

## Evaluation of stabilized soil by *Bacillus* sp. HAI4 in different conditions through Taguchi method

Mehran Alavi\*

PhD Candidate of Nano-Biotechnology, Microbiology Laboratory, Biology Department, Razi university, Kermanshah, Iran, mehranbio83@gmail.com

Mojtaba Taran

Associate Professor of Microbiology, Razi University, Kermanshah, Iran, mojtabataran@yahoo.com

Arezoo Berimavandi

MSc of Microbiology, Microbiology Laboratory, Biology Department, Razi University, Kermanshah, Iran, arezoo.berimavandi@gmail.com

### Abstract

**Introduction:** The process of calcite precipitation resulted from metabolic activities by microorganisms is biocalcification. In this process, calcite ( $\text{CaCO}_3$ ) is deposited on the soil grains by metabolic activities of microorganisms resulting in increased stiffness/strength and reduced erodibility of soil.

**Materials and methods:** In this study, urea hydrolysis and calcite precipitation rates in soil stabilization process were evaluated by the bacterium *Bacillus* sp. HAI4 through Taguchi method. Also, it was designed a wind tunnel for analysis of soil stabilization rate.

**Results:** In this investigation, three factors ( $\text{NH}_4\text{Cl}$ , urea and molasses) were surveyed in optimization of soil stabilization. Results of this study illustrated that Taguchi method is one of effective methods for optimization of effective factors in soil stabilization process. In this case, urea concentration had higher effect on soil stabilization and calcite sedimentation by *Bacillus* sp. Also, evaluating factors interaction showed relationship of factors together. Three levels (0.5, 1 and 1.5 g/L), were determined for  $\text{NH}_4\text{Cl}$  factor in which the highest effect (4.353%) in soil stabilization was related to level 3. Increasing of  $\text{NH}_4\text{Cl}$  resulted in high soil stabilization rate. Three levels 20, 30, and 40 g/L of urea were applied. Results illustrated that effective level was 3 with 4.313%. Also, Taguchi design was in this study, as three levels of molasses (30, 40 and 50 gr/L) illustrated maximum soil stabilization in level 2 by 3.873%. In fact, by increasing of molasses, soil stabilization firstly increased and then decreased.

**Discussion and conclusion:** Totally, this study shows two factors ( $\text{NH}_4\text{Cl}$  and urea) that have a major effect in urea hydrolysis and calcite precipitation rates.

**Key words:** Biocalcification, Soil stabilization, *Bacillus* sp., Urea hydrolysis, Calcite precipitation, Ammonium chloride

---

\*Corresponding Author

## Introduction

Biocalcification has illustrated some promise in soil cementation by microbially induced calcite precipitation (1, 2). In this process, calcite ( $\text{CaCO}_3$ ) is deposited on the soil grains by metabolic activities of microorganisms resulting in increased stiffness/strength and reduced erodibility of soil. In this case, urea hydrolysis by some bacteria such as *Bacillus pasteurii* or *sporosarcina pasteurii* can lead to calcite precipitation as crystals (3-5).

Using this method has illustrated a promise in several fields such as strengthening of concrete and cracks remediation (6, 7), remediation of copper-contaminated soil (8), improving of strength and durability of fly ash-amended concrete (9), Biomineralization based remediation of As(III) contaminated soil (10, 11), Bioremediation of Pb-contaminated soil (12), improvement in the durability of cement mortar (13) and improving of the stiffness/strength of sandy soil (14).

Biomineralization as production of minerals by living organisms (14) is resulted from metabolic activities of some organisms including invertebrates (carbonate), vertebrates (carbonates, phosphates and calcium), algae and diatoms (silicate) (14, 15). In the case of biologically induced mineralization (BIM), the minerals are produced extracellular by metabolic activities of the living organisms (16).

Four important factors which affect calcite precipitation include 1) concentration of the calcium, 2) dissolved inorganic carbon (DIC) concentration, 3) the pH and (4) the availability of nucleation sites (17-19). Different bacterial species can precipitate carbonates in environment with high pH and  $\text{Ca}^{2+}$  ions. In addition, various mechanisms can stimulate this

process by bacteria (17, 20). Ability of bacteria to create environment with alkaline condition are important mechanisms. Carbonate precipitation can be developed as extracellular by successive stratification and it leads to growing carbonate crystals on the bacteria surface (21-23).

In order to calcium carbonate production, several studies applied urea and  $\text{CaCl}_2$  combinations. For example, different combinations of 0.33 and 0.67 M urea and 0.0025, 0.025 and 0.25M  $\text{Ca}^{2+}$  solution were used by Okwada and coworkers (2010). The results of this study, illustrate that the rate of ureolysis increases with bacterial cell concentration, and the bacterial cell concentration had a greater influence on  $k(\text{urea})$  than initial urea concentration (24). Also, application of lower chemical amounts used over multiple injections resulted in more uniform cementation (25). In this study, urea hydrolysis and calcite precipitation rates in soil stabilization process were evaluated for the bacterium *Bacillus* sp. HAI4 by Taguchi method in three levels of three factors ( $\text{NH}_4\text{Cl}$ , urea and molasses).

## Materials and methods

**Particle size analysis of soil:** Sufficient aggregated samples were obtained from Wetland Hooralazim of Khuzestan province in Iran. In order to analyze soil particle size, a gradation test was performed on a soil sample in a laboratory(26). In this case, a determined weighed sample was poured into the top sieve which had a set of sieves with progressively smaller openings (Fig. 1). A mechanical shaker was used for shaking of sieve column. The results were demonstrated in a graph of percent passing versus the diameter of sieves (4.75, 2.36, 2, 1.4, 1.18, 0.85, 0.71, 0.6, 0.425, 0.3, 0.25, 0.15, 0.075) (Table 1).



Fig. 1- Used columns of laboratory sieve in this study

Table 1- Sieve analysis results are generally expressed as the percentage of the total weight of soil that passed through different sieves

Sieve number	Diameter (mm)	Mass of soil retained on each sieve (g)	Percent retained (%)	Cumulative passed (%)
4	4.75	18.53	18.53	81.47
8	2.36	11.9	30.43	69.57
10	2	2.87	33.3	66.7
14	1.4	8.51	41.81	58.19
16	1.118	3.45	45.26	54.74
20	0.85	4.3	49.56	50.44
25	0.71	5.44	55	45
30	0.6	0.178	55.178	44.822
40	0.425	7.866	63.044	36.956
50	0.3	4.58	67.624	32.376
60	0.25	1.54	69.164	30.836
100	0.15	9.36	78.524	21.476
200	0.075	8.018	86.542	13.458
pan	pan	13.458	100	0
		100		

**Bacteria preparation and treatment:** *Bacillus* sp. HAI4 species were isolated from Wetland Hooralazim of Khuzestan province in Iran. Species were grown in broth nutrient at 30C for 24 hours in incubator. In order to bacterial calcite precipitation, nutrient broth-urea containing

30 g urea, 30 g molasses, 5 g NH<sub>4</sub>Cl and 5 g milk powder per liter (10pH) was autoclaved, cooled and combined immediately with desired concentration of filtered (0.2 Mm) and sterilized CaCl<sub>2</sub> before application onto soil. Culture medium was incubated at 30 °C for 48 hours. After time incubation, prepared bacteria were colored by methylene blue.

**Optimization of soil stabilization by Bacteria:** For obtain optimum condition of soil stabilization by bacteria, Taguchi method was applied in three levels for three factors (NH<sub>4</sub>Cl, urea and molasses) (Table 2). In this case, nine experiments were designed (Table 3). Milk powder concentration (0.1 g/L) was fixed for each experiment.

Table 2- Design of three factors with three levels by Taguchi method

Factors (g/L)	Level 1	Level 2	Level 3
NH <sub>4</sub> Cl	0.5	1	1.5
Urea	20	30	40
Molasses	30	40	50

Table 3- Nine experiments were designed by Taguchi method

Experiment number	NH <sub>4</sub> Cl (Level):(g/L)	Urea (Level):(g/L)	Molasses (Level):(g/L)
1	1= 0.5	1= 20	1= 30
2	1= 0.5	2= 30	2= 40
3	1= 0.5	3= 40	3= 50
4	2= 1	1= 20	2= 40
5	2= 1	2= 30	3= 50
6	2= 1	3= 40	1= 30
7	3= 1.5	1= 20	3= 50
8	3= 1.5	2= 30	1= 30
9	3= 1.5	3= 40	2= 40

In addition to nine designed experiments, two experiments were designed as control (Table 4). One experiment, number ten, contains treatment solution without bacteria and other experiment contains only bacteria without treatment solution.

Table 4- Control experiment designed by Taguchi Method

Experiment number	NH <sub>4</sub> Cl (g/L)	Urea (Level):(g/L)	Molasses (Level):(g/L)
10	2= 1	2= 30	2= 40

On the first day, eleven flasks contain 100 g of soil were prepared and 70 ml of bacteria with 0.5 McFarland was added to all flasks apart from control flask with number 10. Afterwards, 100 ml of treatment solutions were prepared and since the second day, 20ml of solutions were added to soils apart from controls for five days. Every day, after adding solutions, soils were incubated at 30C for 24 hours.

**Biological cementation:** Treatment solutions (100 mm) were prepared with adding 2.2g calcium chloride (CaCl<sub>2</sub>). Afterwards, 20 mm of treatment solution were added to each soil for 5 days (apart from control without bacteria). Due to complete drying, after five days, all soils were incubated for one month at 30 C.

**Wind tunnel testing:** To analyze soil fixation, wind velocity was measured by design wind tunnel (27). Wind velocity was evaluated by the following formula:

$$v^2 = \frac{mg \tan\theta}{\frac{1}{2} \rho A}$$

Where v as velocity

m=7.5×10<sup>-3</sup> kg (mass of bullet)

g=9.8 m/s<sup>2</sup>

tanθ as angle of deviation

C as constant of integration equals 0/6

ρ as air density equals 1.3kg/m<sup>3</sup>

A as area of bullet.

In this case, two experiments at two different angles (8° and 12°) were designed.

After this stage, each of weighted soil samples was exposed to wind velocity by 14.56 km/h. Then, reduced weight of soil samples was measured through weigh again of samples (Table 5). This process was applied for wind velocity by 18km/h.

$$\tan 8^\circ = 0.14$$

$$v_1^2 = \frac{7/5 \times 10^{-3} \times 9/8 \times \tan 8^\circ}{1/2 \times 0/6 \times 1/3 \times 16 \times 10^{-4}} = 16/55 \text{ m}^2/\text{s}^2$$

$$\Rightarrow v_1 = 4/1 \text{ m/s} = 14/56 \text{ km/h}$$

$$\tan 12^\circ = 0/21$$

$$v_2^2 = \frac{7/5 \times 10^{-3} \times 9/8 \times \tan 12^\circ}{1/2 \times 0/6 \times 1/3 \times 16 \times 10^{-4}} = 25 \text{ m}^2/\text{s}^2$$

$$\Rightarrow v_2 = 5 \text{ m/s} = 18 \text{ km/h}$$



Fig. 2- Applied wind tunnel in this study

## Results

**Soil fixation:** Interaction of factors and optimum conditions in soil stabilization was evaluated by Qualitek-4 software. Soil stabilization rate in 11 experiments illustrates those bacteria in medium by 1g/L ammonium Chloride, 40 g/L urea and 30 g/L molasses, had maximum of soil stabilization in experiment number 6.

Table 6, demonstrates effect of each factors and their levels on soil fixation. In this case, best level is for ammonium chloride at third level (1.5 g/L), Urea at third level (40g/L), molasses at second level (40g/L).

Table 5- Amount of reduced weight of soil samples in v=18km/h

Sample number	Weight of sample before wind tunnel testing	Weight of sample after wind tunnel testing	Reduction percent
1	23.5	22.85	2.77
2	15	14.7	2
3	35	34	1.45
4	31	30.7	0.97
5	27	26.2	2.96
6	25	24.7	3.61
7	18	16.5	12.5
8	16.3	15.7	3.68
9	20.3	18.7	7.88
10 (first control)	29.2	28.4	2.74
11(second control)	33	30.3	8.18

Table 6- Effect of different level of factors on soil stabilization by *Bacillus* sp.

Factors	Level 1	Level 2	Level 3
NH <sub>4</sub> Cl	2.523	2.513	4.353
Urea	1.49	3.136	4.313
Molasses	3.096	3.873	1.969

Figure 3a shows effect of ammonium chloride (nitrogen source) on soil fixation. There was a low amount of soil stabilization (2.073) in level 1, and this amount raised (2.513) in level 2, and there was a maximum amount by 4.353 in level 3.

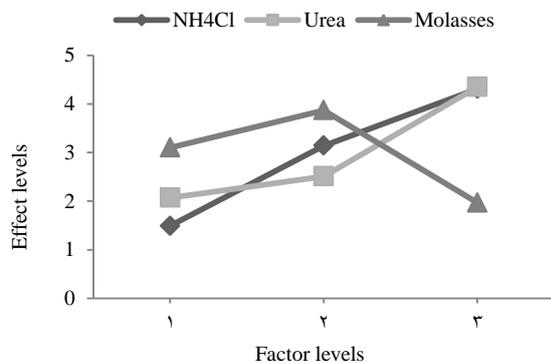


Fig. 3- Effect of different levels of NH<sub>4</sub>Cl, urea and molasses on soil stabilization by *Bacillus* sp.

Effect of urea factor on soil stabilization is illustrated in figure 3b. Amount of soil stabilization increased form level 1 by 1.49 to level 3 by 4.313. This result indicates that urea has direct effect on soil fixation. Also, effect of molasses on soil stabilization is demonstrated in figure 3c. In this case, there was increasing of soil

stabilization form level 1 (3.096) to level 2 (3.873), but in level 3 (1.969), reduction of this process was observed.

**Interaction of factors on soil stabilization by *Bacillus* sp. HAI4:** Table 7 shows factors interaction on soil stabilization. Interactions of paired factors were evaluated based on amount of selected two factors which was changeable form 0.86 to 24.67. Maximum interaction was 24.67 for NH<sub>4</sub>Cl×molasses that NH<sub>4</sub>Cl was in level 3 and molasses was in level 2. Also, Interaction of urea×molasses had minimum intensity of interaction (0.86) as urea in level 3 and molasses in level 2. NH<sub>4</sub>Cl and urea in level 3 had 8.82 of interaction.

**Variance analysis (ANOVA) for soil stabilization by *Bacillus* sp. HAI4:** Variance analysis (ANOVA) illustrates effective parameters on soil stabilization by *Bacillus* sp HAI4. Table 8 demonstrates molasses factor had not effect but NH<sub>4</sub>Cl and urea factors had higher effect by 1.869 and 11.412 respectively. Maximum effect is related to urea which shows a major role of nitrogen in soil fixation.

**Estimation of optimum conditions for soil stabilization:** Table 9 illustrates share of each factor in soil stabilization. Based on these results, urea and NH<sub>4</sub>Cl factors have higher and lower shares. Expected result in optimum conditions of soil stabilization was -0.428%. NH<sub>4</sub>Cl and urea in level 1 and molasses in level 3 had suitable levels.

Table 7- Interaction effect of paired factors on soil stabilization by *Bacillus* sp.

Number	Effect of paired factors	Interaction of factors	Intensity of interaction factors	Column	Optimum conditions
NH <sub>4</sub> Cl×molasses	1	1×3	24.67	2	2.2
NH <sub>4</sub> Cl×urea	2	1×2	8.82	3	2.1
Urea×Molasses	3	2×3	0.86	1	1.2

Table 8- Variance analysis in soil stabilization by *Bacillus* sp.

Factors	DOF (f)	Sum of squares (S)	Variance (V)	F-Ratio (F)	Pure Sum (S)	Percent (%)
NH <sub>4</sub> Cl	2	8.777	4.388	1.076	0.644	1.869
Urea	2	12.067	6.033	1.483	3.934	11.412
Molasses	2	5.495	2.747	0.675	0	0
Total	8	34.473				100%

Table 9- Optimum conditions for soil stabilization by *Bacillus* sp. HAI4

Column/Factor	Level description	Level	Contribution
NH <sub>4</sub> Cl	Factor A-Level 1	1	-0.907
Urea	Factor B-Level 1	1	-1.49
Molasses	Factor C-Level3	3	-1.011
Total contribution from all factors			-3.409
Current grand average of performance			2.98
Expected result at optimum condition			-0.428

## Discussion and conclusion

Today, stabilization of soil and dust by microorganisms is important and because of climate change, desertification and dust storm can effect on environment and human health (28). Stabilization of soils by microorganisms can be effected by different factors such as calcium, pH, and temperature and urea concentration. Therefore, improvement of soil stabilization by microorganisms can lead to increasing microorganisms capacity in soil stabilization (1).

Results of this study illustrated that Taguchi method is one of the effective methods for optimization of effective factors in soil stabilization process. In this method, three factors (NH<sub>4</sub>Cl, urea and molasses) were surveyed in soil fixation optimization. In this case, urea concentration had higher effect on soil stabilization and calcite sedimentation by *Bacillus* sp HAI4. Also, interaction of two factors shows dependence of factors together.

Meyer and coworkers (2011) used

NH<sub>4</sub>Cl with 10g/L concentration as nitrogen source for *Sarcina pasteurii* in soil stabilization (29). This group observed that soil samples as a treatment in wind tunnel by 40 km/h wind velocity had higher mass than controls. In this investigation, three levels (0.5, 1 and 1.5 g/L) were determined for this factor in which the highest effect (4.353) in soil stabilization was related to level 3 of NH<sub>4</sub>Cl (Table 6). Therefore, by increasing NH<sub>4</sub>Cl concentration, soil stabilization increased (Fig. 3). The reason of this change is high ammonium anion for rising of pH, urea hydrolysis and calcite sedimentation.

An important process in ecosystems ammonium oxidation is carried by autotrophic ammonia-oxidizing bacteria (AOB) and change in diversity and quantity of total bacteria is resulted from the application of urea to the soil (30). Also, Neg and coworkers (2012) applied 0.25M of urea for *Bacillus megaterium* in order to stabilize soil samples. In our study, three levels 20, 30, and 40 g/L of urea were utilized. Results illustrated that effective

level was 3 with 4.313% (14). Therefore, it can result in a way that by increasing urea concentration, soil stabilization increases. Hereon, urea hydrolysis was augmented and leads to calcite sedimentation. Similarly, Dejong et al (2006) illustrated suitable result of soil stabilization in sandy samples by using 20 g/L of urea for *Sarcina pasteurii* (31). Amount of 250 mmol in groundwater samples for *Sarcina pasteurii* was used in order to close fracture and porosity in stones for inhibition of groundwater flows and contaminant transport.

Molasses is another factor that was evaluated in soil fixation. Molasses with 1 g/L was applied for *Sarcina pasteurii* in order to prevent water leakage in groundwater. Taguchi design in this study, as three levels of molasses (30, 40 and 50 g/L) illustrated maximum soil stabilization in level 2 by 3.873%. In fact, by increasing of molasses, soil stabilization firstly increased and then decreased (Figure 3). Before initiation of urea hydrolysis, molasses resulted in biomass augmentation; therefore this process causes pH decrease. Therefore, molasses increasing does not have an effective role in soil fixation.

### Acknowledgements

The authors gratefully acknowledge the financial and logistic assistance provided by biology department of Razi University.

### References

- (1) Dhama NK, Reddy MS, Mukherjee A. Biomineralization of calcium carbonates and their engineered applications: a review. *Frontiers in Microbiology*. 2013 10/2907/31/received10/05/accepted;4:314. PubMed PMID: PMC3810791.
- (2) Ebrahimian M, Motamedi H, Shafiei M. *Citrobacter farmeri* phas32, an isolate from bean (*Phaseolus vulgaris*) farm soil with high phytase production. *Biological Journal of Microorganism* 2017 12/22;6(24):55-65. en.
- (3) Achal V, Mukerjee A, Sudhakara Reddy M. Biogenic treatment improves the durability and remediates the cracks of concrete structures. *Construction and Building Materials*. 2013 11//;48:1-5.
- (4) Achal V, Mukherjee A, Reddy M. Microbial Concrete: Way to Enhance the Durability of Building Structures. *Journal of Materials in Civil Engineering*. 2010 2011/06/01;23(6):730-4.
- (5) Achal V, Mukherjee A, Basu PC, Reddy MS. Strain improvement of *Sporosarcina pasteurii* for enhanced urease and calcite production. *Journal of industrial microbiology & biotechnology*. 2009 Jul;36(7):981-8. PubMed PMID: 19408027. Epub 2009/05/02. Eng.
- (6) Achal V, Mukherjee A, Reddy MS. Effect of calcifying bacteria on permeation properties of concrete structures. *Journal of industrial microbiology & biotechnology*. 2011 Sep;38(9):1229-34. PubMed PMID: 21104104. Epub 2010/11/26. Eng.
- (7) Park SJ, Park YM, Chun WY, Kim WJ, Ghim SY. Calcite-forming bacteria for compressive strength improvement in mortar. *Journal of microbiology and biotechnology*. 2010 Apr;20(4):782-8. PubMed PMID: 20467254. Epub 2010/05/15. Eng.
- (8) Achal V, Pan X, Zhang D. Remediation of copper-contaminated soil by *Kocuria flava* CR1, based on microbially induced calcite precipitation. *Ecological Engineering*. 2011 10//;37(10):1601-5.
- (9) Achal V, Pan X, Özyurt N. Improved strength and durability of fly ash-amended concrete by microbial calcite precipitation. *Ecological Engineering*. 2011 4//;37(4):554-9.
- (10) Achal V, Pan X, Fu Q, Zhang D. Biomineralization based remediation of As(III) contaminated soil by *Sporosarcina ginsengisoli*. *Journal of hazardous materials*.

- 2012 Jan 30;201-202:178-84. PubMed PMID: 22154871. Epub 2011/12/14. Eng.
- (11) Karimi N, Alavi M. Arsenic contamination and accumulation in soil, groundwater and wild plant species from Qorveh County, Iran. *Biharean Biologist* 2016; 10(2): 69-73.
- (12) Achal V, Pan X, Zhang D, Fu Q. Bioremediation of Pb-contaminated soil based on microbially induced calcite precipitation. *Journal of microbiology and biotechnology*. 2012 Feb;22(2):244-7. PubMed PMID: 22370357. Epub 2012/03/01. Eng.
- (13) Park SJ, Park JM, Kim WJ, Ghim SY. Application of *Bacillus subtilis* 168 as a multifunctional agent for improvement of the durability of cement mortar. *Journal of microbiology and biotechnology*. 2012 Nov;22(11):1568-74. PubMed PMID: 23124349. Epub 2012/11/06. Eng.
- (14) Anbu P, Kang C-H, Shin Y-J, So J-S. Formations of calcium carbonate minerals by bacteria and its multiple applications. SpringerPlus. 2016 03/0110/21/received02/16/accepted;5:250. PubMed PMID: PMC4771655.
- (15) Obata T, Fernie AR, Nunes-Nesi A. The Central Carbon and Energy Metabolism of Marine Diatoms. *Metabolites*. 2013 05/0703/04/received04/25/revise04/25/accepted;3(2):325-46. PubMed PMID: PMC3901268.
- (16) Rodriguez-Navarro C, Jroundi F, Schiro M, Ruiz-Agudo E, González-Muñoz MT. Influence of Substrate Mineralogy on Bacterial Mineralization of Calcium Carbonate: Implications for Stone Conservation. *Applied and Environmental Microbiology*. 2012 09/30/received03/12/accepted;78(11):4017-29. PubMed PMID: PMC3346411.
- (17) Zhu T, Dittrich M. Carbonate Precipitation through Microbial Activities in Natural Environment, and Their Potential in Biotechnology: A Review. *Frontiers in Bioengineering and Biotechnology*. 2016 01/20 11/30/received 01/07/accepted;4:4. PubMed PMID: PMC4718973.
- (18) Baumgartner LK, Dupraz C, Buckley DH, Spear JR, Pace NR, Visscher PT. Microbial species richness and metabolic activities in hypersaline microbial mats: insight into biosignature formation through lithification. *Astrobiology*. 2009 Nov;9(9):861-74. PubMed PMID: 19968463. Epub 2009/12/09. Eng.
- (19) Bosak T, Newman DK. Microbial nucleation of calcium carbonate in the Precambrian. *Geology*. 2003;31(7):577-80.
- (20) Abe F, Kato C, Horikoshi K. Pressure-regulated metabolism in microorganisms. *Trends in microbiology*. 1999 Nov;7(11):447-53. PubMed PMID: 10542424. Epub 1999/11/05. Eng.
- (21) Dhama NK, Reddy MS, Mukherjee A. Application of calcifying bacteria for remediation of stones and cultural heritages. *Frontiers in Microbiology*. 2014 06/26 04/01/received 06/03/accepted;5:304. PubMed PMID: PMC4071612.
- (22) Dhama NK, Reddy MS, Mukherjee A. *Bacillus megaterium* mediated mineralization of calcium carbonate as biogenic surface treatment of green building materials. *World journal of microbiology & biotechnology*. 2013 Dec;29(12):2397-406. PubMed PMID: 23793943. Epub 2013/06/25. Eng.
- (23) Dhama NK, Reddy MS, Mukherjee A. Biomineralization of calcium carbonate polymorphs by the bacterial strains isolated from calcareous sites. *Journal of microbiology and biotechnology*. 2013 May;23(5):707-14. PubMed PMID: 23648862. Epub 2013/05/08. Eng.
- (24) Okwadha GD, Li J. Optimum conditions for microbial carbonate precipitation. *Chemosphere*. 2010 Nov;81(9):1143-8. PubMed PMID: 20947128. Epub 2010/10/16. Eng.
- (25) Yasuhara H, Hayashi K, Okamura M. Evolution in Mechanical and Hydraulic

- Properties of Calcite-Cemented Sand Mediated by Biocatalyst. *Geo-Frontiers* 2011: American Society of Civil Engineers; 2011. p. 3984-92.
- (26) Gao G-L, Ding G-D, Wu B, Zhang Y-Q, Qin S-G, Zhao Y-Y, et al. Fractal Scaling of Particle Size Distribution and Relationships with Topsoil Properties Affected by Biological Soil Crusts. *PLoS ONE*. 2014 02/0702/17/received01/09/accepted;9(2):e88559. PubMed PMID: PMC3917891.
- (27) Kukačka L, Nosek Š, Kellnerová R, Jurčáková K, Jaňour Z. Wind Tunnel Measurement of Turbulent and Advective Scalar Fluxes: A Case Study on Intersection Ventilation. *The Scientific World Journal*. 201201/1610/31/received12/18/accepted;2012:381357. PubMed PMID: PMC3352249.
- (28) Griffin DW. Atmospheric Movement of Microorganisms in Clouds of Desert Dust and Implications for Human Health. *Clinical Microbiology Reviews*. 2007;20(3):459-77. PubMed PMID: PMC1932751.
- (29) Meyer F, Bang S, Min S, Stetler L, Bang S. Microbiologically-Induced Soil Stabilization: Application of *Sporosarcina pasteurii* for Fugitive Dust Control. *Geo-Frontiers* 2011: American Society of Civil Engineers; 2011. p. 4002-11.
- (30) Dong L, Córdova-Kreylos AL, Yang J, Yuan H, Scow KM. Humic acids buffer the effects of urea on soil ammonia oxidizers and potential nitrification. *Soil biology & biochemistry*. 2009;41(8):1612-21. PubMed PMID: PMC3260537.
- (31) DeJong J, Fritzes M, Nüsslein K. Microbially Induced Cementation to Control Sand Response to Undrained Shear. *Journal of Geotechnical and Geoenvironmental Engineering*. 2006 2006/11/01;132(11):1381-92.

## ارزیابی خاک پایدار شده بوسیله *Bacillus sp. HAI4* در شرایط مختلف از طریق روش تاگوچی

**مهران علوی\*:** دانشجوی دکتری نانوزیست فناوری، آزمایشگاه میکروبی‌شناسی، دانشگاه رازی، کرمانشاه، ایران، mehranbio83@gmail.com  
**مجتبی تاران:** دانشیار میکروبی‌شناسی، دانشگاه رازی، کرمانشاه، ایران، mojtabatan@yahoo.com  
**آرزو بریموندی:** کارشناسی ارشد میکروبی‌شناسی، دانشگاه رازی، کرمانشاه، ایران، arezoo.berimavandi@gmail.com

### چکیده

**مقدمه:** فرایند رسوب کلسیت، در نتیجه فعالیت‌های متابولیکی میکروارگانیسم‌ها، کلسیفیکاسیون زیستی نامیده می‌شود. در این فرایند، کلسیت ( $\text{CaCO}_3$ ) به وسیله فعالیت‌های میکروارگانیسم‌ها بر روی دانه‌های خاک ته‌نشین شده و سبب افزایش سختی/استحکام و کاهش فرسایش خاک می‌شود.

**مواد و روش‌ها:** در این مطالعه، میزان هیدرولیز اوره و ته‌نشینی کلسیت در فرایند تثبیت خاک، بوسیله باکتری *Bacillus sp. HAI4* از طریق روش تاگوچی مورد ارزیابی قرار گرفته است. همچنین، تونل باد برای اندازه‌گیری میزان پایداری خاک، طراحی شد.

**نتایج:** در این پژوهش، سه فاکتور (کلرید آمونیوم، اوره و ملاس) برای بررسی تثبیت خاک، بررسی شدند. نتایج این مطالعه، نشان داد که روش تاگوچی یکی از کارآمدترین روش‌ها برای بررسی فاکتورهای موثر در فرایند پایداری خاک است. در این مورد، غلظت اوره، دارای بیشترین تاثیر در رسوب‌دهی کلسیت و تثبیت خاک بوسیله این باکتری بود. همچنین، بررسی اندرکنش فاکتورها، ارتباط فاکتورها را با هم نشان داد. در مورد فاکتور کلرید آمونیوم با سه سطح (۰/۵، ۱ و ۱/۵ گرم بر لیتر)، در سطح سوم، بیشترین تاثیر (۴/۳۵۳٪) را در فرایند تثبیت خاک داشت. افزایش کلرید آمونیوم، باعث افزایش میزان پایداری خاک شد. سه سطح ۲۰، ۳۰ و ۴۰ گرم بر لیتر اوره نیز مورد بررسی قرار گرفت. در این مورد نیز، سطح سوم با میزان ۴/۳۱۳٪ بیشترین تاثیر را داشت. همچنین، در این مطالعه، طراحی تاگوچی، به صورت سه سطح ملاس (۳۰، ۴۰ و ۵۰ گرم بر لیتر)، بیشینه تثبیت خاک را در سطح دوم با میزان ۳/۸۷۳٪ نشان داد. در حقیقت، ابتدا با افزایش ملاس، تثبیت خاک، افزایش و سپس کاهش یافت.

**بحث و نتیجه‌گیری:** در حالت کلی، این مطالعه نشان داد که دو فاکتور کلرید آمونیوم و اوره، تاثیر بیشتری در میزان هیدرولیز اوره و رسوب کلسیت دارند.

**واژه‌های کلیدی:** کلسیفیکاسیون زیستی، پایداری خاک، *Bacillus sp.*، رسوب کلسیت، آمونیوم کلراید

\* نویسنده مسؤول مکاتبات