Application of biocementation technique using Bacillus sphaericus for stabilization of soil surface and dust storm control (Postprint)

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Abstract

Abstract: Dust emission and wind erosion are widespread phenomena in arid and semi-arid regions, which have far-reaching harmful effects to the environment. This study aimed to use microbial induced carbonate precipitation (MICP) method with Bacillus sphaericus to reduce soil losses that occur in a dust-producing area due to wind erosion in the Ilam Province, Iran. Soil samples at the 0–30 cm depth were used and sterilized in an autoclave for 2 h at 121°C and 103 kPa. Approximately 3 kg soils were weighed and poured in the 35 cm$\times 35 cm \times$3 cm trays. Different treatments included two levels of B. sphaericus (0.0 and 0.5 OD), three levels of suspension volume (123, 264, and 369 mL), two levels of urea-chloride cementation solution (0.0 and 0.5 M), and two levels of bacterial spray (once and twice spray). After 28 d, soil properties such as soil mass loss, penetration resistance, and aggregate stability were measured. The results showed a low soil mass loss (1 g) in F14 formulation (twice bacterial spray+264 mL suspension volume+without cementation solution) and a high soil mass loss (246 g) in F5 formulation (without bacteria+264 mL suspension volume+0.5 M cementation solution). The highest (42.55%) and the lowest (19.47%) aggregate stabilities were observed in F16 and F7 formulations, respectively, and the highest penetration resistance (3.328 kg/cm2) was observed in F18 formulation. According to the final results, we recommended the formulation with twice bacterial spray, 0.5 M cementation solution, and 269 mL suspension volume as the best combination for soil surface stabilization. Furthermore, this method is environmentally friendly because it has no adverse effects on soil, water, and plants, thus, it would be an efficient approach to stabilize soil surface.

Full Text

Preamble

Application of Biocementation Technique Using *Bacillus sphaericus* for Stabilization of Soil Surface and Dust Storm Control

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Abstract: Dust emission and wind erosion are widespread phenomena in arid and semi-arid regions that exert far-reaching harmful effects on the environment. This study aimed to use the microbial induced carbonate precipitation (MICP) method with *Bacillus sphaericus* to reduce soil losses occurring in a dust-producing area due to wind erosion in Ilam Province, Iran. Soil samples from the 0–30 cm depth were collected and sterilized in an autoclave for 2 h at 121°C and 103 kPa. Approximately 3 kg of soil was weighed and placed in 35 cm × 35 cm × 3 cm trays. Different treatments included two levels of *B. sphaericus* (0.0 and 0.5 OD), three levels of suspension volume (123, 264, and 369 mL), two levels of urea-chloride cementation solution (0.0 and 0.5 M), and two levels of bacterial spray (once and twice). After 28 days, soil properties such as soil mass loss, penetration resistance, and aggregate stability were measured. The results showed minimal soil mass loss (1 g) in the F14 formulation (twice bacterial spray + 264 mL suspension volume + without cementation solution) and maximal soil mass loss (246 g) in the F5 formulation (without bacteria + 264 mL suspension volume + 0.5 M cementation solution). The highest (42.55%) and lowest (19.47%) aggregate stabilities were observed in F16 and F7 formulations, respectively, while the highest penetration resistance (3.328 kg/cm^2) was observed in the F18 formulation. Based on the final results, we recommend the formulation with twice bacterial spray, 0.5 M cementation solution, and 269 mL suspension volume as the optimal combination for soil surface stabilization. Furthermore, this method is environmentally friendly because it has no adverse effects on soil, water, and plants, making it an efficient approach for stabilizing soil surfaces.

Keywords: soil stabilization; microbial cementation; calcium carbonate; bacteria; penetration resistance

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1 Introduction

Wind erosion and dust storms have become increasingly common in recent years [?, ?]. Wind erosion adversely affects visibility, air and ground transportation systems, and human health [?, ?, ?, ?]. The abundance of dust in the environment has also become a global concern due to its harmful effects on human health and functional ecosystems [?, ?].

Approaches to dust reduction and soil stabilization are not always efficient according to studies, thus requiring a combination of biological and mechanical treatments. Soil stabilization and wind erosion control are accomplished using various techniques, including biological (vegetation and microorganisms), mechanical, physical (windbreaks), and chemical (polymers and petroleum products) methods [?, ?, ?, ?]. These traditional methods have several drawbacks, including improper plant growth due to poor soil physical and chemical conditions [?, ?]. Additionally, the inability to reset and move windbreaks [?, ?], environmental concerns regarding chemical stabilizers [?, ?], and the infeasibility of engineering methods [?, ?] raise concerns.

In recent decades, microbial induced carbonate precipitation (MICP), also known as microbial cementation, has been developed. The MICP method, compared with traditional methods, is less likely to create the aforementioned concerns [?, ?, ?] and has been employed as a biological mulching method for aeolian erosion mitigation [?, ?, ?]. It improves the properties of different soil types [?, ?], enhances soil erosion resistance [?, ?], and stabilizes fine particles [?, ?, ?], while simultaneously minimizing dust storms [?, ?] and improving soil mechanical properties such as hardness, strength, and permeability [?, ?, ?]. The application of MICP is particularly helpful for soil stabilization due to the in situ generation of cementing agents such as calcium carbonate or calcite [?, ?, ?, ?, ?].

The main process of this method involves the adherence of soil and sand particles to the cementing agent through microbial and carbonate activity [?, ?]. Aside from being low-cost, this method is also environmentally friendly because a wide variety of microorganisms can be utilized without causing harm to the ecosystem [?, ?]. The cementation process of MICP is influenced by numerous factors, including soil physical and chemical properties, ambient and climatic conditions, permeability, and structure [?, ?, ?, ?]. The following three groups of microorganisms have the ability to precipitate calcium carbonate: microorganisms such as cyanobacteria and algae that can stabilize CO_2, sulfate-reducing bacteria, and microorganisms effective in nitrogen transformation [?, ?, ?].

Bacteria are the most commonly used microorganisms for this method and are predominant in soil [?, ?, ?]. These microorganisms help improve soil physical properties due to their small size and vast numbers. A series of soil bacteria such as *B. sphaericus* from the Bacillaceae family represent one of the most abundant groups of soil bacteria that play an important role in soil cementation by producing urease and hydrolyzing urea. Hydrolyzing urea through this approach is approximately 10^{14} times faster than the natural process [?, ?]. Thus, urease-producing bacteria serve two purposes: they produce enzymes that hydrolyze urea and they act as nucleation sites [?, ?]. During the latter process, the ammonium produced increases the pH and, subsequently, in the presence of carbonate ions, calcium carbonate crystals are formed [?, ?, ?, ?]. By coating soil grains with these crystals, soil particles bond together, and the strength and resistance of aggregates improve [?, ?, ?]. Overall, it is widely recognized that carbonate precipitation has enormous potential as a biomineralization approach [?, ?, ?].

Many studies have employed the MICP method. For instance, [?, ?] developed a new method for creating natural calcite deposition where sand was strengthened by utilizing *Sporosarcina pasteurii* with urea and calcium chloride as a cementation solution. [?, ?] measured calcium carbonate synthesis using bacteria in a basic medium containing two grams of yeast extract per liter, finding that the highest amount of calcium carbonate was produced at pH 8.5 and 25°C. Moreover, [?, ?] used *S. pasteurii* with high urease activity and high resistance to produce bio-cement, identifying *S. pasteurii* as the best bacteria for hydrolyzing urea and reducing pH. [?, ?] also observed that microbial polymers can improve tensile strength as a cement between soil particles while being environmentally friendly.

Most studies in Iran focus on combating dust storms using polymers and petroleum product mulches, while surprisingly little is known about biological mulches. Therefore, this study aimed to assess the potential of a microbial induced calcium carbonate precipitation process using *B. sphaericus* to reduce wind erosion and airborne dust derived from silty soils in a dust-producing area of Ilam Province, Iran. For this purpose, experiments were conducted on the surface layer of samples using a mixture of cementation solutions. The stabilization performance of this method was evaluated through aggregate stability and penetration resistance tests on the crustal soil layer, as well as soil mass loss measurements.

2.1 Study Area

Sampling was conducted in one of the dust-prone areas in Ilam Province (32°03 34 N, 45°40 48 E; 237 m a.s.l.), western Iran, in 2020. The province has an average annual precipitation of 120 mm, and the annual average temperature ranges from -13.6°C to 41.2°C. According to the Köppen climate classification, the region has an arid climate [?, ?]. After selecting the study area, we collected approximately 500 kg of soil samples at a depth of 0–30 cm. The following soil physical and chemical characteristics were measured:

soil texture [?, ?], EC [?, ?], pH [?, ?], organic carbon [?, ?], and lime [?, ?]. Equivalent calcium carbonate was also determined by the titration method [?, ?].

2.2 Preparation of Bacteria and Experimental Design

The *B. sphaericus* PTCC (Persian Type Culture Collection) 1487 (CIP S25 001) was obtained as a pure culture from the Iranian Research Organization for Science and Technology (IROST). Bacteria were cultivated on sterile Nutrient Agar (NA) and incubated at 25.0°C for 5-7 days. The bacteria were then cultivated in sterile liquid culture media, Nutrient Broth (Merck®, Germany), and incubated at 28.5°C with shaking at 200 r/min to achieve the required bacterial concentration. The concentration of bacteria in the liquid culture medium used in the experiment was determined by measuring optical density using an M550 UV/VIS spectrophotometer at a wavelength of 600 nm [?, ?, ?]. Sterile Nutrient Broth culture medium was used as a control for optical density readings, and each sample was read three times. The maximum optical density obtained was 0.5 cd/m^2. The experiment was performed with this same concentration of the desired bacterial strain. We calculated the predicted mathematical correlation between the measured data of CFU (colony forming units)/mL (the total number of bacteria) and OD600 (the optical density of a sample measured at a wavelength of 600 nm) for *B. sphaericus* based on Equation 1 [?, ?]:

$$18.57 CFU 4.25e12(OD)mL$$

where the bacterial population at OD=0.5 was 6.42$×10^{6}$ cells/mL.

Bacteria preparation followed a randomized factorial design with four factors: two levels of bacteria (0.0 and 0.5 OD), two levels of urea-chloride cementation solution (0.0 and 0.5 M), three levels of suspension volume (123, 264, and 369 mL), and two levels of bacterial spray (once and twice spray) with three replications (Table 1). Nutrient broth (3 g/L), ammonium chloride (10 g/L), and sodium bicarbonate (2.12 g/L) were used to prepare the 0.5 M urea-chloride cementation solution [?, ?].

2.3 Preparation of Trays

Soil samples were sieved through a 2-mm sieve after physical and chemical analysis. To sterilize the samples, we autoclaved them for 2 h at 121°C and 103 kPa. After cooling, approximately 3 kg of soil was weighed and placed in 35 cm × 35 cm × 3 cm trays (Fig. 1a [Figure 1: see original paper]). The aforementioned suspension was sprayed evenly on the soil surface before placement in an incubator at 25°C for 28 days (Fig. 1b). To keep the soil surface moist, we sprayed sterile water equally on it every 3 days. Following this process, the trays were placed under ambient conditions, and all samples were tested for penetration resistance, aggregate stability, and soil mass loss using the wind tunnel [?, ?].

2.4.1 Wind Tunnel Test

A portable wind tunnel was used to test soil surface stabilization under simulated laboratory conditions. The wind tunnel consisted of two main parts: a jet fan blower and a working section. Air movement was driven by a high-pressure axial fan typically used in industrial ventilation systems. A diesel generator with 15 kW power ran the fan at 2800 r/min, generating wind speeds of 0.3–20.0 m/s at a height of 0.30 m. In this test, the wind speed used was 15.6 m/s. A conic section installed in front of the tunnel entrance allowed for more controlled airflow into the test area. As air flowed, a 25-mm square honeycomb diffuser laminarized the airstream to eliminate unexpected artificial turbulence. The total length of the working section from the diffuser end was 3.4 m with a cross-sectional area of 0.35 m × 0.35 m (Fig. 2 [Figure 2: see original paper]). Wind speed was measured using a hot-wire anemometer Model AN-4330 with two miniature glass-bead thermostats that provide better accuracy at low speeds. Holes installed at the top of the testing section allowed measurement of wind speed and dust. Samples were first weighed before placement in the designated part of the device and weighed again after 15 min of testing. Loss of soil weight in trays indicated soil mass loss or soil erosion by wind [**?**, **?**].

2.4.2 Penetration Resistance Measurement

Penetration resistance is an important physical (mechanical) property affecting other intrinsic soil properties such as erodibility. In this study, the penetration resistance of samples was measured three times after a period of 28 days (depending on favorable moisture content) at 24-hour intervals using a Mortar Penetration Resistance Apparatus (H-4137, Humboldt Mfg. Co., USA). During testing, the penetrometer with a flat end was gradually placed on the surface of the mulched soil samples in the trays. Different locations on each tray were penetrated for each test, and their average values were calculated. Three replications were performed for the various soil treatments and then averaged [**?**, **?**].

2.4.3 Aggregate Stability (AS) Test

Aggregate stability is an important parameter for assessing the effectiveness of soil surface stability in arid and semi-arid areas. The wet sieving apparatus 08.13 (Eijkelkamp Soil & Water, USA) was used to determine the AS of the trays. For this purpose, wet sieving apparatus with 8 sieve sizes of 2.000, 1.000, 0.500, 0.250, 0.125, 0.064, 0.053, and 0.046 mm were used. The AS index (Eq. 2) equals the weight of soil particles obtained in the dispersing solution cans (2 g sodium hexametaphosphate/L) divided by the sum of weights obtained from the distilled water cans and the dispersing solution cans. The closer the index is to 1, the greater the stability of the aggregates.

$$818811MsAS100\%, MsMw111$$

where AS is the aggregate stability index (%); Ms is the weight of soil particles inside cans with dispersing solution (g); and Mw is the weight of soil particles inside cans with distilled water (g).

2.5 Data Analysis

Data analysis was performed using SPSS v. 22.0 and R v. 3.6.0 statistical software [**?**, **?**]. We conducted analysis of variance based on a completely randomized factorial design. After determining differences between treatments (based on analysis of variance table), least significant difference and Duncan multiple range tests at the P<0.05 level were used to determine mean differences.

3.1 Soil Mass Loss

Wind tunnel test results indicated that increasing urea-chloride cementation solution levels had no significant effect on soil mass loss (P<0.05), as shown in Figure 3 [Figure 3: see original paper]. Although soil mass loss increased at the 0.5 M cementation solution level compared with the control, the changes in overall soil weight in each tray were negligible. Solution volume also had no effect on soil mass loss, with no significant difference between various levels at 0.5 M concentration. Relatively high soil mass loss occurred with the solution volume of 264 mL (58.33 g), while the least soil mass loss was observed in the treatment with 123 mL solution volume (23.42 g), as shown in Figure 3. The rate of soil mass loss decreased significantly (P<0.05) as bacterial concentration increased. Soil mass loss on the surface with twice bacterial spray (8.58 g) was less than that with once spray (23.58 g) and without bacteria (76.00 g), as presented in Figure 4 [Figure 4: see original paper]. Results of different formulations (interaction of bacteria, cementation solution, and solution volume) showed significant differences (P<0.05) in soil mass loss.

The F14 formulation (twice spray, 264 mL solution volume, and without cementation solution) had the minimum rate of soil mass loss (1.00 g), while the F5 formulation (without bacteria, 264 mL solution volume, and 0.5 M cementation solution) had the highest rate (246.00 g; Fig. 5 [Figure 5: see original paper]). Overall, results demonstrated that as bacterial concentration increased, the rate of soil mass loss decreased. Therefore, soil mass loss with twice spray was lower than that with once spray.

3.2 Aggregate Stability (AS)

The effect of two cementation solution levels (0.0 and 0.5 M) on AS was not statistically significant (P<0.05), despite an increase in AS with 0.5 M cementation solution (31.01%), as shown in Figure 6 [Figure 6: see original paper]. Results also indicated that solution volume effect on AS was not significant (P<0.05; Fig. 6). The solution volume of 369 mL (WC3t) yielded the best AS with a value of 29.72%. AS increased exponentially as solution volume increased, indicating the effectiveness of solution quantity on stability. Bacterial concentration effect on AS was significant ($P<0.05$); high stability of 33.94% was observed in the BS2t treatment, while low stability of 23.48% was observed in the WBt treatment (Fig. 7 [Figure 7: see original paper]). AS increased as bacteria increased, confirming that increased bacterial concentration could improve AS.

Results of various formulations indicated significant differences between them ($P<0.05$). The F16 and F7 formulations had the highest (42.55%) and lowest (18.00%) AS, respectively (Fig. 8 [Figure 8: see original paper]).

3.3 Penetration Resistance

The use of 0.5 M cementation solution concentration resulted in a penetration resistance of 1.684 kg/cm^2, enhancing soil penetration resistance more than the control (1.308 kg/cm^2), as shown in Figure 8. Although penetration resistance increased with solution amount, differences between these levels were not significant at the 5% level. The solution volume of 369 mL (WC3t) had the highest penetration resistance (1.737 kg/cm^2), while 123 mL volume (WC1t) had the lowest value (1.166 kg/cm^2) (Fig. 9 [Figure 9: see original paper]). Penetration resistance test results at various cementation solution concentrations indicated significant effects on penetration resistance. Bacterial concentration effect on penetration resistance was significant ($P<0.05$), with penetration resistance increasing as bacterial concentration increased (Fig. 10 [Figure 10: see original paper]). Twice bacterial spray (BS2t) had the maximum penetration resistance (2.334 kg/cm^2), while the without-bacteria treatment had the lowest value (0.722 kg/cm^2). Results of different formulations showed significant differences among them ($P<0.05$). Penetration resistance increased with increasing bacteria and cementation solution. The highest penetration resistance (3.328 kg/cm^2) was observed in the F18 formulation, whereas the lowest (0.416 kg/cm^2) occurred in the F3 formulation (Fig. 11 [Figure 11: see original paper]). Overall, results of various formulations indicated that twice bacterial spray with 0.5 M cementation solution and 264 mL solution volume (BS2t+C1t+WC2t) are recommended as the best formulation for improving soil penetration resistance.

4 Discussion

This study conducted a comprehensive laboratory evaluation using the MICP method, including soil wind erosion, penetration resistance, and aggregate stability tests. Using MICP, a combination of bacteria and cementation solution was applied to soil samples, and calcium carbonate was produced from calcium and carbonate ions using hydrolyzing ammonium urease-producing bacteria. According to findings, high microbial concentration (twice spray) reduces soil mass loss and enhances penetration resistance while strengthening aggregate stability. Additionally, increasing the cementation solution (0.5 M) amplifies crust layer thickness and penetration resistance.

A correlation existed between the rate of soil mass loss and calcite precipitation. Comparison of soil mass loss rates by different formulations at 15.6 m/s wind speed indicated that average soil mass loss in F14 and F17 formulations was 1 and 3 g, respectively, whereas the control treatment (e.g., F5) was 246 g. Therefore, high levels of bacteria and cementation solution resulted in a significant decrease in soil mass loss ($P<0.05$). The deposition of additional calcite material that reduced the rate of soil erosion was attributed to bacterial activity [?, ?, ?, ?, ?, ?]. Aggregate stability test results showed that increases in cementation solution and solution volume had no significant impact. As solution volume increased, the trend of aggregate stability increased exponentially, indicating that solution volume is effective in preserving stability. Penetration resistance greater than 1.52 kg/cm^2 with twice spray (Fig. 11) confirmed that increased calcium carbonate deposition crystals between soil particles act as bridges, improving soil stability and resistance to penetration [?, ?, ?, ?, ?]. Thus, increasing the spray number of the MICP method results in increased soil surface penetration resistance. As penetration resistance of the soil surface improves, the rate of soil erosion decreases, confirming that the MICP method is effective for soil erosion control [?, ?, ?, ?, ?].

In this study, twice bacterial spray with 0.5 M cementation solution and 269 mL solution volume (BS2t+C1t+WC2t) was found to be the best formulation for soil surface stabilization. Thus, it would be an efficient approach to stabilize soil surface, mitigate wind erosion, and provide acceptable improvement in soil penetration resistance [?, ?, ?, ?, ?, ?].

Other researchers' findings are consistent with this study. [?, ?] discovered that after 3 days, soil penetration resistance could reach a maximum of 459.9 kPa, and surface soil loss could be reduced to 0 after 30 days. [?, ?] found that increasing treatment duration using the MICP method improves calcite content percentage, crust thickness, and penetrating resistance. The quantity of erosion decreased significantly as the amount of calcite sediment increased [?, ?]. Moreover, [?, ?] showed that wind erosion and soil loss could be withstood with a 1 M cementation solution treatment at a wind speed of 55 km/h.

The MICP method has several advantages, including in situ application [?, ?, ?], environmental friendliness, requiring minimal temperature for bio-cement production [?, ?, ?], and industrial applicability [?, ?, ?]. However, this method also has disadvantages, including the presence of high ammonia concentrations in aquatic environments [?, ?], its inapplicability for soils with less than 1 mm particle size [?, ?], cost-effectiveness issues at large scale due to high costs of enzymes and bacterial nutrients, and the possibility of contaminating air and drinking water when employing this technology at scale [?, ?, ?, ?].

5 Conclusions

Overall, the MICP method is an efficient approach for dust suppression in areas sensitive to wind erosion. Furthermore, applying the MICP method to soil surfaces can be an effective choice for wind erosion control, particularly at higher velocities. The percentage of carbonate precipitation, which is related to reduced particle loss, was the important factor in MICP' s erosion control efficiency. Urease activity and the quantity of suspended bacteria have a considerable positive relationship; when urease activity and bacteria are higher, more urea is hydrolyzed, resulting in higher amounts of calcite precipitation. The amount of soil mass loss and bacterial concentration in different treatments had a strong relationship. This relationship demonstrates that by forming aggregate structure and increasing penetration resistance, the MICP method can greatly reduce wind erosion and provide sand dune stabilization for dust control. The BS2t+C1t+WC2t treatment provided the strongest hardness and penetration resistance. In addition, this technology is ideal for soil surface stabilization and thus represents a better substitute for existing technologies. The primary reason for the technique' s viability is that it is considered an environmentally friendly approach to geotechnical engineering application, and it does not use any chemical cementing agents. Generally, the MICP method may be used to mitigate and limit harmful wind erosion and dust emissions as an alternative to existing methods. However, soil type and environmental elements have a significant impact on the efficacy of this strategy. Therefore, further research is needed to understand the impact of this technique on different soil types as well as the physical properties of treated soil.

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Note: Figure translations are in progress. See original paper for figures.