A laboratory investigation on suppression of dust from wind erosion using biocementation with *Bacillus amyloliquefaciens*

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Abstract

Dust events are among the serious environmental challenges in some countries. Sustainable solutions can be applied to tackle this problem by considering soil as a living ecosystem. Biocementation based on production of carbonates by heterotrophic bacteria is one of the favored methods to suppress the dust from wind erosion because this type of bacteria produces calcium carbonate (main product) as well as water and carbon dioxide (by-products). In present research, bacterial species of Bacillus amyloliquefaciens was used. First, bacteria were cultivated to reach to a pre-determined concentration. Next, bacterial cells and nutrients in the form of solution were sprayed on the soil surface. Then, samples were tested in a closed circuit wind tunnel. Three main groups of samples were tested: without sand bombardment and undisturbed soil surface, with sand bombardment and undisturbed soil surface, and without sand bombardment and with disturbed soil surface. The results show that the implemented method for stabilization of soil was efficient. Moreover, based on the results of second group of tests, curing duration, amount of water, temperature-water interaction and water-bacterial cells interaction were found to be of considerable significance.

Keywords: wind erosion, dust control, wind tunnel, biocementation, calcium carbonate, Bacillus amyloliquefaciens
1. Introduction

Dust events due to soil wind erosion are one of the important sources of air pollution and environmental challenges. Dust phenomena have several effects on human life: it influences the atmospheric radiation balance directly or indirectly and hence, leads to global climatic variations (Shao [1]); it causes air pollution and consequential illnesses such as meningitis, valley fever, asthma (Shahsavani et al. [2]); it is accompanied by economical aspects such as reduction of agricultural production, reduction of sight distance and corresponding dangers and stoppage of economic activity.

Methods of controlling and suppressing dust from wind erosion can be categorized into two main groups. The first group focuses on separating dust from the air, while the second group tries to suppress particles at source. The former group suffers from some limitations. The most important limitation is lack of efficiency to control suspended particles. The latter group tries to stabilize the soil at source which is more appreciated by researchers.

Stabilization of the susceptible soils in the source can be categorized to: correct land use (depending on the potential of land), retention of soil moisture, vegetation, leaving stubble on farm, windbreak (living and artificial), artificial covers (such as gravel and cobble, oil mulch and resin emulsions) and use of microorganism (Refahi [3]). In some methods such as the three first methods and living windbreak, a minimum of moisture and suitable conditions for plant growth are necessary. Furthermore, leaving stubble is restricted to farm lands.

When dust is originated from vast regions, the use of artificial covers such as gravel, cobble and fiber are very expensive and uneconomic. Today’s growing attention to sustainable development and environmental protection makes it challenging to use oil mulch, resin emulsions and chemical additives.

In recent years, biotechnology has made it possible to use microorganisms to stabilize the soil with considering minimum adverse effects on environment. Microorganism application on suppression of dust needs a reasonable amount of moisture which is simultaneously available when microorganism is sprayed on the soil.

Microorganism-based methods are able to cover vast regions in sufficiently acceptable time by using aircraft sprayers. These methods could improve wide range of soil gradation; examples are data of Maleki et al. [4], Mortensen et al. [5], Rebata-Landa [6]. From an economic viewpoint, some of the previous studies published in the literature show the potential of using microorganisms to compete with other stabilization methods. For instance, Ivanov and Chu [7] stated that the costs of the raw materials for the chemical soil grouting are in the range of $2 to $72 per cubic meters of soil, whereas the costs of the raw materials for the microbial grouting lie in the range of $0.5 to $9.0 per cubic meters of soil. In addition, Dejong et al. [8] indicated that actual costs of various improvement processes are ambiguous because of rare field applications. However, they compared alternative applications and their potential, considering implementation feasibility, probability of success, cost/viability, and social acceptance of biogeochemical process in the form of a table. In this comparison, dust mitigation control using biogeochemical process has 18 out of a total score of 20 in general and a score of 4 out of 5 for economic aspect which is considered as the highest ranking.

Some of the previous main researches in which soil stabilization was carried out using bacteria for dust suppression are Bang et al. [9], Stabnikov et al. [10], Anderson et al. [11], Liu et al. [12], Meyer et al. [13] and
O’Brien and Neuman[14]. There also exist other studies in which algae, fungi and enzymes were added to soil for stabilization. Examples are data of Cuadros et al. [15], Neuman and Maxwell [16], Knorr [17], Alsanad [18] and Strong [19]. These types of crusts have several advantages such as flexibility compared to biochemical crusts. However, the main drawback of these techniques is their long and repetitive treatments. For example, O’Brien and Neuman [14] treated samples by Nostoc Commune cyanobacteria for a five week period and the crusts were fed weekly with Bold Basal Medium and watered daily. The repetition of treatment for large scale application is obviously time consuming and uneconomical. Moreover, based on Strong [19], the cyanobacteria crusts were weakened in the case of infrequent small rainfall events which led to increase in vulnerability of soils to wind erosion. These restrictions are serious limitations for long term and large scale application of above-mentioned researches. In return, present method does not suffer from these limitations.

One of the overlooked aspects of previous researches in the field of bacteria application for stabilization of soil and suppression of dust is by-products of reactions. For instance, in several of these investigations, Bacillus sphaericus species of bacteria had been used and the fatal ammonia by-product was obtained. Also, cyanobacteria, algae and fungi were found to reside at the depth in the soil profile until sufficient moisture was received (Strong 2007).

In the present study, Bacillus amyloliquefaciens bacteria are used. These bacteria produce calcium carbonate biocementation as the main product and water and carbon dioxide as by products as follows:

\[
(CH_3COO)_2Ca + 4O_2 \rightarrow CaCO_3 \downarrow +3CO_2 \uparrow +3H_2O
\]

(1)

It is evident that carbon dioxide is acceptable compared to ammonia by-product. Also, for the proposed method in this research, the required moisture will be provided simultaneously while bacteria is being sprayed. Furthermore, an attempt has been made to enrich this research by proper design of experiments to select all significant factors, while the relative importance and influence of these parameters on the mass loss due to wind erosion were obtained. Moreover, the samples were weighted continuously during wind erosion tests in wind tunnel by a 3-component balance to have a better simulation.

2. Materials and Methods

2.1. Materials

Soil was obtained from a critical wind erosion hotspot located in the south of Iran (Khoormoj 90 Km south east of Bushehr). The gradation curve of soil is illustrated in Fig. 1a. The soil is classified as SP-SM based on unified soil classification system and is originated from carbonate rocks. All soil samples were autoclaved before treatment to ensure that the changes in wind erosion potential detected in the tests are solely from biocementation by the specified bacteria. The exception was a control sample which was prepared with unautoclaved soil.

Silica sand with grain distribution between 425 µm and 595 µm was used for sand bombardment. Several researchers (Bagnold [20]; Scott [21]; Shao et al. [22]) have estimated that suspended moving particles in the air are in the range of 70-500µm. Therefore, the selected sand bombardment material lies in a conservative range.
Distilled water was used for soil treatments. Before sample preparation, water was autoclaved to ensure no sign of bacteria in water.

The *B. amyloliquefaciens* bacterium was selected for this research. This type of bacterium exists naturally in soil. However, for the present study the lyophilized powder of the bacterial biomass, strain PTCC No. 1732 (other collection No. DSM7, ATCC23350), was prepared from the Iranian Research Organization for Science and Technology (IROST). This strain is aerobic one with optimum temperature for growth of 30ºC. As stated previously, this type of bacteria has the advantage over other types from the aspect of having environmental friendly by-products and the risk group of *B. amyloliquefaciens* is 1, unlikely to cause disease in human, animals, plants or fungi, according to German Technical Rules Biological Agents (TBRA) classification. The bacteria were cultured in Luria-Bertani(LB) medium which involves 1% tryptone (Merck®), 0.5% yeast extract (Merck®) and 0.1%NaCl (Merck®). Calcium acetate (Merck®) was used as a source of energy.

### 2.2. Methods

#### 2.2.1. Bacteria culture

*B. amyloliquefaciens* bacteria were cultured in LB liquid medium inside shaker incubator with 110 rpm and 28ºC. Bacterial growth curve is shown in Fig.1b. Optical density (O.D.) of bacteria solution was determined by spectrophotometer (Optima model sp-3000 plus) which was set at a wavelength of 600 nm.

#### 2.2.2. Sample preparation and treatment

Soil specimens were prepared in 200×200×50 mm boxes made of transparent Plexiglas plates. The measured insitu density of soil was about 15.7 kN/m³. Soil samples were compacted to a density close to insitu condition. Initial water content of untreated soil was 0.5±0.1%. Next, the predetermined amounts of bacterial cells (O.D.= 0.75 and 1.5 in the form of 40 g solution) and nutrient solution(concentration= 0.05 and 0.1 g/lit , equivalent to 2 and 4 g of nutrient in 20 and 40 g solution) were sprayed on the surface of soil samples. All the water sprayed on samples penetrated in the soil and no run off was observed. Finally, samples were cured at designed temperature and duration. The remained water contents after treatment of samples were 0.2 to 0.4%. Due to treatment condition of samples, most of added water was evaporated during first 24hr of curing time. After wind tunnel tests, the crust thickness were 1 to 2 cm.

#### 2.2.3. Wind tunnel tests

A closed circuit tunnel was used for simulation of wind erosion in laboratory. This tunnel is ISI model 6407 ZAF with800x800 mm test section, maximum wind speed of 100 m/s and total dimension of 18x6.5x3.8 m. The tunnel is equipped by a 3-component balance which allows the specimen’s weight to be recorded continuously during the test.

Seven spires were designed based on Irwin [23] to simulate boundary layer and wind velocity profile in the wind tunnel similar to land surface. The spires were installed 87.7 cm upstream of soil sample. The generation of desired vertical wind velocity profile and the transversal uniformity of wind profile at test section were verified by pitot tube measurements at several points.

In order to have continual measurement of soil erosion during wind tunnel test, the sample box was kept on balance so that the sample weight could be measured continuously without any influence of lift force.
Three main groups of tests were carried out: without sand bombardment and undisturbed soil surface (group A), with sand bombardment and undisturbed soil surface (group B) and without sand bombardment and disturbed soil surface (group C).

2.2.3.1. Group A tests
In these tests, first wind velocity was raised to 30 m/s, and then soil samples were exposed to this flow for 10 minutes. Typical wind velocity graphs for all triple groups of tests are illustrated in Fig. 2.

2.2.3.2. Group B tests
Several researches used different mass fluxes for sand bombardment, for instance, Neuman et al. [24] 0.007 kg/m/s, Langston and Neuman [25] 0.015 kg/m/s, Rice and McEwan [26] 0.002 kg/m²/s, Zobeck [27] 0.01, 0.025 and 0.05 kg/m/s; Houser and Nickling [28] 0.0026 to 0.023 kg/m/s and Neuman and Maxwell [16] 0.014 kg/m/s.

In the present research, due to selected wind velocity for wind erosion test (14 to 15 m/s), the mass flux of sand bombardment was considered about 0.01 kg/m/s (120 gr/min for each sample).

As mentioned earlier, the grain diameter of sand bombardment material ranged between 425 µm and 595 µm. The bombardment was performed by a separating funnel, a connector steel tube and a modified upholstery nozzle. Details of the sand bombardment system were determined from a few preliminary tests.

In this group of tests, at first, wind velocity was raised to 29 m/s and the soil samples were exposed to this flow for 2 minutes. Subsequently, the wind velocity was lowered to 14 m/s followed by sand bombardment for a period of 9 minutes at a wind velocity of 14 to 15 m/s. At the end of sand bombardment stage, the wind velocity was raised again to 28~29 m/s and the soil samples were exposed to this flow for 3 minutes before the test ends. Variation of wind velocity is illustrated in Fig. 2.

2.2.3.3. Group C tests
For the C group, wind velocity was raised stepwise to 25, 27 and 29 m/s (see Fig. 2). Then wind velocity was lowered to zero. A disturbance was created at the center of sample surface by a pocket vane shear apparatus (Gilson® model HM-504A). Finally, the velocity was raised again in four steps (as shown in Fig. 2) and each step lasted for 2 minutes.

2.2.4. Design of experiments
Five main factors to consider for performance evaluation of biological calcium carbonate precipitation at soil surface are: temperature, bacterial population per unit surface area (concentration and volume of bacterial solution), amount of available nutrient for bacteria, moisture at the soil surface and curing time (available time for calcium carbonate precipitation by bacteria before soil is exposed to wind shear force). For determination of levels of each factor, the following considerations were taken into account:

1- Temperature levels: Optimum temperature for cultivation of *B. amyloliquefaciens* bacteria is 30°C. Sources of dust are often located in arid and desert regions where the occurrence of temperatures higher than the optimum temperature is probable. The above mentioned reasons led to temperature levels being considered: 28°C (near to optimum temperature of bacterial growth cultivation) and 37°C. Additional temperature levels were considered for group B experiments to study high temperature effects (50°C) and outdoor conditions (7°C to 18°C).
2- Bacterial concentration: Soil samples were treated in 0.75 and 1.5 levels of optical density (O.D.) and 40 cc bacterial solution per sample. As Fig.1b shows 0.75 O.D. is a concentration level which can be obtained earlier than 1 day and 1.5 O.D. is a level of concentration in which bacterial concentration is stable during a considerable period of time. The former level can be used as an alternative for urgent use in field applications while the latter level can be applied when a more stable level in a vast region is intended.

3- Nutrient: In terms of cost, the most expensive component is the nutrient (calcium acetate). It was tried to make this method a more competitive alternate against other methods by decreasing the amount of nutrient per unit area. Thus, for treatments of samples, nutrient levels were considered as low as 0.05 and 0.1 kg/m² (2 and 4 g/sample).

4- Soil moisture: Water not only facilitates penetration of bacteria and nutrient into soil pores, but also prepares an appropriate medium for bacteria to live. Although a huge amount of water can lead to an uneconomic design in terms of required water to carry and spray. In some traditional methods such as oil mulches, 2 lit/m² is a normal dosage, so total amount of sprayed water on the soil surface was selected at 2 levels of 1.5 and 2 lit/m² (60 and 80 cc/sample).

5- Curing time: The short curing time of treated samples results in partial stabilization and finally overestimation of wind erosion. On the other hand, the long curing time underestimates the amount of laboratory erosion compared to field wind erosion, owing to the controlled temperature and other conditions in the laboratory. Today, thanks to advances in meteorology which has made it possible to forecast the weather for the next 7 to 10 days, field application can be undertaken while the probability of occurrence of imminent strong wind is low. With the abovementioned explanation, two levels of 3 and 7 days were considered to cover all the possibilities.

Based on the aforementioned factors and their levels, a full factorial design of experiments led to 32 tests in each group. A proper statistical design can reduce the required tests while it keeps the results of remained tests valid. The L12 array of Taguchi was chosen as an experiment design in this research. Therefore, the tests were reduced from 32 to 12. It should be noted that for “group A” only 8 samples were tested since nominal erosions were observed in this group. Moreover, in group B of tests, few lime treated samples were tested to compare performance of the two methods.

2.2.5. Complementary tests
In order to ensure that changes in erodibility of tested soil samples are due to biological carbonate cementation, two sets of tests were carried out on treated and untreated samples: 1) Calcium carbonate equivalent (CCE) tests based on ASTM-D4373 standard; 2) Energy dispersive x-ray diffraction (XRD) analysis. Moreover, Scanning electron microscope (SEM) images which can capture a better understanding of bonding formation between soil particles were adopted. For this purpose, SEM of Cambridge model S360 was employed.

3. Results and discussion
Table 1 indicates the results of “group A” tests. The small amount of erosion in these tests (smaller than 0.0125 g/cm²) proves the efficiency of the proposed treatments for this group of tests. Remediation is defined as:

\[
\text{Remediation(\%) = \frac{\text{Untreated Mass Loss} - \text{Treated Mass Loss}}{\text{Untreated Mass Loss}} \times 100}
\]

Using this definition and knowing mass loss of untreated sample is 6.825 g/cm², percent remediations are shown in last column of Table 1.

Table 2 demonstrates the results of wind erosion tests with sand bombardment (group B). In this table, 12 main samples (rows 1 to 12), 6 negative control samples (rows 13 to 18), 7 samples covering different curing conditions (rows 19 to 25) and 2 samples focusing on lime stabilization method(rows 26 and 27) are addressed.

Table 3 lists the analysis of variance (ANOVA) for 12 main samples and contributors. From this table, among the contributors including five treatment factors and two-factor interactions, curing time, water, temperature-water interaction and bacteria-water interaction are significant with p-value less than 0.05. The Normal probability plot of residuals is demonstrated in Fig.3a. The points on this plot lie reasonably close to a straight line, lending support to the conclusion that the abovementioned factors and interactions are the only significant effects and the underlying assumption of the analysis are satisfied. Furthermore, three normality tests including Anderson-Darling, Ryan-Joiner and Kolmogrov-Smirnov tests confirm quantitatively the normality hypothesis again. Plot of residuals versus predicted mass loss, shown in Fig.3b, is satisfactory and the assumption of variance equality is confirmed.

Each factor was coded according to the equation:

\[
\bar{x_i} = \frac{X_i - (X_{i,max} + X_{i,min})/2}{(X_{i,max} - X_{i,min})/2}
\]

where \(\bar{x_i}\) is the coded value of \(X_i\) factor; \(X_{i,max}\) and \(X_{i,min}\) are the values of factor at maximum and minimum levels, respectively. The range of values are 28°C to 37°C for temperature (T), 0.75 O.D. to 1.5 O.D. for bacteria concentration (B), 60 g/sample to 80 g/sample for water (W) and 3 days to 7 days for curing time (C).

The following relationship defines the role of contributors to mass loss based on statistical analysis in terms of coded factors:

\[
\text{massloss(gr/cm²)} = 0.210 + 0.020 \times \bar{T} - 0.019 \times \bar{B} + 0.081 \times \bar{W} - 0.15 \times \bar{C} - 0.079 \times \bar{T} \times \bar{W} - 0.120 \times \bar{B} \times \bar{W}
\]

where \(\bar{T}, \bar{B}, \bar{W}\) and \(\bar{C}\) are temperature, bacteria concentration, water and curing time factors in coded form, respectively.

Expressing factors as coded has the advantage that their coefficients would be meaningful and their relative impact can be assessed by comparing them directly. The following points are deduced from this relationship:

1. Curing time factor has the most significant effect on wind erosion reduction in the present method.
2- Water shows its contributions not only in the form of a single factor, but also in the form of its interactions with temperature and bacteria. Thus, for accurate interpretation of water effects, it is necessary to incorporate interactions:

2-1- Fig. 4a illustrates the temperature-water interaction graph which demonstrates that raising temperature does not have a negative effect on soil stabilization provided the amount of applied water is close to its upper limit, given in table 2. On the contrary, increasing temperature leads to greater mass loss and lower efficiency as long as water used in near its lower limit.

2-2- Bacteria-water interaction is illustrated in Fig. 4b, which indicates that in case of using upper range of water factor, increasing bacteria causes decreasing mass loss while in case of using lower range of water factor, increasing bacteria also leads to increase of mass loss.

Negative control tests, samples 39-1M and 39-2M in table 2, which had no bacteria and nutrient experienced 2.4250g/cm² and 3.3900g/cm² mass loss which are substantially larger than the mass loss of treated samples.

The meaningful differences of wind erosion in treated and untreated samples can be visualized by redrawing bacteria-water interaction diagram, as shown in Fig. 4c. This finding reemphasizes the efficiency of the proposed approach. Negative control tests, samples 35-1M and 37-1M (samples without bacteria) demonstrated 0.9475g/cm² and 1.0375g/cm² mass loss while negative control tests, samples 44-1M and 45-1M (samples without nutrient) showed 0.1950g/cm² and 0.1375g/cm² mass loss, respectively. These tests confirm the results of ANOVA in which bacteria is a contributor factor while nutrient factor is not statistically a significant factor.

Samples 27H and 29H were similar to 27-2M and 29-2M, respectively, but the two former samples’ curing temperature was 50°C instead of 37°C. The amount of erosion in samples 27H and 27-2M (with high level of water factor) was 0.1475g/cm² and 0.2400g/cm² while the mass loss in samples 29H and 29-2M (with low level of water factor) were 1.1600g/cm² and 0.5475g/cm², respectively. Therefore, with the higher amount of water, increase in temperature gave rise to a reduction in mass loss in sample 27H compared to 27-2M. However, with the lower amount of water, increase in temperature led to increase in mass loss in sample 29H compared to 29-2M. These could be expected based on the interpretation of temperature-water interaction stated previously.

It is very important that the applicability of proposed method be investigated in the field, thus additional experiments were planned and samples 27-1F, 27-2F, 29-1F and 29-2F were tested. Samples 27-1F and 27-2F are the same as sample 27-2M but cured in outdoor condition. Also, samples 29-1F and 29-2F are treated in the same condition as 29-2M but with outdoor condition. For outdoor condition, the samples were exposed to the ambient condition with temperature varying from 7°C to 18°C. Samples 27-1F and 27-2F experienced 0.2775 g/cm² and 0.2900g/cm² mass loss, respectively, compared to 0.2400g/cm² for sample 27-2M. This trend was observed in samples 29-1F and 29-2F compared to sample 29-2M. These experiments also confirm the potential of proposed technique for field applications.

As mentioned earlier, soil was autoclaved before sample preparation in order to ensure that the observed changes in wind erosion potential are solely from biocementation by the specified bacteria. The only exception was sample 27U which was prepared similar to sample 27-2M but with unautoclaved soil. The results indicated a mass loss of 0.1250g/cm² for sample 27U compared with 0.2400g/cm² reported for sample 27-2M. This can be
as an indication to encourage further research to examine effect of different treatment conditions on unautoclaved samples.

Considering the fact that *B. amyloliquefaciens* stabilizes the soil by calcium carbonate crystals formation, two samples were treated with lime in order to compare the efficiency of the two methods. Using hydrated lime, the following reaction is expected:

$$\text{Ca(OH)}_2 + \text{CO}_2 \rightarrow \text{CaCO}_3 \downarrow + 3\text{H}_2\text{O} \quad (5)$$

Hence, samples 27A and 29A were treated by spraying 2 g and 4 g of hydrated lime with 80 cc and 60 cc of water, respectively (other conditions are as illustrated in Table 2). Having performed wind tunnel erosion tests with sand bombardment on samples 27A and 29A, their mass loss was 5.4600 g/cm² and 1.7950 g/cm², respectively. This large amount of eroded soil may relate to the difference in nature of surface biocementation bonding compared to hydrated lime bonding.

The results of wind erosion tests with disturbed soil surface (group C tests) are illustrated in Table 4. This table includes results of 24 main samples (rows 1 to 24) and 9 negative control samples (rows 25 to 33). This group of tests was carried out to investigate whether it is feasible to compare the results of wind erosion tests on samples with disturbed soil surface (group C tests) with the results of tests on undisturbed samples with sand bombardment (group B tests). Unfortunately, considerable variation in mass loss was observed for identical samples in group C. For instance, samples 1-1, 1-2, 2-1 and 2-2 (which are samples with same treatment) show 0.2325 g/cm², 0.6825 g/cm², 0.2675 g/cm² and 0.1100 g/cm² mass loss, respectively. This variation can only be attributed to different disturbance condition of soil surface. Consequently, correlation between group C results and group B results sounds difficult. However, other results can be inferred from group C tests as follow:

1- Untreated samples U1 and U2 with 2.7575 g/cm² and 2.9375 g/cm² mass loss, respectively, experienced highest erosion in this group of tests. From comparison of these values with treated samples in the same group, it can be concluded that formation of surface crust from biocementation in the proposed method prevents enlargement of disturbed area and thus considerably reduces the wind erosion potential.

2- Samples 35-2 (negative control of bacteria factor) and 39-1 and 39-2 (negative control of bacteria and nutrient factors) had 2.6800 g/cm², 2.4275 g/cm² and 1.3425 g/cm² mass loss, respectively. These mass losses are significantly larger than typical mass losses in main samples (between 0.0250 g/cm² to 0.6825 g/cm²). Again, efficiency of the proposed method is confirmed in the case of disturbed soil surface.

The formation of calcium carbonate in soil samples was explored by XRD analysis. The results are shown in Fig. 5 (and Fig. A1 and Fig. A2). Comparison of carbonate peaks (2Ө= 29.5, 47.6, 48.6) for treated and untreated samples clearly shows higher calcium carbonate content in the treated.

To further explore the formation of calcium carbonate in soil samples, several calcium carbonate equivalent (CCE) tests were performed. Percent carbonate calcium equivalent determined were 62.5, 62.75 and 68.5 for samples 23-1, 14-1M and 1-1 before treatment, respectively, and the corresponding values for the same samples after treatment were 63.25, 64.5 and 68.75. The results indicated that the treated samples have relatively higher calcium carbonate content.
Furthermore, scanning electron microscopy (SEM) was employed to investigate the role of biocement and bonding between soil particles. Fig. 6 illustrates the images of a treated and untreated sample with x100 and x500 magnification. These images confirm formation of carbonate on surface as well as at particle contacts.

4. Conclusion

An attempt was made to study the performance of biological calcium carbonate cementation as a soil stabilization technique using B. amyloliquefaciens heterotrophic bacteria. The bacteria used in present investigation has the advantage of producing environmental friendly by-products compared to some other bacteria used in past. The proposed method was tested at laboratory scale using a closed circuit wind tunnel. Three main groups of samples were tested: undisturbed soil surface and without sand bombardment (group A), undisturbed soil surface and with sand bombardment (group B), and with disturbed soil surface and without sand bombardment (group C). Group A tests showed that treated soils were stabilized against erosion with wind velocities up to 30 m/s. The maximum amount of erosion measured for this group of tests was about 0.01 g/cm². From Group B tests, the following conclusions are drawn:

1. Generally, the biocement calcium carbonate crusts preserved their structure under sand bombardment condition with wind velocities up to 14 m/s to 15 m/s with minor surface erosion. Sand bombardment flux was kept at 120 g/min during all tests. Therefore, the authors believe that the proposed technique has the potential of field application.

2. Among the five factors, temperature, bacteria concentration, amount of nutrient, amount of water and curing time as well as two-factor interactions, curing time, water, temperature-water, and bacteria-water were statistically significant factors and interactions to suppress wind erosion.

3. For the investigated range of curing time (3 to 7 days), the increase in curing time led to decrease in mass loss. Therefore, the field application of present method will be efficient if there is a suitable safe margin of time between treatment application and possible erosive wind.

4. Two levels for water solution were adopted and soil samples were sprayed with water solution of 1.5 and 2 lit/m² which is equal to 60 and 80 cc/sample. From test results, the followings can be concluded:

4-1- In the case of 80 cc water solution, the increase in temperature had no negative effect on performance of the proposed method. However, in the case of lower amount of water solution (60 cc/sample), increase in temperature led to an increase in soil erosion.

4-2- Using the upper level for water solution, soil erodibility was reduced with increase in bacteria concentration. In contrary, for the lower level of water solution, the increase in bacteria concentration did not improve performance of the proposed technique.
5- Negative control tests in this group which included bacteria and nutrient negative control, bacteria negative control and also nutrient negative control, confirmed results of main tests that bacteria contribute as a controlling factor while nutrient not.

6- Results of tests on outdoor treated samples as well as test results from laboratory samples cured at temperatures as high as 50ºC, indicate that uncontrolled field temperature may not be considered as a limitation for the proposed method.

7- Mass loss in lime treated samples was very significant and therefore, lime treatment was not considered as an effective technique for the soil studied in this investigation.

Group C tests indicated that surface biocementation crusts could reduce wind erosion of soil even in the case of partial disturbance.

To sum up, based on laboratory tests, it is concluded that the proposed method can serve as a promising technique to suppress wind erosion sand dust susceptible zones. However, research is encouraged to carry out pilot field tests to study the influence of ambient condition and long term duration on performance of the method.

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Fig. 1: a) Soil gradation curve b) Bacterial growth curve
Fig. 2: Typical wind velocity during different groups of tests
Fig. 3: a) Normal probability plot of residuals of group B main samples b) Plot of residuals versus predicted mass loss of group B main samples
Fig. 4: a) Temperature-water interaction diagram of mass loss of main samples in group B b) Bacteria-water interaction diagram of mass loss of main samples in group B c) Bacteria-water interaction diagram of mass loss of untreated and main samples in group B
Fig. 5: XRD analysis a) example of XRD peaks (upper: sample 18-2 before treatment, middle:sample 18-2 after treatment, lower: reference code of calcite) b) main peak (2θ = 29.5) of calcite before and after treatment for 18-2 sample c) two main peaks (2θ = 47.6, 48.6) of calcite before and after treatment for 18-2 sample
Fig. 6: SEM images of a treated soil sample, magnification a) x100 b) x500 and untreated soil sample, magnification c) x100 and d) x500
Fig. A1: XRD analysis (upper: sample 18-1 before treatment, middle: sample 18-1 after treatment, lower: reference code of calcite)
Fig. A2: XRD analysis (upper: sample 1-2 before treatment, middle: sample 1-2 after treatment, lower: reference code of calcite)

Table 1: Specifications and results of group A tests
Table 2: Specifications and results of group B tests
Table 3: Analysis of variance (ANOVA) for main samples of group B
Table 4: Specifications and results of group C tests
Fig. 1a

Fig. 1b

Fig. 2
Fig. 3a

Internally Studentized Residuals

Fig. 3b

Predicted
Fig. 4a

Fig. 4b

Fig. 4c
Fig. 5a

Fig. 5b
Fig. 5c

Fig. 6a
Fig. 6b

Fig. 6c
Fig. 6d

Fig. A1
Fig. A2
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<th>Sample No.</th>
<th>Temperature (°C)</th>
<th>Bacteria Concentration (O.D.)</th>
<th>Nutrient (g/sample)</th>
<th>Water (g/sample)</th>
<th>Curing (days)</th>
<th>Mass Loss (g/cm²)</th>
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Table 1
| Sample No. | Temperature (ºC) | Bacteria Concentration (O.D.) | Nutrient (g/sample) | Water (g/sample) | Curing (days) | Mass Loss (g/cm²) | Remediation (%) | Comment         |
|------------|------------------|-------------------------------|--------------------|----------------|--------------|------------------|----------------|----------------|----------------|
| 1          | 18-2M            | 28                            | 0.75               | 2              | 60           | 3                | 0.0825          | 97.6           | Main Test       |
| 2          | 18-1M            | 28                            | 0.75               | 2              | 60           | 3                | 0.0650          | 98.1           | Main Test       |
| 3          | 24-2             | 28                            | 0.75               | 4              | 80           | 7                | 0.3375          | 90.0           | Main Test       |
| 4          | 12-1M            | 28                            | 1.5                | 2              | 80           | 7                | 0.1175          | 96.5           | Main Test       |
| 5          | 14-1M            | 28                            | 1.5                | 4              | 60           | 7                | 0.0050          | 99.9           | Main Test       |
| 6          | 15-M             | 28                            | 1.5                | 4              | 80           | 3                | 0.3150          | 90.7           | Main Test       |
| 7          | 20-1M            | 37                            | 0.75               | 2              | 80           | 7                | 0.2250          | 93.4           | Main Test       |
| 8          | 6-2              | 37                            | 0.75               | 4              | 60           | 7                | 0              | 100.0          | Main Test       |
| 9          | 23-M             | 37                            | 0.75               | 4              | 80           | 3                | 0.5350          | 84.2           | Main Test       |
| 10         | 10-2             | 37                            | 1.5                | 2              | 60           | 7                | 0.1025          | 97.0           | Main Test       |
| 12         | 27-2M            | 37                            | 1.5                | 2              | 80           | 3                | 0.2400          | 92.9           | Main Test       |
| 12         | 29-2M            | 37                            | 1.5                | 4              | 60           | 3                | 0.5475          | 83.8           | Main Test       |
| 13         | 39-1M            | 37                            | 0                  | 0              | 80           | 3                | 2.4250          | 28.5           | Negative Control|
| 14         | 39-2M            | 37                            | 0                  | 0              | 80           | 3                | 3.3900          | 0.0            | Negative Control|
| 15         | 35-1M            | 37                            | 0                  | 2              | 80           | 3                | 0.9475          | 72.1           | Negative Control|
| 16         | 37-1M            | 37                            | 0                  | 4              | 80           | 3                | 1.0375          | 69.4           | Negative Control|
| 17         | 44-1M            | 37                            | 0.75               | 0              | 80           | 3                | 0.1950          | 94.2           | Negative Control|
| 18         | 45-1M            | 37                            | 1.5                | 0              | 80           | 3                | 0.1375          | 95.9           | Negative Control|
| 19         | 27H              | 50                            | 1.5                | 2              | 80           | 3                | 0.1475          | 95.6           | High Temperature Condition |
| 20         | 29H              | 50                            | 1.5                | 4              | 60           | 3                | 1.1600          | 65.8           | High Temperature Condition |
| 21         | 27-1F            | *                             | 1.5                | 2              | 80           | 3                | 0.2775          | 91.8           | Outdoor Condition |
| 22         | 27-2F            | *                             | 1.5                | 2              | 80           | 3                | 0.2900          | 91.4           | Outdoor Condition |
| 23         | 29-1F            | *                             | 1.5                | 4              | 60           | 3                | 0.5900          | 82.6           | Outdoor Condition |
| 24         | 29-2F            | *                             | 1.5                | 4              | 60           | 3                | 0.4750          | 86.0           | Outdoor Condition |
| 25         | 27U              | 37                            | 1.5                | 2              | 80           | 3                | 0.1250          | 96.3           | Unautoclaved Soil |
| 26         | 27A              | 37                            | 0                  | 2              | 80           | 3                | 5.4600          | (-61.1)        | Lime Treatment |
| 27         | 29A              | 37                            | 0                  | 2              | 60           | 3                | 1.7950          | 47.1           | Lime Treatment |

1: Outdoor condition, temperature varying from 7ºC to 18ºC
2: Lime content for lime treated samples
3: Table 2

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Mohammad Mehdi Mohebbi received his M.S. in Geomechanics from Tehran University in 2004 and has been working on his Ph.D. thesis since 2013. He has published 5 papers in credited journals and international conference proceedings.

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