

Article



Biocementation of Calcareous Beach Sand Using Enzymatic Calcium Carbonate Precipitation

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Abstract: Beach sands are composed of a variety of minerals including quartz and different carbonate minerals. Seawater in beach sand contains several ions such as sodium, magnesium, calcium, chloride, sulfate, and potassium. These variations in mineralogy and the presence of salts in beach sand may affect the treatment via enzyme-induced carbonate precipitation (EICP). In this study, set test tube experiments were conducted to evaluate the precipitation kinetics and mineral phase of the precipitates in the presence of zero, five, and ten percent seawater (v/v). The kinetics were studied by measuring electrical conductivity (EC), pH, ammonium concentration, and carbonate precipitation mass in EICP solution at different time intervals. A beach sand was also treated using EICP solution containing zero and ten percent seawater at one, two, and three cycles of treatment. Unconfined compressive strength (UCS), carbonate content, and mineralogy of the precipitates in the treated specimens were evaluated. The kinetics study showed that the rate of urea hydrolysis and the rate of precipitation for zero, five, and ten percent seawater were similar within the first 16 h of the reaction. After 16 h, it was observed that the rates dropped in the solution containing seawater, which might be attributed to the faster decay rate of urease enzyme when seawater is present. All the precipitates from the test tube experiments contained calcite and vaterite, with an increase in vaterite content by increasing the amount of seawater. The presence of ten percent seawater was found to not significantly affect the UCS, carbonate content, and mineralogy of the precipitates of the treated beach sand.

Keywords: biocementation; EICP; calcium carbonate; beach sand

1. Introduction

Enzyme-induced carbonate precipitation (EICP) is an emerging biologically-based ground improvement technique that precipitates calcium carbonate within the pores of a granular soil via hydrolysis of urea catalyzed by free urease enzyme. Calcium carbonate (CaCO₃) can cement the soil particles by precipitating at the particle-to-particle contact points or increase the particle roughness by precipitating at the particle surface. EICP has been studied for different applications, such as strengthening granular soil [1–7], reducing the hydraulic conductivity of granular soil [8–10], fugitive dust mitigation [11–13], sealing cracks in concrete [14], and removal of heavy metal ions [15].

Despite many studies on using microbially induced calcite precipitation MICP and EICP for soil improvement, there are only a few MICP studies using this technique for the treatment of beach sand [16–23]. Shanahan and Montoya [16] created a bench model simulating a coastal sand dune using a clean fine sand, typical of coastal dune deposits. They applied MICP treatment to the sand dune model and then assessed the resistance of the model to erosion under simulated wave actions. They reported that the samples treated with MICP had high resistance to erosion as compared to untreated

soil. The angle of repose of the treated sand increased remarkably as well. Daryono et al. [22] studied the feasibility of constructing artificial beach rocks using MICP and beach sand as a proposed method for coastal protection against water erosion. The results indicated that artificial bedrocks have an unconfined compressive strength of up to around six MPa. This study, however, did not address the effect of seawater on precipitation efficiency. Other studies using EICP for soil strengthening, except for Oliveira et al. [24], who evaluated the effect of organic matter content on the efficiency of EICP that used clean base sands with no or insignificant amount of fines content and salts. No study was performed to explore EICP treatment of beach sands.

Beach sands are composed of a variety of minerals including quartz, feldspar, and different carbonate minerals. They also contain pore seawater, which contains several ions such as sodium (Na^+) , magnesium (Mg^{2+}) , calcium (Ca^{2+}) , chloride (Cl^-) , sulfate (SO_4^{2-}) , and potassium (K^+) . These variations in mineralogy and the presence of seawater can affect soil treatment via MICP and EICP. Bacterial cell attachment to soil particles surface can be affected by soil mineralogy [25]. Pre-existing carbonate particles (such as shell fragments) can act as homogenous nucleation points for carbonate precipitation. It was reported by van Paassen [26] that different morphologies exist of calcium carbonate between the precipitates on the surface of carbonate particle and the surface of quartz sand particles. Presence of seawater can also influence the morphology of the precipitates. It results in co-precipitation of various inorganic compounds depending on its composition, salinity, and temperature [27–29]. An increase in ionic strength (salinity) may increase the rate of calcium carbonate precipitation [28]. Natural inorganic seawater constituents, especially magnesium and sulfate ions, hinder calcite precipitation at a given ionic strength [27,28]. It is well known that morphology of microbial CaCO₃ in seawater is mainly controlled by the Mg:Ca ratio in seawater [30]. Contrary to calcium ions, magnesium ions stabilize unstable, hydrated, and amorphous phases [31]. The adsorption and incorporation of foreign ions like magnesium into a biogenic calcite crystal structure are significantly correlated with temperature and total ionic strength of parent solution [28,29,32]. This may affect the mineralogy of CaCO₃ by inhibiting the formation of rhombohedral calcite crystals [32]. Khodadadi Tirkolaei [33] demonstrated the change in morphology of the microbially-induced calcium carbonate precipitates in the presence of seawater. He showed that aragonite precipitated together with calcite in MICP solution containing seawater. Seawater might be also used as a source of calcium for the biologically-induced calcium carbonate precipitation [34]. Salinity of seawater may also affect the chemical efficiency of microbially- and enzyme-induced carbonate precipitation [4,33]. High ionic strength of solution may also lead to precipitation of enzyme proteins (i.e., salt out) [35]. Due to the scarcity of previous studies considering the applicability of using EICP in the marine environment, the current study aimed to evaluate applying this technique to the strengthening of calcareous beach sand and exploring the effects of seawater on the efficiency and mineralogy of the enzymatic calcium carbonate precipitations.

2. Materials and Methods

2.1. Beach Sand

The sand used in this study was natural beach sand obtained from Famagusta Bay, North Cyprus. The particle size distribution parameters (D_{10} , D_{50} , C_u , and C_c) were obtained using the standard test method for particle-size distribution of soils by dry sieving test, using a set of standard sieves and a mechanical sieve shaker (ASTM D6913) [36]. The minimum and maximum void ratios have been calculated according to JIS A1224 [37], and the CaCO₃ percentage was quantified as described in Section 2.3. The beach sand was classified according to the Unified Soil Classification System (USCS) [38]. Table 1 illustrates the basic geotechnical properties of the sand.

Property	Value	
Effective diameter, D ₁₀	0.14	
Median diameter, D ₅₀	0.7	
Iniformity coefficient, C _u	6.42	
oefficient of curvature, C _c	1.4	
linimum void ratio (e _{min})	0.562	
aximum void ratio (e _{max})	0.880	
CaCO ₃ (%)	62.50	
Specific Gravity, G _s	2.70	
USCS Classification	SP	
CaCO ₃ (%) Specific Gravity, G_s USCS Classification	0.880 62.50 2.70 SP	

Table 1. Physical properties of sand.

2.2. Aqueous Medium Experiments

2.2.1. Enzyme Concentration Determination

Test tube experiments were conducted to determine the appropriate concentration of enzyme yielding a higher mass of precipitation. For this purpose, EICP solution containing 1 M urea, 0.67 M calcium chloride anhydrous, 4 g/L non-fat dry milk powder, and different concentrations (15 mL/L, 11 mL/L, 10 mL/L, 5 mL/L, 2.5 mL/L, and 1.25 mL/L) of crude urease extract (with urease activity of \approx 465 U/mL) were prepared in 50-mL test tubes. The substrates concentration was selected based on Almajed et al. [4]. The urease enzyme was crudely extracted from sword jack bean (Canavalia *Gladiata*) according to the method of Khodadadi Tirkolaei et al. [39]. Activity of the urease enzyme was determined using Nessler's reagent as described by Khodadadi Tirkolaei et al. [36]. Each tube was cured at room temperature for 4 days. After curing, each tube was centrifuged at 3500 rpm for 5 min and the supernatant was discarded. The remaining precipitates were rinsed twice by adding 30 mL distilled water. After each rinsing, supernatant was removed via centrifugation at 3500 rpm for 5 min. Finally, the precipitates were dried at 50 °C until constant mass achieved. The mass of precipitates in each tube was measured by subtracting the mass of each empty tube from the mass of the tube after the final centrifugation. All the tests were conducted in triplicate. At the end, the enzyme concentration resulting in the highest calcium carbonate precipitation ratio (Pr) was selected for beach sand treatment. Precipitation ratio was defined as the precipitation mass over the theoretical maximum precipitation that can be stoichiometrically obtained.

2.2.2. Kinetics of EICP in Presence of Seawater

EICP solutions were prepared at two different concentrations of seawater (5% and 10% (v/v)). Salinity and composition of the seawater are presented in Table 2. Electrical conductivity (EC) and pH of the EICP solutions were measured at different time intervals (0, 0.5, 1, 2, 4, 8, 16, 32, 48, and 72 h), using a Thermo Orion 3-star pH meter and WTW EC meter. The pH and conductivity meters were both calibrated prior to each set of measurements. Rate of carbonate precipitation was determined by measuring the mass of precipitation at the same time intervals and in the same way as explained in Section 2.2.1. Rate of urea hydrolysis in each tube was also estimated using direct colorimetric determination of ammonium using Nessler's reagent (each one mole of hydrolyzed urea will liberate 2 moles of ammonium). In this method, 1 mL of EICP solution was taken at different times (the same time intervals as indicated above) and added into 99 mL DI water (i.e., 100 times dilution). Then, 2 mL of the diluted solution was poured into a cuvette containing 100 μ L Nessler's reagent. After 2 min, the cuvette was placed in a spectrophotometer (Shimadzu—UV-2450) to measure the color absorbance at 410 nm. The same measurements were performed for seawater-free EICP solution (i.e., EICP solution with 0% seawater) as a baseline solution. All the tests were conducted in triplicate.

Ingredient	Seawater
Cl-	28200 ppm
Na ⁺	26250 ppm
SO_4^{2-}	5150 ppm
Mg ²⁺	7100 ppm
K ⁺	461 ppm
Ca ²⁺	400 ppm
pH	6.7
EC	63.3 mS/cm

Table 2. Major ions concentrations, pH and electrical conductivity of the seawater.

2.3. Beach Sand Treatment

Eighteen sand columns of 5-cm diameter and 10-cm height were treated in PVC tubes using two different EICP solutions of 0% (baseline solution) and 10% (v/v%) seawater at one, two, and three cycles of treatment. Each treatment condition was conducted in triplicate. Then, 267 g of beach sand was poured into each tube in three equal layers, each layer being tamped slightly from four sides to achieve a relative density of 60%. One pore volume of treatment solution (i.e., 68 mL of EICP solution) was percolated through the columns at each cycle. The samples were cured for 4 days per treatment cycle at room temperature. After curing, each column was stirred in a water bath for 2 min and soaked for two hours, in order to remove soluble compounds. After washing, specimens were oven-dried at 50 °C until a constant mass was achieved and then leveled by gently polishing the surface with sandpaper and a mini spirit level. An unconfined compressive strength test was performed to measure the strength of each treated sand column. A strain rate of 1 mm/min was applied for all UCS tests.

Carbonate content of the treated specimens was measured by gravimetric acid digestion. In this method, three 20-g chunks of each specimen were separately soaked in 100 mL of 1 M hydrochloric acid (HCl) solution. The sand suspension was stirred until no seething was observed. Ten mL of acid solution was added into the beaker under stirring. This step was repeated until the seething stopped. The suspension was left for 3 h until the sediment completely settled at the bottom of the beaker and then the acid solution was collected using a 50-mL syringe with minimal agitation. Following this, 400 mL of distilled water was added into the beaker and stirred, and then left to settle again for 2 h. After collecting the water using a 50-mL syringe, this step was repeated more times. In the end, the beaker containing the wet sand was put into an oven at 50 °C until a constant mass was achieved. To determine the mass of precipitation obtained by EICP treatment, the non-treated sand was also digested in HCl and its carbonate content was subtracted from the carbonate content of the treated specimens. The mass of enzyme-induced precipitates over the dry mass of sand was reported as enzymatic carbonated content.

2.4. Microscale Identification

Scanning electron microscopy (SEM) JEOL JSM-6610LV was employed to observe the beach sand particles before and after EICP treatment. The samples were coated with carbon using SC7620 Mini Sputter Coater. Crystal phase of the sand particles and the precipitates were identified using X-ray powder diffraction analysis (Rigaku Ultima iv X-ray powder diffractometer) with Cu-K α radiation (40 kV and 30 mA) and a scanning speed of 2°/min, which ranges from 10–90° 20. All samples were thoroughly ground and passed through a number 50 sieve. The Fourier-transform infrared spectroscopy (FTIR, Spectrum Two, PerkinElmer) was also used to determine if the precipitates contain amorphous calcium carbonate.

The following equation was used to quantify calcite and vaterite content of each precipitate using its XRD spectrum [40]:

$$X_C = X_V \frac{I_C}{I_V \times 4.0} \tag{1}$$

where X_C and X_V are the mole fraction of calcite and vaterite, respectively. I_C and I_V are the integrated intensity for a selected peak at $2\theta = 29.5^{\circ}$ for calcite, $2\theta = 25.0^{\circ}$ for vaterite. The integration of peak intensity was calculated by OriginPro (2018) software. This method is used for the precipitate that comprises of only calcite and vaterite crystal phases (i.e., $X_C + X_V = 1$).

3. Results

3.1. Aqueous Medium Experiments

Table 3 shows the results of test tube experiments for enzyme concentration determination. As shown, mass of precipitation and precipitation ratio increased by increasing the enzyme concentration to 10 mL/L above which no significant increase in precipitation mass and ratio was observed. Therefore, enzyme concentration of 10 mL/L, which is equivalent to 4650 U/L, was selected for EICP solution preparation in the aqueous medium and beach sand treatment experiments in this study. This result is consistent with Neupane et al. [8] and Almajed et al. [4] who reported a similar pattern of precipitation ratio versus enzyme concentration.

Tube No.	Engume Concentration mI/I	Enzymatic CaCO ₃ Mass, g	Precipitation Efficiency *		
	Enzyme Concentration, InL/L		Efficiency, %	Mean, STD	
T1	1.25	1.213	36.32		
T2	1.25	1.119	33.50	33.53-2.76	
T3	1.25	1.028	30.78		
T4	2.5	1.334	39.94		
T5	2.5	1.384	41.44	42.81-3.75	
T6	2.5	1.572	47.07		
Τ7	5	2.154	64.49		
T8	5	2.167	64.88	64.37-0.57	
T9	5	2.129	63.74		
T10	10	3.022	90.48		
T11	10	2.956	88.50	90.12-1.167	
T12	10	3.025	90.57		
T13	11	2.898	86.77		
T14	11	2.951	88.35	87.77-1	
T15	11	2.891	86.56		
T16	15	2.894	86.65		
T17	15	2.901	86.86	85.89-1.49	
T18	15	2.811	84.16		

Table 3. I	Results	of	test	tube	experiments.
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* Precipitation efficiency: precipitation mass over the theoretical maximum precipitation, which can be stoichiometrically obtained.

Kinetics of reaction in EICP solutions with 0%, 5%, and 10% (*v*/*v*) seawater was monitored by measuring ammonium concentration, precipitation mass, pH, and EC at different time intervals. Figure 1 shows ammonium concentration and EC versus time. As shown, the rate of ammonium production (i.e., urea hydrolysis) in the three solutions are similar until around 32 h after beginning the reactions, beyond which the rate drops faster in the solutions containing a higher concentration of seawater. The lower rate of urea hydrolysis in the solutions containing seawater after 32 h can indicate a faster rate of decay of the urease enzyme in the presence of the seawater, which caused an increase in the ionic strength of the EICP solution. EC measurements demonstrated the higher ionic strength of EICP solutions containing seawater during the reactions. The increase in EC also verifies ammonium production in each solution; however, the rate of increase in EC did not precisely represent the rate of urea hydrolysis as EC measurement gives only an indication of the ammonium ion concentration in the solution and not the ammonia off-gassed from the solution due to pH increase.



Figure 1. Comparison between electrical conductivity and ammonium concentration at different time durations.

Figure 2 shows that the change in the amount of precipitation occured in a similar pattern as urea hydrolysis versus time. As shown, the final amount of precipitation and the precipitation ratio decreased when the concentration of seawater in EICP solution increased. This can also be attributed to the decay of enzyme in the presence of seawater, resulting in an incomplete hydrolysis of urea and consequently a smaller amount of precipitation. Figure 3 shows the change in pH of each solution during the precipitation reactions. It was observed that the initial pH of 6.6–6.8 spiked to 8.5–8.8 after the addition of an enzyme into the substrate solution. This sudden increase in the pH was because of the fast rate of urea hydrolysis at the beginning of the reaction until the solution become supersaturated. When the first nucleation occured at supersaturation, the pH drops to below 8 until all the calcium ions were consumed. Van Paassen [26] reported a similar pH pattern for microbially-induced carbonate precipitation.



Figure 2. Precipitated calcium carbonate ions at different time durations (dashed line showing the chemical efficiency PR%).



Figure 3. pH values of calcium carbonate precipitation.

Mineralogy of the precipitates obtained from EICP solution containing 0%, 5%, and 10% (v/v) seawater were also examined. As shown in Figure 4a, the precipitates obtained from the three solutions contained calcite and vaterite crystal phases. Presence of vaterite in the plain EICP solution (i.e., 0% seawater) can be ascribed to the organic compounds of milk and crude urease extract [39]. The amount of vaterite in the precipitates increased by increasing the concentration of seawater. This increase in vaterite concentration can be attributed to the effect of inhibiting ions coming from seawater into EICP solution; for instance, magnesium and sulfate were reported to suppress formation rhombohedral calcite and stabilize vaterite in a calcium carbonate precipitation system [41,42]. FTIR spectra verifies the presence of calcite and vaterite in the precipitates obtained from the three solutions (see Figure 4b). It shows the characteristic peaks of calcite (i.e., 713 and 874 cm⁻¹) and vaterite (i.e., 744, 876, and 1087 cm⁻¹) [43]. No peaks associated with the presence of amorphous calcium carbonate were identified. SEM micrographs in Figure 5 illustrate the morphology of the precipitates from each solution. As shown, the precipitates mainly contain agglomerates of spherical crystals and poorly developed rhombohedral crystals. The spherical crystals can be attributed to spherical calcite and vaterite.



(b)

Figure 4. The enzymatic CaCO₃ precipitates obtained from a test tube for (**a**) XRD patterns and (**b**) FTIR patterns.



Figure 5. SEM micrographs of enzymatic CaCO₃ precipitates: (a) 0% of S.W (b); 5% of seawater; (c) 10% of seawater.

3.2. EICP Treatment of Beach Sand

The UCS and carbonate content corresponding to each test condition (0% and 10% seawater of v/v EICP solution) and different treatment cycles are plotted in Figure 6a,b. As expected, carbonate content and consequently UCS of the treated specimens increased with increasing the number of treatment cycles for both 0% and 10% seawater. The schematic in Figure 7 illustrates how increasing the number of treatment cycles may result in the CaCO₃ bond formation between sand particles. For 0% seawater, with increase in the number of treatment cycles from one to three, the average carbonate content increased from 2 to 3.7%, respectively, whereas for 10% seawater the corresponding average carbonate content increased from 1.95 to 3% respectively. Although it was theoretically expected to observe a higher carbonate content in the presence of seawater as seawater adds an additional calcium ions and other cations into the EICP solution, the less average carbonate content for 0% seawater may be attributed to the faster decay rate of urease enzyme in the presence of seawater as shown in the previous section. At the end of three cycles of treatment, for 0% seawater, the average UCS of around 0.263 MPa was obtained (Figure 6a), whereas the average UCS of 0.224 MPa was achieved for 10% seawater. However, considering the standard deviation of the measurements, no statistically significant difference between the carbonate content and UCS of the treated specimens at 0% and 10% seawater was observed.



Figure 6. Beach sand treatment results of unconfined compressive strength and enzymatic carbonate content per treatment cycle (**a**) treated with enzyme-induced carbonate precipitation (EICP) solution prepared with distilled water only (**b**) by adding 10% seawater to the EICP solution (*v*/*v*).



Figure 7. Schematic illustration of inter-particle binding at different cycles of treatment (solid arrows point out the bonds, which become stronger after each cycle and dashed arrows point out the bond development between particles with a higher inter-particle distance) [7].

XRD of the treated specimens are presented in Figure 8. As shown, except for the precipitates deposited on the surface of the treated specimens, the mineral phases detected in the body of the treated specimens for both 0% and 10% seawater were the same as non-treated beach sand. The beach sand used in the present study contains calcite as well as quartz, which makes it hard to distinguish the pre-existing calcite from the enzyme-induced calcite precipitates in the treated specimens using XRD. However, the lack of peaks corresponding to other phases of calcium carbonate (e.g., aragonite and vaterite) in the samples taken from the body of the treated specimens indicates that calcite was the predominant crystal phase of the enzyme-induced precipitation in the treated specimens. Detection of vaterite in the XRD of the precipitates deposited on the surface of the treated specimens is attributed to the vaterite minerals

formed in the treatment solution standing on the sand surface in the treatment columns (see Figure 9). The level of treatment solution added into each column was slightly above the sand surface during these experiments. Presence of vaterite was also demonstrated in the precipitates obtained from EICP solution in the test tube experiments (see Section 3.1). Khodadadi Tirkolaei et al. [39] also reported the same mineral phases for the enzymatic precipitates obtained from bulk solution and in the treated sand. These investigators reported that the presence of organic compounds with carboxyl, hydroxyl, and phosphate groups coming from urease enzyme and milk powder can suppress crystallization of calcite in the bulk solution, whereas the same compounds can promote calcite crystallization when they are adsorbed on a sand surface in the solution. Presence of pre-existing calcite crystals in the beach sand may also facilitate crystallization of calcite [44].



Figure 8. X-ray powder diffraction pattern of pure beach sand, cemented sand with 0% seawater EICP solution, cemented sand with 10% seawater EICP solution, and the crust layer.





Figure 9. EICP-treated specimens: (**a**) one cycle treatment; (**b**) three cycles treatment (the arrow points out the precipitates deposited on the surface of the treated specimen).

Figure 10 shows the SEM images of the bare sand and the treated specimens. The precipitates were observed at inter-particle contacts and on particles' surfaces. The rhombohedral crystals in the treated specimens demonstrated the presence of calcite.



(c)

Figure 10. SEM micrographs of (**a**) pure beach sand, (**b**) cemented sand with 0% seawater EICP solution, (**c**) cemented sand with 10% seawater EICP solution. (red arrows show the precipitated calcite minerals).

4. Conclusions

The study addressed the effects of seawater on the kinetics of EICP solution and strength of the bio-cemented beach sand. The results of the kinetic study revealed that the rate of urea hydrolysis and the rate of $CaCO_3$ precipitation for zero, five, and ten percent seawater were similar within the first 16 h of the reaction. After 16 h, it was observed that the rates dropped in the solution containing seawater, which might be attributed to the faster decay rate of urease enzyme when seawater was added. Calcite

and vaterite crystal phases were identified in the precipitates obtained from all test-tube experiments. It was observed that the vaterite amount increased when seawater concentration increased, which can be attributed to the effect of inhibiting ions coming from seawater into an EICP solution. Presence of seawater was observed not to have a significant effect on the UCS, carbonate content, and mineralogy of the precipitates of the treated beach sand. At the end of three cycles of treatment, a strengthened average UCS of around 0.255 MPa was obtained for the specimens treated with EICP solution containing no seawater, whereas about 0.224 MPa was achieved for those treated with ten percent seawater. The XRD and SEM results of the treated beach sand showed the presence of calcite as the predominant crystal phase of the enzyme-induced carbonate precipitates in the treated specimens.

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