	**100%**

Preparation and Treatment of Bacteria

This study utilized *Sporosarcina pasteurii* ATCC 11859 purchased from the American Type Culture Collection (Bethesda, MD). The culture of *S. pasteurii* grown in ATCC 1832 medium was harvested, washed, and quantified as previously described (Bang et al. 2001). For microbial calcite precipitation experiments, urea-

nutrient broth (NB) containing 3 g NB, 20 g urea, and 10 g NH_4Cl per liter (pH 6.0) was autoclaved, cooled, and combined with a desired volume or concentration of cells and filter-sterilized $CaCl_2$ immediately before application onto soil. The final concentration of $CaCl_2$ used in this study was 1.5% (100 mM), approximately 25 times less than the concentration (38%) currently allowed to use commercially (Lohnes and Coree 2002). In addition, the concentrations of NH_4Cl (1%) and urea (2%) were much lower than a urea content allowed (43%) for commercial use as a frost protectant pesticide with no evidence of adverse chronic effects (EPA, 2002). Samples were sprayed with a 50 mL handheld syringe fitted with a nozzle (Delovan, Inc., Minneapolis, MN) at approximately 80 mm above each sample. All applications were performed and samples maintained at room temperature with 20% humidity unless otherwise noted.

Analyses of Bio-Based Dust Suppression

Soil wind erosion tests were performed using a large-scale wind tunnel (Pietersma et al. 1996), with a height of 0.51 m, a width of 0.51 m, and a length of 8.5 m. Wind speed was measured using a handheld Turbo Meter wind speed detector (Davis Instruments, Inc., Hayward, CA) to determine the correct blower input voltage correlating with 40 km/hr. Incoming air was filtered by attaching fiberglass filters (Flanders, Inc., Washington, NC) to the panel in front of the blower fan. Three replicate samples were placed side-by-side at 3.1 m downstream of the blower fan in the center of the wind tunnel, with 20 mm spacing between each. To account for uneven erosion across the soil surface, 80 mm tall spires were constructed at 0.46 m in front of the soil cups to disperse the wind flow. Samples were subjected to 40 km/hr wind for 3 min. Immediately before the wind tunnel was activated, a Dust Trak 8520 dust detector (TSI, Inc., Shoreview, MN) was turned on to measure the density (mg/m^3) of emitted particulates less than 10 μm in size. The air sampling tube was placed at 5.3 m downstream of the soil cups and at 0.36 m above the base, and particulate density values (mg/m^3) were recorded at each second for 3 min. This data was converted into the specific aerosol production rate ($mg/m^3/s/m^2$) by accounting for the time (180 seconds) and the combined surface areas of the three soil cups (approximately 0.01 m^2). Samples were wind tunnel tested at intervals of 1, 2, 4, 7, and 14 days after the bacterial treatment. Following wind tunnel testing, final masses were recorded. Mass loss of the soil sample was calculated by taking the difference between the masses measured before and after wind tunnel testing. Treated soil percent mass loss was determined by dividing the treated soil mass loss by the untreated soil mass loss and then multiplying by 100.

RESULTS

Effects of Cell Concentration and Application Volume on Unwashed Soil

To determine the impact of bacterial concentration on unwashed soil mass loss, soil samples were treated with 5 mL of medium containing five different cell concentrations ranging from 1×10^5 to 1×10^9 cells/mL (with an increment of one

order of magnitude) and subjected to wind tunnel testing at five intervals, i.e., 1, 2, 4, 7, and 14 days after treatment. As compared to the mass loss of the untreated soil (4.0 – 6.0 g), the highest percent mass loss among treated samples approached 3.5% while the lowest mass loss did approximately 1.6% (Figure 2).

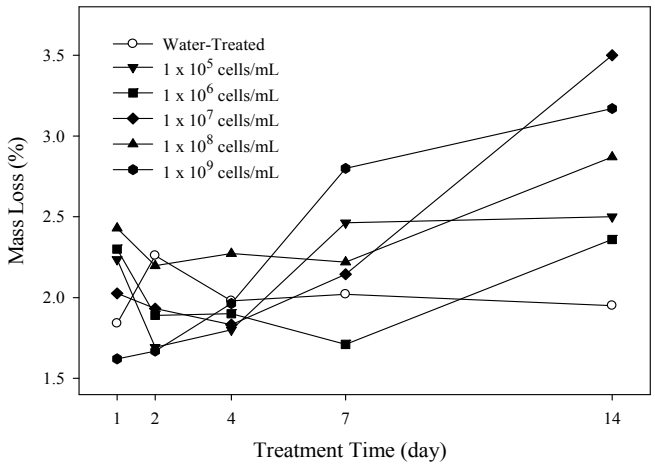

Figure 2. Percent mass losses among soil samples treated with water and five different concentrations of bacteria.

The soil samples treated with a bacterial concentration of 1×10^8 cells/mL or 1×10^9 cells/mL generally lost comparable or even higher amounts of mass when compared to the soil samples treated with a bacterial concentration of 1×10^7 cells/mL with the notable exception of day 14. It is interesting to note that water-treated soil mass losses were similar with bacteria-treated soil mass losses, which were not observed with other soil types such as the silt, clay, and commercial sandblasting sand (Bang et al. 2009). It was speculated that certain chemical constituents including iron oxide (Fe_2O_3) of this specific soil might form a hardened layer upon contact with water. This was the main reason why the washed soil was also investigated in this study. Results of the study on washed soil are included in a latter section.

As shown in Figure 2, the dust suppression effects of a bacterial concentration of 1×10^7 cells/mL were comparable to those of higher bacterial concentrations of 1×10^8 cells/mL and 1×10^9 cells/mL. Therefore, the 1×10^7 cells/mL concentration was selected to examine the effect of different medium application volumes which varied from 1 to 5 mL with an increment of 1 mL. These correspond to the surface coverage of 3.0×10^3 to 1.5×10^4 cells/mm^2. Percent mass loss was calculated in the same manner as described previously. Overall, application of 1 or 2 mL of medium solution resulted in greater soil mass loss than 3, 4, or 5 mL (Figure 3). Soil mass losses did not vary significantly between 3, 4, and 5 mL application volumes. This data indicates that 3 mL at a bacterial concentration of 1×10^7 cells/mL is an adequate volume for the treatment of unwashed soil.

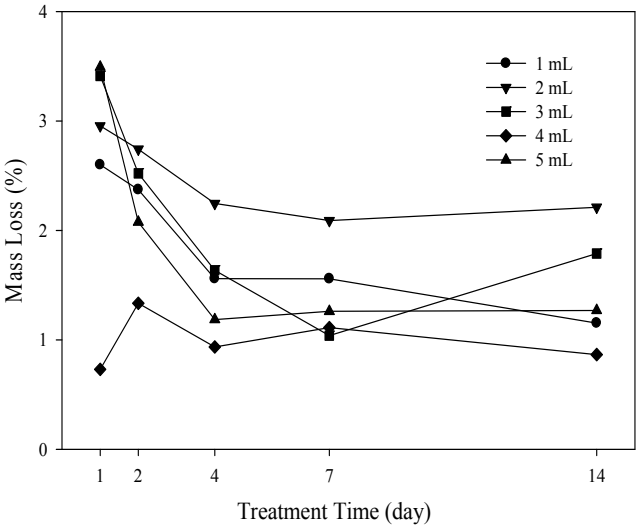

Figure 3. Percent mass losses among soil samples treated with five different application volumes containing bacteria at a concentration of 1×10^7 cells/mL.

Effects of Temperature and Humidity on Unwashed Soil

To study the effects of temperature variations on the treatment of unwashed soil, the samples were maintained at a constant humidity of 20%, but at three different temperatures of 20°C (room temperature), 35°C, and 45°C. All samples were treated with 3 mL of medium containing bacterial cells at 1×10^7 cells/mL, equivalent to a surface coverage of 9.0×10^3 cells/mm^2. At relatively low humidity (20%), as the temperature increased, the percent mass loss decreased in soil treated with bacterial cells (Figure 4). This data suggested that bacterial dust suppression might work more effectively at high temperatures.

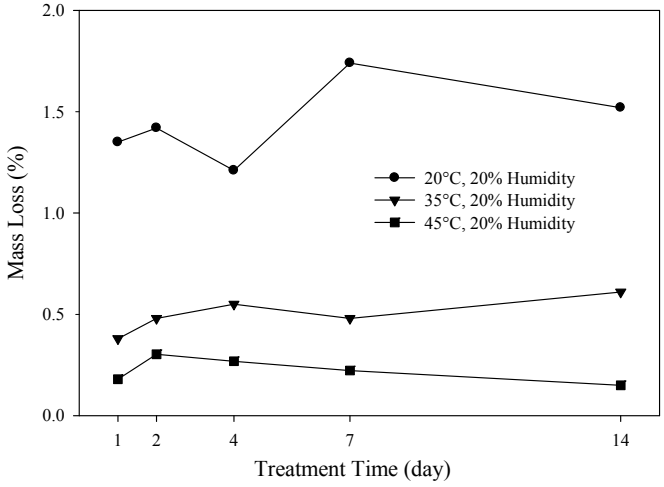

Figure 4. Percent mass losses of samples maintained at 20°C, 35°C, and 45°C (20% humidity).

For the study of humidity effects, the unwashed soil samples were tested at 20°C and at two different humidity levels, 20% and 100%. The soil samples were untreated or treated with 3 mL of medium containing bacterial cells of 1×10^7 cells/mL (9.0×10^3 cells/mm^2) (Figure 5). Bacterial dust suppression was examined by percent mass loss and Dust Trak analysis.

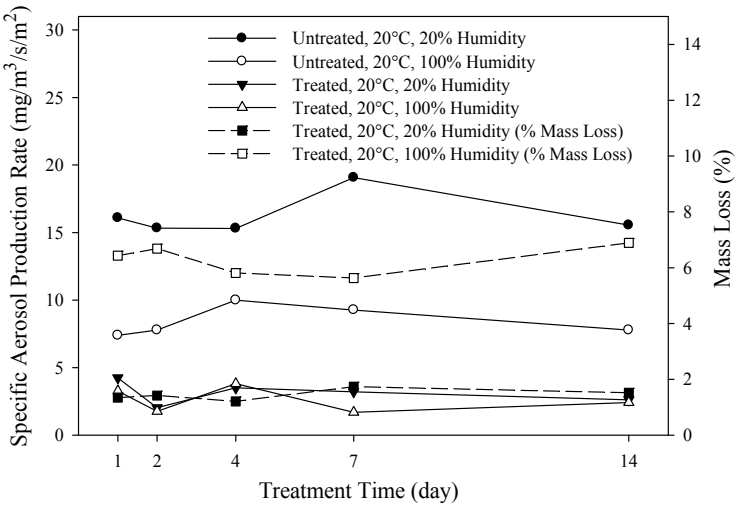

Figure 5. Comparison of specific aerosol production rates (solid line) for both treated and untreated samples and percent mass losses (dotted line) of treated samples kept at 20% and 100% humidity (20°C).

The percent mass loss of treated soil decreased when samples were maintained at a lower humidity. In Dust Trak analyses, untreated soil samples kept at 20% humidity generated approximately twofold higher specific aerosol production rates than untreated samples kept at 100% humidity (Figure 5). However, bacteria-treated samples maintained at both 20% and 100% humidity had nearly equal specific aerosol production rates, despite much greater soil mass losses among untreated samples kept at 100% humidity. Samples maintained at 100% humidity most likely lost most of their mass when larger-sized particles were blown out of the cup but were too large to become airborne, or were excluded by the Dust Trak detector that only captured particles with diameters less than 10 μm. In either case, applying bacteria as a dust control agent is more effective in low-humidity, high-temperature environments.

Effects of Cell Concentration and Application Volume on Washed Soil

Unwashed soil, when treated with water, formed significant surface crust and lost as little soil mass as unwashed, bacteria-treated soil did (Figure 2), which led to a speculation that a water-soluble component, Fe_2O_3, contributed to this phenomenon. Understanding that the content of Fe_2O_3 is variable in soils, it was hypothesized that the application of *S. pasteurii* would most likely be more effective than the water

treatment, mainly because microbiologically-induced calcite precipitation would continue for an extended period of time as *S. pasteurii* grew under appropriate environmental conditions. Therefore, we investigated how washing soil affects both water-treated and bacteria-treated soil mass losses and specific aerosol production rates. The average specific aerosol production rate value of washed, untreated soil was three times less than that of unwashed, untreated soil (4.6 and 14.0 $mg/m^3/s/m^2$, respectively), while washed, untreated soil lost an average of approximately 6.0 g, comparable to unwashed, untreated soil mass losses (data not shown). This is mainly because washing has removed the majority of fine particles from the soil that contributes to more dust production, but to less weight loss.

However, samples treated with either 3 mL water or 3 mL of 1×10^7 cells/mL solution (9.0×10^3 cells/mm^2) lost an average of approximately 4.5 g, which was almost 4 to 20 times higher than the mass losses of other washed soil samples treated with different application volumes and bacterial concentrations (Figure 6). Increasing the application volume to 5 mL while maintaining a cell concentration at 1×10^7 cells/mL (1.5×10^4 cells/mm^2) reduced soil mass losses significantly. Applying either 3 or 5 mL at 1×10^9 cells/mL concentration (9.0×10^5 cells/mm^2 and 1.5×10^6 cells/mm^2, respectively) resulted in even lower soil mass losses, generally less than 0.5 g.

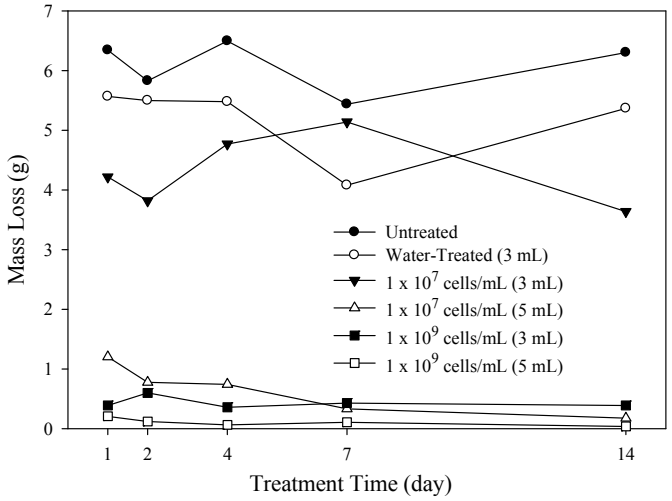

Figure 6. Comparison of soil mass losses (g) among untreated, water-treated, and bacteria-treated samples with different application volumes and bacterial concentrations.

CONCLUSION

Dust palliatives have been extensively studied for unpaved road-based applications (Sanders et al. 1997) and to a lesser extent for construction site materials (Nij et al. 2003). While currently used dust suppressants are either corrosive, short-lived, or environmentally toxic, the calcite-forming quality that *S. pasteurii* possesses potentially makes it suitable as a long-term, environmentally-friendly means to

suppress airborne dust particles. From our study, microbial dust control has shown the potential to be very effective. However, the soil type and environmental conditions (e.g. temperature and humidity) play a significant role in determining this efficacy. For unwashed soil with a large quantity of fine particles, optimal dust suppression occurred under low humidity, high temperature environments similar to arid deserts.

Soil washing has removed not only fine soil particles that may result in an increase of the average soil particle size, but also certain water-soluble chemicals that may harden upon wetting. For washed soil, increasing either the application volume or the bacterial concentration significantly reduced mass losses, suggesting that both factors might influence the level of *S. pasteurii*-mediated dust suppression on a unique type of well graded soil.

Direct application of these findings to other types of soils may not warrant similar results. Additional studies therefore need to be conducted for various types of soils so that optimum bacterial concentrations and associated application volumes specifically applicable to individual soils can be identified.

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