Int. J. Econ. Environ. Geol. Vol. 14 (4) 20-27 2023
Journal home page: www.econ-environ-geol.org

Open Access
ISSN: 2223-957X

Sand Quality Improvement Using Watermelon (*Citrullus Lanatus*) Seeds -Derived Urease-Induced Calcium Carbonate Precipitation

Sara Zahid*, Shahnaz Choudhry

[1,2]Department of Biotechnology, Kinnaird College for Women, Lahore, Punjab, Pakistan

*Email: sarazahid203@gmail.com

Received: *16 March, 2024* ***Accepted:*** *14 May, 2024*

Abstract In Pakistan, the global warming and liquefaction problems have increased due to CO_2 emissions during the cement manufacturing process and the water absorption ability of the cement, respectively. Recently, enzyme-induced carbonate precipitation (EICP) has become increasingly popular due to its eco-friendly nature and mechanical properties. This study is aimed to extract the crude urease enzyme from watermelon seeds, considered "food waste," to test its effects on biocementation and produce a building material with more strength and less permeability for water absorption. Watermelon seeds were used in dry and germinated conditions to investigate urease activity and calcium carbonate formation. The EICP-coated brick showed less weight change than the control brick. The bricks were added in cycles (1, 7, 14 times); the 14-cycle brick was firmer than the other two bricks, but still wasn't sufficiently durable. Therefore to overcome this problem, another EICP method was used, which is the one-phase method, in which a large volume of EICP solution was mixed with sand in the same brick, and compacted in a mold again. The brick was incubated at 35–37 ˚C and cured for 14 days, resulting in a hardened brick. A water absorption test was conducted on the sample, and it was found that the bio brick could not endure the water conditions for a long period of time. Scanning Electron Microscopy (SEM) confirmed that major vaterites were formed instead of calcite, which was the cause of some disintegration of the bio brick. However, it is suggested that the EICP solution can be used as a binder in crack-filling.

Keywords; Watermelon seeds, urease, EICP, calcium carbonate, bio cement.

Introduction

Geotechnical engineering, a specialty within civil engineering, utilizes natural geological substances in engineered systems to design, construct, maintain, and protect public and private infrastructure in the natural environment. Soil and rock play a crucial role in civil engineering structures, serving as the most abundant and cost-effective building materials on Earth. It is essential to comprehend soil texture, climatic associations, and reactivity for accurate geomaterial design and execution (Abdulhameed et al., 2023; Manning et al., 2024; Stabnikov et al., 2015).

Present ground improvement technology encompasses various methods such as cement, chemicals, compression, fracture, jet, micro piles, jacked piers, driven piers, ground anchors, shore, soil dominance, and concrete columns (Baig et al., 2024; Dilrukshi et al., 2016). Microbial Induced Carbonate Precipitation (MICP) is a technology that involves the production of bio-cement by hydrolyzing urea using urease enzymes to yield ammonium and carbonate ions. This process binds soil particles together by producing calcite ($CaCO_3$) through bacterial metabolic pathways, thereby enhancing soil strength and reducing porosity. A general chemical equation for urea hydrolysis by microbial urease is provided below (Fang et al., 2024; Meenakshi et al.,2023; Mohsin et al., 2016; Behere et al., 2021).

Microbial Urease

Microorganisms, including various species such as *Bacillus sphericus, Sporosercina pasteuria, Sporosarcina psychophila, Proteus vulgaris, Mycococcus santhus, Sporosarcina urea, Pararodobacter spp. Bacillus paramycoids. Citrobacter sedalki, Enterobacter, Procella, Bacteria, Enterobacter, Procella, Enterobacter, Procella, Enterobacter, Prosilia, Lecobacter spp* etc. are utilized for MICP, with *Bacillus* species being prominent (Avramenko et al., 2024; Dubey et al., 2021; Garg et al., 2024). While MICP is an effective method for producing bio-cement, the main ingredient, urease enzyme, can also be derived from plants rich in urease, such as jack beans (*Canavalia aniformis*) and soybeans (*Glycine max*), chickpeas (*Caesar aritinum L.*), watermelon seeds (*Citrullus lanatus*), mikawa seeds (*Acer palmatum*) pigweed (*Chenopodium album*) and mulberry leaf (*Moras alba*), and cabbage leaves etc. This alternative method, known as Enzyme Induced Carbonate Precipitation (EICP), involves using an enzyme to induce precipitation in cement (Albenayyan et al., 2023; Dubey et al., 2021; Iqbal et al., 2021; Yuan et al., 2024; Mohamad et al., 2022; Saif et al., 2022;).

Microbial urease

$$CO(NH_2)_2 + H_2O \xrightarrow{\qquad} 2NH_4^+ + CO_2^{-3}$$

$$Ca^{2+} + CO_2^{-3} \xrightarrow{\qquad} CaCO_3 \downarrow$$

Materials and Methods

Urease Enzyme Extraction from Resting Seeds

Watermelon seeds were bought from the seeds shop, Roshan Seeds in Allama Iqbal Town, Lahore and brought to the Kinnaird College Biotechnology laboratory for further investigation. 5 grams of watermelon seeds were taken and washed, first with tap water (2-3 times) then with distilled water (2-3) times, and dried at room temperature. Seeds were ground with the mortar and pestle and then blended in the kitchen blender by adding 100 ml of distilled water. The solution was stirred at 600 rpm on a magnetic stirrer for 1 hr. The solution was divided into 2 portions; one portion was used for the direct measurement of urease activity and the other was used for filtration.

As the solution was thick, a tea strainer was used to remove large particles**,** then muslin cloth was used to remove all the thick material from the crude solution. The solution was filtrated through muslin cloth at least 2 times. The filter paper was used for the filtration of the solution.

After filtration centrifugation was done at 10,000rpm at 25 ºC for 5 minutes. The supernatant was taken out and the pellet was removed. Refiltration was performed to remove a fatty layer present on the supernatant. The pH of the unfiltered and filtrate (crude urease solution) was maintained. Non-treated crude extract and treated crude extract, and 0.1 M urea solution were kept in the water bath at 30 ºC (Faiad et al., 2019; Al Imran et al., 2021; Javadi et al., 2018).

Indophenol Test

After that indophenol was performed (Imran et al., 2021). In this test, the pH of the treated and non-treated urease extract was adjusted to 7.5, and the pH of the phosphate buffer was adjusted to 7 (Al Imran et al., 2021).1 ml of non-treated urease extract and treated urease extract was added to 0.1 M urea solution in phosphate buffer. 2 ml of the solution was taken out from urease - urea mixture solution at different time intervals, 0 min, 5min, 10 min, 15 min. The extraction of solution at 0 min. was done immediately after the addition of the urease solution. The reaction was stopped by the addition of 4ml of phenol reagent, and 4 ml of sodium hypochlorite solution was vortexed so that all the reagents and solution got mixed**.** The tubes were incubated for 10 min. at 50°C. Solutions were made in ratio 1: 3 (one portion of solution and 2 portions of deionized water) Readings of absorbance at 630 nm were taken. (Ch et al., 2021; Al Imran et al., 2021; Javadi et al., 2018; Dilrukshi et al., 2018; Reddy et al., 2013). The standard in this case contained everything except the watermelon seeds crude solution and absorbance was multiplied with the dilution factor. Find the reading of the unknown by using the standard calibration curve.

Standard Calibration Curve

A standard calibration curve was made by using the different ppm (mg/L(1000ml) 0.1, 10, 20, 30, 40, 50 of a solution made from the stock solution of 1000ppm of NH_4Cl.

Extraction of urease from 2 and 4 days germinated watermelon seeds

Washed seeds were placed on a wet paper towel and placed in the incubator at 30-35 ºC for 48 hrs. (2 days) and 96 hrs. (4 days), respectively. After drying crush, them and repeat the protocol mentioned above.

Urease activity calculation formula

One unit of enzyme activity is defined as the amount of enzyme that liberates one µ mole of ammonia per minute**.**

The rate of NH_4^+ generation, r_1 (mg/L/min) can be obtained from graphs 8,9,10,11,12,13, and urease activity was calculated as urease activity will be calculated by the formula given below:

The rate of $NH_4{}^+$ generation (mg /L/ml)

Rate of NH_4 + generation-lmolNH_4^+/min = $r_1 * 0.1 * 1/18 * 10^3 = r_2$

Urease activity = mol urea hydrolyzed/min = [U/mL]

$= r_2 * ½$

Test Tube Experiment

The rate of precipitation of calcium carbonate under different combinations of urease, urea, and $CaCl_2$ was evaluated in a transparent 15 ml Falcon tube to understand the process of solidification in sand specimens.A total of 10ml equimolar solutions of urea-$CaCl_2$, with a concentration of 0.5 mol/L (M), 0.7, and 1M were prepared separately in watermelon seeds extract with known concentrations of 2.5g/100ml, 3.5g/100ml, and 5g/100ml. The urease activity obtained was 2.312 U/ml, 3.107 U /ml, and 5 U/ml, respectively. The reaction was initiated by mixing the required amount of $CaCl_2$ with the $CaCl_2$-urease solution. The tubes were placed in an incubator at 35-37 ºC for 48 hours, through the Whatman filter paper grade 42. For the separation of the $CaCO_3$ precipitated and to remove other impurities the solution was washed with deionized water. Drying $CaCO_3$ in a hot air oven was done for 1 hour and weight was measured (Behere et al., 2021; Yuan et al., 2024; Mohamad et al., 2022; Mohsin et al., 2016).

Table 1. Urea amount and $CaCl_2$ amount

Chemicals	0.5 M	0.7M	1M
$CaCl_2$	0.55g	0.77g	1.1g
Urea	0.3g	0.42g	1.8g

$CaCO_3$ confirmation test

A biochemical test was performed to confirm the presence of $CaCO_3$ in the test tubes for this purpose 1 g of white powder was obtained from the test tube experiment and 5ml concentrated HCl was added to it.

Formation of bio bricks

2 types of experiments were run.

EICP Spray Experiment

A block of cement and sand (Sand, cement, and water in ratio 3:2:1) was made and sprayed with 50 ml of EICP solution incubated at 35- 37 ºC for 14 days, and then its water absorption capacity was checked by immersing the bricks in 100 ml water overnight. The dry weight of the bricks was measured, and then the wet weight of the bricks was measured. The control in this case is made up of cement and sand.

Bio cement brick

The sand was taken from the sides of river Ravi. The sand was placed under the sunlight for 2 days so that it became completely dry. 1M EICP solution containing urea and $CaCl_2$ and dry skimmed milk (organic stabilizer) (1.5 g) was prepared. The bricks were made in cycles, the number of cycles given below indicates the number of times EICP solution was added to bricks (Behere et al., 2021; Yuan et al., 2024; Mohamad et al., 2022; Mohsin et al., 2016).

1, 7 and 14 Cycles

In 1 cycle brick ,100-gram sand was taken and mixed with 50ml of EICP solution containing crude urease extract, urea, calcium chloride, and skimmed milk (1-2 grams) was added to the brick (100g sand and 50 ml EICP solution) were mixed and compacted, and then put in the square molds. In 7 cycle, the above experiment was repeated with the addition of 10 ml EICP solution after every 48 hrs upto 7 times. In 14 cycle, same process was repeated upto 14 times.

The bricks were placed in an incubator at 35-37℃ and cured for 28 days, and all the experiments were run in triplicates.

Additional large volume EICP solution

After the failure of the cycle's method, to overcome this problem one of the bricks of 14 cycles was tested, in which sand containing $CaCO_3$ was taken out in a beaker. A large volume of EICP solution (210 ml) was added to it. It was mixed with sand and compacted in the mold again. It was incubated for about 14 days at 35-37˚C.

Water absorption test

In order to check the water absorption capacity of bricks, a small portion of equal weight 12g was taken from both bio cement brick and control (cement and sand) brick. Those pieces were immersed in 50 ml water for 24 hrs. Wet weight was taken and a change in weight was observed.

Scanning electron microscopy

The precipitated crystals formed in bio brick were analyzed through the Scanning electron microscope. The SEM analysis helped in the determination of the morphology of $CaCO_3$.

Results and Discussion

Crude Urease Extraction- its Urease Activity

In the first part of the study, the extraction of urease enzyme from watermelon seeds having different germinating conditions (dry, 2 days, 4 days germinating seeds) was done. A summary of urease activity (U /ml) of the extracted crude enzyme from watermelon seeds is presented in Table 2. According to the results, the urease activity of the non-treated watermelon seeds crude extract is higher than the treated crude extract. In the case of resting seeds, the urease activity of non-treated crude (4.3 U/ml) solution is higher than treated crude solution (4 U/ml). In the case of 2 days of germinated seeds, the urease activity of non-treated crude solution (5.2 U/ml) was enhanced than treated crude solution (5 U /ml). The urease activity of 4 days germinated non-treated crude solution (3.58U /ml) was more than in treated crude solution (3.50 U/ml). Comparison among urease activity of resting seeds, 2 days and 4 days germinated seeds is shown in Figure 1 which indicates that urease activity of dry seeds was (4 U/ml) gradually increased after 48 h germination (5 U/ml) and then declined after 96h (3.5 U/ml).

The fluctuation of the urease activity was influenced by some metabolic factors, biological reactions, and fluctuational proteins. (Nhung et al., 2019; Jain et al., 2024). Variety of cereal, type of enzymes, the response of seeds towards water content, respiration changes and hormonal changes are also factors (Almajed et al., 2018; Castro-Camba et al., 2022; Guzmán-Ortiz et al., 2019), which trigger the enzymatic activity of the seeds. In this study, the urease activity obtained in the case of dry seeds (4 U/ml) was nearly equal to some previous studies which mainly focused on the crude solution extracted from crushed watermelon seeds (Dilrukshi, 2016).

Table 2. Urease activity of treated and non-treated watermelon seeds crude extract on different days.

Germination period	Urease activity of treated solution (U/ml)	Urease activity of non-treated solution (U/ml)
Resting seeds	4 .00	4.3
2 Days Germinated seeds	5	5.2
4 Days Germinated seeds	3.50	3.58

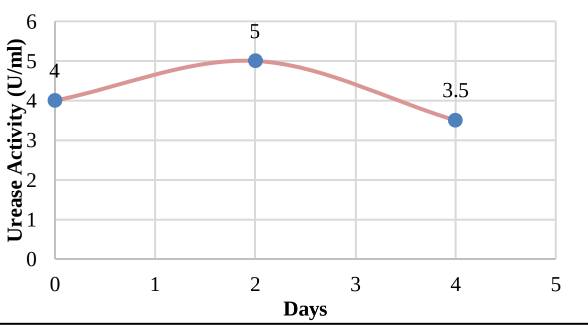

Fig. 1 Urease activity of dry,2, and 4 days of germinating seeds.

Relationship between concentration of NH_{4+} and time

Figure 2 indicates that NH_4^+ concentration in both treated and non-treated crude solutions increases with time (0min-15min). According to the results of resting seeds in treated crude solution, the concentration of NH_4^+ at 0 min is 5 mg/L and gradually increases up to 25 mg/. However, in the case of non-treated crude solution concentration increased from 9mg/L to 32mg/L and represented the straight-line graph. For 2 days and 4 days, germinated seeds Figures 3, and 4 also represent the straight-line graph between the time and concentration of NH_4^+ respectively. In this part of the study, results have shown that NH_4^+ concentration in non-treated crude enzyme solution is slightly higher as compared to the treated crude enzyme solution. This shows that a non-treated crude enzyme solution containing suspended particles of seeds possesses urease activity. The results obtained regarding the high urease activity of non-treated crude enzyme solution are similar to previous studies. The main reason for higher urease activity is the presence of a trace amount of urease enzyme in the seed coat and cotyledon membrane (Francoz et al., 2018). The results have also revealed that by increasing time from 0-15 min at 30°C temperature and pH 7, the concentration of NH_4^+ increases due to an increase in the urease activity.

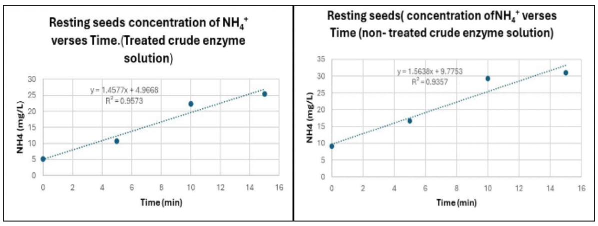

Fig. 2 Concentration of NH_4^+ with time (a) in treated crude enzyme solution (b) in a non-treated solution of resting seeds

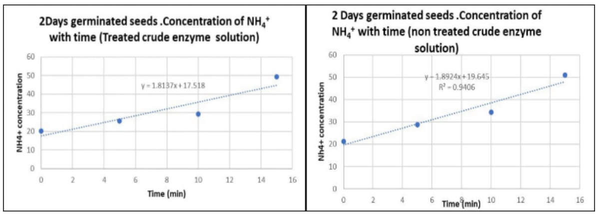

Fig. 3 Concentration of NH_4^+ with time (a) in treated crude enzyme solution (b) in a non-treated solution of 2 days germinated seeds.

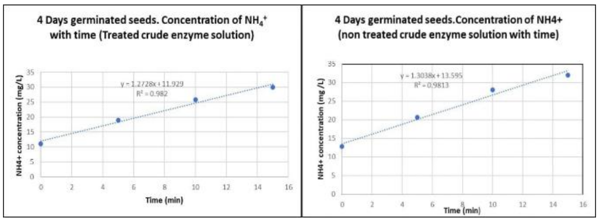

Fig. 4 Concentration of NH_4^+ with time (a) in treated crude enzyme solution (b) in a non-treated solution of 4 days germinated seeds.

Test Tube Experiment

Test tubes containing EICP solution of different molarities (0.5M, 0.7 M, and 1M) that have formed calcium carbonate at the bottom of the test tubes. It indicates that the weight of $CaCO_3$ of 1 M EICP solution was (1.52g), 0.5 M (0.73 g) and 0.7 M (1.16g). The test tube containing 1 M EICP solution formed more $CaCO_3$ crystals as compared to the other 0.5 M and 0.7 M which indicates that the amount of increase in a product has a positive direct relation with the amount of substrate. It has also revealed that the enzyme activity increases due to an increase in the substrate concentration. The comparison of this study with the previous studies shows probably similar results when 0.3, 0.5, and 0.7 M solutions were used and the highest amount of $CaCO_3$ was obtained in 0.7 M. This has confirmed that in the EICP process, the crystal precipitation amount is closely related with $CaCl_2$ -urea concentration, and the $CaCO_3$ crystals started to form immediately because of the accessibility of free urease enzyme (Albenayyan et al., 2023; Al Imran et al., 2021; Dilrukshi et al., 2018; Javadi et al., 2018; Zhang et al., 2023). The biochemical test has also approved the confirmation of the presence of $CaCO_3$ by the effervescence of CO_2 confirming the presence of $CaCO_3$ in the test tubes.

EICP Spray Experiment

Results have shown that the final weight of the sample brick (cement and sand) increased as compared to the control brick weight (Table 3) showing that there is a 4g change in the weight of the sample brick in contrast to the weight change of 18g control brick. The cement and sand brick coated with EICP crude solution had less weight change after the water absorption experiment as compared to the control brick. This is due to the formation of calcium carbonate precipitates on the surface of the brick, due to which the brick did not absorb water. Moreover, the initial weight of the bio brick was less than when coated with EICP solution, and the reason is the same, the formation of calcium carbonate crystals which increase the weight of the brick. In the previous studies, the urease-containing *Bacillus* species were coated on the cement brick, water absorption test was performed which showed the same results, less change in weight as compared to the control (Cheng et al., 2019; Dubey et al., 2021; Yuan et al., 2024; Fang et al., 2024).

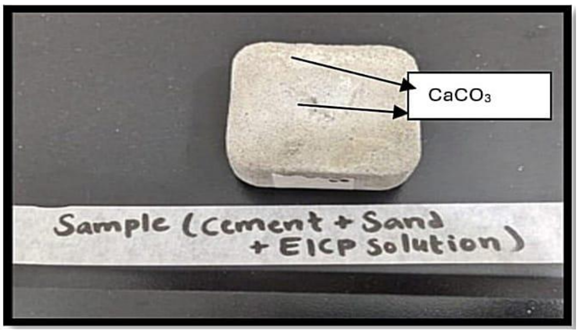

Fig. 5 Formation of $CaCO_3$ on sample brick.

Table 3. Weight of the sand cement brick.

Brick	Dry Weight (Before immersion in water) W1	Weight after immersion in water overnight W2	Change in weight W2-W1
Control (Cement and Sand)	100g	118g	18g
Sample Brick (Cement, sand and EICP solution)	109g	113g	4 g

Bio cement brick formation

At the bio cement brick formation step, the brick with 1-cycle containing 50ml of EICP solution was not hardened because a very small amount of calcium carbonate was made. However, the 7-cycle brick (120ml of EICP solution) has more calcium carbonate than 1 cycle due to hardening at some points but still, not enough, and 14 cycle brick (190 ml of EICP solution) got a little hardened from the edges and center but still, there was loose sand present.

Fig. 6 Bricks with different cycles.

Additional large volume EICP solution

The large volume of EICP solution (210ml) was added to one of the 14-cycle bricks mixed, compacted, and then incubated for 14 days, the brick got hard. The main reason is the formation of calcium carbonate. In the previous studies cycle methods have shown good results as compared to the mix-and-compact method. But in some studies, the mix-and-compact / one-phase method has shown better results (Cheng et al., 2019; Yu et al., 2023) However, it is to be noted that in these studies ratio of sand and EICP was 1:1 whereas in this case ratio of sand is 1:4. The reason according to the researchers is to apply this method to eliminate the cycles and make a one-phase method instead of the number of phases.so, it is useful for the real-life application of this technology. The length and width of the brick were 5cm, height was 2cm. The weight of the brick was 106 grams.

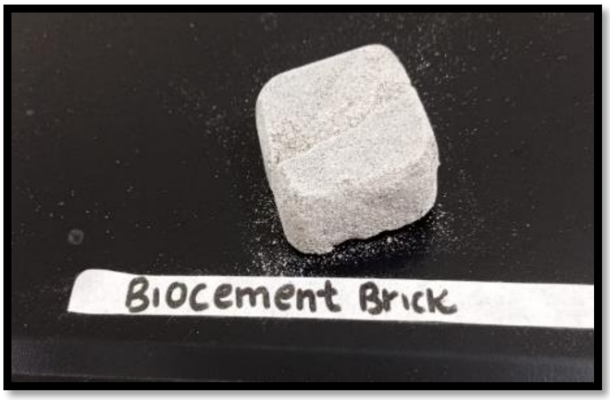

Fig. 7 Bio cement brick.

Water absorption test

In the final phase a water absorption test is applied on a small portion of control brick and bio brick. The weight of the piece of cement brick was enhanced from 12g - 14g however, the bio cement brick weight decreased from 12 g to 8 g due to dispersion of brick in the water. The main reason was that the efficiency of bio-cemented samples depends on the type and structure of the precipitated $CaCO_3$ polymorphs i.e. vaterite or calcite (Lai et al., 2024; Nhung et al., 2019).

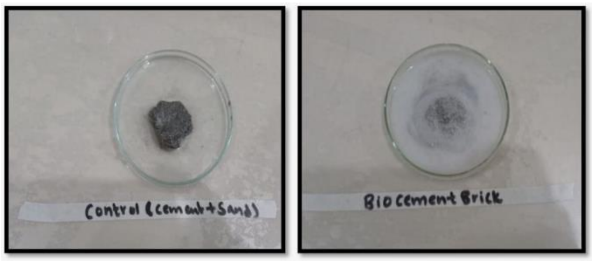

Fig. 8 Comparison of water absorption in control brick (cement and sand) and bio cement brick.

Scanning Electron Microscopy Analysis

The results clearly showed the formation of $CaCO_3$ in bio brick due to urease activity with urea and $CaCl_2$, whereas in control sand there is no $CaCO_3$ (Fig. 9). From a previous study, it was revealed that fast urea hydrolysis by urease enzyme causes a disintegrated phase due to which although $CaCO_3$ formed in calcite crystals form is much less and vaterite form is more (Baig et al., 2024). It was also confirmed by the SEM analysis that vaterite is formed in this case which is not firm calcium carbonate precipitate as calcite and loose sand at some point**.** Vaterite is the least stable polymorph, when it is exposed to water, slowly dissolves and recrystallizes to a stable form, possesses large porosity, and rapid disintegration under mild conditions whereas calcite possesses crystalline nature, and is thermodynamically stable with no disintegration (Dilrukshi et al., 2018; Feng et al., 2024).

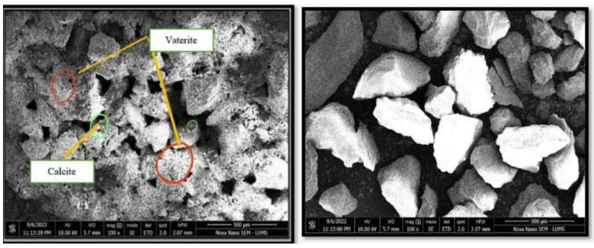

Fig. 9 (a) Vaterites and calcites in biobrick and (b) Control sand without $CaCO_3$.

Moreover, sand type also matters, according to research (Yadav et al., 2021; Arab et al., 2021) it has been mentioned that the EICP solution works best not in all soils, clays, and sands due to different porosity, chemical, and physical properties of sands, soil, and clay. It is essential to check the effect of EICP solution on different sands at the laboratory level before applying it at a large level. Recent research has proved that the addition of $MgCl_2$ in the EICP solution slows down the hydrolysis of the urea process and stops the disintegration phase due to which vaterite formation will be constricted and calcite crystals can be formed (Baig et al., 2024).

Although adding more EICP solution hardened the brick and cemented the sand particles together, it was still not hard enough to apply a strength test to the brick, such as an unconfined compressive strength test, hence the strength was not assessed. It is recommended that this EICP solution obtained from watermelon cannot be used as the main binding agent in making bricks due to its low urease activity, therefore the very large volume of EICP solution will be required as compared to other plants such as soya bean, jack bean in which the urease activity is very high therefore less amount of EICP solution is enough to make good strength brick. However, it can be used as a spray to fill concrete or soil cracks. It is also recommended to further check the crystalline nature of $CaCO_3$, the physical properties of sand and soil, and the addition of $MgCl_2$ in EICP solution to improve brickmaking.

Conclusion

The possibility of utilizing the enzyme-induced carbonate precipitation (EICP) method as an efficacious and eco-friendly bio-inspired technique for soil improvement has been assessed in this study. Successful extraction of urease enzyme from watermelon seeds was made in which 2 days germinated seeds showed higher urease activity than dry and 4 days germinated seeds. The increase in $CaCl_2$-urea concentration caused an increase in $CaCO_3$ formation. Cement and sand brick coated with EICP solution showed less permeability towards the water as compared to control brick. The large volume of EICP solution added to the brick containing 14 cycles of EICP solution hardened the brick, which indicated that the one phase (mix-and-compact) method can also form bio brick and eliminate the repeated cycle procedure. However, the brick was not strong enough due to more vaterite (unstable) polymorph than calcite (stable) polymorph of calcium carbonate, which ultimately lead to dispersion of bio brick in water. However, it is recommended to use the EICP solution as an effective crack-filler spray to fill the minor cracks in soil and concrete buildings.

References

Al Imran, M., Nakashima, K., Evelpidou, N., Kawasaki, S. (2021). Improvement of using crude extract urease from watermelon seeds for bioce mentation technology. *Geomaterial Journal,* **20** (78), 142-147.

Almajed, A., Khodadadi Tirkolaei, H., Kavazanjian Jr, E. (2018). Baseline investigation on enzyme-induced calcium carbonate precipitation. *Journal of Geotechnical and Geoenvironmental Enginee ring,* **144** (11), 29-36.

Arab, M. G., Alsodi, R., Almajed, A., Yasuhara, H., Zeiada, W., Shahin, M. A. (2021). State-of-the-art review of enzyme-induced calcite precipitation (EICP) for ground improvement: Applications and prospects. *Geosciences,* **11**(12), 492-531.

Albenayyan, N., Murtaza, M., Alarifi, S. A., Kamal, M. S., Humam, A., AlAhmari, M. M., Khalil, A., Mahmoud, M. (2023). Optimization of calcium carbonate precipitation during alpha-amylase enzyme-induced calcite precipitation (EICP). *Frontiers in Bioengineering and Biotechnology,* **11**, 1118993-1119003.

Avramenko, M., Nakashima, K., Takano, C., & Kawasaki, S. (2024). Ecofriendly solidification of sand using microbially induced calcium phosphate precipitation. *Scientific Reports,* **14**(1), 12412-12432.

Baig, A. R., Alarifi, S. A., Mahmoud, M., Kamal, M. S., Murtaza, M., AlAhmari, M. M., Alhumam, A. (2024). Experimental investigation of a modified enzyme-induced carbonate precipitation solution for sand production control applications. *SPE Journal,* **29** (5), 2463-2475.

Behere, M., Patil, S. S., Rathod, V. K. (2021). Rapid extraction of watermelon seed proteins using microwave and its functional properties. *Preparative Biochemistry & Biotechnology,* **51** (3), 252-259.

Castro-Camba, R., Sánchez, C., Vidal, N., Vielba, J. M. (2022). Plant development and crop yield: The role of gibberellins. *Plants,* **11**(19), 2650-2676.

Ch, R., Narbaev, K., Aronbaev, D., Aronbaev, S. (2021). Optimization of indophenol complex formation conditions for spectrophotometric determination of ammonium ions. *Norwegian Journal of Development of the International Science,* **3** (77-1), 3-9.

Cheng, L., Shahin, M. A., Chu, J. (2019). Soil bio-cementation using a new one-phase low-pH injection method. *Acta Geotechnica,* **14** (3), 615-626

Dilrukshi, R., Kawasaki, S. (2016). Effective use of plant-derived urease in the field of geoenvir onmental. Geotechnical Engineering. *J. of Civil Environ. Engg.,* **6** (207), 1-13.

Dilrukshi, R., Nakashima, K., Kawasaki, S. (2018). Soil improvement using plant-derived urease-induced calcium carbonate precipitation. *Soils and Foundations,* **58**(4), 894-910.

Dubey, A. A., Ravi, K., Mukherjee, A., Sahoo, L., Abiala, M. A., Dhami, N. K. (2021). Biocementation mediated by native microbes from Brahmaputra riverbank for mitigation of soil erodibility. *Scientific reports,* **11**(1), 15250.

Faiad, S., Naji, E., Hajeej, J. (2019). Optimization for extraction proteins from pulp of watermelon seed. *Tikrit Journal for Agricultural Sciences,* **19**(2), 75-83.

Fang, C., Achal, V. (2024). Enhancing carbon neutrality: A perspective on the role of microbially induced carbonate precipitation (MICP). *Biogeotechnics,* **2** (2), 100083-100089.

Feng, W., Ng, Z. Y., Chen, H., Zhan, J., Xu, D., He, Y., Wei, H., & Dang, L. (2024). Calcium Ion Deposition with Precipitated Calcium Carbonate: Influencing Factors and Mechanism Exploration. *Processes,* **12** (4), 629-644.

Francoz, E., Lepiniec, L., & North, H. M. (2018). Seed coats as an alternative molecular factory: thinking outside the box. *Plant Reproduction,* **31**(3), 327-343.

Garg, R., Garg, R., & Eddy, N. O. (2023). Microbial induced calcite precipitation for self-healing of concrete: a review. *Journal of Sustainable Cement-Based Materials,* **12**(3), 317-330.

Guzmán-Ortiz, F. A., Castro-Rosas, J., Gómez-Aldapa, C. A., Mora-Escobedo, R., Rojas-León, A., Rodríguez-Marín, M. L., Román-Gutiérrez, A. D. (2019). Enzyme activity during germination of different cereals: A review. *Food Reviews International.,* **35** (3), 177-200.

Imran, M. A., Nakashima, K., Kawasaki, S. (2021). Bio-mediated soil improvement using plant derived enzyme in addition to magnesium ion. *Crystals,* **11** (5), 516-530.

Iqbal, D. M., Wong, L. S., Kong, S. Y. (2021). Bio-cementation in construction materials: a review. *Materials,* **14** (9), 2175-2195.

Jain, S. (2024). Influence of biomass and chemicals on kinetics of ureolysis-based carbonate biomineral precipitation. *Environmental Earth Sciences,* **83**(2), 58.

Javadi, N., Khodadadi, H., Hamdan, N., Kavazanjian, E. (2018). EICP Treatment of Soil by Using Urease Enzyme Extracted from Watermelon Seeds. *Geotechnical Special Publication,GSP* **296**,115-124.

Lai, H., Ding, X., Cui, M., Zheng, J., Chu, J., & Chen, Z. (2024). Factors affecting the effectiveness of biocementation of soil. *Biogeotechnics,* **2**(3), 100087-100095.

Mohsin, H., Asif, A., Fatima, W., Nadeem, S., Sabri, A. N. (2016). Assessment of biocalcification by the water absorption test using microorganism from oil contaminated site. *Punjab University Journal of Zoology,* **31** (1), 09-12.

Mohamad, N., Muthusamy, K., Embong, R., Kusbiantoro, A., Hashim, M. H. (2022). Environmental impact of cement production and Solutions: A review. *Materials Today: Proceedings,* **48** (4), 741-746.

Nhung, H. T. N., Loan, H. T. T., Lam, T. B. (2019). Effects of germination time of soybean to the activity of soybean urease enzyme. *Bioresearch Communications-(BRC),* **5** (1), 606-609.

Saif, A., Cuccurullo, A., Gallipoli, D., Perlot, C., Bruno, A. W. (2022). Advances in enzyme induced carbonate precipitation and application to soil improvement: A review. *Materials,* **15** (3), 950-979.

Stabnikov, V., Ivanov, V., Chu, J. (2015). Construction Biotechnology: a new area of biotechnological research and applications. *World Journal of Microbiology and Biotechnology,* **31**(9), 1303-1314.

Yadav, V. K., Yadav, K. K., Cabral-Pinto, M. M., Choudhary, N., Gnanamoorthy, G., Tirth, V. Khan, N. A. (2021). The processing of calcium rich agricultural and industrial waste for recovery of calcium carbonate and calcium oxide and their application for environmental cleanup: A review. *Applied Sciences,* **11**(9), 4212-4236.

Yu, X., Pan, X. (2023). One-phase improvement of sandy soil using seawater-based soybean-induced carbonate precipitation. *Journal of Sustainable Cement-based Materials.,* **12** (8), 962-971.

Yuan, L., Li, G., Liu, J., Wang, P., Liu, C., & Zhang, J. (2024). Study on Mechanical Properties of Sandy Soil Solidified by Enzyme-Induced Calcium Carbonate Precipitation (EICP). *Buildings,* **14** (7), 1977-1991.

Zhang, Y., Hu, X., Wang, Y., & Jiang, N. (2023). A critical review of biomineralization in environmental geotechnics: Applications, trends, and perspectives. *Biogeotechnics,* **1**(1), 100003-1000011.