



An experimental investigation of bacteria-producing calcareous cement in wind erosion prevention

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Abstract

Soil stabilization is essential in different fields, such as the environment, to prevent wind erosion and dust. Microbial-induced carbonate precipitation (MICP) is a soil healing method in which bacteria with CaCO_3 precipitation among soil particles increase the soil erosion resistance against wind erosion. In this study, by culturing a bacterium species with the scientific name of “*Sporosarcina pasteurii*” in the laboratory and preparing the solution with three levels of urea and calcium chloride (concentrations of 0.1, 0.2, and 0.4 M) as a nutrient, the status of CaCO_3 precipitation was investigated. The erosion of the cemented samples was simulated at a speed of 10–20 km h^{-1} at the height of 10 cm from the tunnel bottom using a wind tunnel apparatus. The results showed that the highest CaCO_3 precipitation occurred in treatments of 0.1 M calcium chloride with 0.2 M and 0.4 M urea. At both wind speeds, MICP treatments significantly reduced soil erosion as compared with the control samples. The FTIR test confirmed the CaCO_3 precipitations. Further, the study of CaCO_3 precipitation using XRD and SEM analysis showed that it is more in the form of vitriol crystals, binding together loose soil particles and increasing their resistance to the shear stress of wind.

Keywords Calcium carbonate · FTIR · MICP · Stabilization · *Sporosarcina pasteurii* · XRD

Introduction

Two-thirds of Iran’s area has in dry and semidry climates with over 45 million ha (hectares) of deserts (Ahmadi et al. 2004). Iran has faced a growing drought crisis and increased aerosol in the last two decades, mainly due to human interference in nature (Shokouhi Nia and Rezai Kikhaei 2018). Building excessive dams, grazing exceedingly, drilling unauthorized wells, cultivating and exporting products with high water demand, consuming water and draining underground aquifers excessively, drying wetlands and rivers, besides using plastic materials, are the factors leading to create the aerosol centers in Iran (Barzegar Bafravi et al. 2019).

The aerosol phenomenon has affected Iran, especially the western, southwestern, and central regions (Shahkoui and Rahmani 2018). It has many implications for human health (Chahar Azar et al. 2019), quality of life (Ahani et al. 2019), climate (Moradi and Aprajunqani 2017), and agriculture (Sarani and Rahdari 2019). Thus far, this phenomenon, despite the efforts of the authorities, has not been fully stopped, and it can be observed in many areas, especially the western part of the country (Alipour et al. 2019). Therefore, addressing the aerosol origin is a top priority and on the environmental–organizational agenda.

Research has tended to stabilize prone soils, resisting high-speed wind and preventing the aerosol formation (Ayarán and Kamali 2018). There are many methods to prevent entering dust particles, all of which are done to stabilize the soil. The most common of them is the soil stabilization using the oil, mineral, polymer mulches (Robichaud et al. 2017; Katebi et al. 2018; Kader et al. 2017; Azoogh et al. 2018), and biological mulches of biopolymer and plant (Chen et al. 2018, 2019; Zhang et al. 2019a; Liu et al. 2019; Wieder and Shoop 2018).

A key factor in controlling wind erosion is increasing the soil surface layer resistance to shear stress. Therefore, many

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soil erosion control methods are currently based on soil surface stabilization. In this respect, the formation of a physical crust can greatly reduce wind erosion. The biological process of soil remediation, as a soil stabilization technique with natural concrete, has great potential in geotechnical engineering, such as the slope stabilization and the increase in sand resistance (Jiang and Soga 2019; Zomorodian et al. 2019).

Microbial stabilization of CaCO_3 (MICP) is a new technique for soil remediation that involves ureolytic bacteria, such as *Sporosarcina pasteurii*, to hydrolyze urea in order to form CaCO_3 deposits, causing to fill soil pores (Rahim et al. 2015; Jiang et al. 2016). Urea hydrolysis is a microbial process extremely vital in biotechnological applications (Hammes and Verstraete 2002). MICP is an effective and environmentally friendly technology often used to solve various environmental problems, including soil instability and concrete crack (Anbu et al. 2016). During the MICP process, the ureolytic bacteria convert the urea (nonconductive material) molecule to two ions of ammonium (NH_4^+) and carbonate (CO_3^{2-}) (Anbu et al. 2016; Cuzman et al. 2015), subsequently leading to produce CaCO_3 minerals (CaCO_3) (Jiang et al. 2016). MICP is introduced as a highly desirable method due to its natural accessibility, efficiency, and stability (Al-Thawadi Salwa 2008; Li et al. 2018; Grabiec et al. 2017; Sharaky et al. 2018; Jiang and Soga 2019).

Many studies have shown that the microbial calcite stabilization method plays a vital role in increasing shear strength and decreasing the permeability of soil (Eryürük et al. 2015; Tayebani and Mostofinejad 2019), improving the soil biology (Warren et al. 2010), purifying the wastewater (Hammes et al. 2003), remedying crack and healing concrete resistance (Wu et al. 2019; Tayebani and Mostofinejad 2019; Jongvivatsakul et al. 2019; Ruan et al. 2019), extracting oil (Whitman et al. 1998), strength and hardness of sandy soils (Sharaky et al. 2018), solidifying the soil (Montoya 2012), dust control (Achal et al. 2011), stabilizing the heavy metal (Jiang et al. 2019; Zhang et al. 2019b; Chen and Achal 2019).

Sharaky et al. (2018) used *S. pasteurii* bacteria to enhance the stabilization of loose soil in the large irrigation canal walls of Egypt; he found these bacteria inoculation with a stabilizing solution to be effective in stabilizing the border slopes of these canals. Molares et al. (2019) investigated the replacement of chemical stabilizers with natural cement from microbial CaCO_3 precipitation; they showed that the bacterial effect on clay soils increased the CaCO_3 content, bulk density, and plasticity properties of the soil. Li et al. (2018) examined the desert sand stabilization based on the microbial precipitation rate of generated calcium carbonate. Their results showed that the addition of bacterial and cementing solutions resulted in CaCO_3 precipitation in sand samples. As the concentration

of the cementing solution increased, the amount of CaCO_3 increased, thus increasing density. CaCO_3 reduced the pores between the sand particles and the permeability.

Grabiec et al. (2017) found that the amount of bio- CaCO_3 generated by *S. pasteurii* significantly affected the geotechnical properties of the soil, increasing its shear strength. This study aimed to evaluate the soil stabilization through the biological precipitation of CaCO_3 between soil particles in one of the dust and aerosol hot spots of Iran's central area by *S. pasteurii*.

Materials and methods

This study was conducted in 2018–2019, and data were collected and analyzed in Razi experimental complex of Science and Research Branch, Islamic Azad University, Tehran, Iran.

Soil parameters and location

The studied soil is sampled from the Aran and Bidgol deserts of Isfahan province, one of the active sites of wind erosion in the area located in $34^\circ 00' \text{N}$ and $51^\circ 30' \text{E}$. This city, with an area of 6051 km^2 , is located in 6 km northeast of Kashan in Isfahan province. Table 1 summarizes some of the physical and chemical properties of the studied soil.

Bacteria preparation

In this study, *S. pasteurii* microorganism was used for the biological precipitation of calcium carbonate. The strain of this bacterium was lyophilized from the Iranian Fungal and Industrial Bacteria Collection Center with no. PTTC 1645 (DSM 33). This bacterium is a gram-positive endospore generator grown in alkaline media with urease activity (Li et al. 2018; Omoregie et al. 2019; Jiang et al. 2019).

Table 1 Some of the physical and chemical properties of studied soil

pH	7.54
ECe (dS m^{-1})	1.10
Sand (%)	38.5
Silt (%)	53.5
Clay (%)	8.0
Organic content (%)	0.94
Equivalent CaCO_3 (%)	9.8
Total nitrogen (%)	0.09

ECe saturated electrical conductivity

Preparation of the bacterial suspension

Omeregic et al. (2019) used low-cost culture media to establish microbial precipitation of calcium carbonate. A comparison of food-grade media with laboratory materials showed a significant reduction in the bacterial culture cost by 99.80%. Their findings further showed that yeast extract could be an appropriate choice for bacterial culture in the MICP process concerning cost reduction. For this purpose, yeast extract was used in the LB agar culture medium and then in nutrient broth culture medium (Table 2). Bacteria were grown at 30 °C on a rotary shaker at 130 rpm for 24 h under aerobic conditions. The pH of the culture medium was adjusted to 7.5 before being put into the autoclave with NaOH. Later, 6 g of urea was added to the composition of the culture medium using a sterile filter. Once bacteria were grown properly, the solution of the liquid medium was separated from the suspended bacteria by centrifugation at 200 rpm at 2 °C.

Urease enzyme assay

The soluble electrical conductivity method (dS m^{-1}) was used to measure the urease enzyme activity of bacteria (mM

urine hydrolyzed min^{-1}). Electrical conductivity measurement is a way to determine the amount of enzymatic activity of reaction (Al-Thawadi Salwa 2008; Whiffin et al. 2007; Omeregic et al. 2019). To measure urease activity, 10 ml of the suspension of grown bacteria in liquid culture medium (including bacteria and culture medium of nutrient broth) and 100 ml of urea at various concentrations of 0.4, 0.2, and 0.1 M were mixed. Then, the electrical conductivity of the mixtures was measured at a different times of 5 min, 24 h, and 48 h at 28 ± 2 °C.

Measurement of CaCO_3 precipitation

In the MICP process, *S. pasteurii* plays the role of catalyst in the reaction of urea and calcium chloride, causing urea hydrolysis and CaCO_3 precipitation. Different solutions of urea (NH_2CONH_2) and calcium chloride (CaCl_2) (see Table 3) were prepared to identify their best concentrations that resulted in the highest CaCO_3 precipitation in the MICP process.

The growth temperature was considered to be 37 °C, according to the optimum bacteria growth temperature (Whiffin et al. 2007). Five milliliters of the bacteria

Table 2 Used culture medium and their compositions

Name of culture medium	The composition of culture medium	pH
LB agar	Peptone from casein (10 g l^{-1}), yeast extract (5 g l^{-1}), sodium chloride (10 g l^{-1}), agar-agar (15 g l^{-1})	7.5
Nutrient broth	Peptic digest of animal tissue (5 g l^{-1}), sodium chloride (5 g l^{-1}), beef extract (1.50 g l^{-1}), yeast extract (1.50 g l^{-1})	7.5

Table 3 Treatment of urea and CaCl_2 in CaCO_3 precipitation using MICP method

Treatment	Concentration of urea (M)	Concentration of CaCl_2 (M)	Bacteria suspension	The mount of urea and CaCl_2	Solution volume (cc)
1	0.1	0.1	Broth + 5 cc bacteria yeast + nutrient extract	6 g urea–14.7 g CaCl_2	50
2	0.1	0.2	Broth + 5 cc bacteria + nutrient yeast extract	6 g urea–29.4 g CaCl_2	50
3	0.1	0.4	Broth + 5 cc bacteria + nutrient yeast extract	6 g urea–58.8 g CaCl_2	50
4	0.2	0.1	Broth + 5 cc bacteria + nutrient yeast extract	12 g urea–14.7 g CaCl_2	50
5	0.2	0.2	Broth + 5 cc bacteria + nutrient yeast extract	12 g urea–29.4 g CaCl_2	50
6	0.2	0.4	Broth + 5 cc bacteria + nutrient yeast extract	12 g urea–58.8 g CaCl_2	50
7	0.4	0.1	Broth + 5 cc bacteria + nutrient yeast extract	24 g urea–14.7 g CaCl_2	50
8	0.4	0.2	Broth + 5 cc bacteria + nutrient yeast extract	24 g urea–29.4 g CaCl_2	50
9	0.4	0.4	Broth + 5 cc bacteria + nutrient yeast extract	24 g urea–58.8 g CaCl_2	50



suspension to 50 ml of distilled water containing a mixture of urea and calcium chloride, according to the treatments in Table 3, was added to evaluate the CaCO_3 precipitation produced by the bacteria at the end of the growth logarithmic phase (Arias et al. 2017). Then, erlenmeyers containing the reaction solutions were placed in the incubator without shaking for 24 h at 30 °C. After that, the sample was placed at laboratory temperature. After forming the final CaCO_3 precipitation, the formed precipitation was dried by removing the solution and then measured.

FTIR test

Fourier transform infrared spectroscopy (FTIR) was used to determine the type of formed precipitation by *S. pasteurii* bacteria. FTIR spectra were recorded with a Fourier transform infrared spectroscopy, and Thermo AVATAR spectrometer at a wavelength of 500–4000 cm^{-1} was used to determine the effects of MICP. The mixture in erlens was shaken at 30 °C for 24 h, and then solid precipitations were collected for analysis by FTIR. The precipitates were kept at ambient temperature to dry completely. Produced precipitations in the soluble phase were used to prepare the sample, were thoroughly crushed before testing to powder, and then impregnated with inert materials. Powder samples were transformed into potassium bromide tablets for analysis. To make the tablets, the solid sample was crushed with potassium bromide to obtain powder particles smaller than 2 μm . The powder mixture was then pressed to form the tablet.

The analysis of soil resistance

Columns of PVC with an inner diameter of 6 cm and a height of 20 cm were prepared to assay the soil strength after forming precipitation. Each column was filled with the studied soil (295 g). The used treatments in this test are presented in Table 3. Each treatment was repeated three times (nine treatments), and one treatment contained bacteria without cementing solution; totally, 30 columns were prepared. Tubes were tested to evaluate the penetration resistance index of each treatment in vertical displacement at soil surfaces. The liquid inside the column naturally moved under the gravitational force.

First, 50 ml of bacterial culture medium was added to each column; after 12 h, 50 ml of cementing solution was added, according to the treatments in Table 3. This method helps to keep the bacteria in the soil. All columns were then kept at 28 ± 2 °C for 15 days. After this time, soil samples were extracted from the columns and dried at 140 °C for 48 h to evaluate the surface soil resistance at the dry state. Resistance intensity of the treated

surface soil was measured using a penetrometer. The penetrometer could read a scale of 0–700 psi (0–4.83 MPa). The soil surface resistance was measured by pressing the tip of this device to the surface layer of the columns.

SEM and XRD analysis

To determine the elemental composition and the crystalline phase of the precipitation, four samples of MICP and one control sample were selected for X-ray diffraction (XRD) analysis. The dried samples were first passed through a sieve with 500 mesh and then transferred to a 50 cc Falcon tube. Apparatus with XRD 3003 PTS SEIFERT (Germany) was used for XRD. The XRD spectra were obtained using powdered samples from 5° to 80° with Cu anode with parameters of 40 kV and 35 mA. Scanning electron microscopy (SEM) images were examined using a CamScan MV2300 microscope (Canada) to investigate the CaCO_3 precipitation distribution in the specimens and ensure its presence in 1-cm pores of soil surface. The dried sample was cut from 1 cm of soil surface.

The measurement of soil erosion in the wind tunnel

First, trays with dimensions of 40 × 30 cm and height of 4 cm were prepared to measure the soil wind erosion, and then they were filled with the soil under study. The MICP solution was added to each tray (30 trays), equivalent to 1000 cc of bacteria suspension of *S. pasteurii* and 1000 cc of cementing solution of urea and calcium chloride. It was uniformly sprayed onto the soil using a sprayer. The prepared trays were completely dried in the open air for 15 days. The control treatment was sprayed with the same volume but without the MICP solution.

Next, an apparatus of the wind tunnel was simulated at the site, consisting of two main wind generators with rates of 10 m s^{-1} and 20 m s^{-1} and a wind flow transmission chamber. Using a canal with 2 m length and 50 cm width, the wind was transferred to the surface of the soil trays, and soil wind erosion was assessed in each tray. The tray was positioned in the middle of the chamber with a distance of 30 cm and a height of 10 cm from the generation site of flow so that the wind speed at the soil surface entry was set at 10 or 20 m s^{-1} . To measure the value of the soil wind erosion, the weight of the trays was first measured and then placed inside the wind tunnel apparatus for 20 min. Next, the weight of the trays was measured again. Tray weight loss showed a high rate of soil wind erosion.



The measurement of CaCO_3 pH and EC of treated soils

After measuring the soil resistance in the columns, pH and EC in extract 1:2 water and soil samples were investigated. The pH measurement was performed using the GLP-meter 22 pH ISE CRISON pH apparatus, and the EC measurement was done by the EC-meter GLP 31 + CRISON apparatus. CaCO_3 content was measured by titration using phenolphthalein.

Data analysis

Data were statistically analyzed by SPSS and MSTATC software packages, and data significance was evaluated at 5% level. The diagrams were plotted with EXCEL software.

Results and discussion

CaCO_3 precipitation in liquid medium

Experimental results showed that *S. pasteurii* bacteria produced CaCO_3 precipitation in the culture medium of nutrient broth and yeast extract after 28 h at 28 °C (Figs. 1, 2). The bacteria activity is indicated by the presence of CaCO_3 precipitations, urea usage as a food source, and conversion of calcium chloride to CaCO_3 . Musa Zadeh Moghadampour et al. (2016) also used two culture media of YU and LB to investigate the maximum amount of CaCO_3 precipitation. The results showed that *S. pasteurii* bacteria had more precipitation in the medium with yeast extract. The highest CaCO_3 precipitation occurred in treatments of 0.1 M calcium chloride and 0.1 mM urea, calcium chloride of 0.1 mM and 0.2 mM urea, 0.4 mM urea and 0.1 calcium chloride, besides 0.4 M of calcium chloride.

Fig. 1 Image of control sample surface (a) and cementing sample by MICP technique (b)

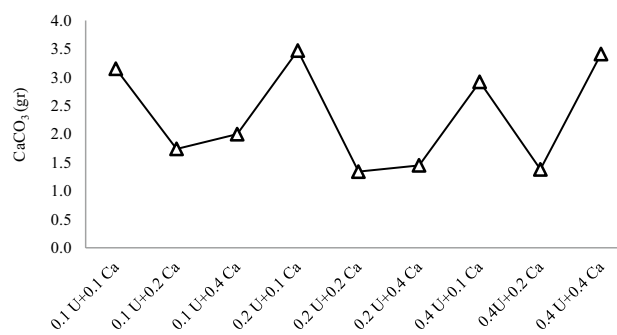
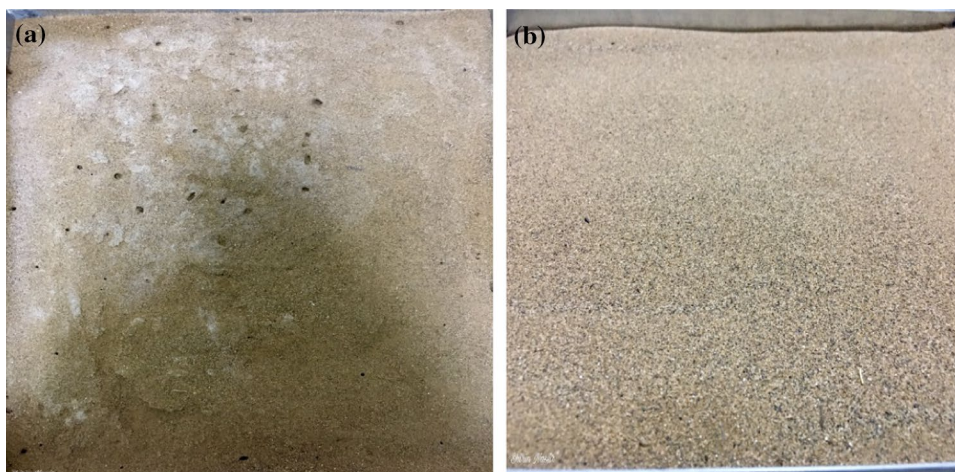


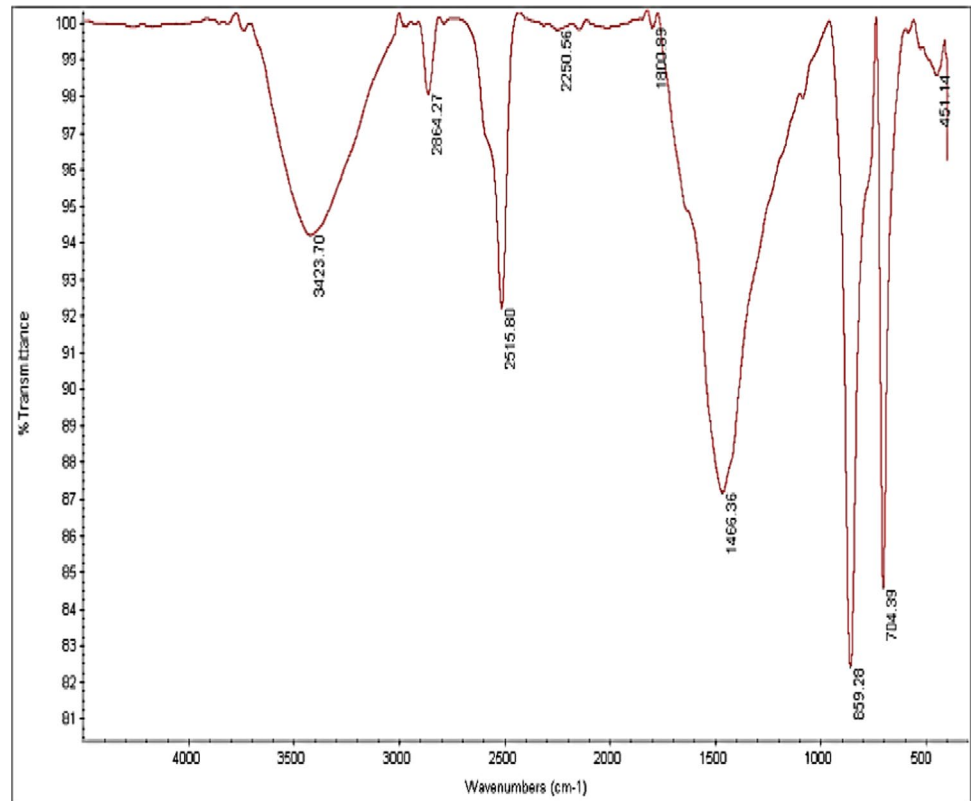
Fig. 2 Formation of CaCO_3 precipitation in liquid culture medium

FTIR and XRD analysis

Infrared spectroscopy (FTIR) was performed to determine the type of formed precipitations by the bacteria in the presence of calcium chloride and urea solution (Fig. 3). The FTIR test showed absorption peaks around 704.39 cm^{-1} and 859.28 cm^{-1} , indicating the calcite phase. The results of the XRD test also confirmed the presence of CaCO_3 crystals in the soil (Fig. 4). FTIR is a precious test used repeatedly to confirm the presence of calcium carbonate.

Achal et al. (2012) investigated the effect of *Sporosarcina ginsengisoli* bacteria on arsenic-contaminated soils, confirming the production of CaCO_3 by bacteria. Ha Nguyen et al. (2019) also verified the microbial CaCO_3 precipitations in concrete by performing FTIR analysis under the inoculation of *S. pasteurii*. CaCO_3 has different sizes in nature, and it consists mainly of three different types of calcite, aragonite, and vitrite crystals (Molares et al. 2019). The XRD analysis was performed to determine the type of formed precipitations between the soil pores (Fig. 4). Analysis of the MICP sample showed the highest peak at 2θ of 29.8° , indicating formed CaCO_3 polymorphs by bacteria. The value of precipitated crystals by bacterial cells was 100% calcite.

Fig. 3 FTIR test of collected precipitations from a mixture of bacteria suspension with urea–chloride calcium solution



SEM analysis

To confirm the formation of CaCO_3 particles by *S. pasteurii* bacteria, samples were taken from control soil and three treated columns and then images were prepared by SEM (Fig. 5). Electron microscopy images showed that the CaCO_3 content in the main soil sample was very low; thus, the soil particles were scattered (Fig. 5a). Examination of the soil thin sections showed that the presence of CaCO_3 precipitation among the soil particles caused the correlation between the soil grains, thereby increasing the soil hardness and resistance (Fig. 5b, c, d). The SEM analysis shows the formation of the observed crystal, similar to the reports by Wasim and Basit (2016) and Qiu et al. (2014), which confirm the formation of calcite with a similar crystalline structure.

Evaluation of pH and EC of treated soils

ANOVA results (Table 4) showed that the effect of treatments on soil pH and EC, CaCO_3 formation, and soil wind erosion (eroded soil) was significant at 1% level. pH is a key parameter in CaCO_3 precipitation since it determines the amount of bacterial activity in the soil. *S. pasteurii* bacterium has the highest activity at neutral pH (Hammes and Verstraete 2002); however, ambient pH

enhances by increased activity and urea decomposition (Dejong et al. 2006). The general increasing trend of the pH of the treated soil samples from 7 to about 7.7 is for this reason, as shown in Fig. 6. pH on modified soils by the MICP process was tested by Omoregie et al. (2017), Henze and Randall (2019), and Ruan et al. (2019); it showed that CaCO_3 formation was directly related to pH, and the higher rate of CaCO_3 stabilization was observed at higher pH. The data analysis (Fig. 6) showed that the control soil had the lowest EC compared to the treated soil. In the treated soils, soil EC increased due to the high bacterial activity.

Precipitation of calcium carbonate

At the end of the stabilization of soil particles, samples of control and MICP treatments were used to measure the amount of formed CaCO_3 in the soil (Fig. 7). Results showed that the amount of CaCO_3 in bacteria precipitation increased by about 20% compared to the control soil. The increase of calcite crystals in the soil pores and the aggregation of soil particles explained the resistance of the samples in two tests of penetration resistance (“The measurement of penetration resistance” section) and wind tunnel. Li et al. (2018) showed that the amount of CaCO_3 in soil samples gradually increased using the

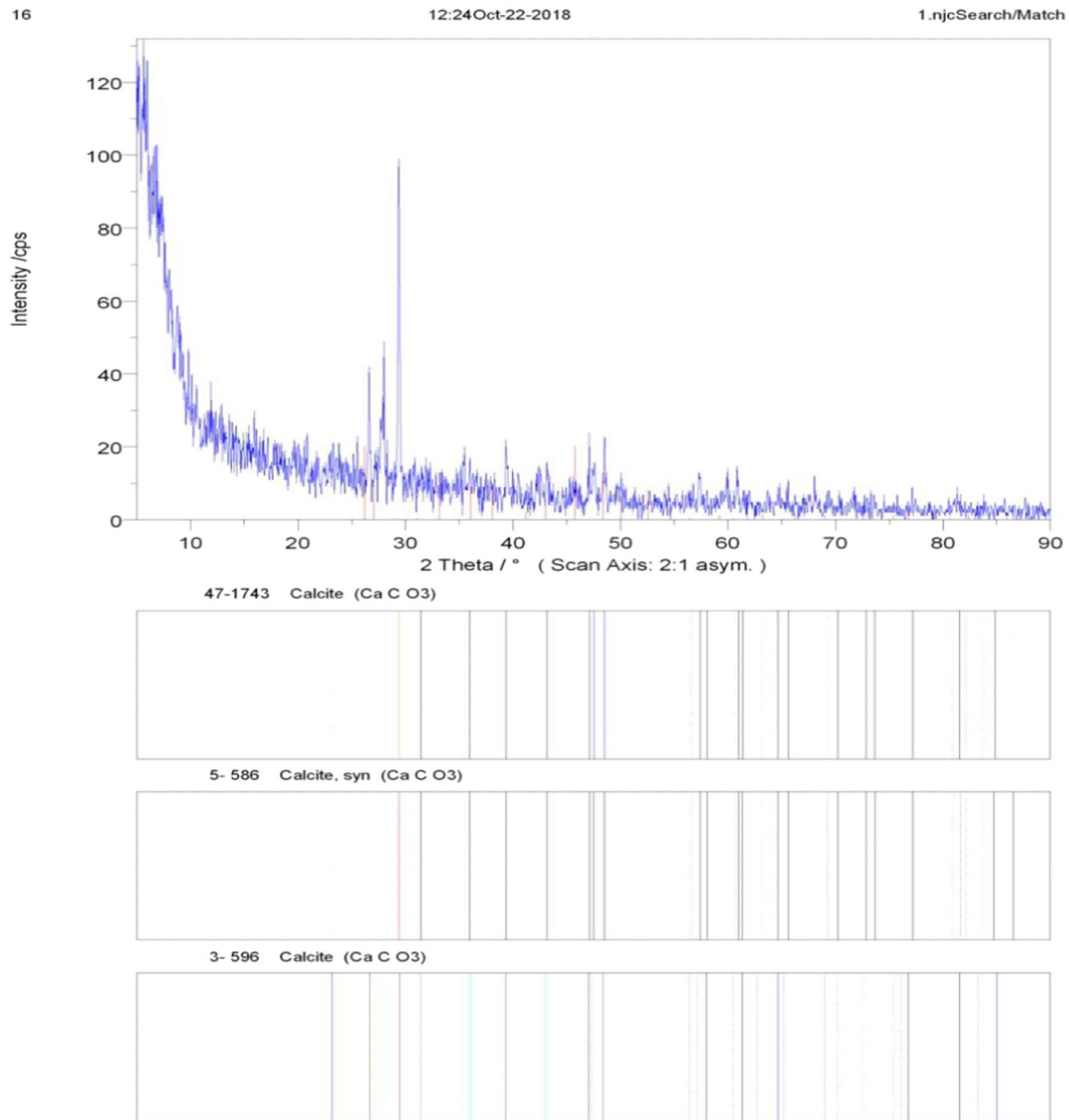


Fig. 4 XRD spectra of MICP sample

MICP process compared to the control soil. According to these results, the formation of CaCO₃ was the leading cause of change in other physical properties.

The measurement of penetration resistance

The MICP effect on compressibility and surface resistance was measured using a penetrometer, with values between 0 and 700 psi (0–4.83 MPa) in dry-air conditions at 1 cm of the samples' surface layer. The results of the experiment showed that all treated columns had the highest measured resistance of 700 psi. A comparison of the control sample

with MICP treatments showed a fourfold increase in penetration resistance.

Many studies have been conducted on the formation of CaCO₃ crystals in sand columns to evaluate the degree of microbial stabilization (Dejong et al. 2010; Stabnikov et al. 2013). Studies have shown that soil microbial remediation by a mixture of sand and microorganisms increases the compressive strength of the treated specimens (Omoregie et al. 2017).

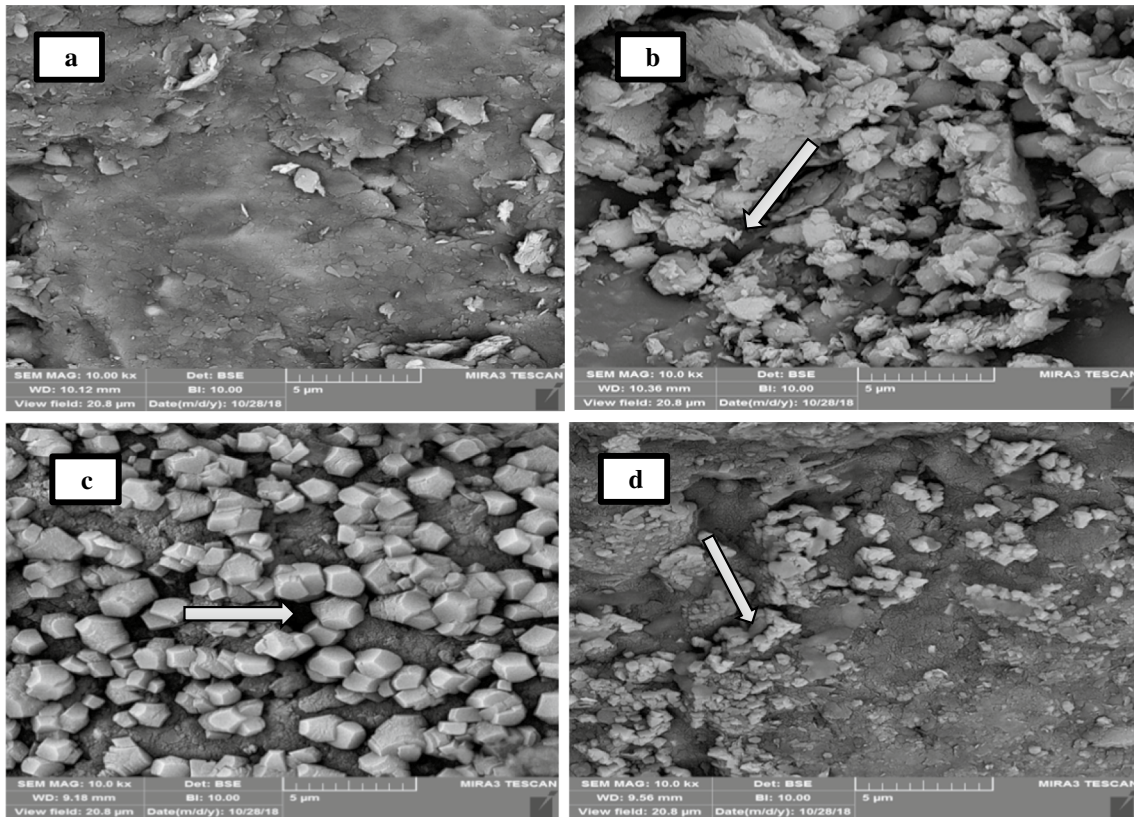


Fig. 5 SEM analysis of sandy soil before MICP treatment with magnification 5μ (a), SEM analysis of sandy soil with MICP treatment (0.2 urea and 0.1 CaCl₂) with magnification of 5 μm (b), SEM analysis

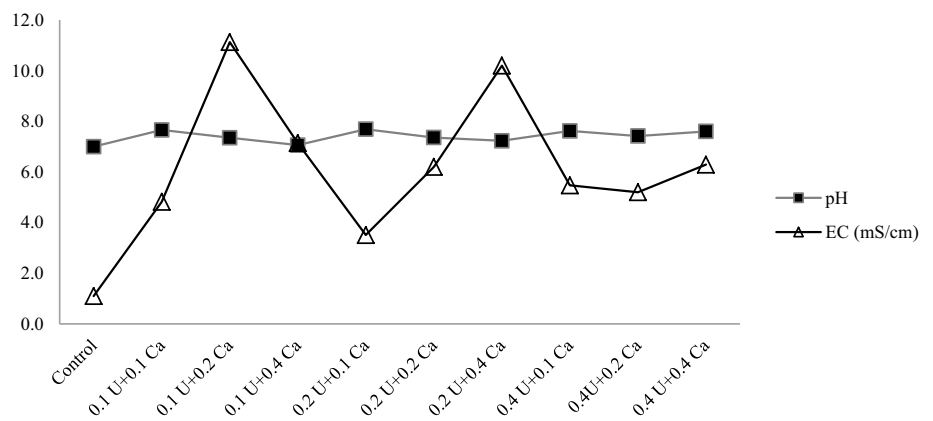
of sandy soils with MICP treatment (0.4 urea and 0.4 CaCl₂) with magnification of 5μ (c), SEM analysis for sand refined by MICP process (0.4 urea and 0.1 CaCl₂) with magnification of 5 μm (d)

Table 4 ANOVA results of the MICP treatments effects on pH, ECe, CaCO₃ formation, and soil wind erosion (eroded soil)

Variations resources	Freedom degree	Mean squared					
		pH	ECe	CaCO ₃	Eroded soil (10 m s ⁻¹)	Eroded soil (20 m s ⁻¹)	Total eroded soil
Treatment	9	3.6**	0.95**	95.8**	278**	127**	456**
Error	18	0.6	0.12	14.2	42.1	22.3	14.8
CV	–	6.5	10.1	8.2	5.4	4.6	7.2

**Significant at 1% level

Fig. 6 pH and EC values for treated and untreated soil samples



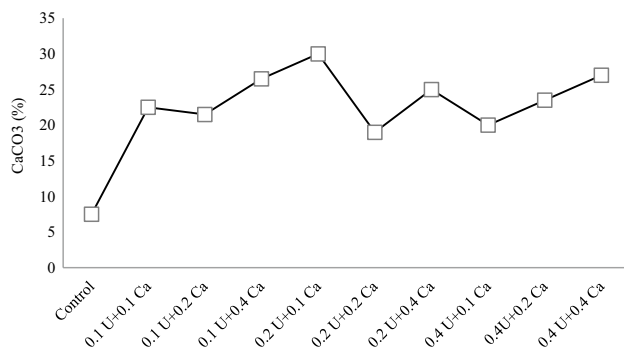


Fig. 7 CaCO₃ content in the treated soil samples

Wind erosion

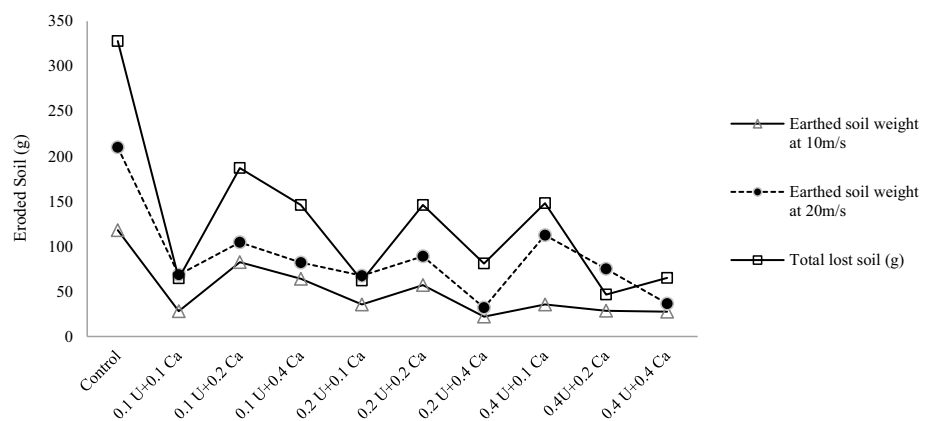
The results of variations in the amount of eroded soil in the two samples of control and MICP treatment were investigated at two wind speeds of 10 m s⁻¹ and 20 m s⁻¹ (Fig. 8). The control soil tray and treated trays were tested by MICP intact after 28 days. The lost soil rate in the wind erosion test was measured by weighing the trays at the end of each experiment. The difference in the amount of erosion between biological treatments and the control soil showed a significant effect of MICP in controlling wind erosion, especially at high velocities. Maximum erosion occurred in the control soil due to the absence of bacterial activity.

The XRD analysis (Fig. 4) confirms that calcite precipitations by *S. pasteurii* bacteria in the treated columns cause pore filling; also, like a bridge, they bind the soil particle together. They are the main cause of the increase in penetration resistance of the MICP samples and, consequently, their significant resistance to wind erosion (Fig. 8). The results showed the main cause of soil resistance to be the presence of calcite crystals, which is the most stable type of CaCO₃ polymorph and causes the aggregation of soil particles. The XRD analysis results on sandy soil samples showed that the formed

precipitations by *S. pasteurii* bacteria in the MICP process were quartz and calcite, in agreement with Dhimi et al. (2017).

Based on the comparison of the SEM analysis (Fig. 5) with the wind tunnel test results (Fig. 8), it was concluded that the soil resistance was due to a significant amount of CaCO₃ crystals formed between the pores of the soil, causing a correlation between the porous media and its soil resistance to high wind speeds. On the other hand, the precipitation of CaCO₃ reduced the open pores of the soil and led to increased penetration resistance. It can be concluded that the increase in the surface layer resistance of the samples is because of the biological cement produced through the MICP process, and the cohesiveness of sand particles is the main reason for the decrease in soil loss. The experiment on soil trays confirms the results of all experiments performed in this study. According to the results of Tobler et al. (2011) and Chen et al. (2008), the bacterial activity becomes better at the higher concentration of urea and calcium chloride. According to Zomorodian et al. (2019), the MICP process can protect soil against erosion at different wind speeds. The least amount of soil erosion occurs in the treatments of 0.1 M CaCl₂ and 0.1 M urea, together with 0.4 M urea and 0.4 mM CaCl₂. As previously shown in Fig. 2, most of the CaCO₃ precipitation by the bacteria occurs in the abovementioned solutions, i.e., 0.1 and 0.4 M concentrations of CaCl₂ and urea. According to Fig. 7, the highest percentage of lime is observed in these treatments, confirming bacterial activity and CaCO₃ precipitation. The highest percentage of soil lime is observed in the treatment of urea 0.2 M and 0.1 M CaCl₂, in agreement with the results of the rate test of CaCO₃ precipitation (Fig. 2) and soil erosion (Fig. 8). Electron microscopy images also confirm the highest formation of CaCO₃ precipitate in the treatment of 0.4 M urea and CaCl₂. The treatment of 0.4 M urea and 0.1 M CaCl₂ has a large amount of CaCO₃ precipitation (Fig. 2); thus, it has little erosion at

Fig. 8 Results of soil erosion supplementary test at two speeds of 10 and 20 m s⁻¹ of wind



the rate of 10 m s^{-1} (Fig. 8); however, the amount of soil erosion increases at the rate of 20 m s^{-1} .

Conclusion

A main object of this study was the stabilization of erosion-prone soils through the biological precipitation of CaCO_3 between soil particles by *S. pasteurii*. The results have shown the highest bacterial activity at concentrations of 0.4 M urea and 0.4 M CaCl_2 ; the highest CaCO_3 precipitation occurs in this treatment. XRD and FTIR analyses confirm the formation of CaCO_3 . Wind erosion is significantly reduced in MICP treatments than the control sample; however, the highest wind erosion reduction occurs in the 0.4 M urea and CaCl_2 treatment. The treatment of 0.1 M urea and CaCl_2 results in the same reduction in wind erosion as the 0.4 M urea and CaCl_2 treatment; economically, due to the need for less raw materials for bacterial activity and precipitation formation, the 0.1 M urea and CaCl_2 treatment is preferred. Results confirmed the use of MICP technique in remediation of erosion-prone areas, but field tests are recommended to validate this method further in soil stabilization and wind erosion prevention.

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