A laboratory investigation on suppression of dust from wind erosion using biotechnology

Etude en laboratoire sur la suppression de l'érosion éolienne à l'aide de la biotechnologie

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ABSTRACT: Dust events are among the serious environmental challenges in some countries. Several methods such as windbreak and artificial covers are available to prevent soil wind erosion, but sustainable and innovative solutions can be applied to tackle this problem by considering soil as a living ecosystem. In present research, bacterial species of Bacillus amyloliquefaciens that live naturally in soil was used. First, bacterial were cultivated until specified concentration. Next, bacterial cells and nutrients in the form of water solution were sprayed on the soil surface to form "soil crust". Different treatments of soil were made on the basis of five varied factors (temperature, bacterial cells concentration, nutrients dosage, amount of water and curing time). Then, 200×200×50 mm samples were tested in a closed circuit wind tunnel having 800×800 mm test section. The implemented method for stabilization of soil was efficient with minimum environmental side effects compared to other biotechnological methods. Moreover, based on the results of tests, among five treatment factors and two-factor interactions, curing duration, water amount, temperature-water interaction and bacterial cells-water interaction were found to be of considerable significance.

RÉSUMÉ Événements de poussière sont parmi les défis environnementaux les plus importants dans certains pays. Plusieurs méthodes telles que les brise-vent et couvertures artificielles sont disponibles pour empêcher l'érosion éolienne des sols. Cependant des solutions innovantes et durables peuvent être appliquées pour résoudre ce problème en considérant le sol comme un écosystème vivant. Dans la présente recherche, des espèces bactériennes de Bacillus amyloliquefaciens qui vivent naturellement dans le sol ont été utilisées. Tout d'abord, les bactéries ont été cultivées selon une concentration spécifiée. Ensuite, les cellules bactériennes et les éléments nutritifs sous forme de solution aqueuse ont été pulvérisés sur la surface du sol pour former « croûte du sol ». Différents traitements de sol ont été appliqués en variant cinq facteurs (température, concentration des bactéries, dosage des substances nutritives, quantité d'eau et le temps de durcissement). Puis, les échantillons de taille 200×200×50 mm ont été testés dans une soufflerie de circuit fermé ayant une section d'essai de 800×800 mm. La méthode implémentée pour la stabilisation du sol a été plus efficace en comparaison avec les autres méthodes biotechnologiques.

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 2008); it is accompanied by economical aspects such as reduction of agricultural production.

In recent years, biotechnology has made it possible to use microorganisms to stabilize the soil with considering minimum adverse effects on environment. Some of the previous main researches in which soil stabilization was carried out using bacteria for dust suppression are Anderson et al. (2012), Liu et al.(2008), Meyer et al. (2011) and O'Brien and Neuman (2012). 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 spharicus species of bacteria had been used and the fatal ammonia by-product was obtained.

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:

$$(CH_3COO)_2Ca + 4O_2 \rightarrow CaCO_3 \downarrow + 3CO_2 \uparrow + 3H_2O \quad (1)$$

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.

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. 1. 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.

Silica sand with grain distribution between 425µm and 595µm was used for sand bombardment. Several researchers (Bagnold 1941; Scott 1995; Shao et al. 1996) 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 bacteria was selected for this research. For the present study, the lyophilized powder of the bacterial biomass, strain PTCC No. 1732, 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 (Mecrk®) was used as a source of energy.

2 .2 *Bacteria culture*

B. amyloliquefaciens bacteria were cultured in LB liquid medium inside shaker incubator with 110 rpm and 28ºC. Optical density (O.D.) of bacteria solution was determined by spectrophotometer which was set at a wavelength of 600 nm.

2 .3 *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/m^3. Soil samples were compacted to a density close to insitu condition. Next, the predetermined amounts of bacterial cells and nutrient solution were sprayed on the surface of soil samples. Finally, samples were cured at designed temperature and duration.

2 .4 *Wind tunnel tests*

A closed circuit tunnel was used for simulation of wind erosion in laboratory. This tunnel is ISI model 6407 ZAF with 800×800 mm test section and maximum wind speed of 100 m/s.

Seven spires were designed based on Irwin (1981) 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.

Several researches used different mass fluxes for sand bombardment, for instance, Neuman et al. (1996) 0.007 kg/m/s, Langston and Neuman (2005) 0.015 kg/m/s and Neuman and Maxwell (1999) 0.014kg/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 (120gr/min for each sample). The bombardment was performed by a separating funnel, a connector steel tube and a modified upholstery nozzle.

In wind tunnel 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 14m/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.

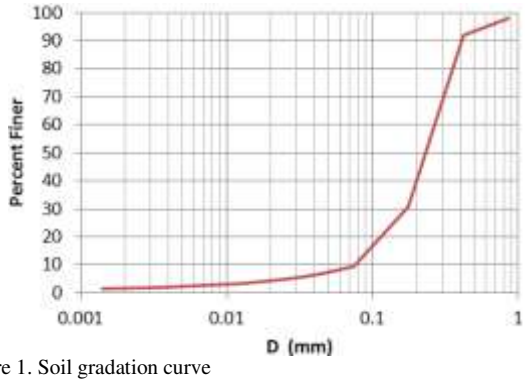

Figure 1. Soil gradation curve

2 .5 *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, amount of available nutrient for bacteria, moisture at the soil surface and curing time. For determination of levels of each factor, the following considerations were taken into account:

a) Temperature levels: Two temperature levels were considered to study temperature effects, namely, 28 ºC and 37 ºC.

b) 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.

c) Nutrient: For treatments of samples, nutrient levels were considered as low as 0.05 and 0.1 kg/m^2 (2 and 4 g/sample).

d) Soil moisture: The total amount of sprayed water on the soil surface was selected at 2 levels of 1.5 and 2 lit/m^2 (60 and 80 cc/sample).

e) Curing time: Two levels of 3 and 7 days were considered to cover short term and long term curing of the soil.

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 main tests were reduced from 32 to 12.

3 RESULTS

Remediation is defined as:

$$Remediation(\%) = \frac{Untreated\ Mass\ Loss - Treated\ Mass\ Loss}{Untreated\ Mass\ Loss} \times 100 \quad (2)$$

Table 1 demonstrates the results of wind erosion tests with sand bombardment. In this table, 12 main samples, 6 negative control samples and 7 samples covering different curing are addressed.

Table 2 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.

Each factor was coded according to the equation:

$$\bar{X}_i = \frac{X_i - (X_{i,\max} + X_{i,\min})/2}{(X_{i,\max} - X_{i,\min})/2} \quad (3)$$

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:

$$mass loss(gr/cm^2) = 0.210 + 0.020 \times \overline{T} - 0.019 \times \overline{B} + 0.081 \times \overline{W} - 0.015 \times \overline{C} - 0.079 \times \overline{T} \times \overline{W} - 0.120 \times \overline{B} \times \overline{W} \quad (4)$$

where $\overline{T}, \overline{W}, \overline{B}$ and $\overline{C}$ 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:

a) Curing time factor has the most significant effect on wind erosion reduction in the present method.

b) 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:

b-1) Fig. 3a 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.

b-2) Bacteria-water interaction is illustrated in Fig. 3b, 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.

c) Negative control tests, samples 39-1M and 39-2M in table 2, which had no bacteria and nutrient experienced 2.4250 g/cm^2 and 3.3900 g/cm^2 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. 3c. This finding reemphasizes the efficiency of the proposed approach. Negative control tests, samples 35-1M and 37-1M (samples without bacteria) demonstrated 0.9475 g/cm^2 and 1.0375 g/cm^2 mass loss while negative control tests, samples 44-1M and 45-1M (samples without nutrient) showed 0.1950 g/cm^2 and 0.1375 g/cm^2 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 50ºC instead of 37ºC. The amount of erosion in samples 27H and 27-2M (with high level of water factor) was 0.1475g/cm^2 and 0.2400g/cm^2 while the mass loss in samples 29H and 29-2M (with low level of water factor) were 1.1600g/cm^2 and 0.5475g/cm^2, 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 7ºC to 18ºC. Samples 27-1F and 27-2F experienced 0.2775g/cm^2 and 0.2900g/cm^2 mass loss, respectively, compared to 0.2400g/cm^2 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.

Table 1. Specifications and results of wind tunnel tests.

Sample No.	Treatment					Mass Loss (g/cm^2)	Remediation (%)	Comment
	Temperature (ºC)	Bacteria Concentration(O.D.)	Nutrient (g/sample)	Water (g/sample)	Curing (days)			
18-2M	28	0.75	2	60	3	0.0825	97.6	M.T.
18-1M	28	0.75	2	60	3	0.0650	98.1	M.T.
24-2	28	0.75	4	80	7	0.3375	90.0	M.T.
12-1M	28	1.5	2	80	7	0.1175	96.5	M.T.
14-1M	28	1.5	4	60	7	0.0050	99.9	M.T.
15-M	28	1.5	4	80	3	0.3150	90.7	M.T.
20-1M	37	0.75	2	80	7	0.2250	93.4	M.T.
6-2	37	0.75	4	60	7	0	100.0	M.T.
23-M	37	0.75	4	80	3	0.5350	84.2	M.T.
10-2	37	1.5	2	60	7	0.1025	97.0	M.T.
27-2M	37	1.5	2	80	3	0.2400	92.9	M.T.
29-2M	37	1.5	4	60	3	0.5475	83.8	M.T.
39-1M	37	0	0	80	3	2.4250	28.5	N.C.
39-2M	37	0	0	80	3	3.3900	0.0	N.C.
35-1M	37	0	2	80	3	0.9475	72.1	N.C.
37-1M	37	0	4	80	3	1.0375	69.4	N.C.
44-1M	37	0.75	0	80	3	0.1950	94.2	N.C.
45-1M	37	1.5	0	80	3	0.1375	95.9	N.C.
27H	50	1.5	2	80	3	0.1475	95.6	H.T.C.
29H	50	1.5	4	60	3	1.1600	65.8	H.T.C.
27-1F	a	1.5	2	80	3	0.2775	91.8	O.C.
27-2F	a	1.5	2	80	3	0.2900	91.4	O.C.
29-1F	a	1.5	4	60	3	0.5900	82.6	O.C.
29-2F	a	1.5	4	60	3	0.4750	86.0	O.C.

M.T.: Main Test, N.C.: Negative Control, H.T.C.: High Temperature Condition, O.C.: Outdoor Condition

[a]: Outdoor condition, temperature varying from 7ºC to 18ºC

[b]: Lime content for lime treated samples

Table 2. Analysis of variance (ANOVA) for main samples of wind tunnel test

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.3741	6	0.0623	18.19	0.0030	Significant
T:Temperature	0.0043	1	0.0043	1.27	0.3114	
B: Bactria	0.0039	1	0.0039	1.14	0.3343	
W: Water	0.0780	1	0.0780	22.76	0.0050	
C: Curing	0.2014	1	0.2014	58.75	0.0006	
T×W	0.0565	1	0.0565	16.48	0.0097	
B×W	0.1334	1	0.1334	38.91	0.0016	
Residual	0.0171	5	0.0034			
Lack of Fit	0.0170	4	0.0042	27.73	0.1414	Not significant
Pure Error	0.0002	1	0.0002			

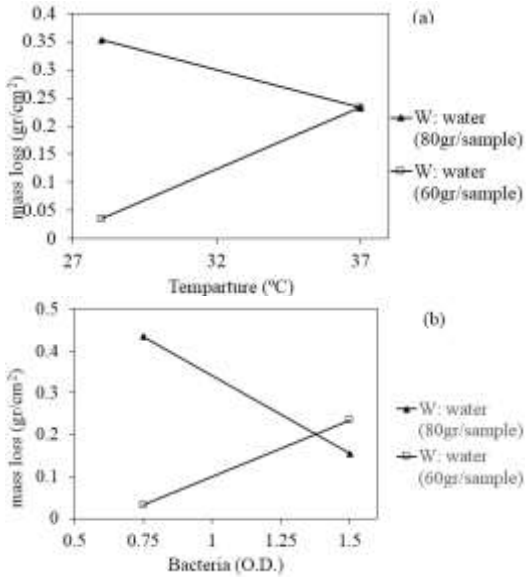

Figure 2. a) Temperature-water interaction diagram of mass loss of main samples b) Bacteria -water interaction diagram of mass loss of main samples

3 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 researches such as Meyer et al. (2011), and Anderson et al. (2012). This comparison is also valid between present method and some enzyme based methods such as Knorr (2014).

Some previous methods used biopolymers as the binding cement to control wind erosion (e.g. Alsanad 2011 and Kavazanjian et al. 2009). Due to degradability of biopolymers, these methods are considered as short term control systems. For instance, Alsanad (2011) explained that "filed experiments indicated that topically applied biopolymer should be durable for a period of at least one or two weeks in the absence of rain fall". Contrary to the above methods, the calcium carbonate biocement produced in this investigation is a stable and durable cementing agent and hence, it may be considered as a long term technique to suppress wind erosion.

The proposed method was tested at laboratory scale using a closed circuit wind tunnel.

From tests, the following conclusions are drawn:

a- 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.

b- 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.

c- 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.

d- 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:

d-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.

d-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.

e- 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.

f- 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.

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