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Author(s)	Yan, Zhen; Nakashima, Kazunori; Takano, Chikara et al.
Citation	Acta Geotechnica, 20(12), 6373-6387 https://doi.org/10.1007/s11440-025-02728-2
Issue Date	2025-12
Doc URL	https://hdl.handle.net/2115/98074
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Type	journal article
File Information	Minor-Revised Manuscript.pdf



**Efficient reduction of coarse sand permeability by waste shellfish powder-assisted
enzyme-induced carbonate precipitation.**

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Abstract

Using biocement represents an innovative approach for controlling permeability. However, achieving the desired effects in coarse sand necessitates multiple treatment cycles. This study aims to enhance the efficiency of permeability reduction by introducing kitchen waste-derived powdered shellfish and modifying precipitation patterns. Experimental findings indicate that the control group, devoid of powdered shellfish, achieved a reduction of approximately 46%, whereas the experimental group, supplemented with powdered shellfish, experienced a reduction of over 90% within the same treatment period. The significance of biocement's precipitation patterns in ameliorating permeability was substantiated through a series of experiments. These discoveries provide novel insights into optimizing biocement technology, with significant implications for potential applications, including hydraulic barrier pollution remediation schemes.

Keywords: *Enzyme-Induced Carbonate Precipitation (EICP); Coarse sand; Permeability reduction; Waste recycling*

1. Introduction

Biocement has garnered significant attention in recent years as a sustainable geotechnical engineering technique¹⁻⁵, due to its potential to reduce energy consumption and mitigate pollution, particularly from the calcination process in conventional cement production⁶⁻¹⁰. It can systematically improve the geotechnical properties of soil in a controlled manner, enabling applications such as foundation reinforcement¹¹, liquefaction mitigation¹², slope stabilization¹³, erosion management¹⁴, and permeability reduction¹⁵. The reduction in soil permeability results from microscale precipitation of calcium carbonate, which decreases voids between particles and thereby slows water flow¹⁶⁻¹⁸. This increases hydraulic resistance and reduces the overall permeability coefficient. As a result, reducing soil permeability can help mitigate leakage in hydraulic engineering and enable targeted in-situ plugging of seepage in underground structures and natural formations.

Despite biocement's potential to reduce permeability, its effectiveness largely depends on the quantity of biomineral precipitation¹⁹⁻²¹. Consequently, multiple treatment cycles are typically necessary to achieve a substantial degree of precipitation and meet the desired reduction targets. Moreover, coarse sand exhibits high porosity, requiring more calcium carbonate to fill the voids, thereby amplifying the challenge of reducing permeability²²⁻²⁴. Studies have shown that different precipitation patterns of calcium carbonate in biocement can impact its performance²⁵⁻²⁹. Adjusting the precipitation pattern to encourage calcium carbonate to more effectively fill the voids between sand particles may lead to a faster reduction in soil permeability under similar precipitation volumes and porosities. This approach could reduce reliance on simply increasing precipitation amounts and extending treatment cycles.

This study explores a novel approach, as illustrated in Figure 1, which employs kitchen waste shellfish powder as an additive. Compared to commonly used EICP additives such as skim milk powder, sorbitol, and sucrose, shellfish powder is more economical and environmentally friendly³⁰⁻³⁴. The waste shellfish is ground into powder and combined with equimolar concentrations of urea, calcium chloride cementation solution, and crude enzymes. During the

initial treatment cycle, the mixture containing shellfish powder is injected into the sand column, followed by subsequent cycles using the mixture without shellfish powder. The inclusion of shellfish powder induces EICP-generated calcium carbonate, which initially coats the sand grain surfaces and then gradually fills the interstitial spaces, using the shellfish powder as a nucleus. This transformation significantly alters the precipitation pattern, narrows pore spaces, increases flow resistance, and more rapidly reduces soil permeability in a small-scale sand column model. The study investigates the mechanisms and potential benefits of shellfish powder-assisted enzyme-induced carbonate precipitation in controlling the permeability of coarse sand, aiming to offer a novel and highly efficient biocement strategy for sustainable engineering and construction.

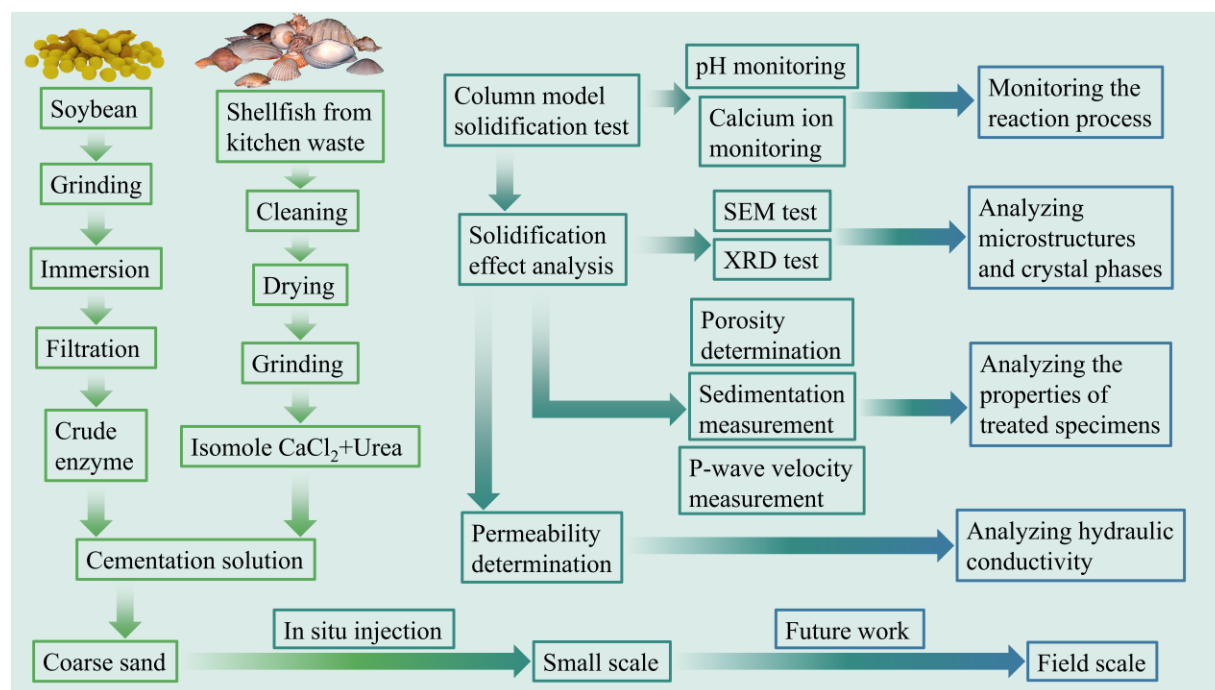


Figure 1. Flow chart of the experiment.

2. Materials and methods

2.1. Used shellfish powder

The present investigation utilized abalone (*Haliotis*) shellfish, as depicted in Figure 2(a), sourced from kitchen waste. These shellfish underwent careful cleaning procedures to remove any extraneous contaminants. Subsequently, they were dried in an oven at 60 °C for 48 hours

to eliminate residual moisture. The shellfish were ground into powder using a grinder and sieved to obtain particles with a diameter of less than 50 μm , in the same batch, for subsequent experiments. Scanning electron microscopy (SEM) images shown in Figure 2(e), obtained using a Hitachi TM3000 Miniscope, showed the angular morphology of the shellfish powder. As shown in Figure 2(b), the weight percentage of chemical components in the shellfish powder, determined through X-ray fluorescence (XRF) analysis using the Hitachi SEA1200VX spectrometer, indicated a calcium oxide concentration exceeding 80%, confirming the high calcium content and aligning with prior research findings^{35–39}. Zeta potential measurements, illustrated in Figure 2(c), were conducted using the Otsuka Electronics ELSZ-1000 Zeta potential analyzer, with the suspension prepared using deionized water at a solid-to-liquid ratio of 1 g/L, demonstrating a negative surface charge on the shellfish powder. X-ray diffraction (XRD) analysis of the shellfish powder was performed using the Rigaku MiniFlex benchtop X-ray diffractometer, as shown in Figure 2(d), which confirmed the predominance of different crystalline forms of calcium carbonate as the main component. Energy-dispersive X-ray spectroscopy (EDS) analysis of the shellfish powder was conducted using the JEOL JSM-IT200 scanning electron microscope, as depicted in Figure 2(f).

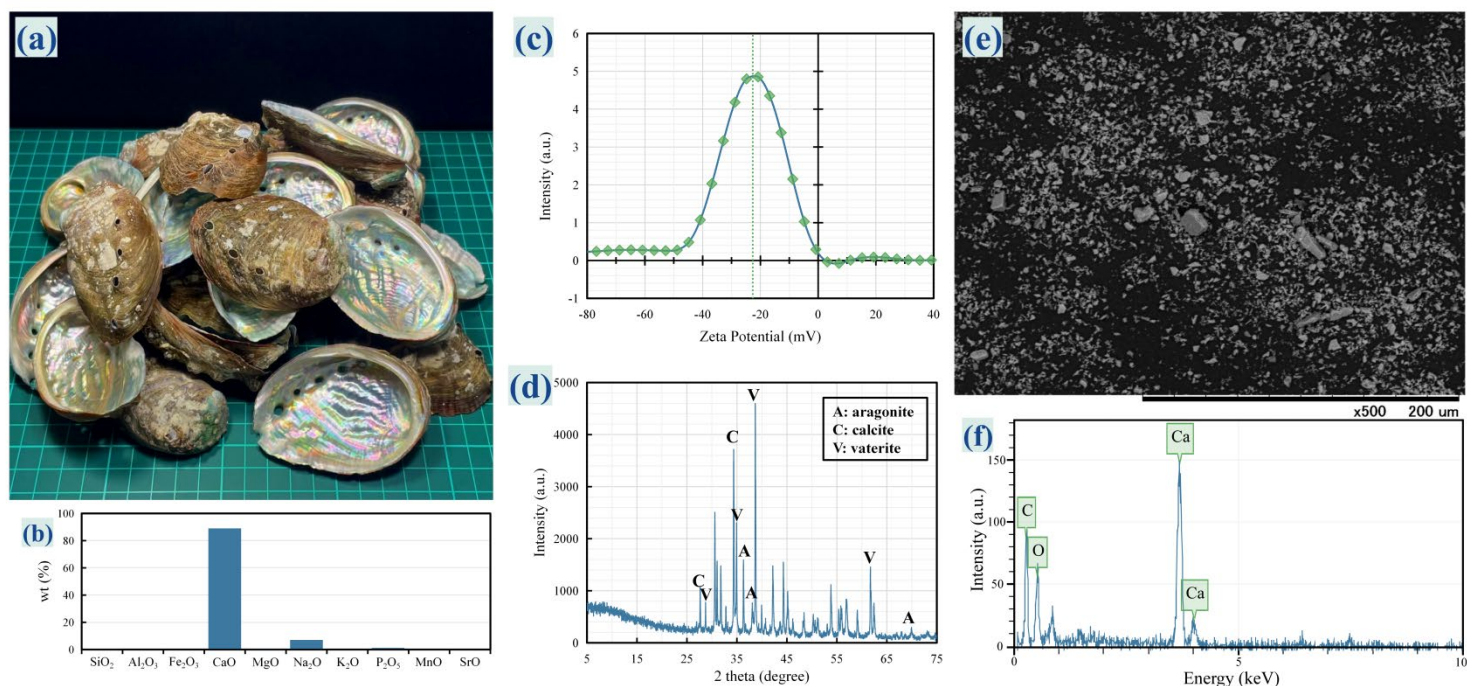


Figure 2. The waste shellfish used is shown in (a) its appearance, (b) XRF analysis, (c) zeta potential analysis, (d) XRD analysis, (e) SEM analysis, and (f) EDS analysis.

2.2. Extraction of crude enzyme and preparation of cementation solution

The soybeans (*Glycine max*) utilized in this study were procured from a local market, as depicted in Figure 3(a). The specific preparation procedure involved grinding the soybeans into powder, followed by mixing the soybean powder with distilled water in a solid-liquid ratio of 1:10, as illustrated in Figure 3(b1), defining it as a soybean urease concentration of 100 g/L. The mixture was stirred for 30 minutes to ensure thorough extraction of urease. Subsequently, the mixture was filtered, and the collected filtrate was utilized as the crude soybean urease solution, as shown in Figure 3(b2). To prevent the decline of urease activity, the urease solution was freshly prepared before use. The crude enzyme activity was determined by measuring the amount of ammonia/ammonium produced from soybean crude enzyme hydrolysis of urea after a 10-minute reaction time under controlled temperature conditions of 25 °C using an AS ONE Thermal Robo TR-1α water bath. Ammonia/ammonium produced from urea hydrolysis reacted with hypochlorite to form chloramine, which then reacted with phenol under alkaline conditions to generate indophenol blue dye. The intensity of the indophenol dye was measured at 630 nanometers using a Jasco V-730 UV-Visible spectrophotometer. Calibration curves were established using different concentrations of ammonium chloride and their corresponding intensities to determine the concentration of ammonia/ammonium, thereby obtaining the crude enzyme activity, denoted as 22.7 U/mL. The consolidating solution was prepared by mixing a 2 M calcium chloride solution and a urea solution with an equal volume of crude enzyme solution, as depicted in Figure 3(b3). Adequate agitation, as illustrated in Figure 3(b4), was necessary prior to injection when adding shellfish powder to prevent its precipitation and ensure uniform injection.

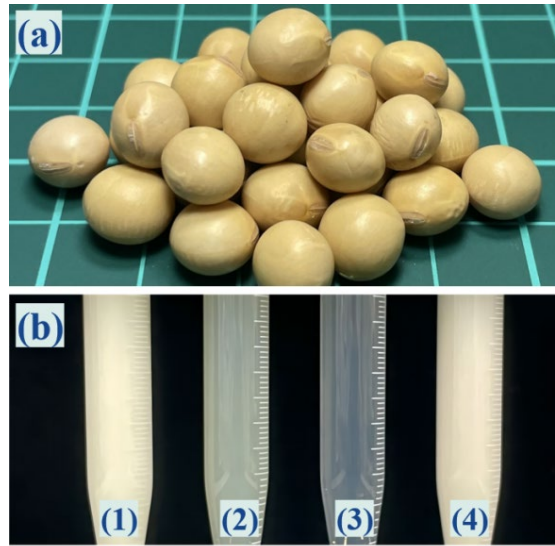


Figure 3. (a) Raw material for crude enzyme extraction. **(b1)** Unfiltered soybean crude enzyme; **(b2)** Filtered soybean crude enzyme; **(b3)** Mixture of soybean crude enzyme and cementation solution; **(b4)** Mixture of soybean crude enzyme, cementation solution, and 50 g/L shellfish powder.

2.3. Used sand

The sand utilized in this study is Mikawa No.4 silica sand, as illustrated in Figure 4(a), and characterized using the same instrumentation as described in Section 2.1. This is a standard silica sand characterized by loose structure, low cohesion, and poor stability. Its relatively uniform characteristics are conducive to minimizing the potential complexities inherent in the bio-cementation process. The SEM image in Figure 4(b) reveals that the particles of the Mikawa No. 4 silica sand exhibit angular to sub-angular shapes, and its particle size distribution is illustrated in Figure 4(c). According to the Unified Soil Classification System, this sand is classified as poorly-graded (SP) sand. The average diameter of the sand grains is 0.87 millimeters, with a uniformity coefficient of 1.27 and a curvature coefficient of 0.97. Furthermore, its particle density is 2.66 grams per cubic centimeter, with a maximum dry density of 1.476 grams per cubic centimeter and a minimum dry density of 1.256 grams per cubic centimeter. Prior to any experimentation, it is subjected to 48 hours of drying at 60 °C to remove residual moisture. Figure 4(d) presents the Zeta potential of the Mikawa No. 4 silica, indicating its negative surface charge. The XRD analysis of Mikawa No. 4 sand, shown in Figure 4(e), identifies silica as its primary component. This finding is further supported by the EDS analysis in Figure 4(f) and the XRF analysis detailed in Figure 4(c).

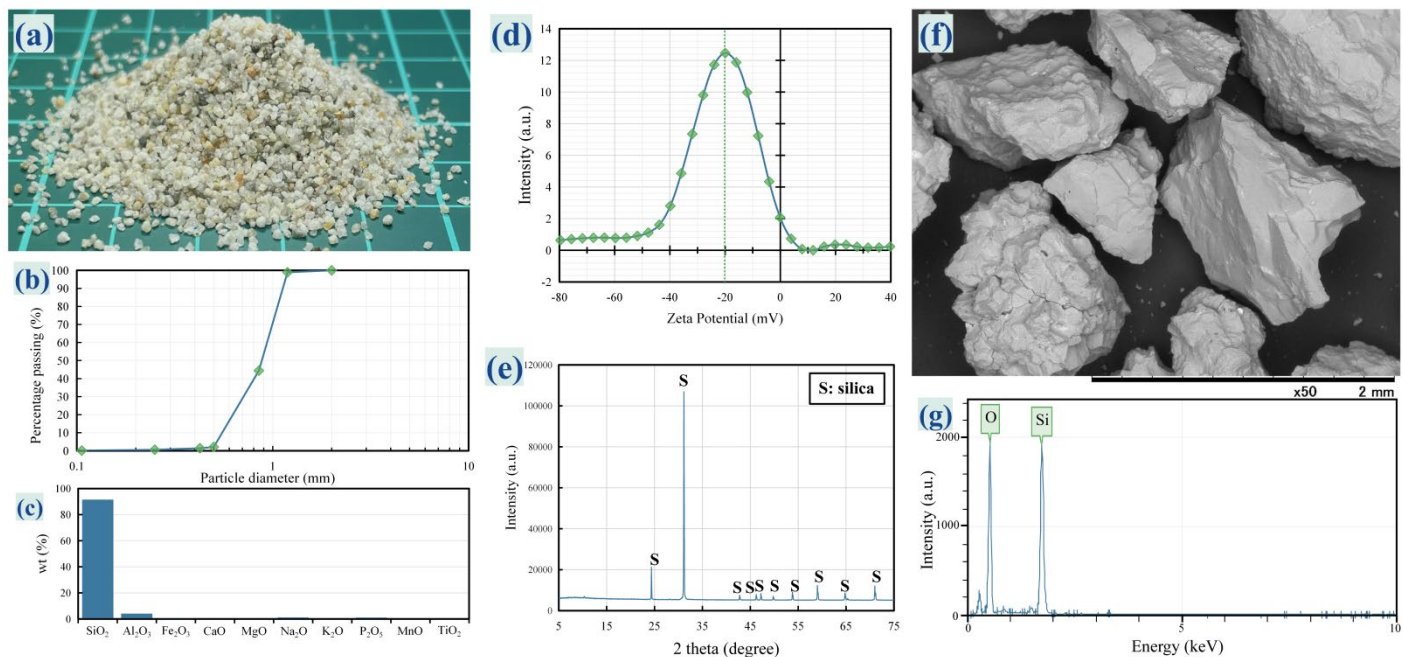


Figure 4. The Mikawa No. 4 silica sand used is shown in (a) its appearance, (b) its particle size distribution, (c) XRF analysis, (d) zeta potential analysis, (e) XRD analysis, (f) SEM analysis, and (g) EDS analysis.

2.4. Column model solidification test

The experimental approach used in this study is a single-phase treatment method, chosen due to its minimal procedural steps, facilitating future scalability. Each experimental run of this study involved the preparation of 30 samples according to the procedures outlined in Table 1, and was repeated five times independently. Sample nomenclature incorporates alphabetical characters denoting experimental groups and numerical values representing the total treatment cycles undergone. Samples were housed within 3D-printed cylindrical membranes. Individual samples were filled with 30 grams of sand, to ensure uniform compaction, with the initial weight recorded as $M_{\text{untreated}}$ prior to treatment. The consolidation procedure involved the direct infusion of a volume of consolidating solution equivalent to the pore volume of the sand column into the top of the column. Under the influence of gravity and capillary action, the solution rapidly permeated the sand, ensuring thorough impregnation. After 24 hours, the effluent solution was completely drained to complete one treatment cycle. Waste solutions from each treatment cycle were monitored for calcium ion concentration using the Horiba LAQUAtwin Ca-11 compact calcium ion meter, facilitating the calculation of calcium ion

consumption, while pH values were monitored using the Horiba LAQUA F-71 benchtop pH meter. During the initial treatment cycle, experimental groups ranged from A to F, with 0 to 0.5 g of shellfish powder added, followed by subsequent treatment cycles employing consolidation solutions devoid of shellfish powder. After all treatment cycles, the samples were thoroughly rinsed with distilled water equal to five times the pore volume of the sand column to remove any residual unreacted chemicals.

The permeability of the samples was tested using the falling head method, and the variation in the permeability coefficient of the samples was calculated using Darcy's law. Each sample underwent five permeability tests, and the average value was calculated. Subsequently, the samples were placed in a drying oven at 60 ° C for 72 hours to ensure complete desiccation. The mass of the samples, including the molds, was recorded as $M_{treated}$, and the difference in weight compared to $M_{untreated}$ was used to calculate the total mass of the biocement. The biocement volume was then calculated from its mass, and the post-treatment sand porosity was determined using the water displacement method.

To preserve the structural integrity of the samples, the molds were carefully cut open, and the consolidated columns were extracted, as illustrated in Supplementary Figure 1. Subsequently, the samples were clamped between the probes of the Oyo Sonicviewer-SX. One end of the device emitted ultrasonic waves at 200 kHz, while the other end, equipped with a receiver, recorded the time required for the waves to pass through the samples, thereby allowing calculation of the compression wave velocity (V_p) of the treated specimens. Prior to testing, both ends of the samples were polished with sandpaper to ensure close contact with the oscillator and receiver. Microscopic and crystallographic characterization was performed using the same instrumentation described in Section 2.1. SEM was utilized to observe the sample morphology at the microscale. The samples were then ground into powder and subjected to XRD analysis to identify the crystalline phases present.

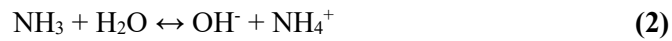
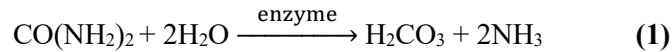
Table 1. Laboratory scale solidification test formulations.

Shellfish powder concentration in first treatment cycle (g/L)	Sample name	Treatment cycle				
		First treatment cycle	Second treatment cycle	Third treatment cycle	Fourth treatment cycle	Fifth treatment cycle
0	A1	treated	untreated	untreated	untreated	untreated
	A2	treated	treated	untreated	untreated	untreated
	A3	treated	treated	treated	untreated	untreated
	A4	treated	treated	treated	treated	untreated
	A5	treated	treated	treated	treated	treated
10	B1	treated	untreated	untreated	untreated	untreated
	B2	treated	treated	untreated	untreated	untreated
	B3	treated	treated	treated	untreated	untreated
	B4	treated	treated	treated	treated	untreated
	B5	treated	treated	treated	treated	treated
20	C1	treated	untreated	untreated	untreated	untreated
	C2	treated	treated	untreated	untreated	untreated
	C3	treated	treated	treated	untreated	untreated
	C4	treated	treated	treated	treated	untreated
	C5	treated	treated	treated	treated	treated
30	D1	treated	untreated	untreated	untreated	untreated
	D2	treated	treated	untreated	untreated	untreated
	D3	treated	treated	treated	untreated	untreated
	D4	treated	treated	treated	treated	untreated
	D5	treated	treated	treated	treated	treated
40	E1	treated	untreated	untreated	untreated	untreated
	E2	treated	treated	untreated	untreated	untreated
	E3	treated	treated	treated	untreated	untreated
	E4	treated	treated	treated	treated	untreated
	E5	treated	treated	treated	treated	treated
50	F1	treated	untreated	untreated	untreated	untreated
	F2	treated	treated	untreated	untreated	untreated
	F3	treated	treated	treated	untreated	untreated
	F4	treated	treated	treated	treated	untreated
	F5	treated	treated	treated	treated	treated

3. Results and discussion

3.1. Reaction monitoring analysis

The examination of calcium ion consumption rates during the reaction process depicted in Figure 5 reveals that there is no significant disparity in the calcium ion consumption rates among different groups. This suggests that the addition of shellfish powder does not impede the reaction process. Moreover, calcium ions in all groups are nearly completely depleted, indicating a high degree of reaction advancement. This is attributed to the decomposition of urea by the soybean crude enzyme, as per Equation 1, followed by the consumption resulting from the precipitation of calcium carbonate, as indicated by Equation 4. The substantial consumption of calcium ions in the treatment solutions is advantageous for future engineering applications. Adequate calcium ion consumption implies that there will be no excessive calcium ion contamination in groundwater and the surrounding ecological environment during future on-site applications.



Monitoring of pH levels reveals that pH values are consistently below 8. In contrast to the high pH levels associated with traditional biocement treatments, the lower pH levels signify reduced ammonia emissions. This is partly due to the substantial precipitation of the treatment solution, which promotes the rightward shift of Equation 3 and Equation 4, leading to the consumption of carbonate ions and consequent self-acidification of the solution system⁴⁰. Additionally, the soybean crude enzyme additive, rich in proteins, likely contributes to buffering effects⁴¹. In this context of neutral to slightly acidic conditions, ammonia tends to convert into ionized forms, thereby aiding in the suppression of uncontrolled ammonia emissions and associated negative impacts compared to traditional biocement treatments with higher pH levels.

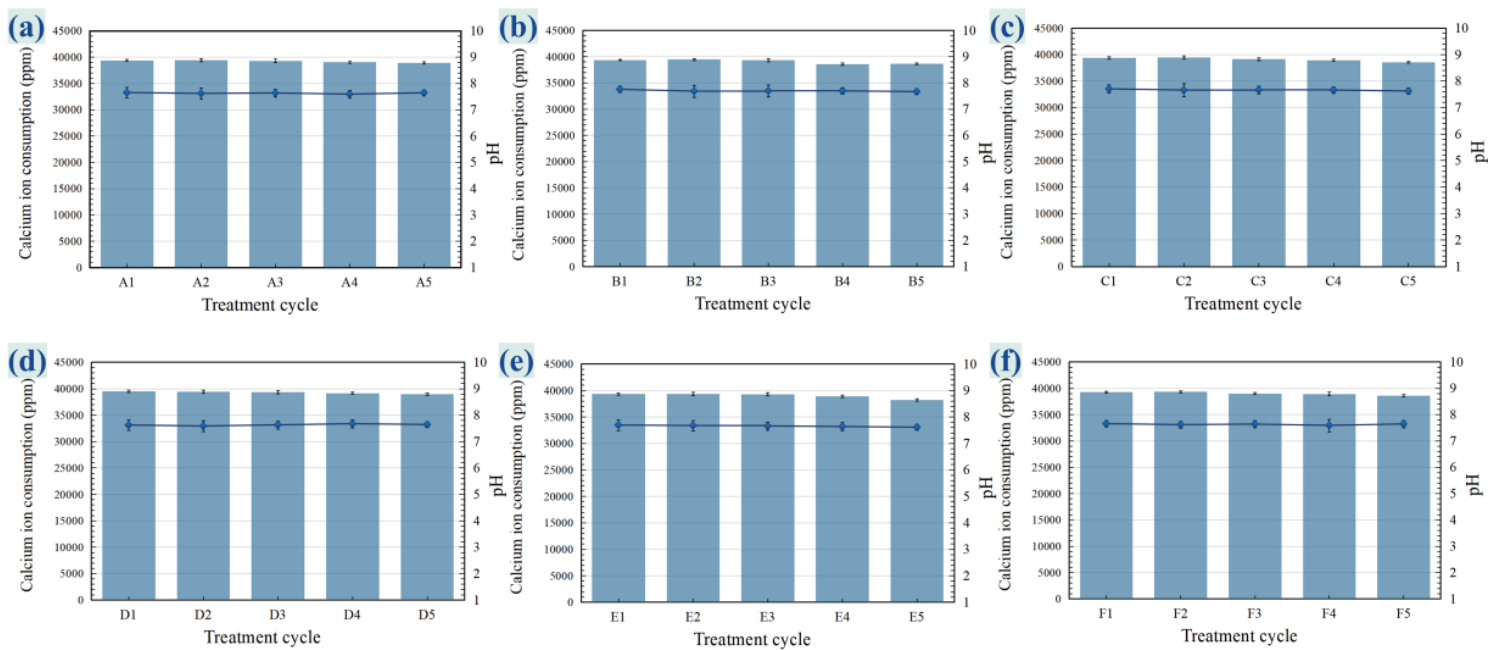
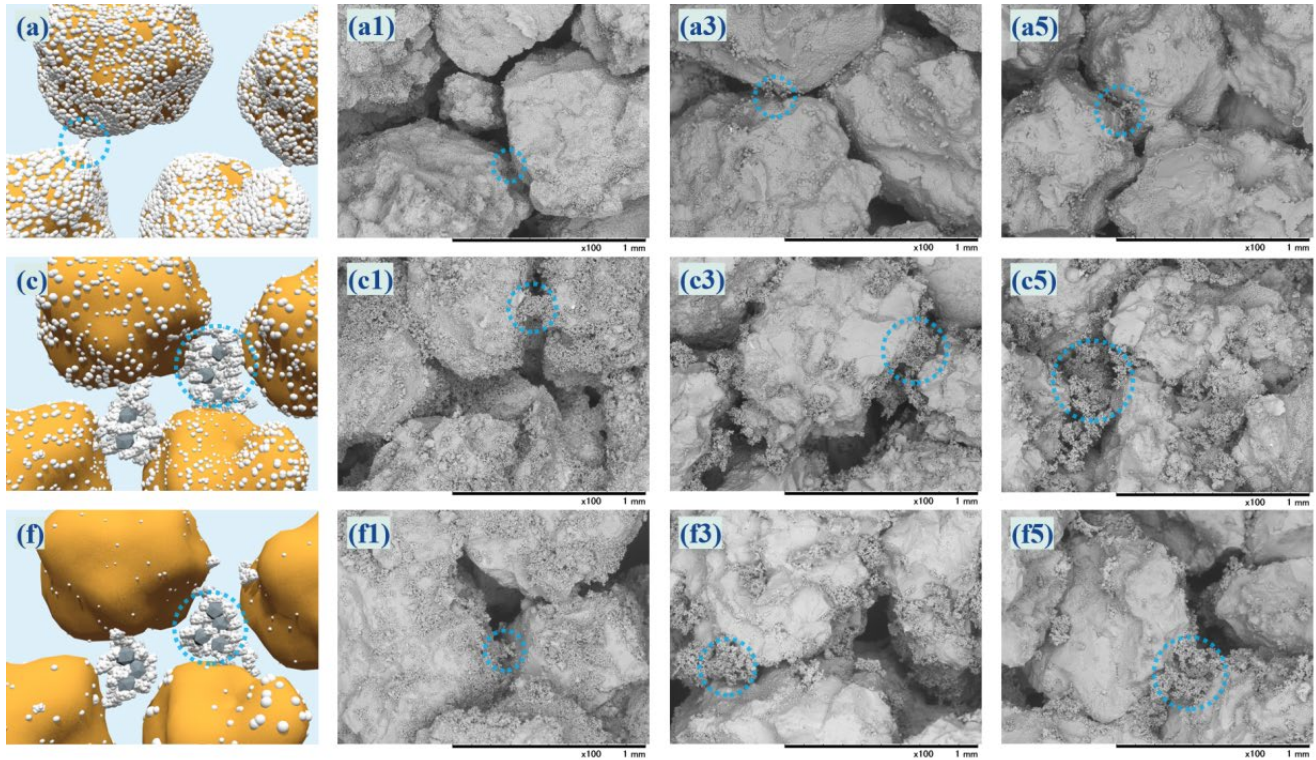


Figure 5. (a) to (f) represent from Group A to Group F, where the bar chart illustrates calcium ion consumption, and the line graph depicts pH. Error bars represent the standard deviation after five independent experiments.

3.2. Microstructural and crystal phase inspection

Combining the mechanistic schematic in Figure 6 with scanning electron micrographs allows the observation of variations in the precipitation modes of calcium carbonate under different conditions. In the absence of any shellfish powder addition, as depicted in Group A of Figure 6, the primary precipitation mode of calcium carbonate is coating. However, upon the addition of an appropriate amount of shellfish powder, as illustrated in Group C of Figure 6, the precipitation modes of calcium carbonate swiftly transition to encapsulating around the shellfish powder core, effectively filling the gaps between sand grains. In the scenario of excessive shellfish powder addition, as shown in Group F of Figure 6, the diminutive particle size and relatively high surface energy of the shellfish powder make it prone to aggregation due to weak interparticle forces. Consequently, the excessive addition of fine shellfish powder particles leads to particle agglomeration, hindering uniform dispersion and thereby compromising the homogeneity of precipitation distribution.



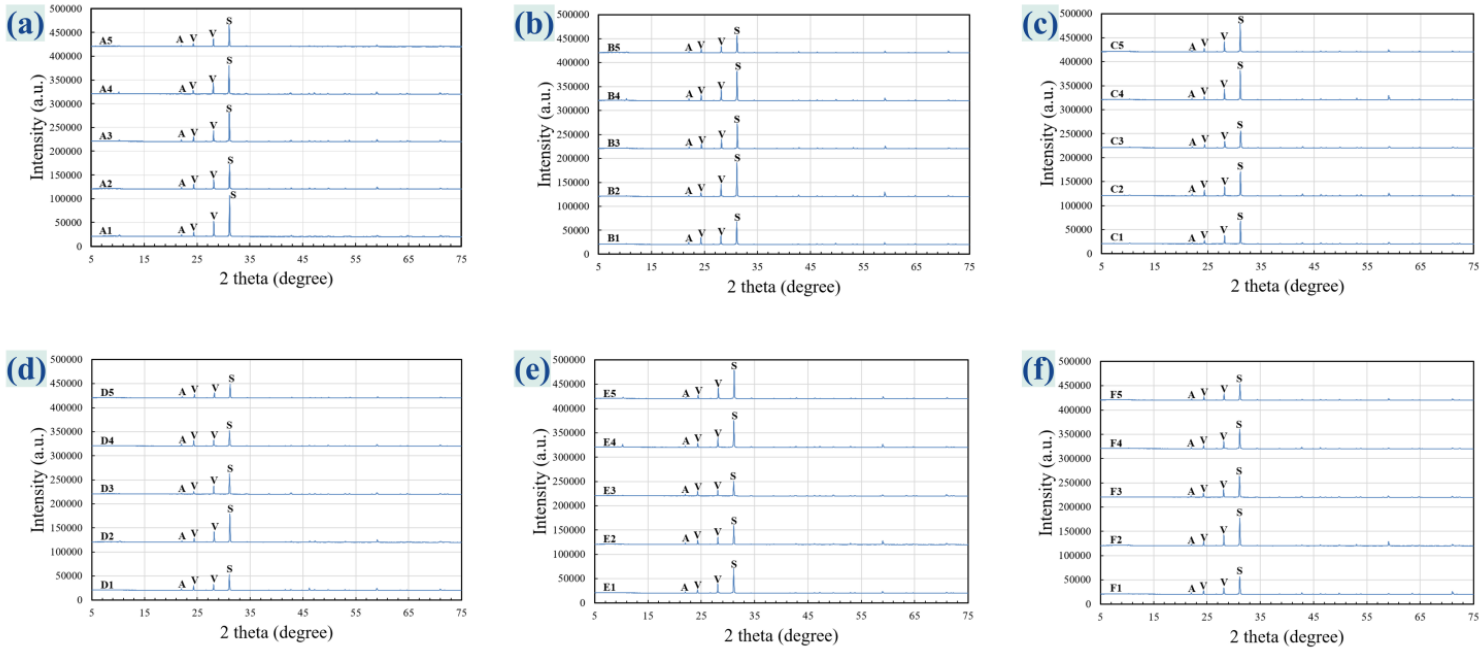
215 **Figure 6.** (a), (c), and (f) respectively depict schematic diagrams of samples from Groups A,
 216 C, and F, followed by SEM images of the corresponding samples in each group.

217 The nucleation effect of shellfish powder is superior to that of sand, potentially explaining the
 218 shift in calcium carbonate precipitation modes following shellfish powder addition. Previous
 219 studies have shown that bacteria involved in urea decomposition can serve as nucleation sites
 220 for calcium carbonate crystals during Microbially Induced Calcium Carbonate Precipitation^{42–}
 221 ⁴⁶. This phenomenon is attributed to the electrostatic attraction of free calcium ions to the
 222 negatively charged surfaces of bacteria, leading to their accumulation on bacterial surfaces,
 223 resulting in surface ion supersaturation that exceeds the solubility product constant, thereby
 224 promoting carbonate ion crystallization. Supersaturation plays a crucial role in determining
 225 the chemical potential at a given temperature and strongly influences nucleation dynamics
 226 and subsequent crystal growth. A high supersaturation index represents a high chemical
 227 potential and substantial thermodynamic driving force, favoring rapid crystal growth⁴⁷. In this
 228 study, the introduction of negatively charged shellfish powder facilitates the adsorption of
 229 calcium ions on shellfish powder particles. Moreover, shellfish powder contains a significant
 230 amount of calcium carbonate, and partially ionized calcium ions can create a localized ion
 231 supersaturation zone around the shellfish powder, surpassing the solubility product constant
 232 and promoting nucleation. Additionally, calcium carbonate in shellfish powder may also serve

as seed crystals, lowering the activation energy required for secondary nucleation, thus facilitating calcium carbonate precipitation on its surface.

Accordingly, when shellfish powder is not added, calcium carbonate primarily precipitates on the surface of sand grains, forming a coating pattern. However, the addition of shellfish powder provides additional competitive nucleation sites, reducing the precipitation mode of calcium carbonate on the surface of sand grains. In the future, combining the use of microfluidic device, which can provide direct visual evidence of mineral nucleation and crystal growth^{48–52}, will facilitate a better intuitive exploration and understanding of how shellfish powder alters the precipitation modes of biocement and its impact on permeability.

The XRD graph in Figure 7 identifies the crystalline phases present in the samples as silica, vaterite, and aragonite. Silica originates from the silica sand matrix, while vaterite and aragonite are polymorphic forms of calcium carbonate. Notably, the crystalline compositions of calcium carbonate are similar across all groups and cases. Recent studies employing ultrasonic oscillation and nanoindentation tests have demonstrated that the elastic modulus, hardness, and ductility of CaCO_3 precipitates are influenced by their crystalline phase composition⁵³. However, the specific influence of these polymorphic forms on permeability remains insufficiently understood, underscoring the need for further investigation.



250 **Figure 7. (a) to (f) represent XRD from Group A to Group F, where A represents aragonite, V**
 251 **represents vaterite, and S represents silica.**

252 3.3. Mineralogical and permeability analysis

253 Figure 8 illustrates the Vp of the samples post-curing, along with the total amount of
 254 precipitates within the samples. Both parameters show an increase with treatment cycles.
 255 Referring to Figure 5, the high calcium ion consumption rates observed across different
 256 groups indicate that the majority of calcium ions are converted into precipitates. Since
 257 additional shellfish powder is only introduced once, the precipitate amounts and trends among
 258 the different groups closely resemble each other.

259 Within each group, Vp shows an increasing trend with treatment cycles. This increase is
 260 attributed to the rise in calcium carbonate content following similar precipitation patterns,
 261 which enhances the degree of cementation and gradually increases the bridging sites at the
 262 contact points of sand grains, thereby facilitating more efficient Vp transmission⁵⁴⁻⁵⁸. Across
 263 different groups, Vp gradually decreases with increasing shellfish powder content. This
 264 phenomenon is primarily influenced by the precipitation mode of calcium carbonate⁵⁹. In the
 265 absence of shellfish powder, calcium carbonate predominantly forms a continuous coating,
 266 efficiently transmitting longitudinal waves, resulting in the highest velocity. With increasing

shellfish powder content, calcium carbonate tends to aggregate around the shellfish powder core, reducing the continuous coating of calcium carbonate, hence resulting in a gradual decrease in V_p .

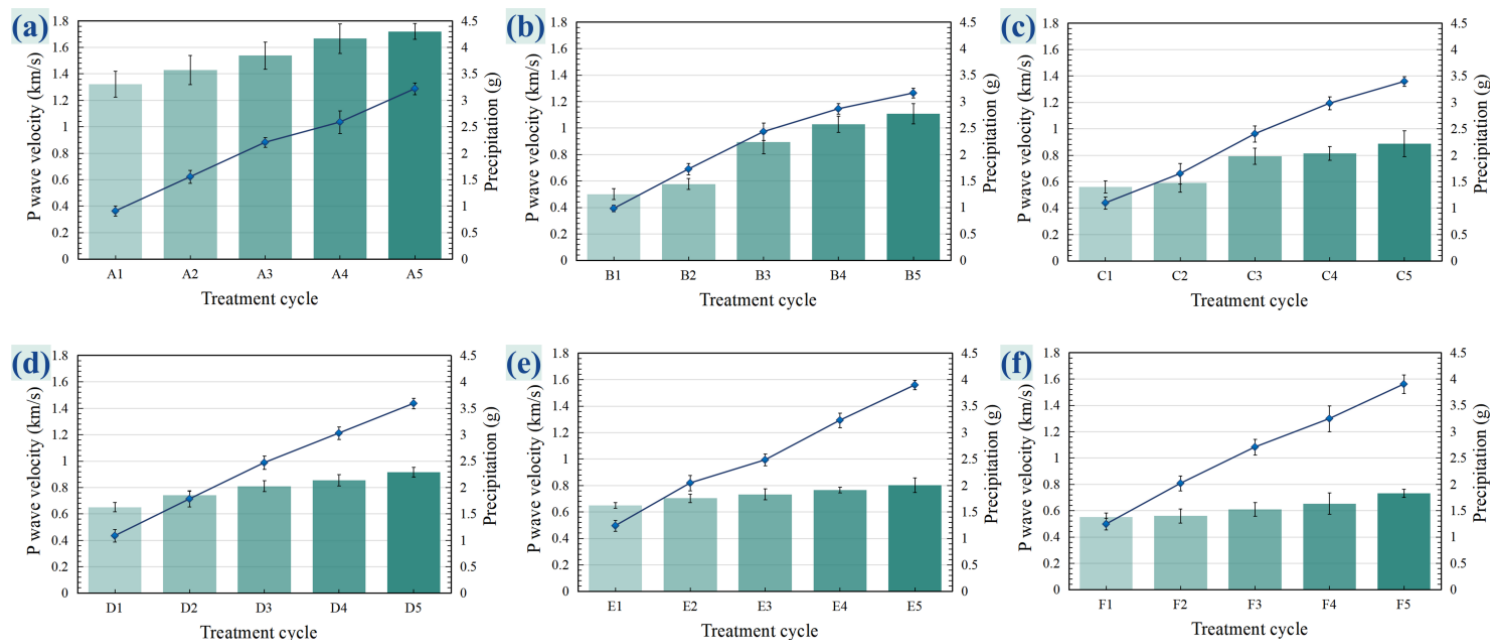


Figure 8. (a) to (f) represent Groups A to F, where bar charts depict P-wave velocity and line graphs represent precipitation quantity. Error bars represent the standard deviation after five independent experiments.

Figure 9 shows the dimensionless relative permeability of the samples, which is the ratio of the permeability measured after treatment to the initial permeability of the sand columns, along with the porosity. It can be observed that both permeability and porosity decrease as the number of treatment cycles increases across all groups. In this study, the reduction in porosity was a result of the combined effects of calcium carbonate precipitation and the addition of shellfish powder. Within the same group, an increase in treatment cycles closely reduced porosity. However, as the amount of precipitate formed in each cycle was similar and the shellfish powder addition was minimal, the overall volume change was small, resulting in a similar reduction in porosity across groups. Porosity plays a key role in influencing permeability. More treatment cycles reduced porosity due to the accumulation of precipitate, which narrowed the pore throats and effective pore sizes, thereby leading to lower permeability. However, the rate of permeability reduction differed across groups, suggesting

that the relationship between porosity and permeability alone cannot fully explain the variations observed. Although the trend of porosity reduction was similar across groups, the rate of permeability reduction increased with rising shellfish powder content. Group C exhibited the fastest decrease in permeability. However, as the shellfish powder content increased further, the rate of permeability reduction began to slow. This suggests that the shellfish powder additive affected permeability in other ways.

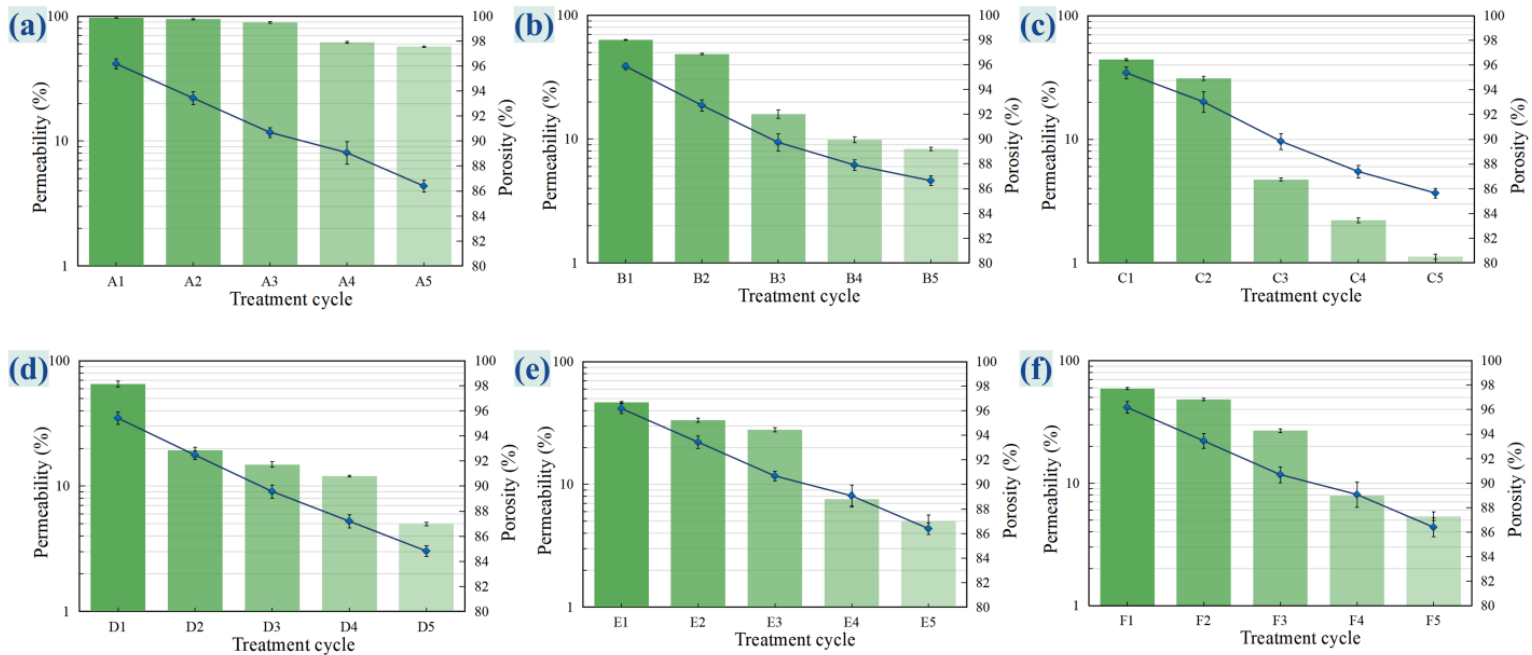


Figure 9. (a) to (f) represents Groups A to F, where the bar charts represent permeability, and the line graphs represent porosity. Error bars represent the standard deviation after five independent experiments.

The changes in permeability among different groups are attributed to alterations in the mode of calcium carbonate precipitation caused by the addition of shellfish powder, leading to notable alterations in micro-pore morphology. Microstructural changes directly influence macroscopic permeability alterations⁶⁰. In samples without any shellfish powder, i.e., ordinary EICP samples, ineffective resistance is provided due to the predominance of encapsulation mode precipitation, resulting in the lowest rate of permeability reduction. Conversely, the addition of appropriate shellfish powder reduces the encapsulation pattern of precipitated calcium carbonate, significantly increasing the number of pore bodies and channels capable of providing effective resistance, hence accelerating the rate of permeability reduction. However,

excessive shellfish powder provides ample nucleation sites, leading to overly concentrated calcium carbonate precipitation, reducing the contact area with the fluid and resistance, thus decreasing the rate of permeability reduction.

In the future, combining X-ray computed microtomography can provide valuable information on sample porosity and specific surface area. Additionally, utilizing simulation techniques to establish more accurate biomineralization permeability models, especially considering calcium carbonate precipitation modes, will facilitate effective fitting and prediction of hydraulic conductivity changes during biocement treatment processes, thereby aiding in practical applications and dissemination.

3.4. Future applications, environmental impacts, and limitations analysis

The expansion of aquaculture has led to a sharp increase in shellfish waste, which, if not properly disposed of, can result in serious environmental pollution^{61–65}. To address this issue, recent studies have explored the utilization of waste shellfish such as oysters, scallops, and conchs as sustainable raw materials in construction applications^{66–70}. This study presents a novel pathway for the integrated management and valorization of shellfish waste, contributing to both environmental protection and material innovation. Moreover, shellfish-derived materials have demonstrated promising potential in environmental remediation, particularly in the removal of heavy metals^{71–75}. However, further investigation is required to evaluate the synergistic application of shellfish powder and EICP in this context. In terms of cost reduction and sustainability, the use of lower-grade or technical-grade feedstocks offers a viable pathway to alleviate economic burdens associated with biocement production^{76–80}. Animal urine, as a renewable nitrogen-rich resource, can serve as an alternative to synthetic urea^{81–85}, thereby reducing both environmental impact and the energy-intensive processes associated with traditional urea synthesis^{86–90}. Similarly, the application of soybean-derived crude enzymes circumvents the need for commercial enzyme production, leading to considerable cost savings^{91–95}. In addition, crude enzyme systems offer enhanced biosafety compared to microbial systems, making them more suitable for controlled applications^{96–98}.

Notably, protein-rich solid residues remaining after crude enzyme extraction may be repurposed as livestock feed, promoting circular resource utilization⁹⁹.

Nevertheless, the use of urea introduces challenges related to ammonia emissions during hydrolysis, as described in Equation (1). Ammonia release contributes to haze formation, nitrogen deposition, soil acidification, eutrophication, and biodiversity loss^{100–104}. Viable mitigation strategies include pH adjustment to convert ammonia to its ionic form, a technique widely used in agriculture through the acidification of slurry using sulfuric acid, which has proven effective in reducing emissions^{105–109}. Similarly, acid addition in biocementation systems has been shown to suppress ammonia volatilization^{110–112}. In this study, the pH was maintained near-neutral during columnar solidification, facilitating the conversion of ammonia to ammonium ions and thus minimizing gaseous emissions while enabling controlled nutrient dissemination. Furthermore, residual urea and ammonium ions can be recovered as fertilizers, thereby integrating nutrient recycling into the biocementation process. The interactions between vegetation and biocement are also worth investigating, as plant roots may work synergistically with biocement to improve soil quality¹¹³, while the hardened layer reduces water evaporation and supports plant growth¹¹⁴. Despite the near-complete consumption of calcium ions, minimizing risks of groundwater pollution, residual salts may elevate soil osmotic pressure, potentially limiting vegetation growth¹¹⁵. Therefore, additional field experiments are needed to comprehensively evaluate plant–soil interactions in biocement-treated environments.

Compared to conventional EICP techniques, the method proposed in this study demonstrates enhanced efficiency in reducing sample permeability, offering broad engineering applicability. Potential fields include sealing high-permeability zones in oil reservoirs to improve recovery, landfill containment to prevent hazardous leachate, and environmental risk mitigation in CO₂ sequestration and nuclear waste storage^{116–120}. As illustrated in Figure 10, the treated material may serve as an in-situ hydraulic barrier, capable of rapid deployment to intercept contaminant migration during emergencies, thereby protecting groundwater resources. However, the current single-phase injection method, despite its simplicity and practicality, is

susceptible to clogging at injection points. Although approaches such as low-temperature or low-pH injection have been employed to mitigate clogging^{121–125}, further optimization is essential to enhance injection efficiency. Moreover, field-scale application must prioritize environmental compatibility and long-term sustainability, particularly in sensitive subsurface environments. Future research should also focus on conducting comprehensive life cycle assessments of biocementation technologies, which will be critical in quantifying economic costs, carbon footprints, and potential ecological burdens^{126–130}.

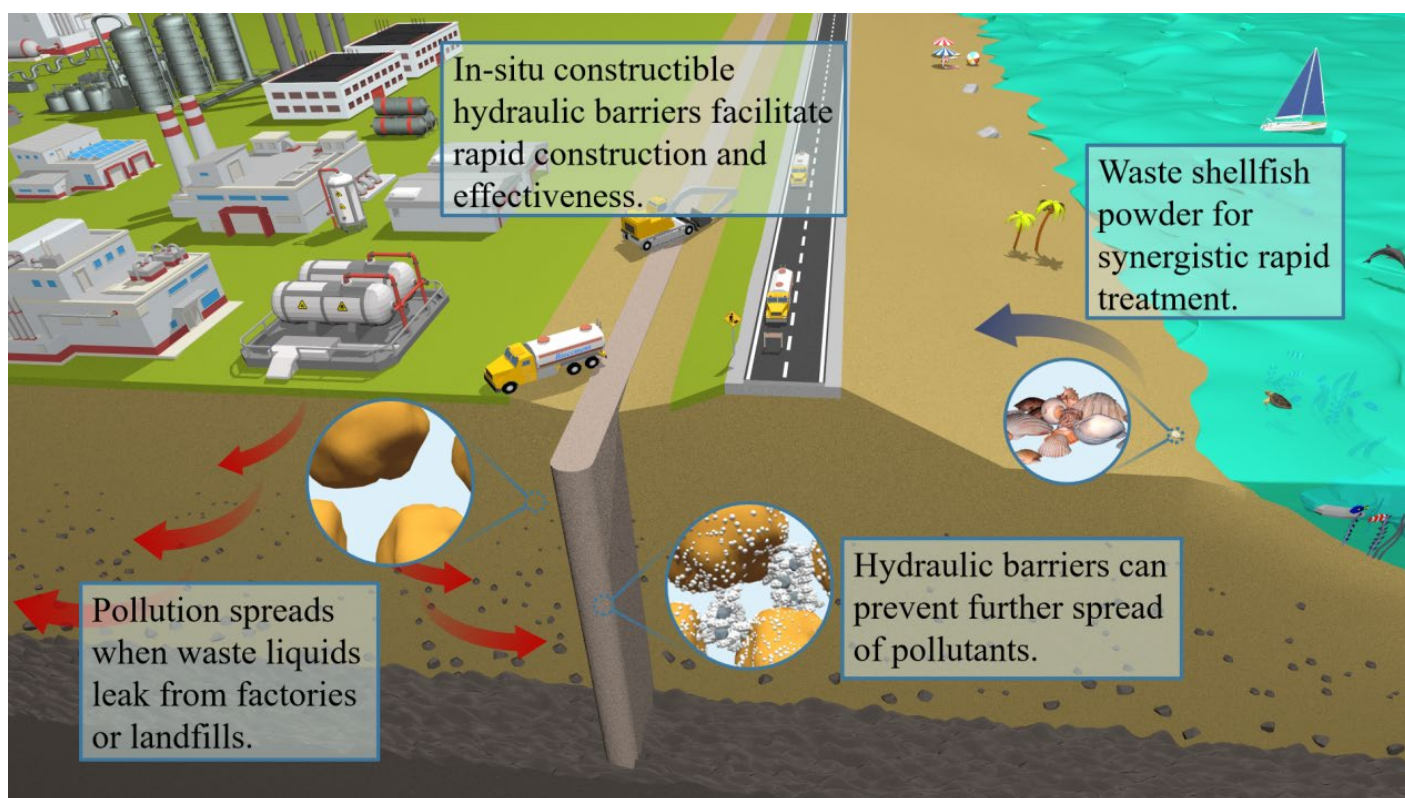


Figure 10. The vision of future solutions for in-situ hydraulic barrier construction using biocement to prevent pollutant migration.

4. Conclusion

The present study conducted a series of experiments on coarse sand with high porosity to investigate the efficiency of reducing permeability, aiming to provide insights into potential applications such as the in-situ establishment of hydraulic barriers. The key findings are summarized as follows:

1. The addition of shellfish powder, which provides nucleation sites, significantly altered the precipitation pattern of biocement. In the same five treatment cycles, the control group without shellfish powder exhibited a reduction of approximately 46 %, while the experimental groups with added shellfish powder consistently achieved reductions of over 90 %. However, excessive shellfish powder, due to excessive aggregation, slightly weakened the efficiency of permeability reduction. In this study, the optimal rate of permeability reduction was achieved with the amount of shellfish powder added in Group C.

2. SEM provided intuitive microstructural evidence showing a transition in the precipitation pattern from predominantly coating-based to aggregation around shellfish powder cores after the addition of shellfish powder. This transition facilitated pore size reduction and increased pore roughness, thereby aiding in permeability reduction.

3. Comparison of the total amount of biocement precipitation and porosity measurements between groups with and without shellfish powder did not reveal significant differences. However, notable distinctions were observed in ultrasonic testing results due to variations in sediment distribution patterns, thus confirming that the precipitation pattern is the primary factor influencing permeability changes in this study.

4. The observed reduction in permeability in this experiment does not conform to the empirical functional relationships established in prior studies with factors such as biocement precipitation and porosity. Further investigation is warranted to quantitatively consider precipitate mode factors and establish corresponding fitting functions.

5. The single-phase injection strategy employed in this study is susceptible to clogging at the injection points. Further research is necessary to optimize this method and assess its performance across various soil types to improve its scalability and suitability for field applications.

397 5. References:

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Acknowledgements

This work was supported by JST SPRING, Grant Number JPMJSP2119, and was partly supported by JSPS KAKENHI Grant Number JP22H01581.

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 statement

All the experimental data that support the findings of this study are available from the corresponding author upon reasonable request through email.

Credit authorship contribution statement

Z.Y.: Conceptualization, experiments investigation, manuscript writing, funding acquisition;
K. N.: Resources;
C. T.: Resources;
S. K.: Resources, funding acquisition.