

Soybean urease-based EICP stabilization of washed recycled sands derived from demolition wastes cured at low temperatures

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ARTICLE INFO

Keywords:

EICP
Recycled sand
Demolition waste
Geotechnical fills
Ground improvement

ABSTRACT

This study evaluated the feasibility and efficiency of stabilizing washed recycled sand (RS) derived from construction and demolition wastes when treated by soybean-based enzyme-induced carbonate precipitation (EICP), cured at a low temperature of 4 °C. Two types of RS were studied, being fine recycled sand (FRS) and coarse recycled sand (CRS). Urease extracted from soybeans was used to catalyze the urea hydrolysis reaction, which led to the precipitation of calcium carbonate, which acts as a soil binder. Such a low-temperature curing condition was found to effectively minimize surface clogging at the same treatment cycles when compared to room-temperature curing due to a comparatively low urease activity. The excess amount of CaCO₃ precipitated on the surface was found to block the effective EICP solution from flowing through the sample and did not contribute to strength improvement. The unconfined compressive strength (UCS) results on CRS depicted a slight strength reduction at low-temperature curing, but the improvement in bio-clogging indicated the potential of involving extra treatment cycles which could further develop the strength. SEM, EDS and XRD analyses further established the existence of the biocement gel triggered by soybean-extracted urease cured at low temperatures within the both CRS and FRS particles. As such, soybean-based EICP solution was found to be an effective green binder for washed RS in terms of the noticeable stabilizing effect, hence was found to be a suitable as a geotechnical fill material for ground improvement works.

1. Introduction

Since the 20th century, an increasing number of engineers and scientists have proposed the concept of recycling and reuse of sustainable construction materials, in order to create a better future for both humans and the Earth. At the same time, land disposal wastes from construction activities have been witnessed to grow dramatically in the past few years, which is a negative impact as a result of increased industrialization and modernization progress. The equilibrium between the waste disposal growth and field capacity is increasingly difficult to maintain,

resulting in the generation of excessive amounts of solid waste. Thus, engineers, especially from the civil and geotechnical engineering fields, have been progressively and continuously seeking sustainable alternatives of virgin materials to be used in a wide range of applications by adopting appropriate processing approaches and technologies. Researchers proposed to use recycled crushed concrete aggregates derived from construction and demolition (C&D) waste to substitute the original aggregates used in the pavement subbase [1]. Reported values of California bearing ratio, Los Angeles Abrasion Loss, and resilient modulus from repeated load triaxial tests concluded the satisfactory strength

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<https://doi.org/10.1016/j.conbuildmat.2024.136735>

Received 24 March 2024; Received in revised form 2 May 2024; Accepted 20 May 2024

Available online 27 May 2024

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Table 1
Cost details of EICP treatments.

Material	Unit price ^a	Typical Dose per L solution ^b
Purified enzyme powder	AUD 156/g (USD 102/g)	0.25 g
Soybean	AUD 0.012/g (USD 0.078/g)	180 g ^c
DM water	AUD 1.10/L (USD 0.72/L)	> 1 L
CaCl ₂ powder	AUD 0.087/g (USD 0.06/g)	110.99 g
Urea powder	AUD 0.058/g (USD 0.04/g)	60.06 g
Skim milk powder	AUD 10/g (USD 6.5/g)	4 g

Note: ^a Unit price with conversion (1 AUD = 0.66 USD).

^b shows the dose used in EICP solution containing 60 g/L sieved soybean powder, 1.0 M CaCl₂ solution and 1.0 M Urea solution.

^c includes the assumption of 30 % sieving rate.

performance and durability of recycled concrete aggregates in pavement applications. Some studies prompted alkali activation on recycled concrete aggregate and recycled aluminium salt slag and discussed the opportunities in terms of being used in semi-rigid inclusion columns [2]. Researchers also carried out a series of strength and environmental tests on olive stone biochar produced via the gasification process, reporting the suitability and potential of the usage as construction fill materials [3].

Washed recycled sand (RS) is an innovative concept within recycled products derived from C&D waste due to the participation of high-pressure watering technology. Such a processing approach makes washed RS different from the unconventional term of recycled sand [4, 5]. The feeding material of the washing plant contains diverse types of C&D waste, washed RS, as one of the final products, which is therefore different from natural sand because of the ingredients. However, they fall into the same particle size ranges, as outlined in the United Soil Classification System (USCS) [6].

The target application of washed RS is as a geotechnical fill materials for ground improvement works, thus it has to be readily compacted [7]. Portland Cement is one of the most widely acknowledged and used chemical stabilizers in the world's construction industry over the last few decades, with various benefits such as strong binding ability, high durability, low permeability, short reaction time, easy accessibility and fair adaptability [8–10]. However, the production process of cement was reported to be one of the largest contributors to carbon dioxide emissions at the same time [11], hence the need to seek other chemical binders with less carbon dioxide footprint from an environmentally sustainable perspective.

Biocementation is an environmentally friendly biochemical urea

hydrolysis process releasing a considerable amount of carbonate ions, which can form inorganic compounds at a later stage. It is believed to be an ideal candidate in terms of cement replacement binder as the precipitated calcium carbonate bonds soil particles together and decreases the permeability by filling up the voids [12,13]. The expected precipitation of calcium carbonate is induced either by free enzyme, which is called enzyme-induced carbonate precipitation (EICP), or microbially bacteria, which is called microbially induced carbonate precipitation (MICP). Both EICP and MICP approaches follow the same working principle which embraces a fundamental step called ureolysis [14]. A source solution containing calcium (Ca²⁺) is vital to form calcium carbonate. Calcium chloride was chosen for this study, considering its easy access from the market as well as its affordable price. Differing from MICP, EICP mainly relies on the urease enzyme's catalyzing function without absorbing the calcium ions [15], thus highlighting the importance of urease activity. The reaction equation is presented as follows [16].



Several studies reported a generally low calcium conversion rate of EICP treatment compared with MICP treatment, indicating extra treatment cycles are expected, and extra cost would be spent [17, 18]. Yet purified urease powder, such as when extracted from jack bean (*Canavalia ensiformis*), was reported to have fairly high activity in numbers [19, 20], which can make it competitive with MICP-treated samples in terms of strength improvement. The price of the purified urease powder

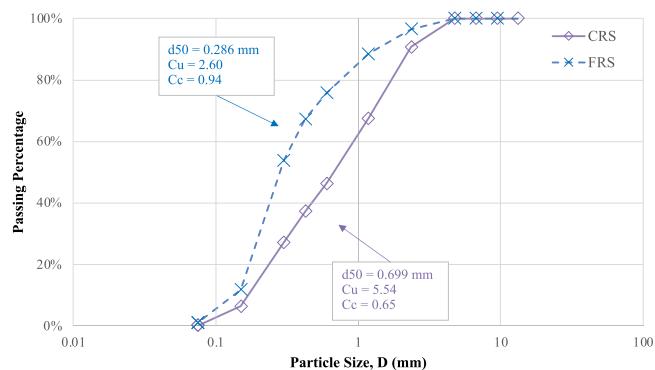


Fig. 1. Gradation curves of CRS and FRS (Modified after [29]).

Table 2
UCS details of EICP treated samples using soybean extracted urease enzyme.

UCS (MPa)	Soybean powder concentration (g/L)	Curing temperature (°C)	ES's pH adjustment	CS's molar concentration (M)	Treatment cycles	Source material	Reference
0.12 ^a	100	20	8	0.75	1	Yellow river silt	[34]
1.8 ^a	80	25	N.A.	1.0	4	Ottawa sand	[21]
3.8	50	20	N.A.	1.0	12	Calcareous sand	[33]
0.6 ^a	100	25	6.5	2.0	2	Ottawa silica sand	[39]
0.623	20	Room temperature	N.A.	1.0	3	Silica sand	[47]
1.5	80	25	N.A.	1.0	10	ASTM C778-graded sand	[48]
0.2	100	25	6.5	0.5	4	River sand	[23]
0.7	30	25	7.3	1.5	1	Clayed sand	[49]
0.8 ^a	60	25	N.A.	0.5	6	ASTM C778-graded sand	[43]
0.1							
0.2 ^a	60	25	N.A.	0.5	8	Ottawa sand	[50]

Note: N.A. = Not adjusted.

^a additives involved in either urease extraction or sample treatment process.

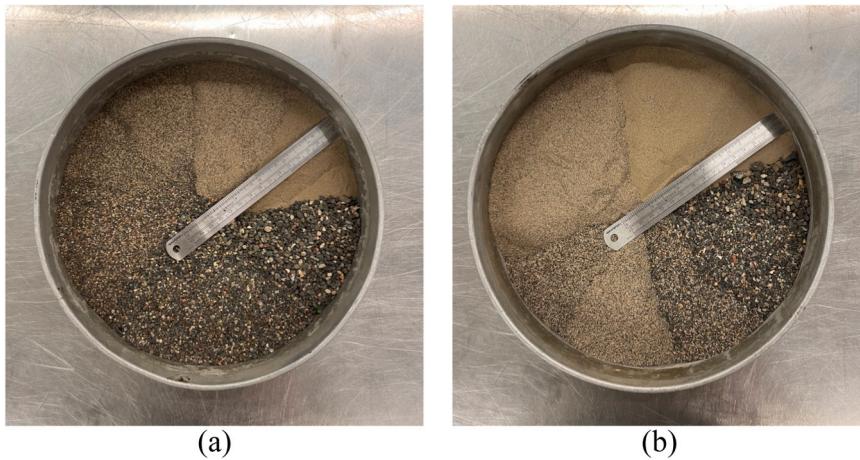


Fig. 2. Physical appearance of (a) CRS; and (b) FRS.

often results in financial pressure, as outlined in [Table 1](#). As such, for one cubic meter RS sample treated with 4 rounds of EICP injections using purified urease powder, equal volume and equimolar of urea and CaCl_2 solutions, and skim milk powder, it is estimated that 80k USD will be spent. Besides, the storage of the purified powder itself could also be an issue, as it was supposed to be sealed and stored at a cool place with a bag of silica gel aside to minimize the presence of any moisture. Extra attentiveness and equipment such as the laboratory scale are expected due to the fact that single dose usage of purified jack bean urease is relatively small (i.e. accurate to a unit of mg). However, it is reported that the general size of plant urease is smaller than that of bacteria, which makes EICP solution less problematic in filling up the pores in finer particles such as sand [\[21\]](#). Moreover, a relatively germ-free working environment and a certain level of biochemical knowledge is required for the adoption of MICP technology, which makes it complex and difficult for the industry to employ on real projects or on large scales. EICP was therefore selected in this research study as an alternative to cement for stabilizing washed RS. Due to the commercial concern on the usage of purified urease powder, several researchers have explored a wide range of plants with naturally existing urease enzyme, such as beans, melons and fungi [\[22\]](#), to stimulate the reaction of EICP at low costs. Among those, soybean (*Glycine max*) has been reported to be of particular interest for years, primarily due to its high urease content and easy accessibility.

Unconfined compressive strength (UCS) is one of the key indicators directly linked to the effectiveness of the EICP treatment method [\[12\]](#). For EICP treatment using soybean extracted enzyme, it was stated that UCS values are predominantly controlled by factors of enzyme activity methods and cycles of treatment, and source material, while the urease enzyme activity is generally controlled by the concentration of soybean powder, temperature and duration of curing condition, pH of enzyme solution (ES), and molar concentration of cementitious solution (CS) [\[21, 23, 24\]](#). [Table 2](#) provides a brief UCS summary of soybean extraction-based EICP stabilized sand samples published in the past five years, considering the aforementioned factors, with a one-phase injection method applied. The majority of the studies reported a room temperature curing (20–25 °C) rather than low-temperature curing (i.e. 4 °C) due to a higher urease activity [\[25–27\]](#). However, EICP is deemed to have a rapid reaction time compared with MICP in terms of the early stage presence of carbonate [\[28\]](#), indicating that the unwelcome carbonate often precipitates on the sample surface before reaching the target site. As such, bio-clogging takes place and prevents the EICP solution from flowing throughout the sample, therefore further resulting in inconsistent calcium carbonate precipitation.

This research study evaluated a low-temperature curing environment for soybean-based EICP, which slowed down the urea hydrolysis

process and aimed to minimize the bio-clogging impact without compromising the amount of calcium carbonate precipitation. The washed RS was suggested to be used previously in unbound conditions for natural sand substitution [\[29\]](#). However, the authors intended to explore the wider possibilities of RS treatment in construction activities, namely in stabilized geotechnical fills. The particular interest in EICP was triggered by a low carbon footprint perspective, as well as study their usage as a stabilized geotechnical fill material, which requires lower strength values which can be met by EICP treatment. The solid-liquid ratio of the soybean powder during the enzyme extraction process and the number of treatment cycles were accordingly modified. Electrical conductivity (EC) was chosen to be the reference parameter which implies the urease activity.

The main intention of this study was to carry out pilot research on EICP treated washed RS cured at low temperatures, considering the innovation of the source material and different point of view in curing temperature with the existing publications. The results indicate the EICP treatment with RS is promising, and at implementation stage further refinements can be developed. Ammonium ions, as the inevitable by-product in the carbonate precipitation process, however, are a major environmental and health concern [\[19\]](#).

2. Material and methods

2.1. Washed recycled sand

The parent soil used in this study, washed recycle sand (RS) was manufactured from a local washing plant in Melbourne, Victoria, and the product itself is commercially available. This is Australia's first C&D washing plant which has adopted high-pressure washing technology to produce washed recycled products. The input ingredients contain few kinds of C&D waste such as clean fill with rock, non-destructive digging soils and ballast. Washed recycled products such as coarse recycled sand (CRS) and fine recycled sand (FRS) are tested to have the following geotechnical characteristics: G_s of 2.65 and 2.66, maximum void ratio of 0.61 and 0.64, minimum void ratio of 0.39 and 0.45, D_{50} of 0.699 mm and 0.286 mm, C_c of 0.65 and 0.94 and C_u of 5.54 and 2.60, individually [\[29\]](#). Both sand products were classified as poorly graded sand (SP), in accordance with USCS [\[6\]](#), with the gradation curves presented in [Fig. 1](#). As presented in [Fig. 2](#) (a) and (b), both CRS and FRS have natural sand-like physical properties.

2.2. Urease enzyme extraction

Soybeans, as seen in [Fig. 3](#) (a), were purchased from a local market and air-dried for a minimum of 48 hours. The dried soybean was fed into

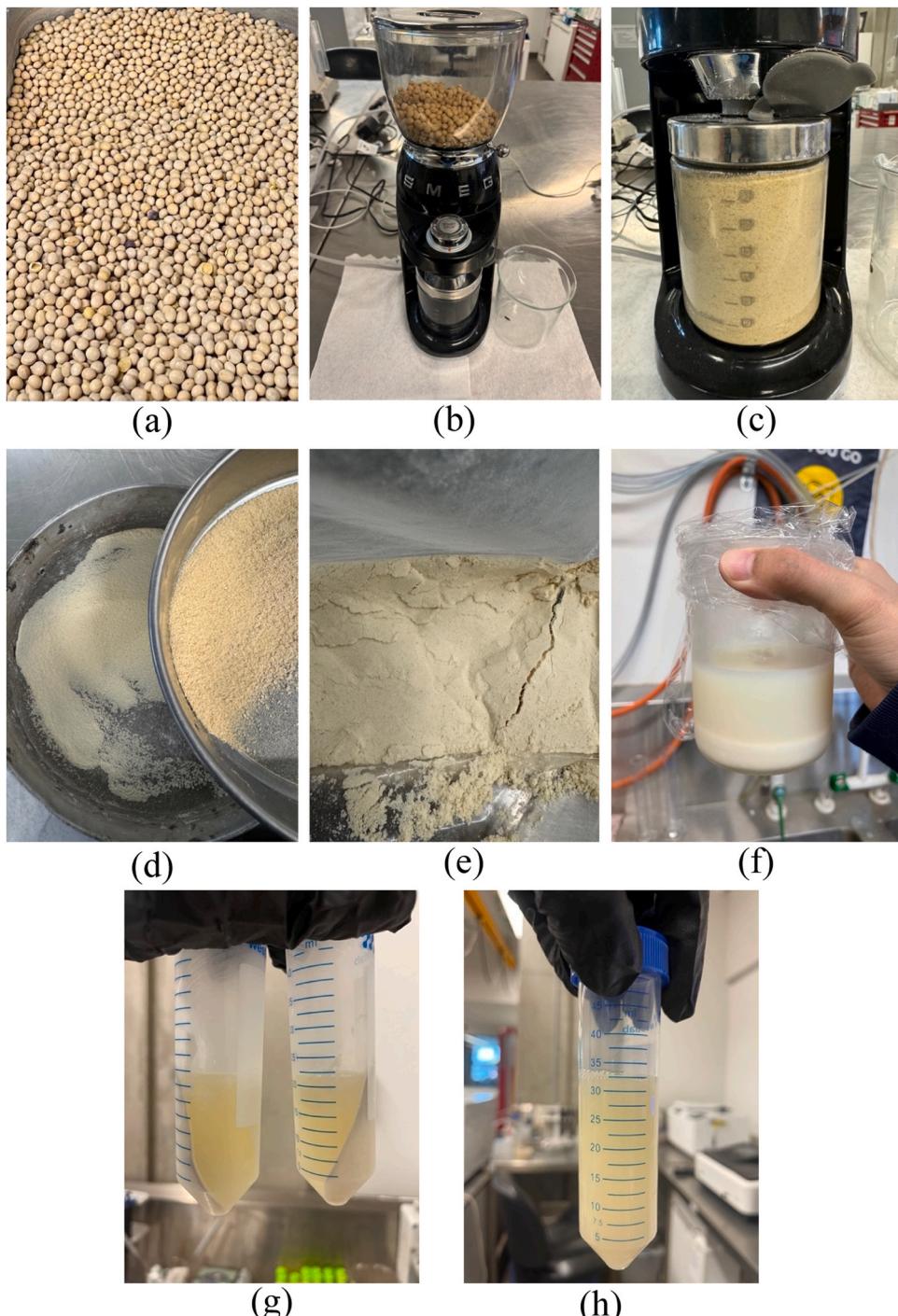


Fig. 3. Soybean enzyme extraction process: (a) soybean purchased from the market; (b) home-grade grinder; (c) soybean powder after grinding; (d) sieving; (e) sieved soybean powder; (f) soybean solution before centrifuging; (g) soybean solution after centrifuging; and (h) collected supernatant as the ready-to-use urease enzyme solution (ES).

a home-grade coffee grinder that was set to the finest mode, referring to Fig. 3 (b) and (c). The soybean powder gained from the grinder was sieved using 300 µm mesh and collected as ready-to-use soybean powder. The purpose of the extra sieving step was to prevent material imperfections that are resulted from large inclusions [24], and an optimum particle size range of 0–250 mm has been conveyed [21]. For every 200 g of the soybean, about 61 g of the powder could be obtained after grinding and sieving, implying a 30 % of the retaining rate.

Sieved soybean powder in Fig. 3 (e) was mixed completely with 1000 mL demineralized water at the concentrations of 20 g/L, 40 g/L,

60 g/L and 80 g/L respectively, using a magnetic stirrer for at least 40 minutes, and being left for 30 minutes at room temperature. The supernatant of the soybean liquid was collected and centrifuged at 6000 rpm for 15 min at 4 °C. As exhibited in Fig. 3 (g) and (h), the supernatant after the centrifugal action, as noted as the soybean extracted urease, was sealed in separate containers and stored in the 4 °C refrigerator. The freshly made soybean-extracted urease could be used directly without any further purification and consumed within four days to ensure enzyme activity.

Laboratory-graded urea and calcium chloride powder were

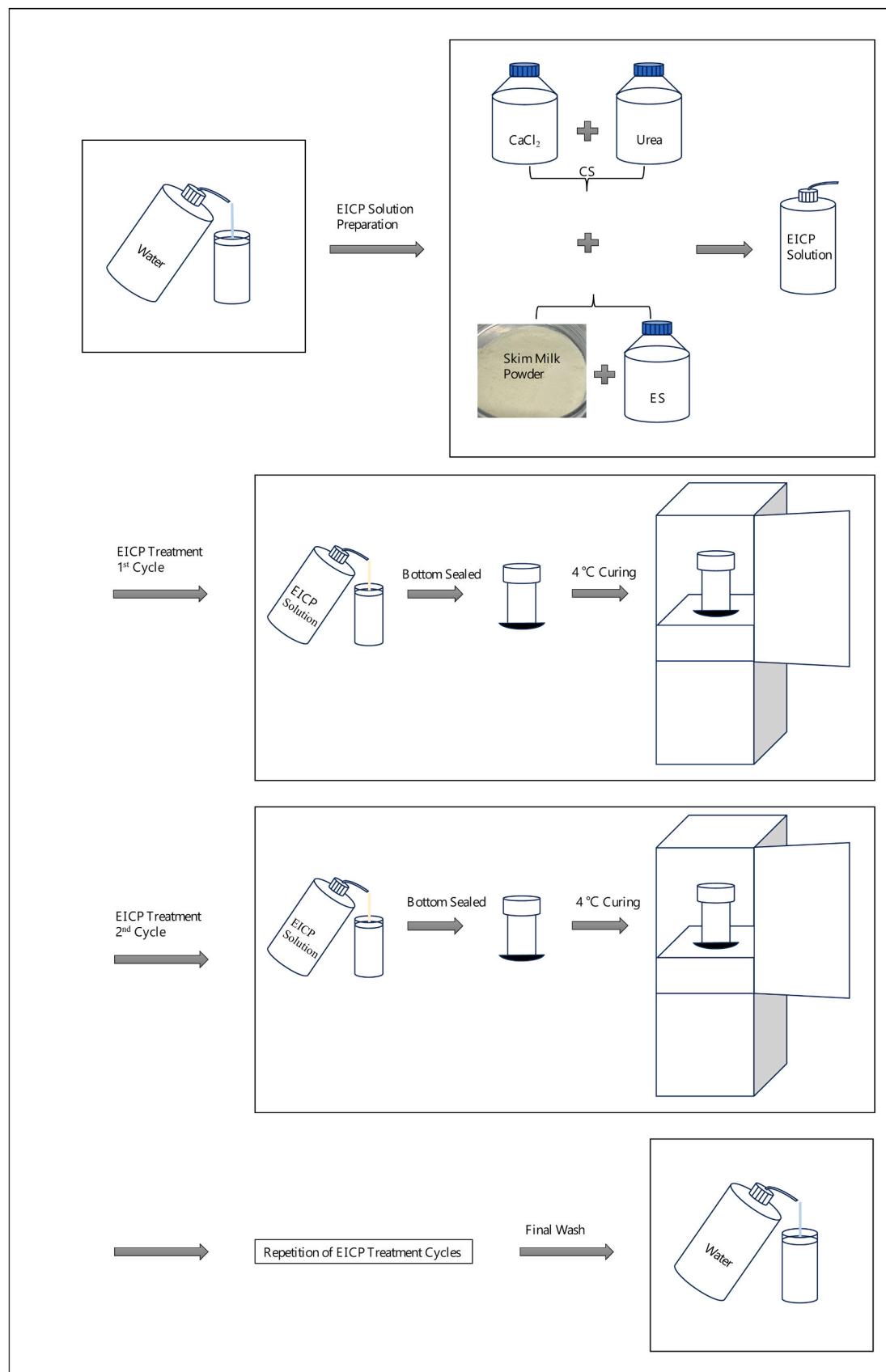
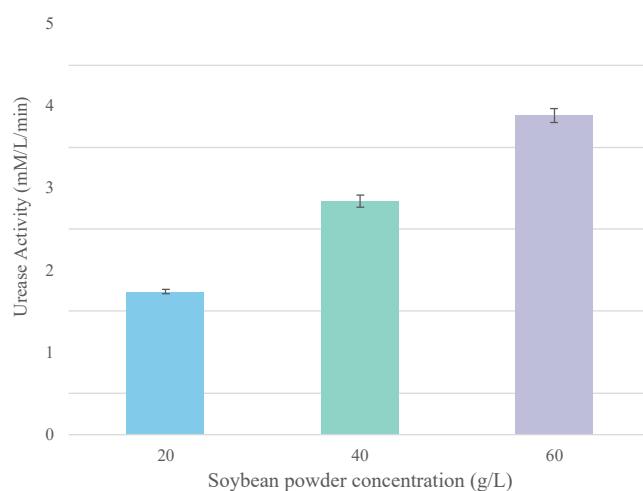
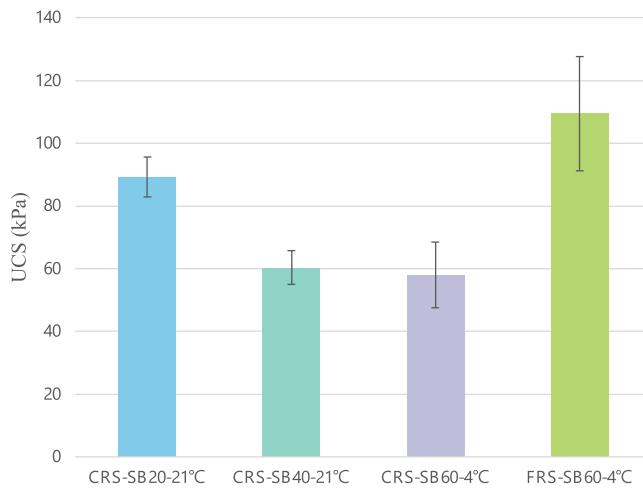


Fig. 4. EICP treatment procedure.

Table 3

Treatment details of UCS samples.

Sample ID	Soil types	Soybean powder concentration (g/L)	Curing temperature	Presence of stabilizer	Treatment cycles
CRS-SB20-21°C	CRS	20	21	N	6 ^a
CRS-SB40-21°C	CRS	40	21	N	4 ^a
CRS-SB60-4°C	CRS	60	4	Y	5
FRS-SB60-4°C	FRS	60	4	Y	5

Note: ^a indicates that the final treatment cycle did not contain full volume solution.**Fig. 5.** Change of urease activity in against to soybean powder concentration.

commercially purchased and fully mixed with demineralized water at the desired molar concentration. 1.0 M and 1.11 M urea solution, and 1.0 M calcium chloride solution were prepared separately in advance and stored at low temperature (4 °C).

2.3. Urease activity measurement

Enzyme activity was measured and calculated to determine the EC by using a compact conductivity meter [30]. A tested solution containing 1 mL of the soybean extracted ES and 9 mL of 1.1 M urea solution was prepared, and left under 21 °C for 30 minutes because the change in EC was found to be unstable right after mixing [21]. The values of EC were hereafter monitored for continuous 5 min and a dilation factor of 10 was used to work out the EC change per minute. 1 mS/cm/min can be considered equivalent to 11.11 mM/L/min in the process of urea

hydrolysis [31]. As such, urease activity presented in this study has the unit of mM/L/min and can be worked out. Triplicate samples were made for the batch tests and the average value was reported.

2.4. UCS sample preparation

The UCS samples were prepared using a split clear acrylic mold with a dimension of 50 mm in inner diameter and 110 mm in height at an ambient temperature of 21 °C. The final height of the soil sample would be 100 mm, and an extra mold height of 10 mm was involved for the convenience of sample compaction prior to the treatment. The gauze was put at the bottom of the sample, and functioned as the filter that allows the solution to drain out freely by gravity without losing the sand particles [32]. A thin plastic vinyl layer was settled around the circular mold. Oven-dried CRS and FRS were compacted respectively using the three-layer compaction method, with the target relative density value of 40 %. From the desired relative density, the total mass of a EICP treated sample was computed. Dried materials were accordingly prepared in three portions with equivalent mass and added to the mold in lifts of 3.33cm each. A manual compaction technique was carried out for each layer, while the sample height after compaction was checked before the addition of the following layer. The surface of each layer was slightly scarified to ensure the uniformity of the overall compaction. A filter paper was put on top of the sample to prevent surface disturbance from the solution-pouring process.

It is widely acknowledged that biocement can be carried out using either the premix or percolation methods, while percolation can be fulfilled by a one-phase or two-phase injection approach [17, 19, 33]. One phase injection method was picked for this study, in which soybean-extracted ES and CS were first mixed together with the volume ratio 1:1, hereafter known as EICP solution, which was poured into the sample as a whole. Considering the density difference, urea solution was added to equal volume of the CaCl₂ solution, followed by the presence of ES. Skim milk powder was also included as a stabilizer at 4 g/L concentration. This is because that soybean extracted urease is generally less stable and therefore, lacks nucleation sites [34]. It is believed that the presence of proteins, organic substances and calcium carbonate ‘seed’ can all contribute to the development of the nucleation items for calcite precipitation [35–37]. Among the current findings, skim milk powder is deemed as a cost-effective and achievable option. The preparation of EICP solution was presented in Fig. 4. The pH of the EICP solution was not adjusted in this study.

Also in Fig. 4, the overall treatment procedure was conducted as follows: (1) 1.2 void volume equivalent of demineralized water was injected into the sample with drainage allowed; (2) 1.2 void volume equivalent EICP solution was poured on the slightly compacted soil samples right after the solution was made, i.e. at low temperature, with drainage allowed; (3) the bottom was sealed when all the EICP solution was poured in and the sample was sent to the fridge for 48 hours; (4) another 1.2 void volume equivalent of EICP solution was injected and the sample was put at the low-temperature environment for 24 hours; (5) step (4) was repeated for another three times such that a total of 5 treatments was fulfilled; (6) the sample was taken out from the fridge after the final treatment and cured at room temperature (21 °C) for two days. The liquid surface stayed higher than the sample to keep the sample as saturated as possible during the whole treatment process. CRS

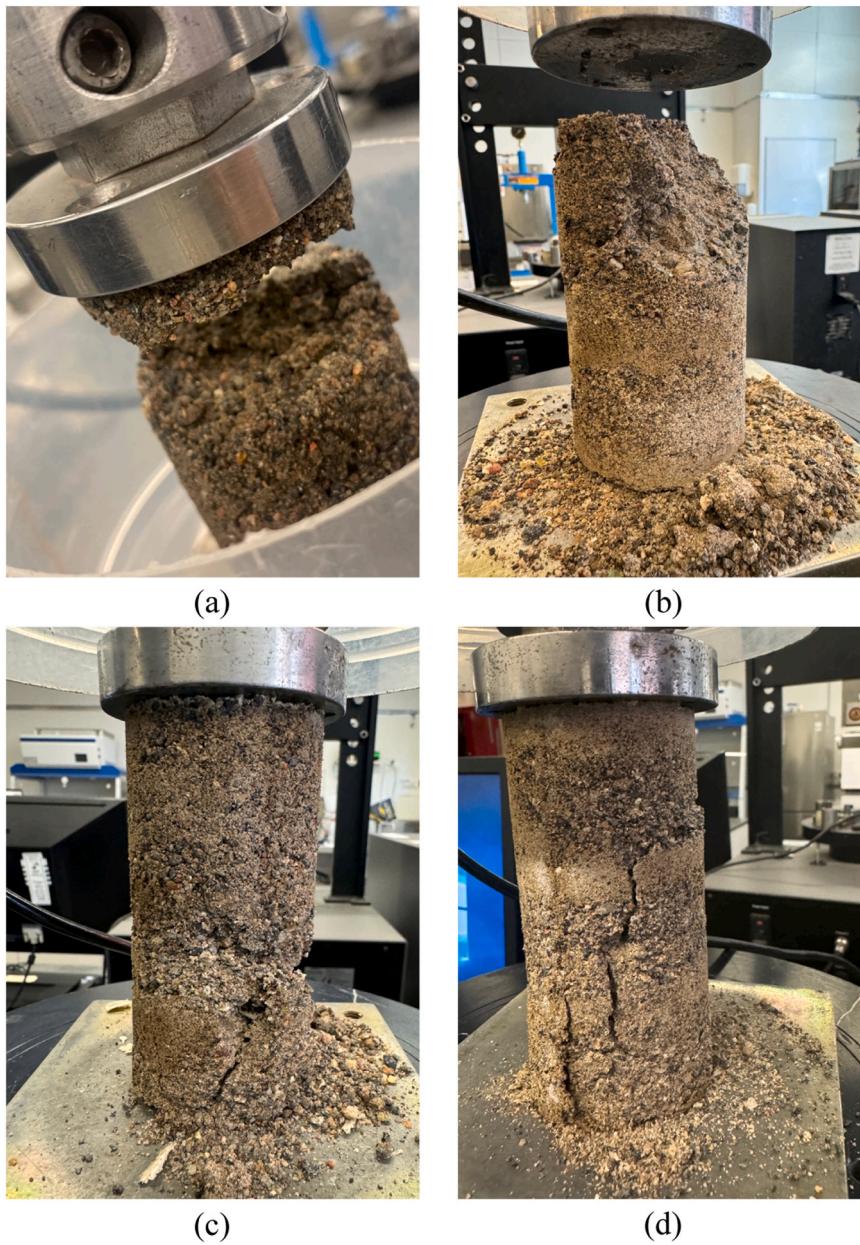


Fig. 7. Failure patterns during UCS test for (a) sample CRS-SB20–21°C; (b) sample CRS-SB40–21°C; (c) sample CRS-SB60–4°C; and (d) sample FRS-SB60–4°C.

samples treated with the same molar concentration of CS but different soybean powder concentration were prepared and cured at room temperature to determine the curing temperature influence on surface bio-clogging. The summary of EICP treatment details is given in Table 3.

The demineralized water with double the void volume was used to rinse the sample. Several studies have mentioned the presence of the residual calcium salt or urea after the biocement treatment [23, 38–40]. It was pointed out that the strength of the soil could be therefore positively impacted, however, leading to the strength overestimation [38]. Hence, the water rinsing / flushing process was suggested by several studies to minimize the existence of the unwanted chemical materials. The fully treated samples were oven-dried at 60 °C until the mass was no longer reduced and hence deemed ready for the UCS test. Triplicate soil samples were prepared.

A deformation rate of 1 mm/min was set for UCS testing as outlined in ASTM D2166 [41]. A sandpaper was utilized to slightly level the sample surface prior to the test, if required.

2.5. Microstructural analysis

Scanning Electron Microscopy (SEM) test was carried out to capture the presence of the biocementation gel in the micro-scale. Energy-dispersive X-ray spectroscopy (EDS) analysis and X-ray powder diffraction (XRD) analysis were performed to determine the chemical components generated along the biocement growth process. The tested fragments for SEM, EDS and XRD tests were collected from samples that underwent UCS tests.

Fragments collected from UCS samples were further pulverized into powder, before being placed into the X-ray diffractometer. For 2θ angles from 10 degrees to 80 degrees, the angular speed of 0.02 degrees and a scanning speed of 4 degrees/min readings were recorded.

2.6. CaCO_3 precipitation measurement

Besides SEM and XRD analyses, fragments gathered from the UCS test were fully soaked in a hydrochloride acid solution with a concen-

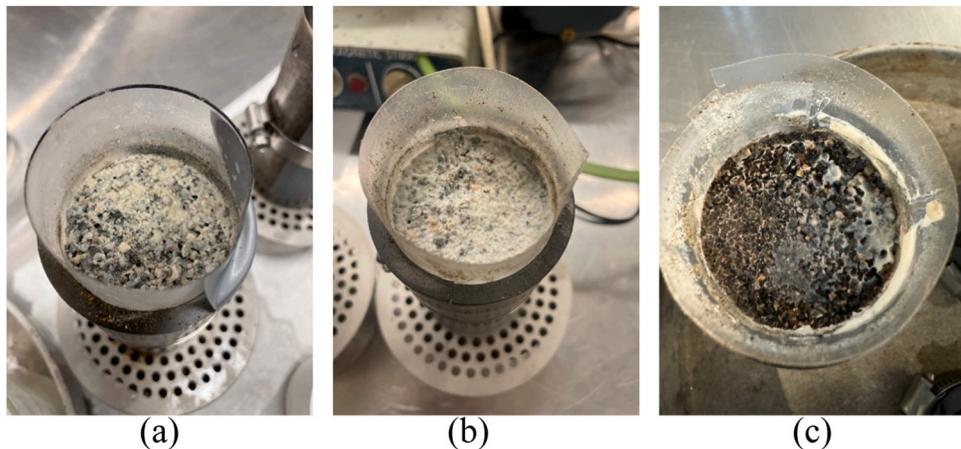


Fig. 8. Surface clogging of (a) sample CRS-SB20–21°C after 5th treatment cycle; (b) sample CRS-SB40–21°C after 3rd treatment cycle; and (c) sample CRS-SB60–4°C after 5th treatment cycle.

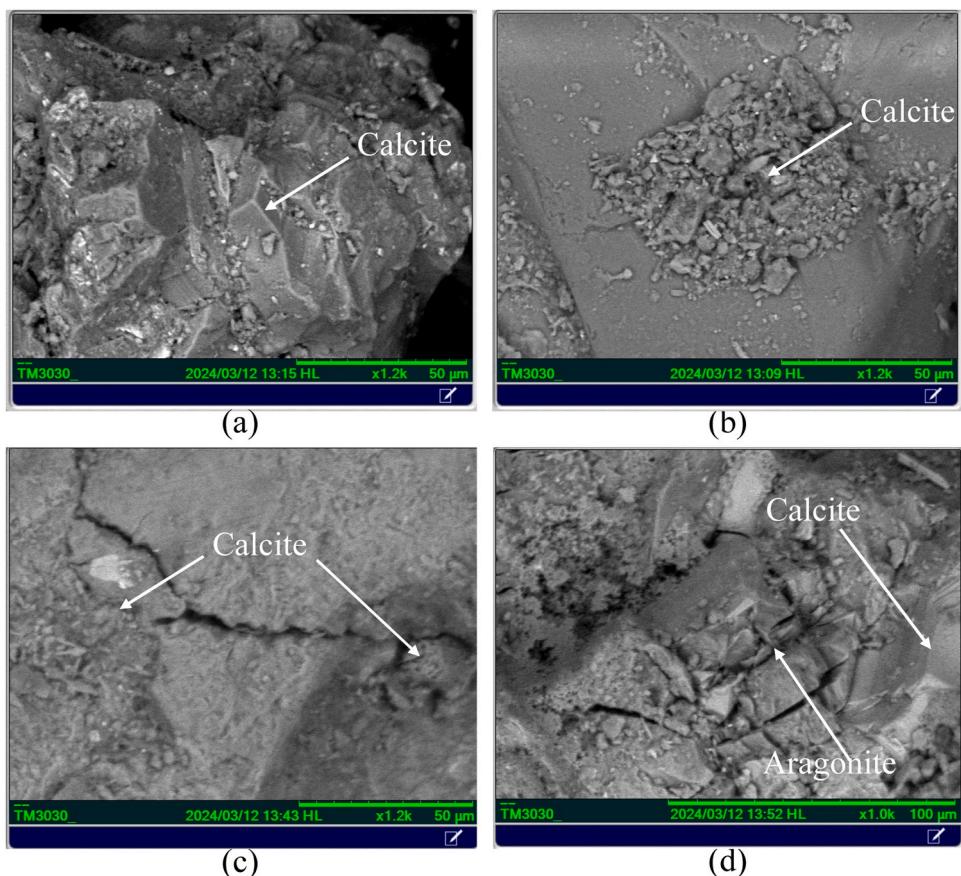


Fig. 9. SEM images of (a-b) sample CRS-SB60–4°C; and (c-d) sample FRS-SB60–4°C.

tration of 1.0 N. To obtain a comprehensive understanding of the average amount of CaCO_3 precipitation, three small pieces of fragments were collected from the bottom, the middle and the top part of each single sample, respectively. Acid-soaked samples were flushed with sufficient DI water when bubbles no longer presented and all large blocks thawed into small pieces, hence dried in the oven at 60°C. As per sample, the pure dry mass of the fragments before (m_1) and after (m_2) acid wash was noted down. The percentage of CaCO_3 precipitation could be then worked out as following [42]:

$$\text{CaCO}_3(\%) = \frac{m_1 - m_2}{m_2} * 100 \quad (4)$$

3. Results and discussion

3.1. Urease activity, UCS and CaCO_3 precipitation measurement

The calculated urease activity at different soybean powder concentrations was depicted in Fig. 5, that 60 g/L of soybean powder was able to provide the most active urease enzyme. The increasing trend of urease

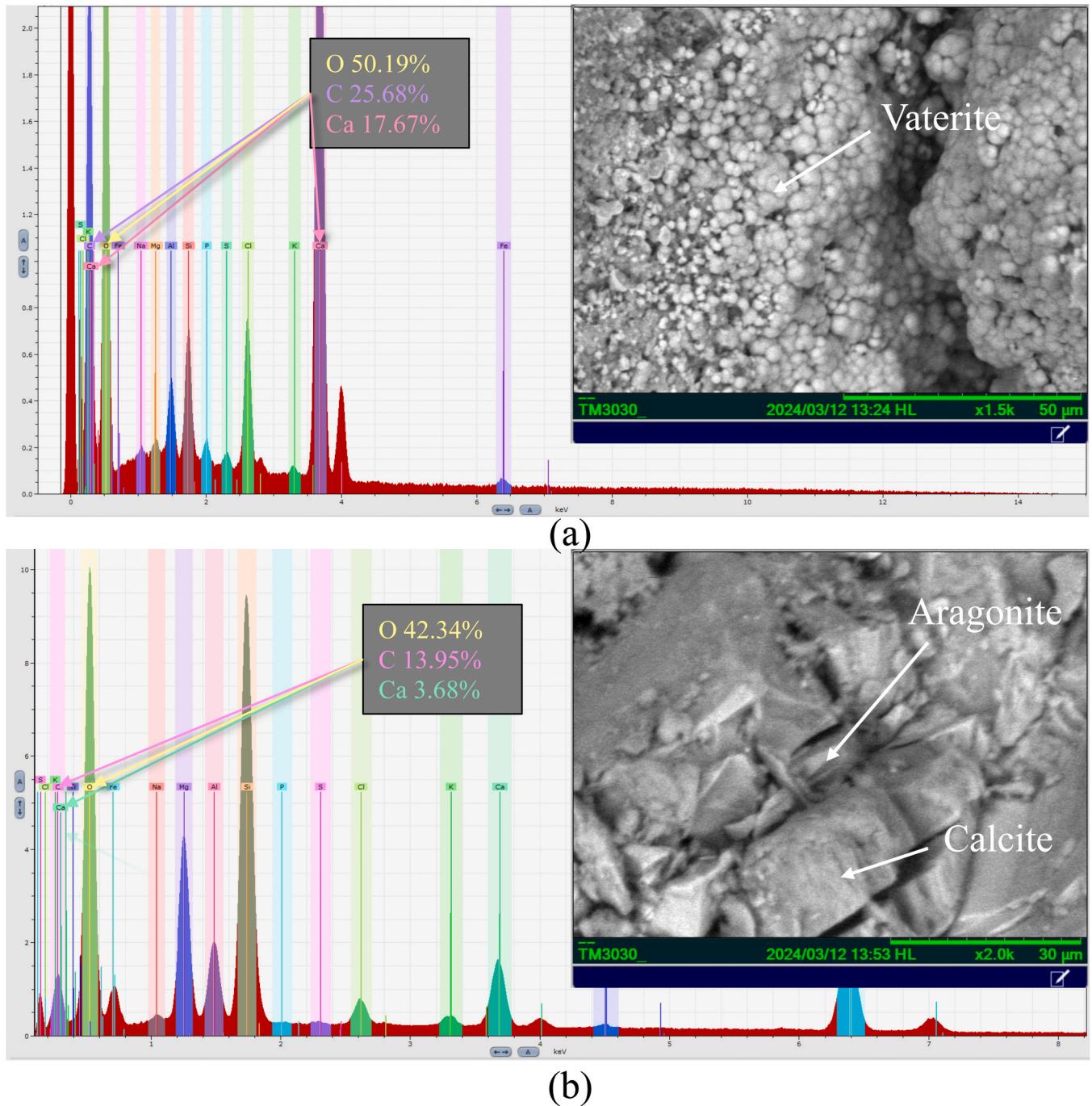


Fig. 10. SEM and EDS results at selected probes of (a) sample CRS-SB60-4°C; and (b) sample FRS-SB60-4°C.

activity in contrast to the soybean powder concentration growth was similarly reported, the corresponding values of urease activity were also found to match with previously reported findings [43]. To offset the negative impact of low-temperature curing on urease activity reduction at the same levels of soybean powder concentration, 60 g/L was picked to extract urease enzyme for CRS-SB60-4°C and FRS-SB60-4°C sample treatments. UCS results are summarized in Fig. 6.

As seen in Fig. 7 (a) and (b), both CRS-SB20-21°C and CRS-SB20-21°C samples split into halves at the failure point, the top part of the CRS-SB40-21°C sample, in particular, broken into small pieces and loose sand. Such failure patterns indicated an uneven distribution of biocement gel throughout the sample. A very limited amount of CaCO_3 was precipitated near the top part of CRS-SB40-21°C, which could be explained by the presence of a white crust layer observed after the

second treatment cycle. The white crust layer tended to be thicker and thicker with the increased treatment cycles, while the EICP solution was found to hardly percolate through, as shown in Fig. 8. The appearance of such a clogging layer was spotted at a later stage of sample CRS-SB20-21°C than of sample CRS-SB40-21°C, due to the lower value of soybean powder concentration at 20 g/L. However, it still disrupted the sixth treatment cycle for sample CRS-SB20-21°C because of the heavy layer on top, as presented in Fig. 8 (a). For EICP containing higher soybean powder concentration (i.e. 40 g/L), there was increased bioclogging, leading to a lower UCS value of 60.67 kPa compared with sample CRS-SB20-21°C (89.39 kPa). This is because CaCO_3 precipitated and accumulated at the sample surface did not contribute to the strength improvement. As previously discussed, the higher the concentration of soybean powder involved, the more macromolecular proteins

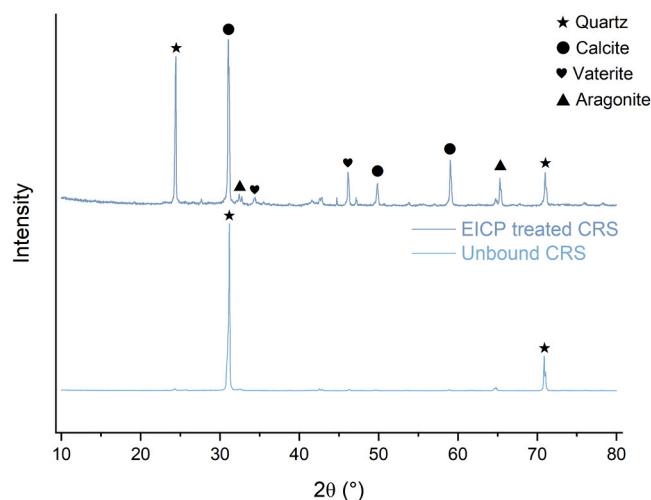


Fig. 11. XRD spectra for CRS.

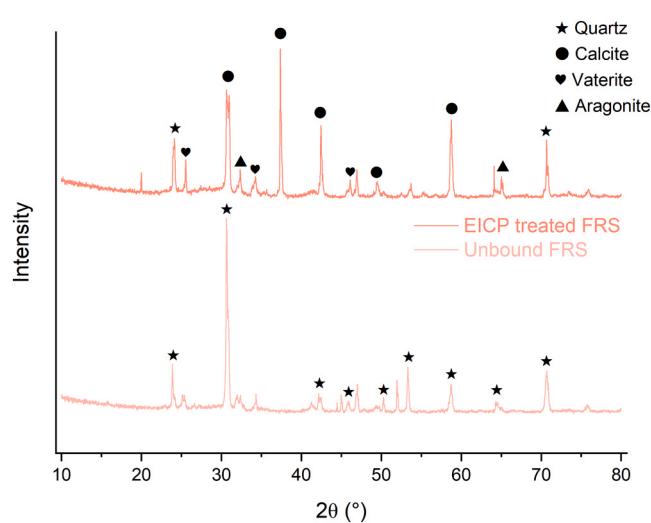


Fig. 12. XRD spectra for FRS.

contained, the higher the likelihood of protein clustered near the surface, the more severe the bio-clogging issue, thus, the less uniform of CaCO_3 distributed through the sample [43].

A decrease in UCS was noticed, as expected, for EICP-treated CRS under low-temperature curing. 52.6 % reduction was found for samples CRS-SB20–21°C and CRS-SB60–4°C, while only 4.6 % was spotted between CRS-SB40–21°C and CRS-SB60–4°C. Meanwhile, the existence of the white crust layer was not observed until the fifth treatment for samples curing at low temperatures, signifying the potential for extra rounds of EICP solution injection. As such, the further development of UCS could be expected.

The shear plane shown in Fig. 7 (d) was with cracks further extended through the entire sample column, specifying a more uniform and homogeneous CaCO_3 precipitation condition, as proved by the highest UCS value reported (109.45 kPa). Meanwhile, sample CRS-SB60–4°C was measured with a lower average CaCO_3 precipitation content of 2.58 % than sample FRS-SB60–4°C (8.49 %), which was in line with their UCS values. This could be explained by the fact that a larger value of void ratio may contribute to a greater amount of CaCO_3 precipitated [23]. FRS was found to be looser in structure and hence had slightly higher void ratio values than CRS [29].

3.2. SEM, EDS and XRD analysis

Microstructural analyses were carried out for samples CRS-SB60–4°C and FRS-SB60–4°C to further investigate the feasibility of applying low-temperature curing EICP technology on washed RS.

SEM images and EDS results of samples CRS-SB60–4°C and FRS-SB60–4°C were shown in Fig. 9 and Fig. 10, where the presence of CaCO_3 crystals was observed, but the linkage in-between was not tight. A mixture structure of calcite and vaterite was observed for CRS, while FRS contained more calcite and a few aragonite crystals. Compared to calcite forms of replace CaCO_3 crystals, aragonite and vaterite were less favoured due to the lack of stability in terms of proving a secondary nucleation site due to their irregular and spherical shapes [44, 45].

EDS results for low-temperature curing samples were conducted at probe locations, providing information on the elemental composition of minerals. As shown in Fig. 10, Oxygen (O), Carbon (C) and Calcium (Ca) were detected in both samples, hence verifying the existence of CaCO_3 . EDS, however, was not able to further determine the morphology of the precipitated crystals, specifying the significance of conducting XRD analysis.

Fig. 11 and Fig. 12 presented the morphology of the CaCO_3 precipitation under XRD analysis for low-temperature cured EICP-treated CRS and FRS, respectively. For both the unbound CRS and FRS, XRD spotted high peaks for quartz only, while calcite, vaterite, as well as aragonite crystals, were traced for EICP-treated samples. The finding depicted that CaCO_3 crystals precipitated in stabilized FRS were mainly calcite, while vaterite also took a spot in stabilized CRS. The intensity of calcite peaks traced by XRD was higher in FRS than in CRS, indicating a stronger bonding. The chemical compositions of both sands (CRS and FRS) were previously tested via the XRF test and reported in earlier publication [29]. SiO_2 is believed to be the major mineral in both CRS (65 %) and FRS (75 %).

4. Conclusions

This research applied soybean-extracted enzyme-based EICP on washed RS. A single-phase injection method was adopted, where ES and CS were premixed at low temperatures and added to the sample directly. 89.39 kPa of UCS value was tested for the CRS sample treated with EICP solution containing 20 g/L soybean powder and 1.0 M of urea and calcium chloride solution and cured at room temperature. However, a bio-clogging phenomenon was observed, and the clogging became worse along with the increased cycles of treatment. This was evident from the UCS reduction for CRS treated with EICP solution, which contained a higher concentration of soybean powder, thus a higher urease activity was expected. A low-temperature curing environment was found to be able to improve surface clogging and lessen the amount of undesired CaCO_3 precipitation during the treatment process. A similar UCS strength of approximately 60 kPa was tested for EICP-treated CRS samples curing at 21 °C to 4 °C, with a slight difference in the applied EICP solutions. Although the UCS should have a positive linear correlation with the number of treatments, serious surface congestion was witnessed for samples cured at room temperature, which stopped the possibility of performing further treatments. For samples cured at low temperatures, however, the unwanted white crust layer appeared at a later stage with a small amount, indicating the potential of the UCS development. Low temperature cured FRS sample was reported to have a higher UCS than CFS, as well as a greater proportion of CaCO_3 precipitation. Meanwhile, microscale analyses, including SEM, EDS and XRD proved the presence of CaCO_3 crystals within the tested washed RS particles.

This study indicated that soybean-extracted urease-based EICP can stabilize washed RS, and a low-temperature curing environment could help with the ease of bio-clogging. As such, EICP stabilized RS could be used as geotechnical fill materials and contribute to ground improvement works. Implementation of the EICP treatment during construction

has been proposed to take place during the winter months, whereby low temperatures of 4 °C are common. It is also recommended that future studies should investigate the optimum parameter of EICP treatment on washed RS to further increase the strength and shorten the overall treatment duration period. Considering the strength achieved, EICP stabilized washed RS could be involved in temporary backfill or pavement subgrade layers where minimum surface load are imposed. The environmental value of practically adopting EICP on top of the presence of unwanted ammonium ions is also supposed to be assessed in the near future. For instance, the emission of ammonia gas could be controlled with the assistance of polyacrylic acid [46].

CRediT authorship contribution statement

Suksun Horpibulsuk: Writing – review & editing, Supervision, Funding acquisition. **Arul Arulrajah:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, Conceptualization. **Jian Chu:** Writing – review & editing, Supervision, Funding acquisition. **Yunxin Xue:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Some or all data, models, or codes that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

This research was supported under Australian Research Council's Linkage Projects funding scheme (project numbers LP220100099). The authors appreciate the kind support of Repurpose It Pty Ltd., Victoria, Australia as the project partner.

References

- [1] A. Arulrajah, J. Piratheepan, M. Ali, M. Bo, Geotechnical properties of recycled concrete aggregate in pavement sub-base applications, *Geotech. Test. J.* 35 (5) (2012) 743–751, <https://doi.org/10.1520/GTJ103402>.
- [2] Y. Lin, F. Maghool, A. Arulrajah, S. Horpibulsuk, Alkali activation of recycled concrete and aluminum salt slag aggregates for semi-rigid column inclusions, *Constr. Build. Mater.* 366 (2023) 130106, <https://doi.org/10.1016/j.conbuildmat.2022.130106>.
- [3] Y. Bai, A. Arulrajah, S. Horpibulsuk, J. Chu, Gasified olive stone biochar as a green construction fill material, *Constr. Build. Mater.* 403 (2023) 133003, <https://doi.org/10.1016/j.conbuildmat.2023.133003>.
- [4] Y. Feng, B. Zhang, J. Xie, Z. Xue, K. Huang, J. Tan, Effects of recycled sand and nanomaterials on ultra-high performance concrete: workability, compressive strength and microstructure, *Constr. Build. Mater.* 378 (2023) 131180, <https://doi.org/10.1016/j.conbuildmat.2023.131180>.
- [5] C. Ulsen, H. Kahn, G. Hawlitschek, E. Masini, S. Angulo, V. John, Production of recycled sand from construction and demolition waste, *Constr. Build. Mater.* 40 (2013) 1168–1173, <https://doi.org/10.1016/j.conbuildmat.2012.02.004>.
- [6] ASTM, Standard Practice for Classification of Soils for Engineering Purposes (Unified Soil Classification System. 2017, West Conshohocken.
- [7] ARTC, Earthworks Materials Specification, 2020, Australian Rail Track Corporation.
- [8] A. Buritatum, A. Suddeepong, S. Horpibulsuk, A. Udomchai, A. Arulrajah, A. Mohammadinia, J. Horpibulsuk, M. Hoy, Improvement of tensile properties of cement-stabilized soil using natural rubber latex, *J. Mater. Civ. Eng.* 34 (4) (2022) 04022028, [https://doi.org/10.1061/\(ASCE\)MT.1943-5533.0004173](https://doi.org/10.1061/(ASCE)MT.1943-5533.0004173).
- [9] M.S. Baghini, A. Ismail, S.S. Naseralavi, A.A. Firooz, Performance evaluation of road base stabilized with styrene-butadiene copolymer latex and Portland cement, *Int. J. Pavement Res. Technol.* 9 (4) (2016) 321–336, <https://doi.org/10.1016/j.ijprt.2016.08.006>.
- [10] N.Q. Tran, M. Hoy, A. Suddeepong, S. Horpibulsuk, K. Kantathum, A. Arulrajah, Improved mechanical and microstructure of cement-stabilized lateritic soil using recycled materials replacement and natural rubber latex for pavement applications, *Constr. Build. Mater.* 347 (2022) 128547, <https://doi.org/10.1016/j.conbuildmat.2022.128547>.
- [11] L. Wang, M.L. Chen, D.C. Tsang, Green remediation by using low-carbon cement-based stabilization/solidification approaches. in: *Sustainable Remediation of Contaminated Soil and Groundwater*, Elsevier, 2020, pp. 93–118.
- [12] I. Ahenkorah, M.M. Rahman, M.R. Karim, S. Beecham, Unconfined compressive strength of MICP and EICP treated sands subjected to cycles of wetting-drying, freezing-thawing and elevated temperature: experimental and EPR modelling, *J. Rock. Mech. Geotech. Eng.* 15 (5) (2023) 1226–1247, <https://doi.org/10.1016/j.jrmge.2022.08.007>.
- [13] M.-J. Cui, H.-J. Lai, T. Hoang, J. Chu, One-phase-low-pH enzyme induced carbonate precipitation (EICP) method for soil improvement, *Acta Geotech.* 16 (2) (2021) 481–489.
- [14] J. He, Y. Liu, L. Liu, B. Yan, L. Li, H. Meng, L. Hang, Y. Qi, M. Wu, Y. Gao, Recent development on optimization of bio-cementation for soil stabilization and wind erosion control, *Biogeotechnics* (2023) 100022, <https://doi.org/10.1016/j.bgtech.2023.100022>.
- [15] Y. Zhang, H. Guo, X. Cheng, Influences of calcium sources on microbially induced carbonate precipitation in porous media, *Mater. Res. Innov.* 18 (sup2) (2014) S2-79–S2-84, <https://doi.org/10.1179/1432891714Z.000000000384>.
- [16] I. Ahenkorah, M.M. Rahman, M.R. Karim, S. Beecham, C. Saint, A review of enzyme induced carbonate precipitation (EICP): the role of enzyme kinetics, *Sustain. Chem.* 2 (1) (2021) 92–114.
- [17] M.-J. Cui, H.-J. Lai, T. Hoang, J. Chu, One-phase-low-pH enzyme induced carbonate precipitation (EICP) method for soil improvement, *Acta Geotech.* 16 (2021) 481–489, <https://doi.org/10.1007/s11440-020-01043-2>.
- [18] T. Hoang, J. Alleman, B. Cetin, K. Ikuma, S.-G. Choi, Sand and silty-sand soil stabilization using bacterial enzyme-induced calcite precipitation (BEICP), *Can. Geotech. J.* 56 (6) (2019) 808–822, <https://doi.org/10.1139/cgj-2018-0191>.
- [19] A. Almajed, H. Khodadadi Tirkolaei, E. Kavazanjian Jr, Baseline investigation on enzyme-induced calcium carbonate precipitation, *J. Geotech. Geoenviron. Eng.* 144 (11) (2018) 04018081, [https://doi.org/10.1061/\(ASCE\)GT.1943-5606.0001973](https://doi.org/10.1061/(ASCE)GT.1943-5606.0001973).
- [20] I. Ahenkorah, M.M. Rahman, M.R. Karim, S. Beecham, Enzyme induced calcium carbonate precipitation and its engineering application: a systematic review and meta-analysis, *Constr. Build. Mater.* 308 (2021) 125000, <https://doi.org/10.1016/j.conbuildmat.2021.125000>.
- [21] S. Shu, B. Yan, B. Ge, S. Li, H. Meng, Factors affecting soybean crude urease extraction and biocementation via enzyme-induced carbonate precipitation (EICP) for soil improvement, *Energies* 15 (15) (2022) 5566, <https://doi.org/10.3390/en15155566>.
- [22] I. Ahenkorah, M.M. Rahman, M.R. Karim, P.R. Teasdale, Optimization of enzyme induced carbonate precipitation (EICP) as a ground improvement technique. in: *Geo-Congress 2020*, American Society of Civil Engineers, Reston, VA, 2020.
- [23] M.-J. Cui, H.-J. Lai, S.-F. Wu, J. Chu, Comparison of soil improvement methods using crude soybean enzyme, bacterial enzyme or bacteria-induced carbonate precipitation, *Géotechnique* 74 (1) (2022) 18–26, <https://doi.org/10.1680/jgeot.21.00131>.
- [24] A. Cuccurullo, D. Gallipoli, A.W. Bruno, C. Augarde, P. Hughes, C. La Borderie, Earth stabilisation via carbonate precipitation by plant-derived urease for building applications, *Geomech. Energy Environ.* 30 (2022) 100230, <https://doi.org/10.1016/j.gete.2020.100230>.
- [25] L. Cheng, M.A. Shahin, D. Mujah, Influence of key environmental conditions on microbially induced cementation for soil stabilization, 04016083, *J. Geotech. Geoenviron. Eng.* 143 (1) (2017), [https://doi.org/10.1061/\(ASCE\) GT.1943-5606.0001586](https://doi.org/10.1061/(ASCE) GT.1943-5606.0001586).
- [26] S.A. Alarifi, A. Mustafa, K. Omarov, A.R. Baig, Z. Tariq, M. Mahmoud, A review of enzyme-induced calcium carbonate precipitation applicability in the oil and gas industry, *Front. Bioeng. Biotechnol.* 10 (2022) 900881, <https://doi.org/10.3389/fbioe.2022.900881>.
- [27] M. Nemat, G. Voordouw, Modification of porous media permeability, using calcium carbonate produced enzymatically in situ, *Enzym. Microb. Technol.* 33 (5) (2003) 635–642, [https://doi.org/10.1016/S0141-0229\(03\)00191-1](https://doi.org/10.1016/S0141-0229(03)00191-1).
- [28] Y. Wang, X. Sun, L. Miao, H. Wang, L. Wu, W. Shi, S. Kawasaki, State-of-the-art review of soil erosion control by MICP and EICP techniques: problems, applications, and prospects, *Sci. Total Environ.* (2023) 169016, <https://doi.org/10.1016/j.scitotenv.2023.169016>.
- [29] Y. Xue, A. Arulrajah, G.A. Narstiloo, S. Horpibulsuk, J. Chu, Washed recycled sand derived from construction and demolition wastes as engineering fill materials, *Constr. Build. Mater.* 358 (2022) 1–12, <https://doi.org/10.1016/j.conbuildmat.2022.129433>.
- [30] Whiffin, V.S., Microbial CaCO₃ Precipitation for the Production of Biocement, 2004, Murdoch University.
- [31] V.S. Whiffin, L.A. Van Paassen, M.P. Harkins, Microbial carbonate precipitation as a soil improvement technique, *Geomicrobiol. J.* 24 (5) (2007) 417–423.
- [32] I. Ahenkorah, M. Rahman, M. Karim, P. Teasdale, A comparison of mechanical responses for microbial-and enzyme-induced cemented sand, *Géotech. Lett.* 10 (4) (2020) 559–567.
- [33] Q. Zhang, W. Ye, Z. Liu, Q. Wang, Y. Chen, Influence of injection methods on calcareous sand cementation by EICP technique, *Constr. Build. Mater.* 363 (2023) 129724 (Influence of injection methods on calcareous sand cementation by EICP technique).
- [34] J. Zhang, X. Wang, L. Shi, Y. Yin, Enzyme-induced carbonate precipitation (EICP) combined with lignin to solidify silt in the Yellow River flood area, *Constr. Build. Mater.* 339 (2022) 127792, <https://doi.org/10.1016/j.conbuildmat.2022.127792>.

- [35] G. Cao, L. Ma, I. Ngo, A.E. Osemudiamhen, Z. Guo, Experimental investigation on the combination of enzyme-induced calcium carbonate precipitation and organic materials for underground backfilling preparation, *Minerals* 14 (2) (2024) 153.
- [36] T. Hamed Khodadadi, E. Kavazanjian, L. van Paassen, J. DeJong, Bio-grout materials: a review, *Grouting 2017* (2017) 1–12.
- [37] A. Robles-Fernández, C. Arejas, D. Daffonchio, V.C. Vahrenkamp, M. Sánchez-Román, The Role of Microorganisms in the Nucleation of Carbonates, *Environmental Implications and Applications*, *Minerals* 12 (12) (2022) 1562.
- [38] J. He, J. Chu, Cementation of sand due to salt precipitation in drying process, *Mar. Georesources Geotechnol.* 35 (3) (2017) 441–445, <https://doi.org/10.1080/1064119X.2016.1168498>.
- [39] H.-J. Lai, M.-J. Cui, S.-F. Wu, Y. Yang, J. Chu, Extraction of crude soybean urease using ethanol and its effect on soil cementation, *Soils Found.* 63 (3) (2023) 101300, <https://doi.org/10.1016/j.sandf.2023.101300>.
- [40] A. Almajed, H. Abbas, M. Arab, A. Alsabhan, W. Hamid, Y. Al-Salloum, Enzyme-Induced Carbonate Precipitation (EICP)-Based methods for ecofriendly stabilization of different types of natural sands, *J. Clean. Prod.* 274 (2020) 122627.
- [41] ASTM, Standard Test Method for Unconfined Compressive Strength of Cohesive Soil. 2016, West Conshohocken.
- [42] J. He, X. Mao, Y. Zhou, Q. Tang, Cementation of sand with enzyme-induced carbonate precipitation (EICP) using concrete-extracted calcium, *Front. Phys.* 9 (2022) 825356, <https://doi.org/10.3389/fphy.2021.825356>.
- [43] B. Yan, Y. Zhou, C. Li, S. Shu, Y. Gao, Modified SICP method to mitigate the effect of bio-clogging by excess protein from soybean crude urease extracts for biocementation process, *Acta Geotech.* 18 (9) (2023) 5047–5062, <https://doi.org/10.1007/s11440-023-01825-4>.
- [44] M. Huang, K. Xu, C. Xu, G. Jin, S. Guo, Micromechanical properties of biocemented shale soils analyzed using nanoindentation test, *J. Geotech. Geoenviron. Eng.* 147 (12) (2021) 04021157, [https://doi.org/10.1061/\(ASCE\)GT.1943-5606.0002653](https://doi.org/10.1061/(ASCE)GT.1943-5606.0002653).
- [45] J. Küther, R. Seshadri, W. Knoll, W. Tremel, Templated growth of calcite, vaterite and aragonite crystals on self-assembled monolayers of substituted alkylthiols on gold, *J. Mater. Chem.* 8 (3) (1998) 641–650, <https://doi.org/10.1039/A705859D>.
- [46] Z. Yan, S. Gowthaman, K. Nakashima, S. Kawasaki, Polymer-assisted enzyme induced carbonate precipitation for non-ammonia emission soil stabilization, *Sci. Rep.* 12 (1) (2022) 8821.
- [47] G.B.S. Pratama, H. Yasuhara, N. Kinoshita, H. Putra, Application of soybean powder as urease enzyme replacement on EICP method for soil improvement technique, in: IOP Conference Series: Earth and Environmental Science, IOP Publishing, 2021.
- [48] H. Meng, S. Shu, Y. Gao, B. Yan, J. He, Multiple-phase enzyme-induced carbonate precipitation (EICP) method for soil improvement, *Eng. Geol.* 294 (2021) 106374, <https://doi.org/10.1016/j.engevo.2021.106374>.
- [49] S. Lee, J. Kim, An experimental study on enzymatic-induced carbonate precipitation using yellow soybeans for soil stabilization, *KSCE J. Civ. Eng.* 24 (7) (2020) 2026–2037, <https://doi.org/10.1007/s12205-020-1659-9>.
- [50] S. Shu, B. Yan, H. Meng, X. Bian, Comparative study of EICP treatment methods on the mechanical properties of sandy soil, *Soils Found.* 62 (6) (2022) 101246, <https://doi.org/10.1016/j.sandf.2022.101246>.