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Exploring the Solidification of Sand Using Biocement Technology with Kitchen Waste



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ABSTRACT

This dissertation presents a comprehensive investigation into a novel biocementation approach that utilizes kitchen waste to solidify sand, aiming to provide a cost-effective and environmentally friendly alternative to traditional cement-based soil stabilization methods. By structuring the study around biocement's economic and environmental advantages, this work offers valuable insights into reducing production costs and maximizing sustainable applications of biocement methods.

The need for sustainable soil stabilization is becoming increasingly critical as the construction industry seeks to reduce carbon emissions and transition toward greener practices. Despite the well-recognized potential of biocement techniques, traditional methods involve high costs and environmental challenges, such as ammonia emissions and the use of costly reagents. To address these issues, this study explores the use of kitchen waste materials in biocement processes, introducing an innovative, lower-cost pathway to achieving biocementation.

Chapter 1 details the organization and novel contributions of this study in the context of sustainable construction and soil improvement.

Chapter 2 reviews current measures for lowering biocement production costs, discussing alternative cultivation media for MICP, plant-based sources for EICP, and various urea and calcium sources. This chapter provides a comprehensive overview of recent advancements in cost-effective biocementation strategies.

Chapter 3 introduces a novel method of improving biocement strength by altering precipitation patterns. Using eggshell waste and soybean crude enzymes, it demonstrates how the nucleation sites from eggshells can shift calcium carbonate precipitation from a coating to a bridging mode, thereby significantly enhancing strength within the same treatment period.

Chapter 4 presents techniques to reduce biocement permeability through modifications in precipitation patterns. Shellfish waste and soybean crude enzymes are used to provide nucleation sites, allowing calcium carbonate precipitates to effectively fill pore spaces, which decreases permeability within the same processing duration.

Chapter 5 investigates how altering the crystal phase of precipitates enhances biocement strength. This approach shows that adjusting crystal phases of calcium phosphate compounds can significantly increase material strength without increasing the precipitation amount, demonstrating a sustainable method for durable biocementation.

Chapter 6 shows the exploration of biocementation under microgravity conditions, marking the first use of crude enzyme-induced calcium phosphate precipitation for biocement production in microgravity environments. This pioneering investigation provides valuable insights for future space construction applications.

Chapter 7 summarizes the study's findings and conclusions for future research directions.

Together, the findings of this thesis highlight the potential of biocement technology, leveraging waste resources to achieve sustainable soil stabilization. This approach holds promise for a wide

range of applications, including terrestrial and extraterrestrial infrastructure, thereby advancing sustainable development in diverse construction contexts.

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CHAPTER 1 INTRODUCTION OF DISSERTATION

1.1 Organization of Dissertation

This dissertation is structured into seven chapters.

Chapter 1 presents the structure and originality of this research.

Chapter 2 provides a comprehensive review of current strategies to reduce biocement production costs. It examines multiple aspects, including the cultivation media required for Microbially Induced Calcium Carbonate Precipitation (MICP), plant-based sources for Enzyme Induced Calcium Carbonate Precipitation (EICP), alternative urea sources, and alternative calcium sources.

Chapter 3 explores strength enhancement in biocement by altering the precipitation mode. Using eggshell kitchen waste and crude soybean enzymes, the study reveals how eggshells can serve as nucleation sites, converting coating-mode calcium carbonate into bridging-mode calcium carbonate, as shown in Figure 1, significantly increasing strength within the same treatment period.

Chapter 4 addresses permeability reduction in biocement by modifying the precipitation pattern. Utilizing shellfish kitchen waste and crude soybean enzymes, the study demonstrates that shell powder, by providing abundant nucleation sites, can modify the precipitated calcium carbonate to effectively fill pore spaces, as depicted in Figure 1, thus achieving rapid permeability reduction within the same treatment period.

Chapter 5 discusses strength enhancement through crystal structure modification. By employing bone kitchen waste and acidic urease, it was discovered that manipulating temperature and pH can transform calcium phosphate precipitates into hydroxyapatite (HA), significantly increasing strength

within the same treatment period.

Chapter 6 explores future applications by employing in-situ resource utilization (ISRU) methods for construction/geotechnical applications using resources available on space stations. Through the use of bone kitchen waste and crude soybean enzymes, preliminary experiments demonstrate calcium phosphate precipitation under microgravity conditions, laying the theoretical groundwork for biocement applications in space.

Chapter 7 provides a comprehensive summary of the findings and conclusions derived from this study.

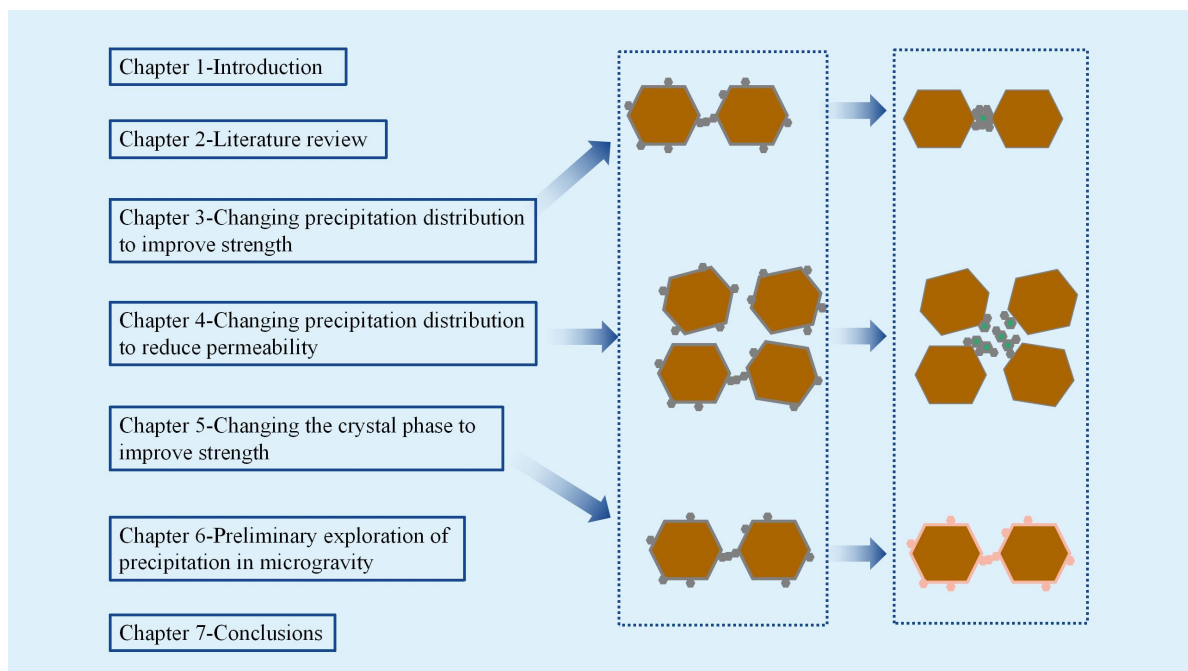


Figure 1.1 dissertation flowchart

1.2 Originality and importance of the study

This study introduces a series of pioneering approaches to enhance biocementation, each with significant implications for cost-efficiency, sustainability, and versatility in applications. The findings include improvements in strength and permeability by modifying precipitation modes, maintaining neutral reaction conditions without acidic additives, and enabling the use of sustainable calcium sources such as waste eggshells and shellfish. Additionally, it introduces a novel approach by altering the crystal phase of precipitates, significantly enhancing strength even with equivalent precipitation volumes. Furthermore, this research explores biocementation under microgravity for the first time, achieving calcium phosphate precipitation with crude enzymes in space-like environments. Together, these innovations position biocement as a promising material for both terrestrial and extraterrestrial applications, supporting sustainable construction and broadening its potential within diverse geotechnical contexts.

Chapter 2:

-This chapter conducts the first comprehensive analysis of various strategies for reducing biocement production costs. It examines factors from multiple perspectives, including the media required for Microbially Induced Calcium Carbonate Precipitation (MICP), plant-based materials for Enzyme Induced Calcium Carbonate Precipitation (EICP), as well as alternative sources for urea and calcium.

Chapter 3:

-Achieves, for the first time, an increase in biocement strength by altering the precipitation mode, allowing for enhanced strength within the same treatment period.

- Establishes a neutral reaction system without the addition of acids.

- Successfully incorporates waste eggshell recycling within the biocementation process.

Chapter 4:

- Achieves, for the first time, a reduction in permeability through changes in the precipitation mode, realizing reduced permeability within the same treatment period.

- Maintains a neutral reaction environment without requiring acid additives.

- Integrates waste shell recycling into the biocement process.

Chapter 5:

- For the first time, enhances strength significantly by modifying the crystal phase of precipitates, achieving this with an equivalent amount of precipitation.

Chapter 6:

- Explores biocementation under microgravity for the first time.

- Achieves initial success in inducing calcium phosphate precipitation using crude enzymes under microgravity conditions.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

In light of the continual global population growth and urbanization, the escalating demand for infrastructure has posed a substantial challenge. Simultaneously, the construction industry has left a considerable environmental footprint on the planet, owing to the consumption of natural resources and energy demand¹⁻⁵. To minimize this environmental burden, researchers have employed various strategies to develop more environmentally friendly and sustainable solutions within the realms of concrete and construction. Notably, Microbially Induced Calcium Carbonate Precipitation (MICP) and Enzyme-Induced Carbonate Precipitation (EICP) have garnered increasing attention and investigation due to their potential to obviate the substantial energy consumption and greenhouse gas emissions associated with the cement production processes frequently utilized in the construction sector⁶⁻¹⁰. As depicted in Figure 2.1, these methods facilitate the adhesion of particles or filling of internal pores and fissures within materials, rendering their applicability across a spectrum of domains including liquefaction alleviation¹¹⁻¹³, slope stabilization¹⁴⁻¹⁶, coastal erosion management¹⁷⁻¹⁹, biobrick manufacturing²⁰⁻²², structural fissure repair²³⁻²⁵, heritage conservation²⁶⁻²⁸, dust suppression²⁹⁻³¹, enhanced petroleum recovery³²⁻³⁴, heavy metal remediation³⁵⁻³⁷, and carbon sequestration³⁸⁻⁴⁰. Nonetheless, the majority of current research remains confined to laboratory and small-scale applications, with large-scale implementation still posing significant limitations. Certain scholars have explored the drivers and barriers of construction, with cost being a predominant constraint in construction projects⁴¹⁻⁴³.

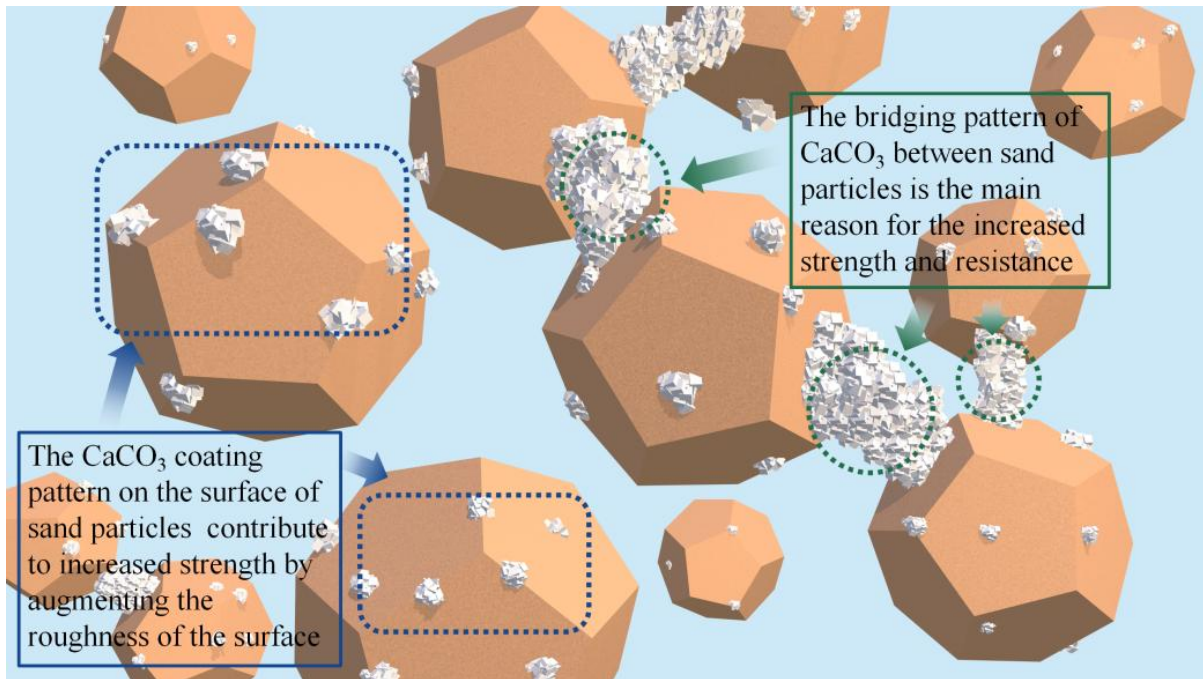


Figure 2.1 Biocement reinforcement mechanism

This review chapter delves into research focused on cost reduction in sustainable construction utilizing biocement, primarily dominated by urease-mediated MICP and EICP. MICP encompasses processes such as ureolysis, photosynthesis, ammonification, denitrification, sulfate reduction, anaerobic sulfide oxidation, and methane oxidation^{44–48}. Among these pathways, urease-mediated MICP, due to its simplicity and ease of control, represents the most extensively studied and applied form of MICP^{49–51}. The biogeochemical reactions involved utilize urease-producing bacteria or direct urease induction to generate calcium carbonate. Urease is employed to catalyze urea hydrolysis, yielding carbonate ions, subsequently precipitating calcium carbonate in the presence of calcium ions. The key determinant for cost control in MICP and EICP, as illustrated in Figure 2.2, hinges upon the accessibility of cost-effective urease-producing bacteria or urease, affordable urea sources, and calcium ion reservoirs.

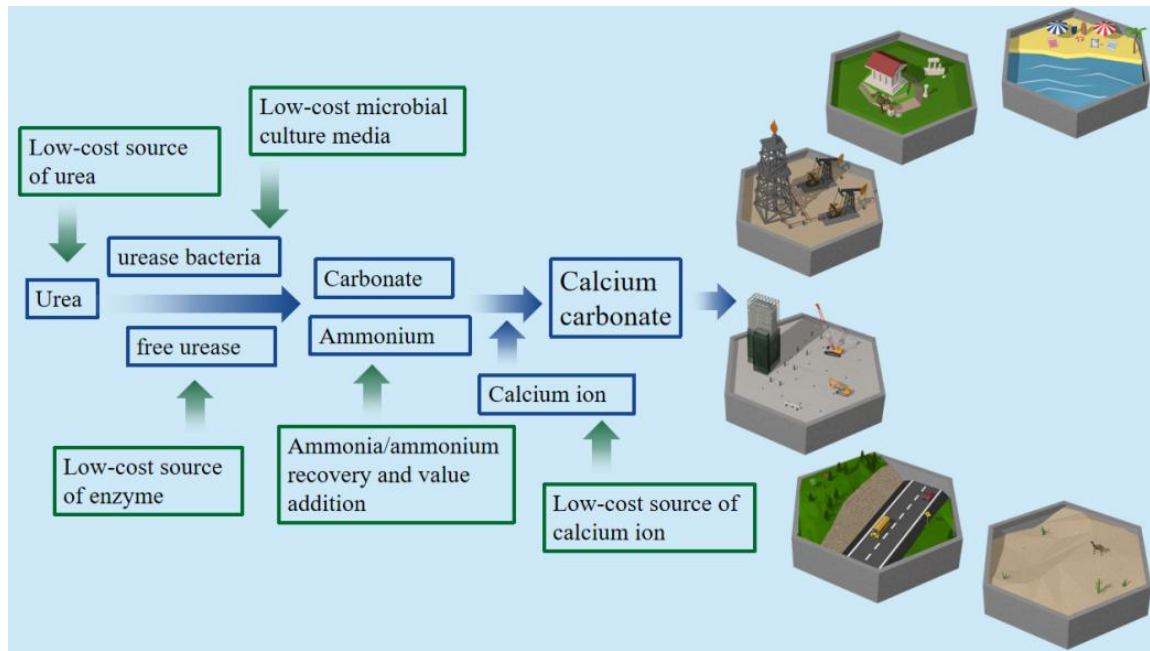


Figure 2.2 Flow chart of the review

2.2 Low-cost Microbial Growth Media for MICP

Thus far, a multitude of microorganisms have been identified for their capability to facilitate MICP through urea degradation, with *Sporosarcina pasteurii* being the most frequently employed due to its often robust urease activity and environmental adaptability^{52–54}. Furthermore, its pronounced negative charge and affinity for calcium ion supplementation facilitate its role as nucleation sites for the generation of calcium carbonate^{55–57}. Regardless of the urase-producing bacteria employed, it was imperative to cultivate them under optimal growth conditions to enable the synthesis of a sufficient quantity of enzymes capable of catalyzing the biomineralization process and to provide an adequate bacterial population for mineralization nucleation sites.

To ensure bacterial growth, it is imperative to provide them with a suitable culture medium containing essential nutrients such as carbon, nitrogen, phosphorus, and other requisite minerals. However, the use of standard culture media is uneconomical for large-scale applications. The selection of cost-effective culture media to supply microorganisms and facilitate a series of precipitation reactions is a critical factor in reducing the production costs of urease-producing bacteria and even biocement. Organic waste from kitchen waste, food industries, agriculture, and livestock directly discharged into the environment leads to various environmental issues, such as eutrophication of water bodies. Recycling these organic waste materials as potential nutrient sources for microbial growth can effectively reduce costs and turn waste into a valuable resource.

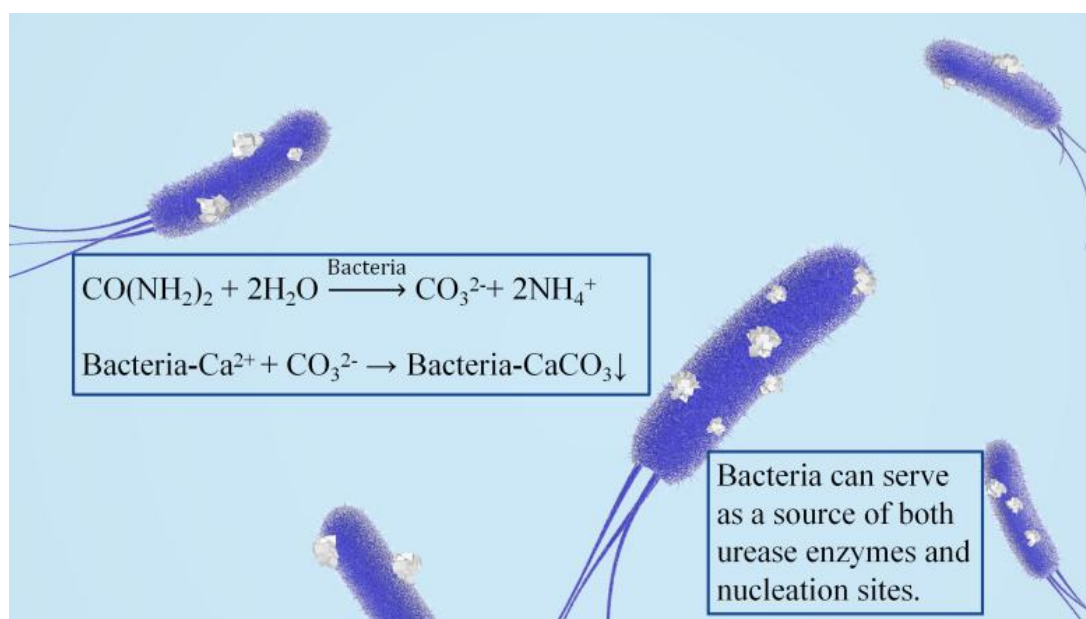


Figure 2.3 The role of bacteria in MICP

As one of the constituents of urban waste, kitchen waste has garnered widespread attention due to the rapid urbanization of global populations and the development of the foodservice industry. The proper management of substantial volumes of kitchen waste remains a global challenge, as improper handling can lead to severe environmental pollution and resource wastage. Kitchen waste primarily comprises starch, polysaccharides, proteins, cellulose, lipids, vitamins, and inorganic salts. It can serve as a nutritive substrate for supporting microbial growth, thus reducing the cost of microbial cultivation during its processing. The research investigated the feasibility of cultivating *Bacillus* spores in kitchen waste and their application in controlling desert soil erosion through microbial-induced carbonate precipitation. Enzymatic hydrolysis was carried out using commercial enzymes to break down long-chain protein molecules into small peptides and free amino acids, enhancing the release and recovery of proteins in kitchen waste, which can subsequently be used for microbial production. After optimizing the conditions, the maximum biomass concentration (OD 600) and urease activity of *Bacillus* spores in the kitchen waste medium reached 4.19 and 14.32 mM urea min⁻¹, respectively, comparable to the results obtained in traditional standard media. The harvested *Bacillus* spores were then employed to catalyze calcium carbonate precipitation in desert soil, and their performance in controlling wind erosion was evaluated through wind tunnel experiments. Even after undergoing 12 cycles of wet-dry or freeze-thaw, microbially mediated calcium carbonate significantly reduced wind erosion losses in desert soil. Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) analysis confirmed the bridging effect of calcium carbonate crystals in the soil matrix. The findings validate the enormous potential of kitchen waste as a cost-effective alternative for bacterial cultivation and carbonate precipitation as nutrients in large-scale applications⁵⁸.

Moreover, the agricultural and food processing industries often generate substantial organic waste, raising concerns regarding potential environmental pollution. The research involved the utilization of composted fertilizers derived from poultry manure, typically employed as organic fertilizers due to their rich organic constituents. Experimental data, obtained through bacterial growth and enzyme activity tests, were compared with standard growth media, affirming the applicability of poultry manure as a potential substitute for nutrient-urea⁵⁹.

Furthermore, the efficacy of anaerobically digested chicken manure wastewater, following biogas processing, has been established as a cost-effective and nutrient-rich growth medium. Owing to its substantial nutrient content, this wastewater serves as a potent stimulant for bacterial growth. In comparison to three other standard growth media, it exhibits the highest urease activity, thereby facilitating calcium carbonate precipitation. Utilizing chicken manure wastewater as a growth medium results in a cost reduction of 88.2% per liter when compared to conventional growth media, underscoring its significant potential for widescale application in biomineralization⁶⁰.

The wastewater from tofu production, known as tofu wastewater (TW), is characterized by a high Chemical Oxygen Demand (COD) concentration exceeding 30,000 milligrams per liter, several hundred times higher than COD discharge standards. The study delves into the utilization of TW as a nutrient growth medium. Researchers conducted a comparative analysis of urease production and bacterial growth between TW and a standard nutrient broth. Importantly, both growth media exhibited no significant differences in bacterial growth. Furthermore, the study meticulously examined the properties of sandstone formed using TW as a growth medium, thereby confirming the feasibility of TW as a growth substrate⁶¹. For instance, cheese production facilities may discharge wastewater containing residual whey. Whey has been validated as a growth medium. Notably,

research demonstrates that substituting disinfected growth medium for sterilized growth medium retains 64% of the optimal urease activity, resulting in reduced energy consumption and investment. Additionally, the utilization of brackish seawater as a solvent in the growth medium does not significantly alter the urease performance of bacteria, thereby contributing to water resource conservation and reducing the cost of the growth medium⁶².

The industrial wastewater in the dairy industry, known as lactose mother liquor (LML), has also been validated as a cost-effective nutritional source for bacteria. Its efficiency was compared to two other standard growth media, yielding similar urease activity in cultured bacteria. Performance in terms of calcium carbonate precipitation and compressive strength improvement was closely aligned with standard media, signifying LML's potential as an alternative growth medium for calcium carbonate precipitation. This offers an environmentally friendly choice for replacing expensive nutrient or yeast-based growth media and facilitates wastewater recycling⁶³. In the realm of agriculture and food industry processing, the substantial generation of organic byproducts has been validated for their potential applications. Byproducts such as Sugarcane molasses and vinasse, originating from sugar and alcohol production, have been investigated as growth substrates in comparison to Tryptic Soy Broth (TSB) for the evaluation of Microbially Induced Carbonate Precipitation (MICP) as an alternative soil conservation method to combat wind erosion. The study reveals that in arid environments, when compared to TSB treatment, Sugarcane molasses and vinasse significantly enhance soil permeability and exhibit robust soil stabilization effects against wind erosion⁶⁴.

Furthermore, the low-cost byproduct of the food industry, corn steep liquor (CSL), derived from corn starch processing, has undergone extensive exploration and validation. Due to its content of amino acids, vitamins, minerals, and proteins, CSL has been recognized by numerous studies as an economical nutritional source for bacterial culture. Research utilizing Microbially Induced Carbonate Precipitation (MICP) for sand consolidation has verified corn steep liquor as an economical nitrogen source in lieu of a portion of soy peptone in the growth medium. Specifically, by substituting 30 g/L of corn steep liquor for 25 g/L of soy peptone, this innovative medium exhibited a 24.21% increase in urease activity, a 52.5% reduction in nitrogen source cost, and an overall 50.5% cost reduction. Studies have also confirmed that the addition of CSL to the growth medium does not adversely affect the rheological properties of cement slurry. Furthermore, observations following bacterial treatment of concrete using CSL have revealed a significant enhancement in compressive strength and permeability performance, augmenting resistance to water and corrosive agents, all without compromising the alkalinity of the concrete. In addition, a modeling approach incorporating artificial intelligence, involving multilinear regression (MLR), adaptive neuro-fuzzy inference system (ANFIS), and genetic programming (GP), was employed for modeling and optimization. The interaction of factors such as CSL-urea concentration and incubation time on urease production was investigated. Subsequently, the results of these models were compared to unveil their performance and determine the optimal conditions for urease production. Through a Hybrid Method Based on Response Surface Methodology and Artificial Neural Networks, it was established that the CSL-urea medium not only provides comparable urease efficiency to conventional yeast extract nutrient media but also presents significant economic advantages.

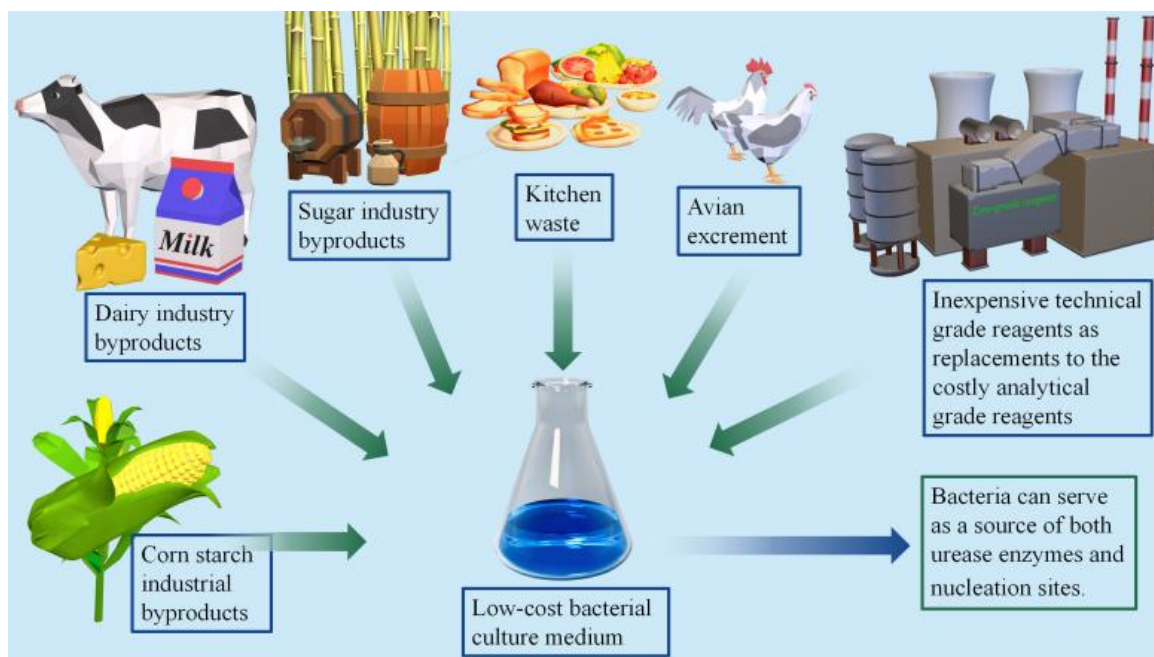


Figure 2.4 Schematic diagram of various cheap microbial cultures

2.3 Plant Extractives as Enzyme Substitutes for Low-cost EICP

In comparison to the incorporation of microorganisms in Microbially Induced Calcium Carbonate Precipitation (MICP), the addition of free urease enzymes often offers a higher degree of biological safety^{65–67}. MICP relying on microbial agents raises numerous issues, such as the necessity to secure governmental approvals and permits, as well as the ongoing monitoring of microorganisms to ensure safety. Furthermore, free enzymes exhibit a decline in activity and functionality over time in engineering applications, naturally degrading and thereby eliminating any long-term ecological impact. In addition, urease enzymes added as free agents can be employed in finer soils. Generally, microbial abundance increases with an increase in soil particle size, and in soils with excessively

small particle sizes, reduced pore space can restrict bacterial growth and movement, consequently affecting cementation effectiveness. The smaller size of free urease enzymes provides a distinct advantage when penetrating exceedingly small pore spaces compared to microorganisms⁶⁸⁻⁷⁰.

Many reported studies on Engineered Induced Calcium Carbonate Precipitation (EICP) have utilized purified urease enzymes. Commercially available purified urease enzymes represent one of the costliest components in the chemical mixture, accounting for approximately 70 to 80% of the total cost^{RRR}. This cost factor can render EICP treatments prohibitively expensive, particularly for large-scale field applications. Some studies have explored the use of crude urease enzyme extracts as a cost-effective enzyme source, offering a feasible avenue for future large-scale applications. Certain research endeavors have involved the cultivation of bacteria and subsequent extraction of bacterial urease⁷¹⁻⁷³, but these methods entail additional processing steps and chemical agents. Hence, this review focuses on the analysis of enzymes derived from plant sources, which offer a more straightforward operational approach and present cost advantages. The pertinent research findings are summarized in Figure 2.5.

Plants known to be rich in urease include sword beans and pumpkin seeds. These plant-derived enzyme extracts not only have high enzyme activity, but also show good catalytic effects under reaction conditions.

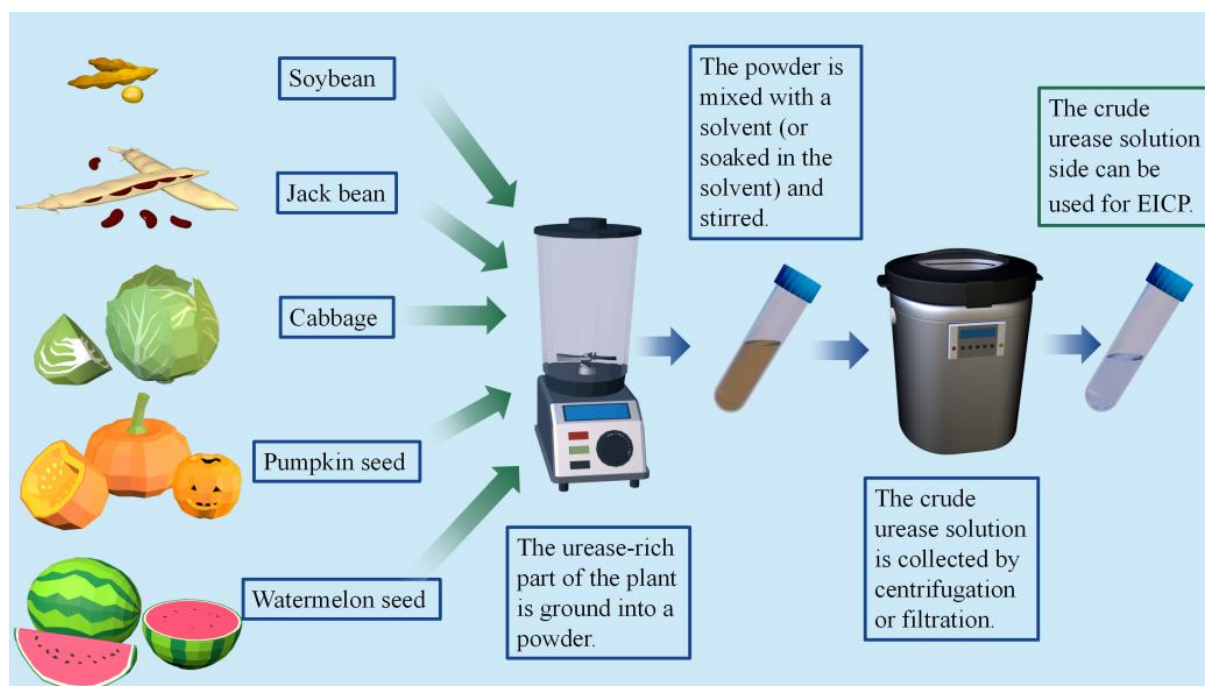


Figure 2.5 Schematic diagram of various crude enzyme extractions

Sword bean (*Canavalia ensiformis*): Sword bean is one of the plants with high urease content, and its crude enzyme extract is easy to obtain and the extraction process is low-cost, so it is widely regarded as a potential enzyme source for the EICP process. In related studies, sword bean-extracted enzymes showed significant calcium carbonate precipitation catalytic effects, which can replace expensive purified enzymes to a certain extent, providing a feasible option for large-scale biocement applications. Pumpkin seeds (*Cucurbita* spp.): Pumpkin seeds are also rich in urease and have high enzyme activity. Its enzyme source cost is much lower than that of purified enzymes, so it is one of the economically feasible EICP alternative enzyme options. Pumpkin seed extracts are easy to obtain, especially suitable for large-scale applications in local resource-rich areas.

Although plant enzyme extracts have advantages in cost and accessibility, their application in EICP still faces some challenges. First, the enzyme activity of plant extracts is usually not as consistent as that of purified enzymes, and plant extracts may contain impurities or other components that may affect the precipitation reaction. In addition, enzymes from different plant sources may differ in composition and activity, which can affect their reaction efficiency in EICP. Therefore, future research should focus on optimizing the plant enzyme extraction process to improve enzyme activity and purity, and systematically screen plant species suitable for EICP.

2.4 Substitutes for urea in biocement

Urea is a core component in current biocementation processes, particularly in techniques like Microbially Induced Calcium Carbonate Precipitation (MICP) and Enzyme-Induced Calcium Carbonate Precipitation (EICP). However, urea production is highly dependent on fossil fuels, resulting in considerable energy consumption and carbon emissions. Given the escalating concerns over global climate change, the research community has been increasingly focused on developing low-carbon materials to achieve more sustainable biocement applications. Therefore, identifying affordable urea sources could not only reduce biocement production costs but also effectively lower its carbon footprint. In pursuit of this goal, researchers have explored a variety of low-cost urea alternatives, including human urine, pig urine, cow urine, and fertilizers, to provide the biocement industry with more economical and eco-friendly options^{74–77}.

Urine as a potential substitute for urea in biocement has garnered increasing attention due to its nitrogen-rich composition, which provides chemical properties similar to urea and can effectively induce calcium carbonate precipitation. Current research primarily focuses on the following urine-based alternatives:

Human Urine: Rich in urea and other organic compounds, human urine has shown potential as a urea substitute in biocement when appropriately processed, with a nitrogen content sufficient to drive calcium carbonate precipitation. However, the collection and treatment of human urine pose challenges related to hygiene and social acceptability. **Livestock Urine:** Urine from livestock, particularly pigs and cows, also presents a potential urea substitute due to its abundant urea content and large-scale availability. Despite these advantages, livestock urine is compositionally complex and may contain high concentrations of organic matter and minerals, which could impact microbial or enzymatic stability. Therefore, further studies are needed to understand its composition and efficacy in biocementation processes. **Agricultural Fertilizers:** Nitrogen-rich fertilizers, especially those containing synthetic urea, offer significant application potential due to their large-scale production and low cost. Using fertilizer-sourced urea as a substitute can reduce dependence on costly analytical-grade urea, and the relatively pure composition of fertilizers allows for easier control. However, fertilizers may introduce additional minerals that could influence the performance of biocement formation.

Using urine and similar substitutes offers sustainability advantages. Firstly, this approach can mitigate the carbon footprint associated with traditional urea production, aligning with carbon neutrality and circular economy principles. Secondly, utilizing waste resources, such as human and livestock urine, not only reduces resource waste but also alleviates wastewater treatment pressures,

providing substantial environmental benefits. However, practical challenges remain in addressing hygiene, collection, transportation, and treatment requirements, necessitating further research. Additionally, the variability in composition and concentration across different urine sources may lead to varying impacts on biocement quality, underscoring the need for empirical studies to validate their applicability and stability.

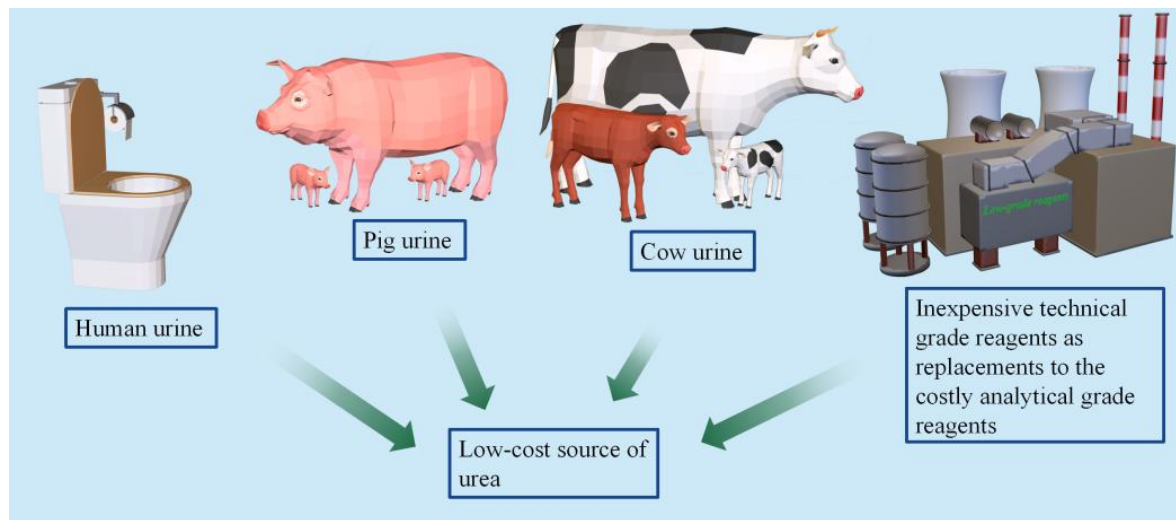


Figure 2.6 Schematic diagram of various urea substitutes

2.5 Affordable Alternatives to Calcium

In the production process of biocement, calcium source is one of the important components, and its cost often significantly affects the overall production cost. Choosing a low-cost calcium source not only helps to reduce production costs, but also conforms to the concept of circular economy and sustainable development. By recovering calcium resources from waste materials, it can effectively

reduce dependence on natural calcium materials and alleviate the pressure of treating construction and domestic waste. In addition, this resource recovery strategy can transform waste into valuable resources, laying the foundation for achieving an economic model of "zero waste" and resource circulation. Therefore, exploring low-cost and environmentally friendly calcium sources is of great significance for the widespread application and promotion of biocement in the future.

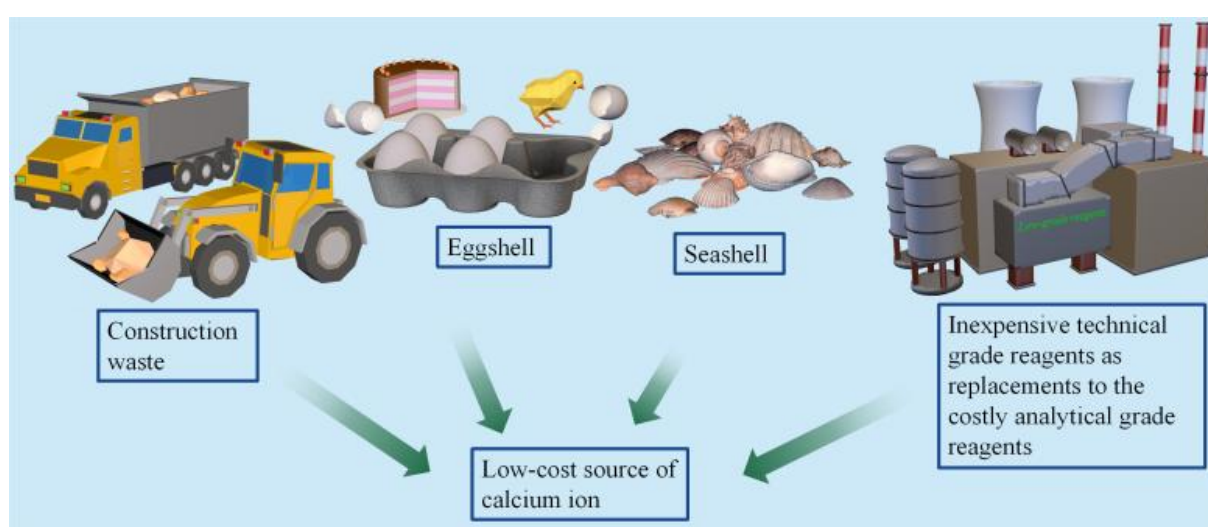


Figure 2.7 Schematic diagram of various calcium source substitutes

Researchers have explored a variety of alternatives to low-cost calcium sources, which are abundant in sources, low in price, and show good reaction effects in biocement applications. The following are the main low-cost calcium sources at present: Construction waste, including concrete, gypsum board, etc., often contains rich calcium components. After crushing, screening and other treatments, construction waste can become an alternative source of calcium in biocement. On the one hand, the use of construction waste can reduce the mining of natural limestone and reduce dependence on

mineral resources; on the other hand, it also provides a sustainable solution for waste management in the construction industry. However, the composition of construction waste is relatively complex and may contain some impurities, so its applicability and safety in biocement applications still need further research. Eggshells and shellfish wastes such as oyster shells and clam shells are rich sources of calcium carbonate and have attracted much attention because their chemical composition is close to that of natural limestone. These wastes can not only provide sufficient calcium content in biocement production, but also have high purity and are suitable for microbial or enzyme-induced precipitation processes. In addition, the recycling of such kitchen waste can effectively reduce the landfill volume of kitchen waste and has important environmental benefits. However, how to improve the treatment efficiency of these wastes and avoid pollution remains a technical challenge in this field. In cold areas, snow removers are mainly used for snow removal and anti-skid of roads and buildings. Their main components are usually calcium chloride or other calcium salts. Snow removers have become potential calcium source substitutes due to their easy availability and low cost. Using snow removers as a calcium source for biocement not only helps to reduce resource waste, but also conforms to the concept of recycling. However, chlorides in deicing agents may affect the durability and microbial activity of biocement, so composition adjustment and process optimization are required in practical applications to ensure its applicability.

The use of low-cost calcium sources can not only significantly reduce the production cost of biocement, but also conform to the concept of circular economy and sustainable development. By recovering calcium resources from construction waste, kitchen waste and industrial by-products, the demand for calcium source mining can be effectively reduced, alleviating the pressure on mineral resources. At the same time, this recycling and reuse strategy helps to reduce waste treatment costs and environmental pollution. In addition, the application of low-cost calcium sources can also

promote the circular economy model of "waste as resource", providing a new path for building a resource-saving society.

2.6 Challenges and future perspectives

The recycling of ammonia nitrogen (ammonia gas and ammonium ions) has significant potential for cost-effectiveness and environmental sustainability in the biocement process. However, current research on ammonia nitrogen recovery and its use in applications such as fertilizers is still limited. The successful application of ammonia nitrogen recovery in actual biocement production can not only reduce material costs, but also promote the resource utilization of waste, which is in line with the concept of green economy and circular economy. Therefore, the research on ammonia nitrogen recovery is expected to bring more cost-effective and environmentally friendly solutions to biocement in the future.

In addition to ammonia nitrogen recovery, cost reduction and sustainable development of the biocement process can also be achieved through a variety of innovative means. The following are some key future development directions:

Immobilization of bacteria and enzymes: Bacteria and enzymes are key active ingredients in biocement production, but their activity and stability are often affected by various environmental factors during the production process. Through immobilization technology, bacteria and enzymes are firmly attached to carrier materials, which can improve their stability in the reaction system and

extend their service life. This technology can not only reduce the cost of supplementing microorganisms and enzymes, but also improve the production efficiency of biocement. However, the immobilization process of bacteria and enzymes still faces problems such as the selection of suitable carrier materials and the optimization of immobilization technology. Future research should focus on solving these technical problems.

Systematic introduction of life cycle assessment (LCA) and life cycle sustainability assessment (LCSA): In order to further reduce the environmental and economic costs of biocement production, life cycle assessment (LCA) and life cycle sustainability assessment (LCSA) provide systematic analysis tools. LCA analysis can comprehensively evaluate the resource consumption, greenhouse gas emissions and waste generation in the production process of biocement, help identify weak links in the production process, and optimize resource utilization efficiency. At the same time, LCSA incorporates environmental, economic and social factors into the assessment framework to provide data support for achieving more comprehensive sustainability. With the increasing maturity of the application of LCA and LCSA in the field of biocement, it is expected to further promote its process improvement and reduce the environmental burden in the future.

Although biocement has shown great potential in cost control and environmental benefits, its large-scale application still depends on continuous technological innovation and research. Future research should further focus on the technical optimization of ammonia nitrogen recovery and its safe application to ensure that biocement products comply with environmental regulations and safety standards. In addition, through the immobilization technology of bacteria and enzymes and evaluation methods such as LCA and LCSA, the sustainability and cost-effectiveness of biocement

production will be significantly improved. These challenges and technological innovations will not only bring new opportunities for the development of biocement, but also promote the construction industry to move towards low-carbon, circular economy and sustainable development.

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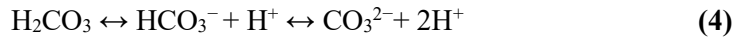
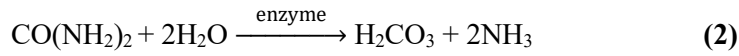
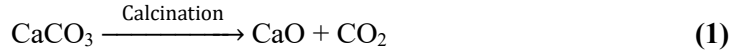
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CHAPTER 3 Sand improvement by altering the distribution pattern of enzyme-induced carbonate precipitation using eggshell waste

3.1 Introduction

Since the advent of the industrial revolution, human activities have led to an unprecedented escalation of greenhouse gas emissions, resulting in an increased greenhouse effect¹⁻⁵, as depicted in Figure 1. These emissions are primarily attributed to the widespread use of fossil fuels, large-scale manufacturing, construction infrastructure, and other factors. The Portland cement industry is one of the most significant sources of anthropogenic carbon dioxide emissions, accounting for approximately 7% of global anthropogenic carbon dioxide emissions⁶⁻¹⁰. The calcination reaction in cement production, as portrayed in Eq. (1), releases carbon dioxide directly. Additionally, the high-temperature reaction conditions also give rise to the generation of carbon dioxide as a byproduct of massive energy consumption¹¹⁻¹⁵. Biocement, as an environmentally friendly and sustainable approach with a smaller carbon footprint, has garnered significant research attention in numerous laboratory and field investigations. This method encompasses microbial-induced carbonate precipitation (MICP) and enzyme-induced carbonate precipitation (EICP), with the chemical reactions involved being elucidated in Eqs. (2)-(5)¹⁶. These investigations have explored various applications, including carbon sequestration¹⁷, contaminant immobilization¹⁸, coastal erosion inhibition¹⁹, fugitive dust suppression²⁰, slope stabilization²¹, liquefaction mitigation²², crack remediation²³, heritage conservation²⁴, bio-brick fabrication²⁵, and enhanced oil recovery²⁶.



At the microscale, the carbonates formed through the aforementioned sequence of biochemical reactions precipitate onto the dispersed sand particles, as illustrated in Figure 1(b), culminating in the formation of the biocemented soil matrix as depicted in Figure 1(c)^{27–29}. On a macroscopic level, these discrete sand particles solidified into the specimen configuration delineated in Figure 1(d). As EICP employs free enzymes, it circumvents the laborious process of cultivating bacteria, resulting in enhanced safety from a biological perspective, reduced energy consumption, and a diminished requirement for monitoring³⁰. Furthermore, owing to the comparatively smaller dimensions of free enzymes in contrast to microorganisms, EICP technology demonstrates effectiveness across a broader spectrum of soils, including fine-grained soil types, by virtue of its capacity to access finer pores³¹. However, due to the absence of microorganisms serving as nucleation sites, there is a potential for adverse effects on the precipitation of calcium carbonate in EICP, which in turn may diminish its treatment efficacy^{32–34}. To augment nucleation sites and enhance treatment efficiency, current researchers are exploring the addition of non-fat milk powder to the baseline EICP solution. It appears that casein within milk precipitates and provides favorable nucleation sites conducive to

the formation and growth of calcite crystals, thereby achieving heightened strength at lower carbonate content³⁵. In experiments employing sorbitol as a nucleation agent, a gradual increase in calcium carbonate yield was observed with the increment of the nucleation agent dosage. Specifically, when the sorbitol content reached 5%, there was a 5.1% augmentation in calcium carbonate yield. Furthermore, the most significant enhancement in calcium carbonate yield, reaching up to 12.3%, was achieved upon the addition of 4% sucrose³⁶. However, it is imperative to acknowledge that both the carbon footprint and cost associated with non-fat milk powder, sorbitol, and sucrose are relatively high. Consequently, the pursuit of more environmentally sustainable and cost-effective nucleation agents continues to be paramount for large-scale applications.

Eggshell waste from kitchen refuse is typically discarded, necessitating considerations that include disposal costs, environmental concerns such as the potential transmission of pathogens, and the availability of disposal facilities^{37–39}. Some researchers have employed eggshells as an economical material for metal adsorption^{40–42}. In the domain of cement research, persistent endeavors have been devoted to the exploration of incorporating eggshells as a supplementary cementitious material, harnessing their capacity to enhance the nucleation process of calcium silicate hydrates^{43–45}. This strategic utilization aims to curtail cement consumption, thereby mitigating carbon dioxide emissions^{46–48}. It is highly probable that eggshells may similarly possess the potential to serve as a nucleation agent in the context of EICP by adsorbing calcium ions. In the realm of biocement, endeavors have been undertaken to investigate alternative reservoirs of commercial calcium salts by means of acid-mediated dissolution from eggshells^{49–51}. However, as delineated in the experimental procedure depicted in Figure 1, this study involves the direct amalgamation of crushed eggshell particles with sand. Microscopic level experimental results affirm that the addition of eggshell

powder conspicuously optimizes the spatial distribution of the precipitated calcium carbonate. Calcium carbonate precipitates at the nucleation points provided by the eggshell powder, thereby facilitating interconnection among soil particles. Furthermore, the introduction of eggshell powder enhances the particle size distribution of the sand grains and fills the voids within the sand grains, resulting in a substantial macroscopic-level increase in the unconfined compressive strength (UCS) of the solidified sand column specimens. This pioneering approach, employing eggshells as additives, offers a novel perspective for enhancing EICP holds significant potential for the widespread application.

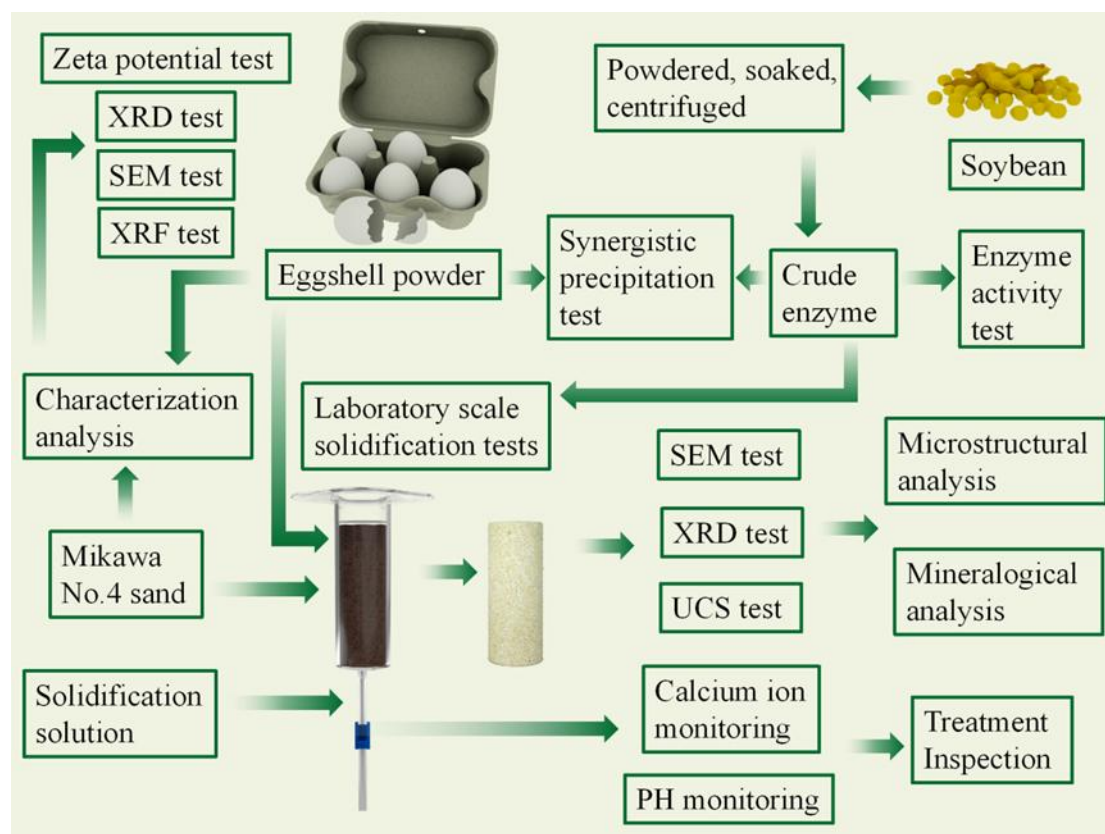


Figure 3.1 Flow chart of the experiment

3.2 Materials and methods

3.2.1 Characterization of Eggshell Powder and Sand

In this experiment, eggshells were sourced from household kitchen waste. However, for prospective large-scale implementation in the future, it is deemed more feasible to consider collecting from prolific sources such as bakeries and poultry farms, where a substantial volume of eggshells is generated. This consideration takes into account the logistical complexities associated with collection, transportation, and segregation. The collected eggshells were meticulously cleaned to eliminate any external contaminants and subjected to a thorough de-shelling process to remove the eggshell membranes. Following this, they underwent a 24-hour drying period in an oven set at a temperature of 110 °C to expunge residual moisture. The eggshells were ground into powder using a grinder and sieved to obtain particle sizes smaller than 150 μm for further experiments, with the final appearance shown in Figure 2(a). The sand used in this study is Mikawa No. 4 silica sand as shown in Figure 2(c), a standard silica sand with loose structure, low cohesion and poor stability. It is poorly graded sand according to the Unified Soil Classification System. Specific properties of the sand include maximum and minimum dry densities of 1.476 and 1.256 g/cm^3 , respectively. Additionally, it has a particle density of 2.66 g/cm^3 and a mean diameter of 0.87 mm. The sand's coefficients of uniformity and curvature are determined to be 1.27 and 0.97, respectively. Before any experimentation, it was also dried in an oven at 110 °C for 24 hours to remove residual moisture. The scanning electron microscope (SEM) images in Figures 2(d) and Figures 2(e), obtained using the Hitachi TM3000 Miniscope, reveal that both the eggshell powder and the Mikawa No. 4 silica sand particles exhibit an angular to subangular morphology. However, the eggshell powder possesses smaller particles, indicating a greater specific surface area. The X-ray fluorescence (XRF) chemical compositions of eggshell powder and Mikawa No. 4 silica sand, obtained using the Hitachi

SEA1200VX X-ray fluorescence analyzer, are presented in Figure 2(b) as weight percentages. Notably, the eggshell powder exhibits a significant calcium content⁵². Additionally, the X-ray diffraction (XRD) analysis of the eggshell powder and Mikawa No. 4 silica sand, conducted using the Rigaku MiniFlex benchtop X-ray diffractometer, is displayed in Figure 2(g). The results indicate that the primary component of the eggshell powder is calcium carbonate in the form of calcite⁵³, while Mikawa No. 4 silica sand is primarily composed of silicon dioxide. Figure 2(f) illustrates the Zeta potentials measured using the Otsuka Electronics ELSZ-1000 Zeta potential analyzer, indicating that both Mikawa No. 4 silica sand and eggshell powder possess negative surface charges.

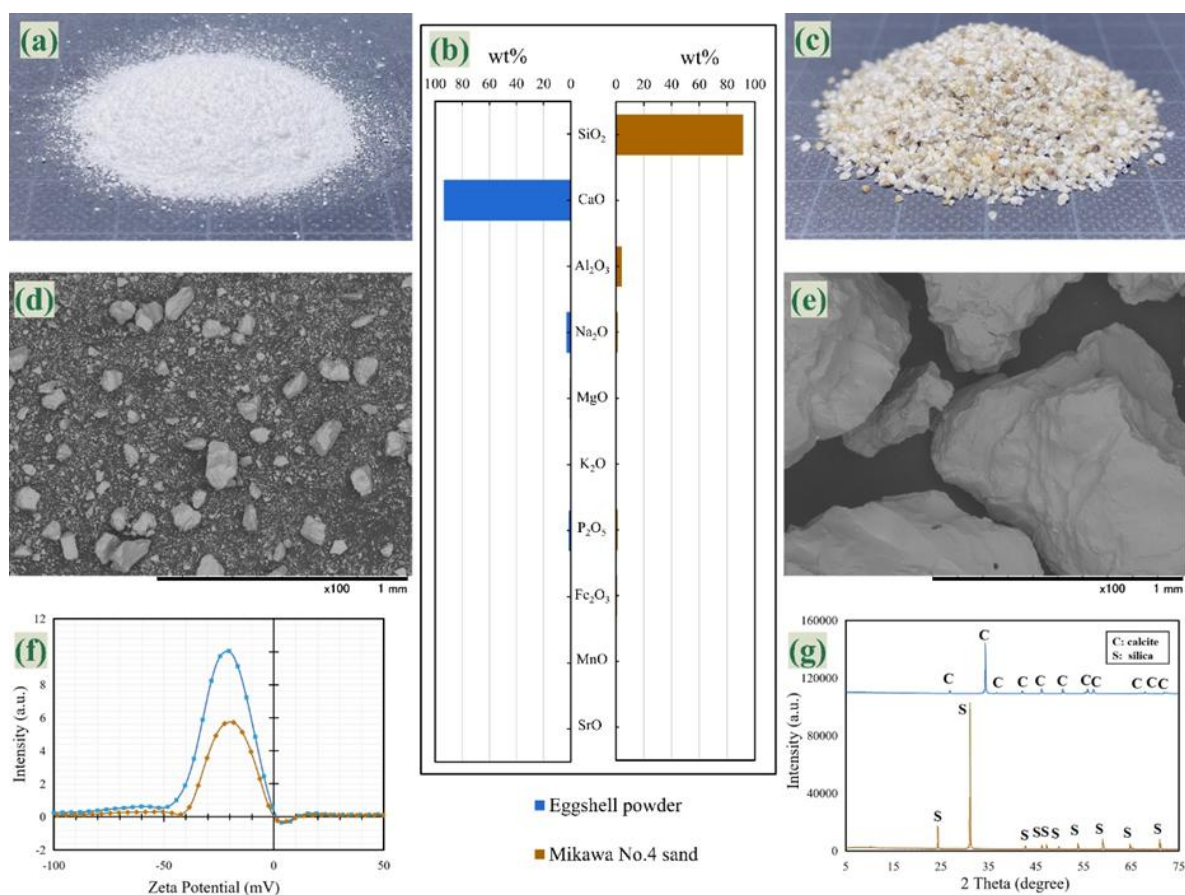


Figure 3.2 Characterization of eggshell Powder and Mikawa No.4 sand: (a) and (c) represent the appearance of eggshell powder and Mikawa No.4 sand, respectively. (d) and (e) depict the SEM images of eggshell powder and Mikawa No.4 sand, while (b), (f), and (g) correspond to their XRF,

zeta potential, and XRD, respectively

3.2.2 Extraction of crude enzyme and evaluation of enzyme activity

Compared to the utilization of expensive purified commercial urease, the extraction of crude urease from soybeans (*Glycine max*) through a simple procedure exhibits notable cost advantages and practical potential^{54–56}. The steps involved in extracting crude soybean urease in this experiment are as follows: Commercially acquired dried soybean was pulverized and sieved through a 150 µm mesh. Subsequently, the soybean powder was added to distilled water, stirred for 10 minutes, and then left to stand at 4 °C for 24 hours, ensuring optimal enzyme extraction. After centrifugation at 10,000 rpm for 10 minutes, the supernatant was collected without further purification to obtain crude soybean urease.

Under temperature-controlled conditions at 25 °C in an AS ONE Thermal Robo TR-1α water bath, this study conducted enzyme activity tests on crude soybean urease at different pH levels, using a dosage of 50 g/L. The aim was to investigate the impact of pH on the activity of crude soybean urease. Furthermore, at pH 7, various concentrations of soybean powder (10 g/L, 30 g/L, 50 g/L, 70 g/L, and 90 g/L) were employed to examine the influence of soybean powder concentration on the activity of crude soybean enzyme.

The crude enzyme activity is determined by measuring the amount of ammonia/ammonium produced from the hydrolysis of urea by soybean crude enzyme after 10 min reaction time. The ammonia content is measured using the indophenol spectrophotometric method based on the

Berthelot reaction. The ammonia/ammonium resulting from urea hydrolysis react with hypochlorite to form monochloramine, which then reacts with phenol in an alkaline medium to produce an indophenol blue dye⁵⁷⁻⁵⁹. The intensity of the indophenol dye is measured at 630 nanometers using the Jasco V-730 UV-Visible spectrophotometer. A calibration curve is established using different concentrations of ammonium chloride and their corresponding intensities to determine the concentration of ammonia/ammonium to obtain the crude enzyme activity.

3.2.3 Synergistic precipitation test

In pursuit of a deeper comprehension of the influence of eggshell powder on soybean crude enzyme-induced carbonate precipitation, a synergistic experiment was conducted. As depicted in Table 1, 1 milliliter of soybean crude enzyme was added to a mixture of 20 milliliters of 0.5 M calcium ions and urea, mimicking the liquid-phase conditions during the solidification test. Eggshell powder was introduced in experiments EP1 to EP4, while sand powder was added in experiments SP1 to SP4. In further exploration of the impact of eggshell powder on soybean crude enzyme, the urea solution was adjusted to 20 milliliters while keeping other conditions constant, with the addition of eggshell powder in experiments EC1 to EC4 and sand powder in experiments SC1 to SC4. All test tubes used were sealed to prevent ammonia gas escape, which could result in a reduction in ammonia/ammonium concentration. The test tubes were stirred at a rate of 160 revolutions per minute for 24 hours at 25 °C, after which the concentrations of ammonia/ammonium and calcium ions in the solutions were measured.

Table 3.1 Synergistic precipitation test formulations

Synergistic precipitation case No.	Formulation		
	Crude enzyme	Added solution	Added Eggshell/Sand powder
C1	1 mL	20 mL CaCl ₂ + urea solution	0 g
EP1/SP1	1 mL	20 mL CaCl ₂ + urea solution	1.4 g
EP2/SP2	1 mL	20 mL CaCl ₂ + urea solution	2.8 g
EP3/SP3	1 mL	20 mL CaCl ₂ + urea solution	4.2g
EP4/SP4	1 mL	20 mL CaCl ₂ + urea solution	5.6 g
C2	1 mL	20 mL urea solution	0 g
EC1/SC1	1 mL	20 mL urea solution	1.4 g
EC2/SC2	1 mL	20 mL urea solution	2.8 g
EC3/SC3	1 mL	20 mL urea solution	4.2g
EC4/SC4	1 mL	20 mL urea solution	5.6 g

3.2.4 Laboratory scale solidification test

The present experiment employed a one-phase processing approach due to its fewer injections⁶⁰, making it more practical for widespread application. The experiment was conducted following the treatment methods outlined in Table 1. Each experimental trial consisted of five sets of samples,

with each set comprising three parallel specimens. The coagulation solution was prepared by mixing 20 mL of 0.5 M calcium chloride and urea solution with 1 mL of crude enzyme. Given a comprehensive consideration of urease activity and cost-effectiveness, the present study employs a concentration of 50 g/L for the crude enzyme.

The specimens were prepared by mixing 70 grams of sand with the amount of eggshell powder as described in Table 2. After thorough mixing, evenly distribute the mixture into syringes with a diameter of 3 centimeters, which will serve as sample molds. Layer the mixture in three increments, lightly tapping ten times after each layer to ensure a tight and consistent fill. The samples underwent a total of 14 treatment cycles, where the specific processing method involved the direct injection of the coagulation solution into the top of the column. The solution permeated through the sand by gravity, and the liquid level was carefully adjusted to be 2 millimeters above the sample surface to ensure thorough impregnation. After a 24-hour period, the effluent solution was drained, and the solution collected from the bottom outlet of the sample was analyzed using the Horiba LAQUAtwin Ca-11 compact Calcium ion meter to measure calcium ion levels and the Horiba LAQUA F-71 benchtop pH meter to determine pH values.

Upon completion of the treatment process, the samples were thoroughly rinsed with distilled water to eliminate any remaining unreacted chemicals. The samples were then placed in a drying oven at 60 °C for 72 hours to ensure complete drying. Subsequently, before proceeding to the next experimental step, the molds were carefully cut, and the samples were extracted from the molds.

Table 3.2 Laboratory scale solidification test formulations

Laboratory scale		Formulation	
solidification case number	Crude enzyme	Added solution	Eggshell powder
S1	1 mL	20 mL CaCl ₂ + urea solution	0 g
S2	1 mL	20 mL CaCl ₂ + urea solution	1.4 g
S3	1 mL	20 mL CaCl ₂ + urea solution	2.8 g
S4	1 mL	20 mL CaCl ₂ + urea solution	4.2g
S5	1 mL	20 mL CaCl ₂ + urea solution	5.6 g

3.2.5 Mineralogical and microstructural inspection

UCS is an important indicator for assessing the consolidation effect of the treatment of biocement curing. In this experiment, the UCS of the treated specimens was measured at three different locations using a Maruto SH-70 Penetrometer. The needle of the instrument was inserted into the bottom, middle, and top positions of the sand specimens, and penetration resistance and depth were recorded according to the needle penetration scale. The UCS of the sample can then be determined from the regression relationship, such as the linear regression provided by the manufacturer.

Utilizing the same instrument as described in Section 2.1 for characterization, the morphology of the samples was examined at the microscale using SEM to assess the bonding effectiveness.

Subsequently, the samples were ground into powder and subjected to XRD to identify the crystalline phases present in the specimens.

3.3 Results and discussion

3.3.1 Crude enzyme activity analysis

Figure 3(a) illustrates the increase in crude enzyme activity with the addition of soybean powder. A similar trend was also observed in a previous study⁶¹. Given the crucial role of enzyme activity in the processing outcome, this implies that in practical engineering applications, the amount of soybean powder can be flexibly adjusted based on actual requirements and cost considerations to regulate the activity of soybean crude enzymes.

Figure 3(b) presents the activity of soybean crude enzymes under different pH conditions. It is evident that soybean crude enzymes exhibit optimal activity under neutral conditions, while excessively acidic or alkaline environments are detrimental to enzyme activity. The observed phenomenon can be attributed to the influence of extreme pH conditions on the protein structure of the enzyme, specifically on the degree of dissociation of crucial functional groups in the active site and the ionization state necessary for proton donors or acceptors within catalytic groups. Additionally, pH values can affect the dissociation of substrates and coenzymes, consequently impacting the enzyme's substrate binding ability and subsequently affecting its activity⁶².

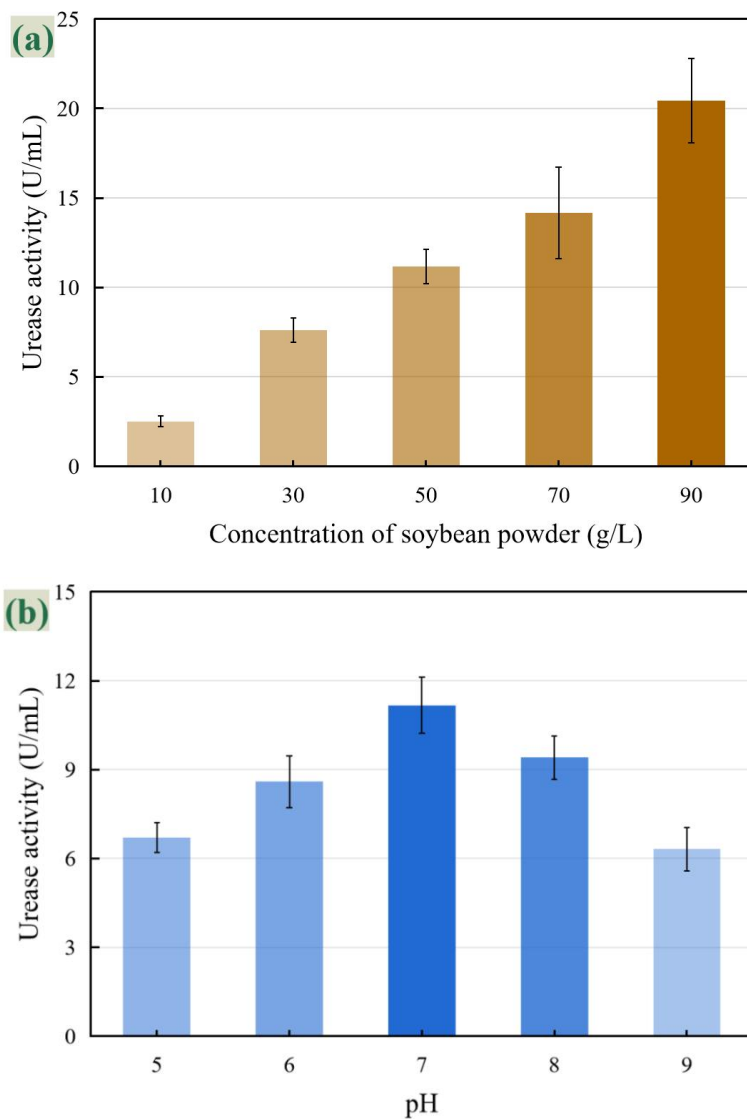


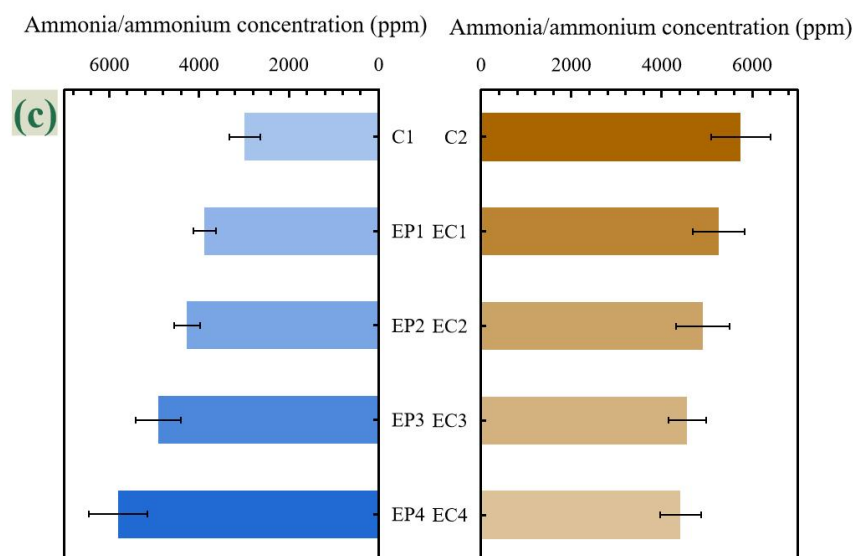
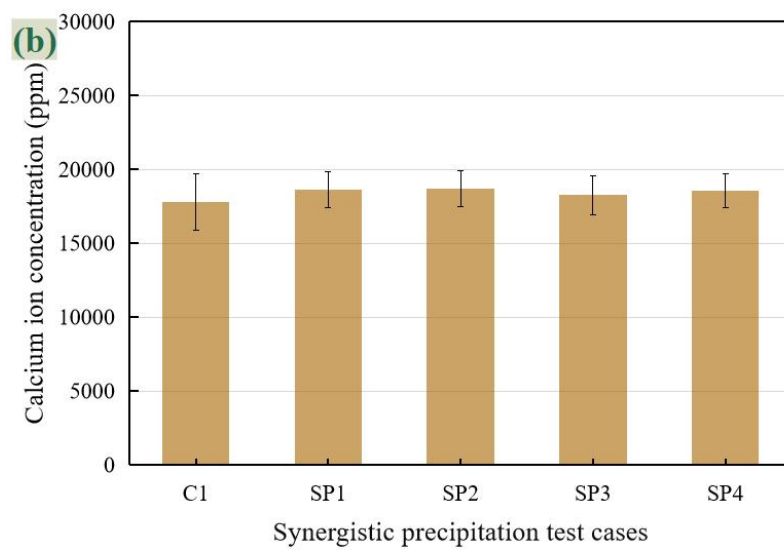
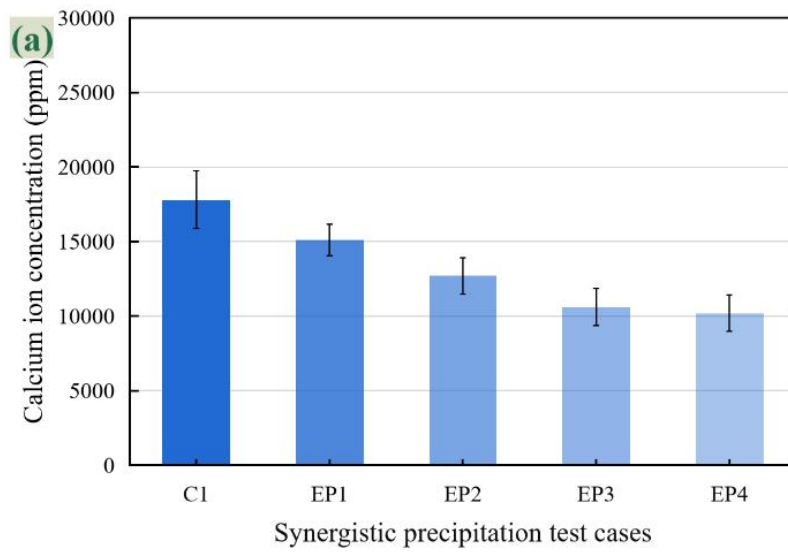
Figure 3.3 Soybean crude enzyme activity: (a) is the activity of soybean crude enzyme at different concentrations; (b) is the activity of soybean crude enzyme at different pH conditions

3.3.2 Synergistic mechanism analysis

Figure 4(a) and Figure 4(b) illustrate the concentration of calcium ions in the synergistic test samples, reflecting the consumption of calcium ions and the precipitation of calcium carbonate in

the liquid phase environment. The consumption of calcium ions increases with the addition of eggshell powder, indicating that eggshell powder facilitates the precipitation of calcium carbonate. Previous studies have demonstrated that eggshell powder exhibits an affinity for calcium ion⁶³, leading to an adsorption effect that facilitates the adhesion of calcium ions to the surface of eggshell powder and consequently promotes the deposition of calcium carbonate on the eggshell powder surface. The high specific surface area of eggshell powder is analogously favorable for its use as a nucleation site for calcium carbonate precipitation. As the amount of eggshell powder provided in the system increases, there will be more heterogeneous nucleation sites available in the system. This results in a higher deposition of calcium carbonate and a greater consumption of calcium ions in the reaction, leading to a lower concentration of calcium ions in the post-reaction solution.

The ammonia/ammonium concentration in the samples of the synergistic precipitation test, as shown in Figure 4(c) and Figure 4(d), serves as a reflection of the extent of urea hydrolysis by the crude enzyme in the liquid phase environment. The observed increase in the ammonia/ammonium concentration in samples EP1 to EP4 could potentially be attributed to the promotion of calcium carbonate precipitation facilitated by the addition of eggshell powder. This significant precipitation of calcium carbonate consumes calcium ions within the system, thereby alleviating the inhibitory effect of calcium ions on urease activity⁶⁴. Therefore, similar increasing trends were observed in ammonia/ammonium concentration and calcium ion consumption.



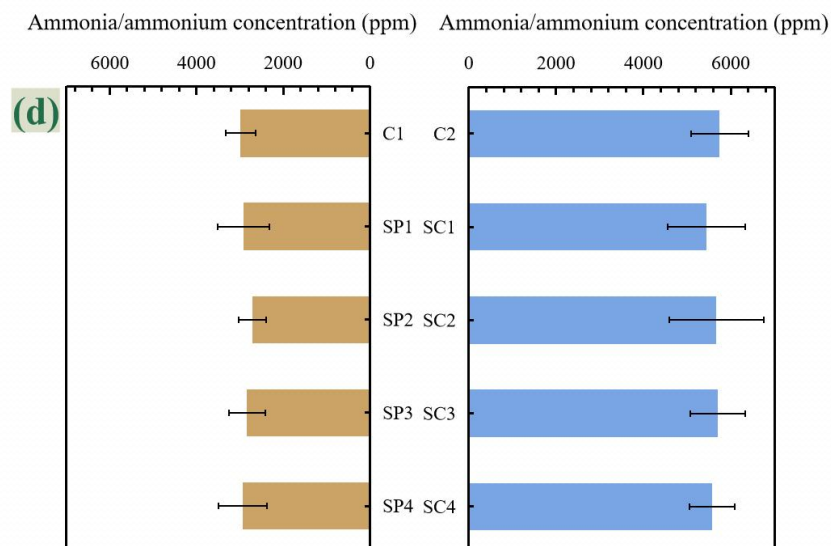


Figure 3.4 (a) and (b) correspond to the calcium ion concentrations for synergistic precipitation experiments with the addition of eggshell powder and sand powder, respectively; (c) and (d) correspond to the ammonia/ammonium concentrations for the synergistic precipitation test with the addition of eggshell powder and sand powder, respectively

In contrast, samples EC1 to EC4 show only a slight decrease in ammonia/ammonium concentration. This phenomenon can be attributed to the release of calcium ions due to the addition of eggshell powder, which moderately inhibits soybean urease activity. Calcium ions have been identified to inhibit soybean urease activity, possibly through the induction of conformational changes in the enzyme's active site, thereby altering its catalytic activity⁶⁵. Urease, responsible for catalyzing the hydrolysis of urea into carbon dioxide and ammonia, harbors a crucial nickel ion within its active site. In the presence of calcium ions, they may compete with nickel ions for binding sites on the enzyme due to their higher affinity. This competition can prevent urea molecules from binding to the active site, ultimately reducing enzymatic activity. Additionally, calcium ions may disrupt the

overall structure and stability of urease, inducing conformational changes in the protein, thereby affecting its folding and diminishing its enzymatic activity.

By conducting a comparative analysis between the ion concentrations following reactions in samples involving the combined addition of calcium chloride and urea, and samples where only urea was introduced, the demonstrable outcome validates the pronounced ability of eggshell powder inclusion to significantly enhance the precipitation of calcium carbonate. This augmentation distinctly contributes to the facilitation of consolidation experiments.

3.3.3 Solidification mechanism analysis

Figure 5(a) portrays the post-cured appearance of soil specimens, while Figure 6(b) delineates the UCS of the samples. Notably, a significant augmentation in strength is discernible in samples fortified with eggshell powder, as compared to their eggshell-absent counterparts. The XRD analysis of the specimens, expounded upon in Figure 6(c), reveals the absence of other calcium carbonate crystalline forms besides the calcite peak. Integrating the analysis of Figure 7 along with the SEM imagery and mechanistic delineation, the augmentative increase in strength is discernibly attributed to the refined spatial distribution of calcium carbonate facilitated by the inclusion of eggshell powder. This observation could be attributed to the propensity of calcium carbonate, post eggshell powder introduction, to nucleate and precipitate at sites, diminishing the influence of the coating-mode calcium carbonate on strength enhancement while notably bolstering the strength-inducing bridging-mode calcium carbonate⁶⁶. Within samples devoid of eggshell powder, the distribution of calcium carbonate delineates two distinct modes: bridging and coating of sand particles. In contrast, specimens supplemented with eggshell powder manifest an absence of calcium

carbonate coating sand particles, resulting in a notably pronounced increase in strength^{67–69}.

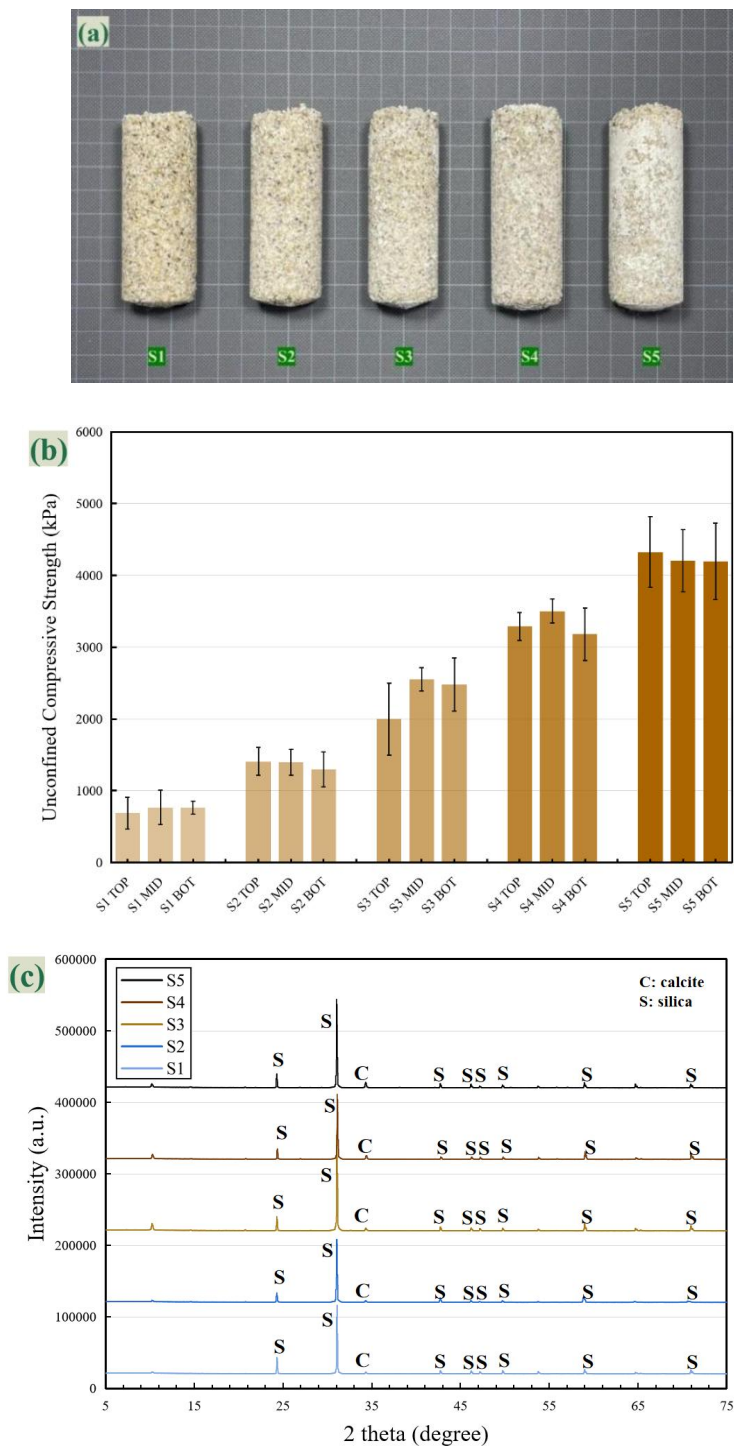


Figure 3.5 (a) is the appearance of the solidified specimen; (b) is the UCS strength of the solidified specimen; (c) is the XRD analysis of the specimen

Previous studies have proposed that in MICP, ureolytic bacteria such as *Sporosarcina pasteurii* serve as nucleation sites for calcium carbonate crystals^{70–72}. This phenomenon occurs because calcium ions are attracted to the bacterial cell surfaces, resulting in the aggregation of calcium ions at these surfaces, followed by their combination with carbonate ions to form crystals. In this experiment, the introduction of powdered eggshell led to a preference for calcium ion adsorption on the eggshell particles over sand grains. This resulted in ion oversaturation near the eggshell particles, which exceeded the solubility product constant, thereby leading to the nucleation and precipitation of calcium carbonate on the eggshell particles⁷³. For a more direct substantiation of the dynamic evolution of amorphous calcium carbonate, in situ characterization is warranted^{74–76}.

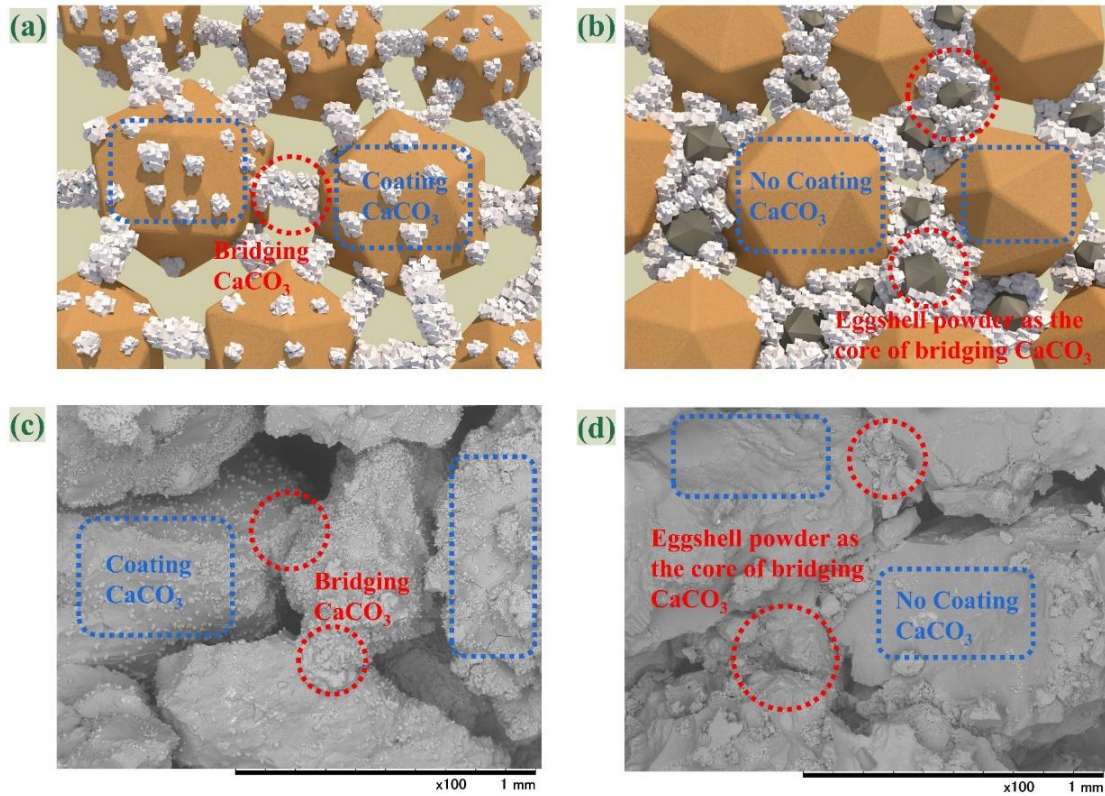


Figure 3.6 (a) and (c) are schematic mechanism and scanning electron microscope images of solidified specimen S1; (b) and (d) are schematic mechanism and scanning electron microscope images of solidified specimen S5

Simultaneously, in conjunction with the depiction of calcium ion concentration in drainage during the process elucidated in Figure 7, an augmentation in eggshell powder dosage precipitates a concomitant diminution in calcium ion concentration, signifying an augmented overall deposition of calcium carbonate within the sand-cementitious fluid system and concomitantly bolstering strength. Furthermore, the incorporation of eggshell powder ameliorates the particle size distribution of the specimens, effectuating interstitial sand particle filling, thereby synergistically amplifying the UCS strength of the samples⁷⁷.

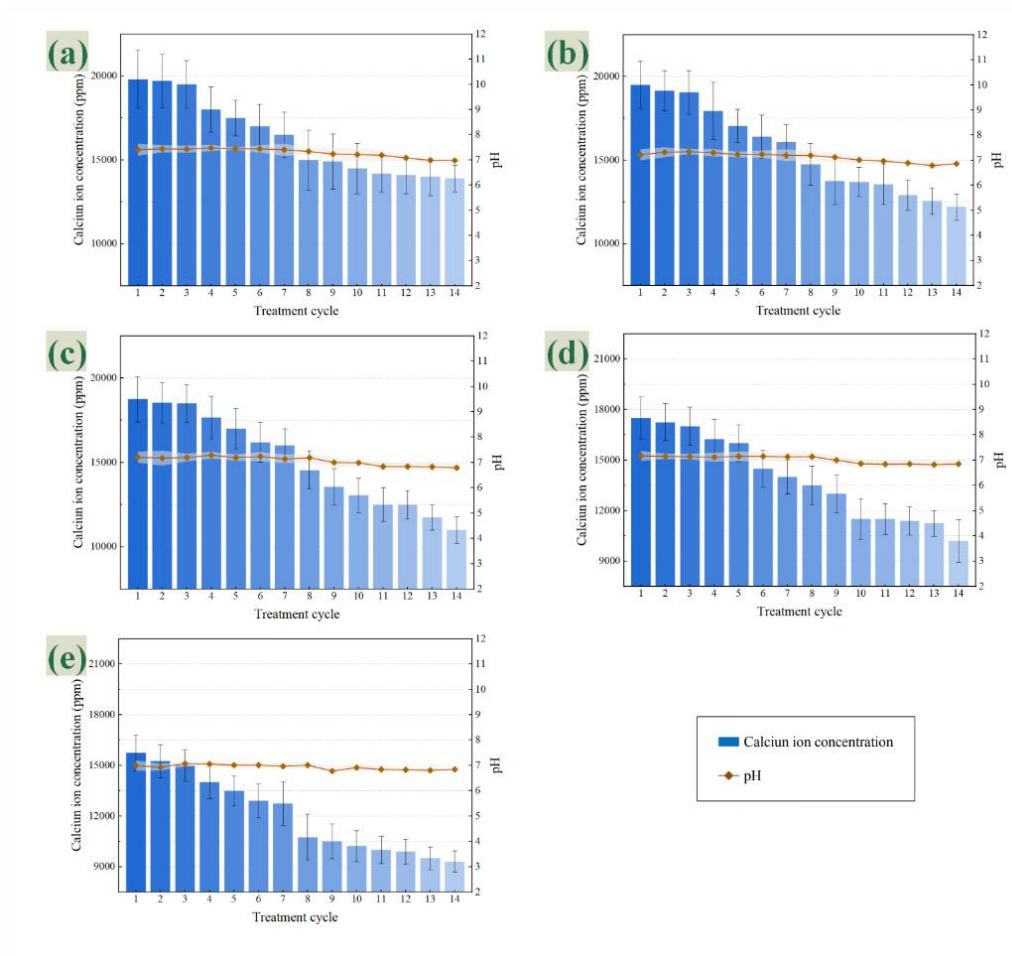


Figure 3.7 Diagrams (a), (b), (c), (d), and (e) respectively depict the conceptual representation of calcium ion concentrations and pH in the drainage during the solidification processes of specimens S1, S2, S3, S4, and S5

As depicted in Figure 7, the monitoring of wastewater pH values during this process reveals that the pH levels across all groups tend to approach neutrality. This may be attributed to the relatively low enzyme dosage employed in this experiment⁷⁸⁻⁸⁰, with the abundant proteins present in crude soybean enzymes potentially exerting a buffering effect⁸¹. Additionally, the supplementary addition of eggshell powder may enhance the predisposition for calcium carbonate precipitation by providing nucleation sites. Consequently, the ensuing consumption of carbonate ions promotes the rightward shift of Equation (4), resulting in the generation of hydrogen ions and the facilitation of solution self-acidification⁸². Within this neutral environment, ammonia transitions into its ionized form, thereby curbing uncontrolled ammonia emissions. Furthermore, the ammonium ions present in the wastewater are more amenable to capture and subsequent retrieval.

3.3.4 Environmental impact analysis

The widespread implementation of MICP and EICP encounters certain challenges due to the emission of ammonia resulting from urea hydrolysis⁸³⁻⁸⁵, as indicated in Equation (2). The release of ammonia engenders a myriad of environmental predicaments, including haze pollution, nitrogen deposition, soil acidification, water eutrophication, and diminished biodiversity⁸⁶⁻⁸⁸. A viable approach to address this issue involves the regulation of pH to diminish ammonia emissions through the conversion of ammonia into its ionized state. For instance, in agricultural sector, a prominent source of ammonia emissions, the large-scale acidification of slurry using sulfuric acid has proven effective in mitigating ammonia emissions⁸⁹⁻⁹¹. In the context of biocementation, the utilization of hydrochloric acid can reduce ammonia emissions by 90%⁹², whereas the application of polyacrylic acid can completely eliminate ammonia emissions⁹³. Nonetheless, it is noteworthy that these

methodologies necessitate the addition of supplementary acids to facilitate pH control. Throughout the entirety of the lab-scale solidification tests, this study maintained pH levels near neutrality. This signifies the absence of a significant amount of ammonia emissions during the entire treatment process, as ammonia was converted into the ionic form of ammonium. Furthermore, as depicted in Figure 8, the recovered ionic ammonium can be directly utilized as fertilizer in agricultural production.

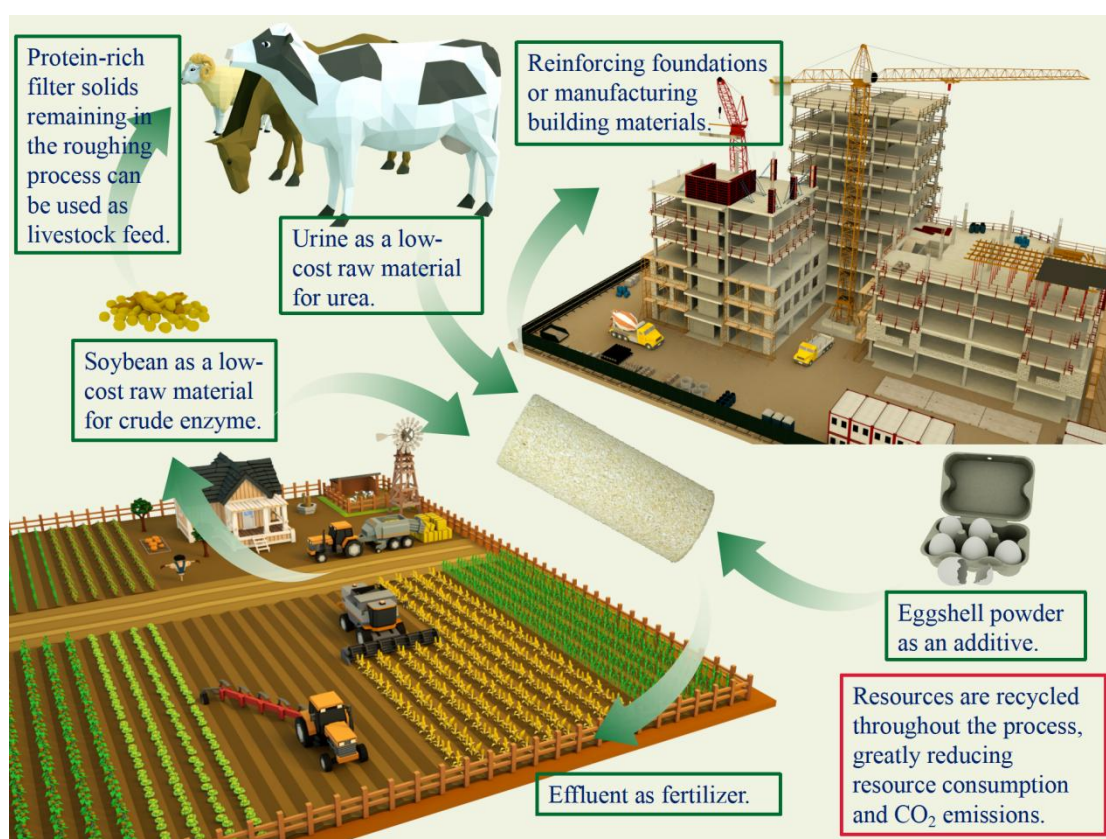


Figure 3.8 Schematic diagram of potential sustainable large-scale application

Moreover, animal urine, such as that of cattle, can serve as a more sustainable source of urea for the production of biocement^{94–96}. This mitigates the environmental impact of animal manure and avoids the energy-intensive and environmentally problematic production of synthetic urea. Additionally, the use of soybean crude enzyme circumvents the resource consumption associated with commercial

pure enzyme production. The solid particles rich in protein that remain during the coarse filtration process can be employed as livestock feed, promoting the recycling of resources⁹⁷.

Simultaneously, the addition of powdered eggshell not only enables the treatment of waste generated by the poultry and food industries but also results in an increase in the strength of the specimens treated via EICP, as evidenced in this study. This offers substantial advantages for reinforcing foundations or manufacturing construction materials. Furthermore, the ability to flexibly select an appropriate reduction in cycle count and treatment duration based on the desired target strength can enhance cost-effectiveness and promote environmental sustainability⁹⁸. Additionally, research has demonstrated the potential application of EICP for heavy metal remediation^{99–101}. Nevertheless, a more extensive inquiry is required to assess the viability of integrating eggshell with EICP for heavy metal remediation.

3.4 Conclusion

This study utilizes biowaste eggshell powder as an additive material in EICP. The high external surface area and affinity of eggshell powder for calcium ions make it a suitable nucleation site for calcium carbonate precipitation. The results of solidification experiments indicate that the incorporation of eggshell powder not only increases the number of nucleation sites, promoting an increase in calcium carbonate precipitation, but also reduces the inhibition of enzyme products and regulates the precipitation pattern of calcium carbonate. This refinement promotes the deposition of calcium carbonate at nucleation sites facilitated by the eggshell powder, thereby fostering bridging among soil particles. The addition of eggshell powder also improves the particle size distribution and fills the voids within the sand grains, greatly enhancing the unconfined compressive strength.

Furthermore, neutral pH is achieved in the reaction system without adding any acid. This successful implementation controls the emission of ammonia. This innovative methodology suggests that using kitchen waste eggshells as additives offers a fresh perspective for increasing strength and effectively eliminating ammonia in the biocement treatment process, which holds vital significance for widespread application.

3.5 References

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CHAPTER 4 Efficient reduction of coarse sand permeability by waste shellfish powder-assisted enzyme-induced carbonate precipitation.

4.1 Introduction

Biocement has garnered significant attention in recent years as a sustainable geotechnical engineering technique¹⁻⁵, owing to its potential to mitigate substantial energy consumption and pollution, particularly arising from the calcination process in conventional cement production⁶⁻¹⁰. It can systematically enhance the geotechnical properties of soil through controlled means to achieve desired responses, such as foundation reinforcement¹¹⁻¹⁵, liquefaction mitigation¹⁶⁻²⁰, slope stabilization²¹⁻²⁵, erosion management²⁶⁻³⁰, and permeability reduction³¹⁻³⁵. The reduction in soil permeability stems from microscale precipitation of calcium carbonate, leading to a decrease in interparticle voids and subsequently impeding water flow³⁶⁻³⁸. This phenomenon elevates hydraulic resistance, thereby macroscopically diminishing the permeability coefficient. Consequently, by orchestrating a reduction in permeability, leakage phenomena in hydraulic engineering can be mitigated, and targeted in-situ plugging of seepage within subterranean structures and natural formations can be achieved. Such techniques find application across various engineering domains, including the closure of highly permeable zones within oil reservoirs to enhance petroleum recovery rates, mitigation of hazardous seepage in landfill sites, and addressing pivotal environmental risks associated with CO₂ geosequestration and nuclear waste disposal projects³⁹⁻⁴³.

However, notwithstanding the potential of biocement to reduce permeability, its effectiveness in permeability reduction primarily depends on the quantity of biomineral precipitation⁴⁴⁻⁴⁶. Consequently, multiple treatments are typically necessary to achieve a substantial degree of precipitation and meet the desired reduction targets in permeability. Moreover, coarse sand exhibits a higher porosity, necessitating a larger amount of calcium carbonate to fill voids, thereby amplifying the challenge of reducing permeability⁴⁷⁻⁴⁹.

The present study investigates a novel approach, as illustrated in Figure 1, which involves grinding kitchen waste shellfish into powder and combining them with coarse enzymes to formulate a cementation solution. During the initial treatment cycle, the shellfish-containing cementation solution is injected into the soil, followed by subsequent treatment cycles using cementation solution devoid of shellfish powder. The inclusion of shellfish powder induces a EICP-generated calcium carbonate, which coats the surface of sand grains, into a calcium carbonate core filling the interstices of sand grains with shellfish powder as the nucleus. This transformation significantly increases flow resistance, consequently reducing soil permeability in small-scale sand column models. The study delves into the mechanisms and potential advantages of shellfish powder-assisted coarse enzyme-induced calcium carbonate in controlling the permeability of coarse sand, aiming to provide a novel and efficient bio-cement alternative for sustainable engineering and construction practices.

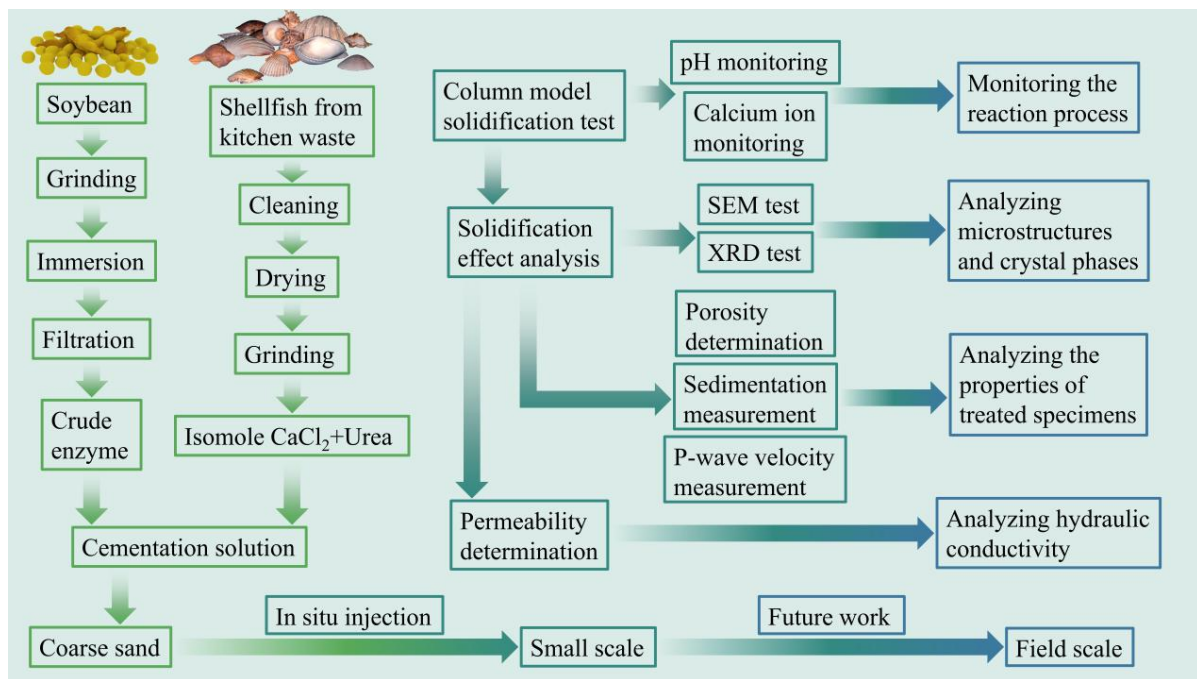


Figure 4.1 Flow chart of the experiment

4.2 Materials and methods

4.2.1 Used shellfish powder

The present investigation utilized abalone (*Haliotis*) shellfish, as depicted in Figure 2(a), sourced from kitchen waste. These shellfish underwent meticulous cleansing procedures to eliminate any extraneous contaminants. Subsequently, they were dried in an oven at 60 °C for 48 hours to remove residual moisture. The shellfish were ground into powder using a grinder and sieved to obtain particles with a diameter less than 50 μm for further experimentation. Scanning electron microscopy (SEM) images in Figure 2(b), obtained using a Hitachi TM3000 Miniscope, revealed the angular morphology of the shellfish powder. Zeta potential measurements, depicted in Figure 2(c), were conducted using the Otsuka Electronics ELSZ-1000 Zeta potential analyzer, indicating 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 constituent. Energy-dispersive X-ray spectroscopy (EDS) analysis of the shellfish powder was conducted using the JEOL JSM-IT200 scanning electron microscope, as illustrated in Figure 2(e), corroborating the significant calcium content in the shellfish powder, consistent with prior research findings^{50–54}.

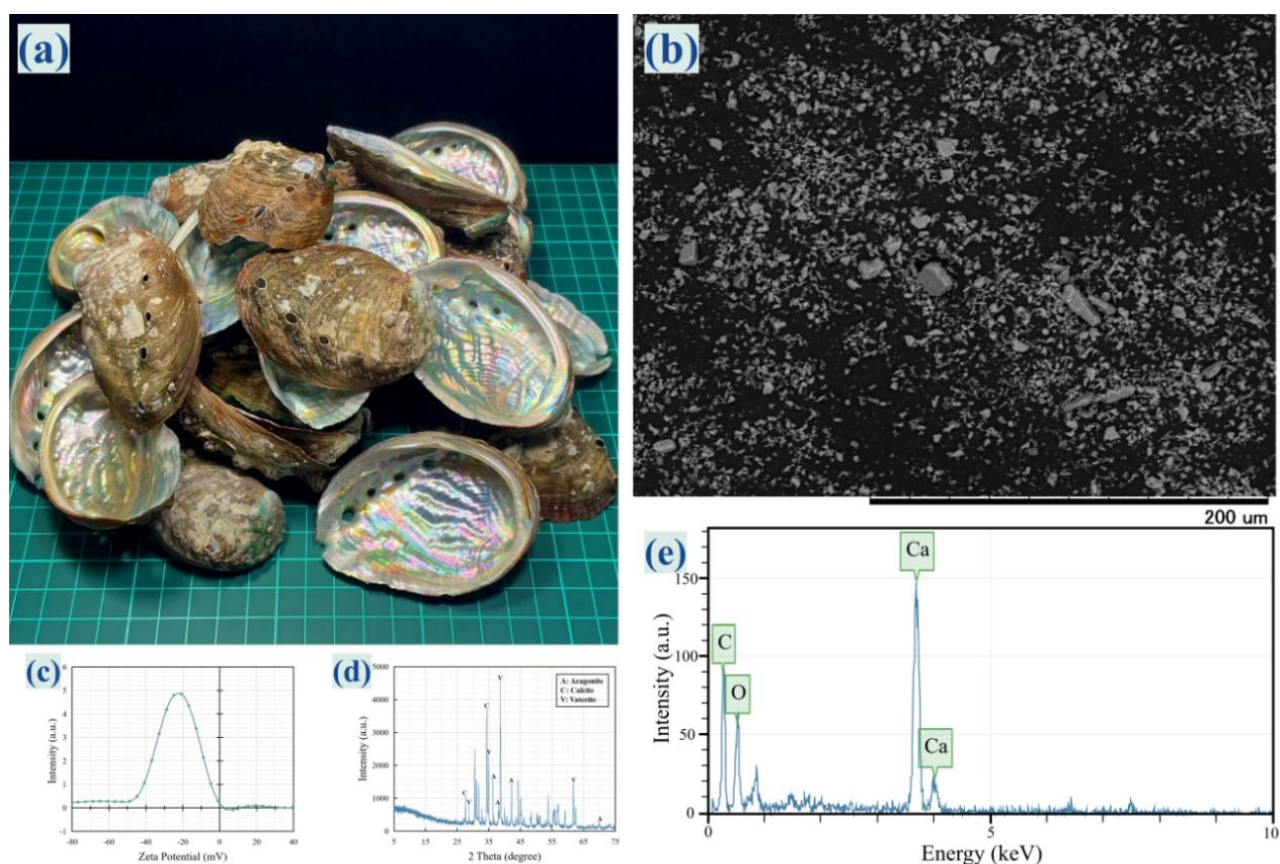


Figure 4.2 (a) depicts the appearance of waste shellfish used, (b) depicts the SEM image of shellfish powder, while (c), (d), and (e) correspond to their zeta potential, XRD, and EDS, respectively

4.2.2 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.

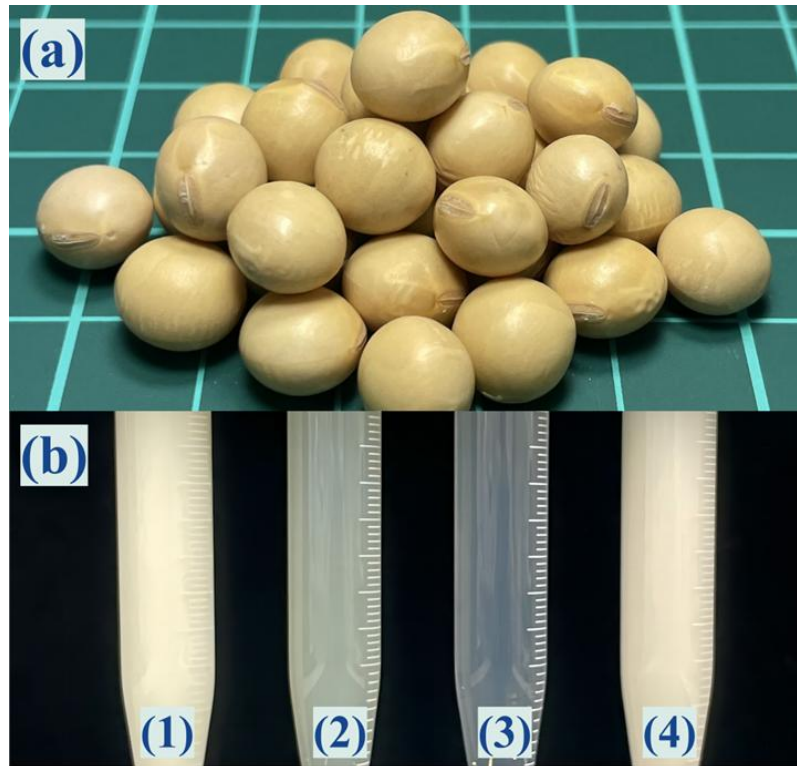


Figure 4.3 (a) illustrates the appearance of the soybeans used, while in (b), b(1), b(2),b(3), and b(4) respectively correspond to unfiltered soybean crude enzyme, filtered soybean crude enzyme, cementation solution, and cementation solution with 50 g/L shellfish powder added

4.2.3 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. XRD analysis of the Mikawa No. 4 silica, as shown in Figure 4(e), reveals that its primary component is silica dioxide, a conclusion further supported by EDS analysis shown in Figure 4(f).

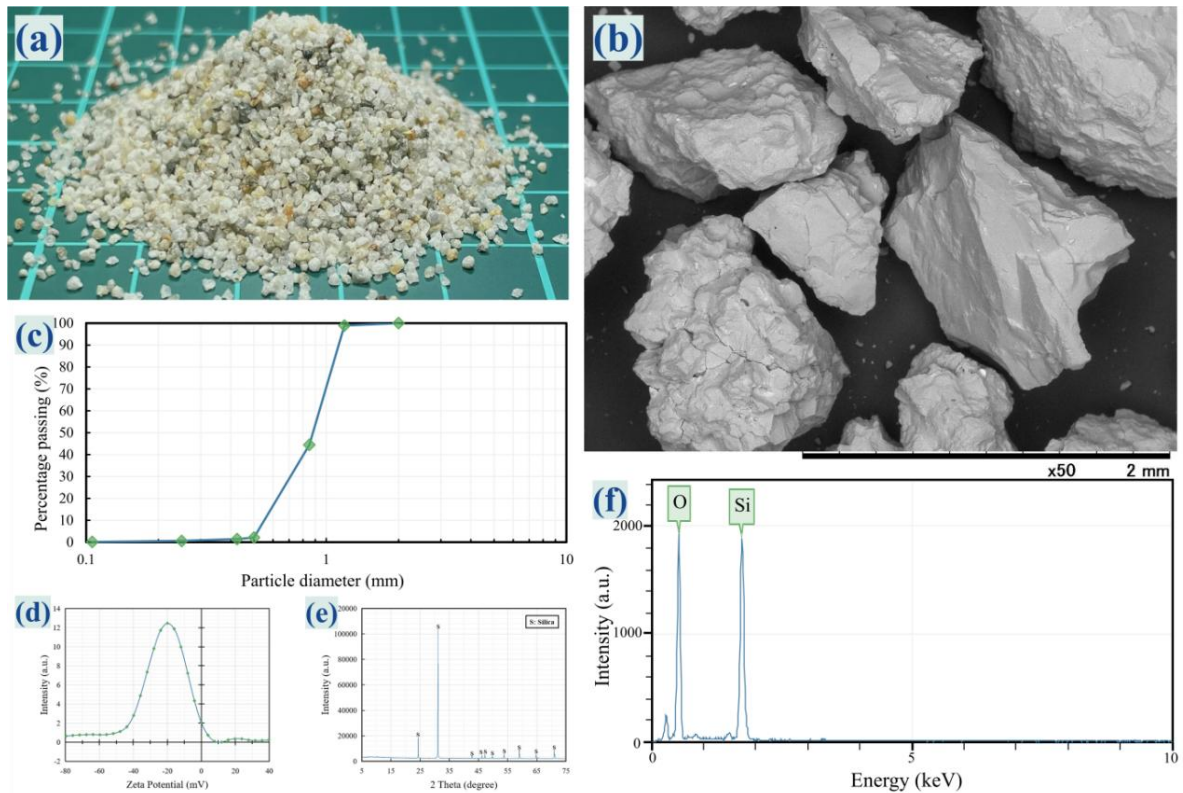


Figure 4.4 (a) depicts the appearance of Mikawa No.4 silica sand used, (b) depicts the SEM image of sand, while (c), (d), (e) and (f) correspond to their particle size distribution, zeta potential, XRD, and EDS, respectively

4.2.4 Column model solidification test

The experimental approach employed herein is a monophasic treatment method, chosen due to its minimal procedural steps, thereby facilitating future scalable deployment. Each experimental iteration 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 cylindrical membranes fabricated via 3D printing. Individual samples were filled with 30 grams of sand, ensuring uniform and compact packing, with the initial weight recorded as $M_{\text{untreated}}$ prior to treatment. The specific consolidation procedure entailed direct infusion of a volume of consolidating solution equivalent to the pore volume of the sand column into the top of the sand 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, marking the completion of one treatment cycle. Waste solutions from each treatment cycle were monitored for calcium ion content using the Horiba LAQUAtwin Ca-11 compact calcium ion analyzer, 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 quantities of shellfish powder ranging from 0 to 0.5 grams added, followed by subsequent treatment cycles employing consolidation solutions devoid of shellfish powder. Upon completion of the treatment regimen, samples were thoroughly rinsed with distilled water equivalent 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 sand soil was measured using Darcy's law. Each sample underwent five permeability experiments, and the averages were 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, along with the molds, was measured as M_{treated} , and the difference in weight compared to $M_{\text{untreated}}$ yielded the total mass of the biocement. Subsequently, the volume of the biocement was calculated based on its total mass, and the sand porosity after treatment was computed based on the sample volume and its pore volume.

Carefully, the molds were then cut open to ensure the integrity of the shape, and the samples were extracted from the molds. Subsequently, the Oyo Sonicviewer-SX was used to clamp the samples between the apparatus. One end of the oscillator generated ultrasonic waves at 200 kHz, while the other end, through the receiver, measured the time taken for the waves to pass through the sample, thus computing the V_p for the respective treated samples. However, before testing, both ends of the samples were polished with sandpaper to ensure snug alignment with the oscillator and receiver.

Microscopic and crystallographic characterization of the samples was conducted using the same instrumentation described in Section 2.1. SEM was employed to examine the morphology of the samples at the microscopic scale to observe their microstructure. Subsequently, the samples were ground into powder and subjected to XRD analysis to determine the crystalline phases present in the samples.

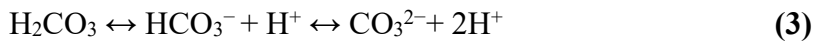
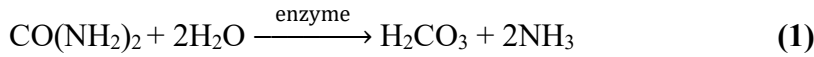
Table 4.1 Laboratory scale solidification test formulations

Shellfish concentration in first treatment cycle (g/L)	Sample name	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	untreated
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	untreated
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	untreated
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	untreated
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	untreated
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	untreated

4.3 Results and discussion

4.3.1 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 system⁵⁵. Additionally, the soybean crude enzyme additive, rich in proteins, likely contributes to buffering effects⁵⁶. 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.

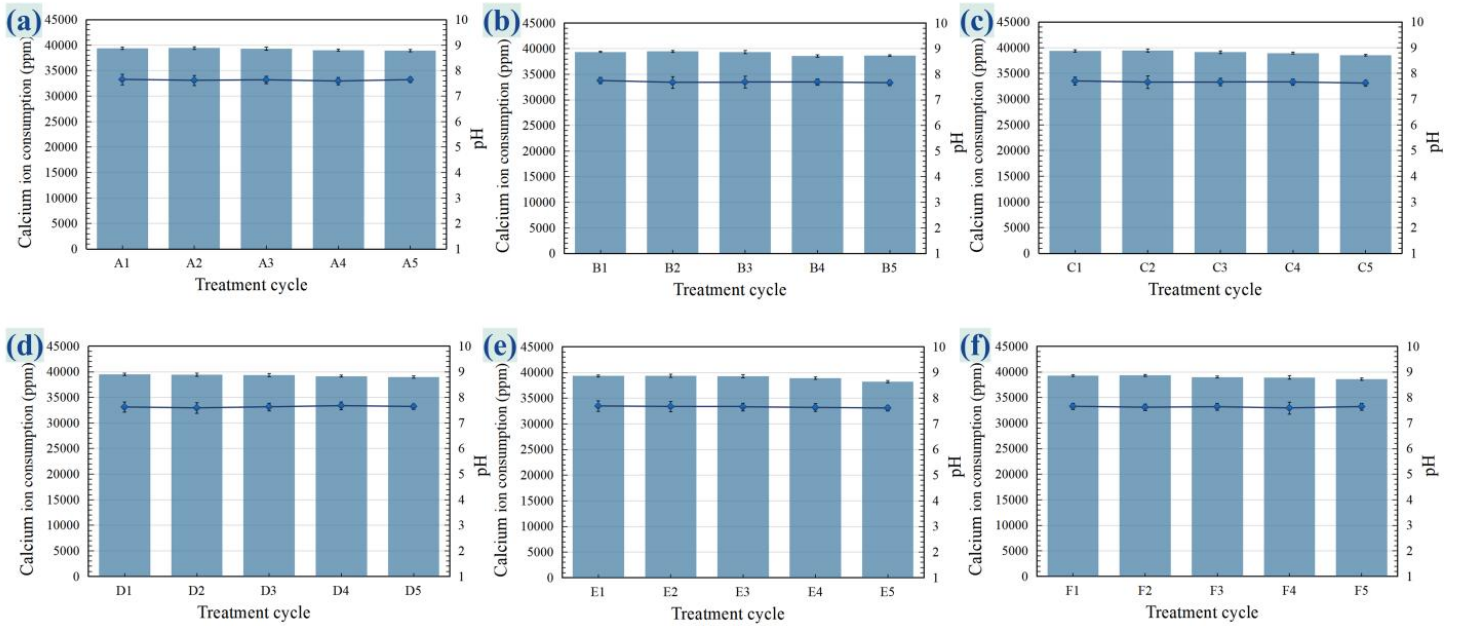


Figure 4.5 (a) to (f) represent from Group A to Group F, where the bar chart illustrates calcium ion consumption, and the line graph depicts pH. Error bars represent the standard deviation after five independent experiments

4.3.2 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 their tendency to coalesce, impeding their uniform dispersion. Thus, the homogeneity of precipitation distribution is compromised.

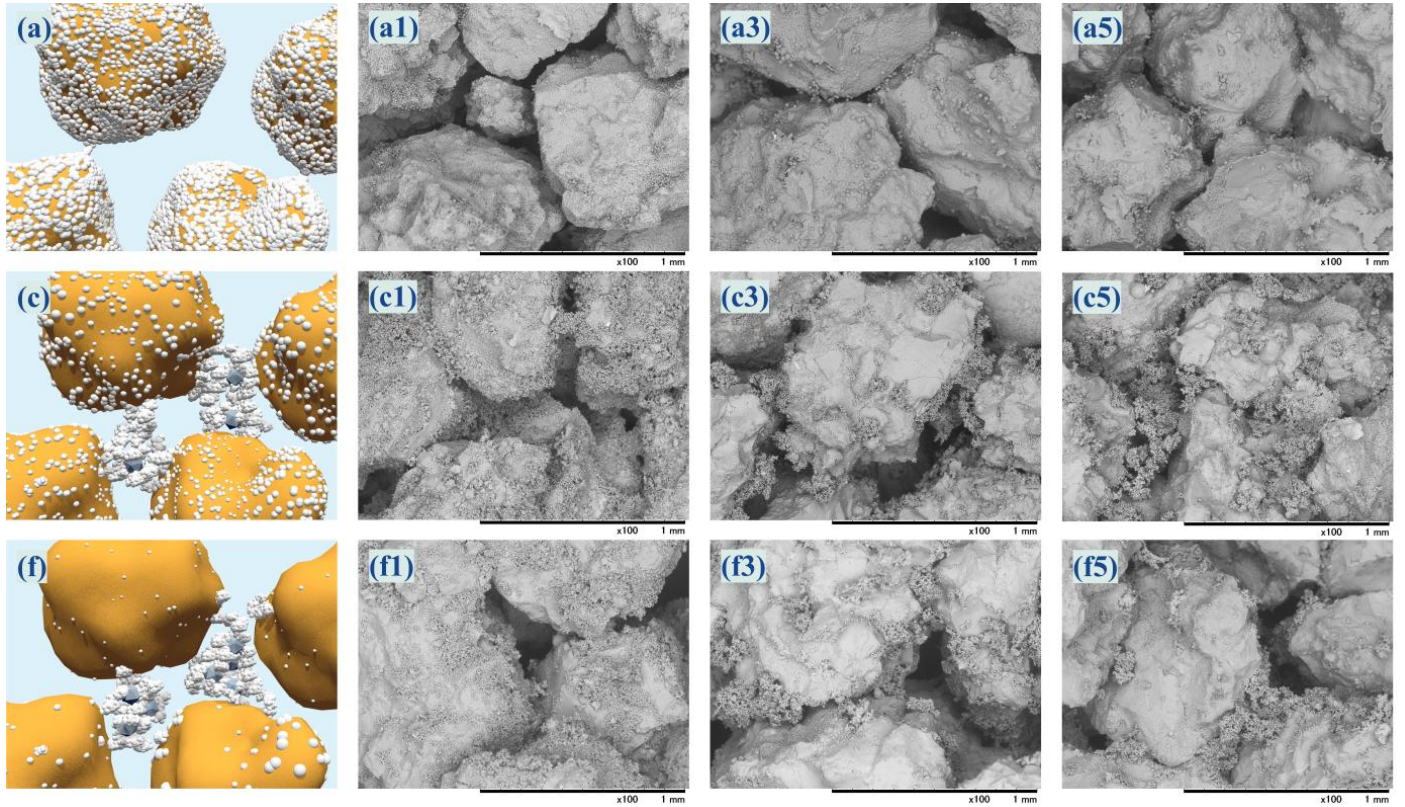


Figure 4.6 (a), (c), and (f) respectively depict schematic diagrams of samples from Groups A, C, and F, followed by SEM images of the corresponding samples in each group

The nucleation effect of shellfish powder surpasses that of sand, which may be the reason for the alteration in calcium carbonate precipitation modes upon shellfish powder addition. Previous studies indicate that bacteria involved in urea decomposition can serve as nucleation sites for calcium carbonate crystals during Microbially Induced Calcium Carbonate Precipitation⁵⁷⁻⁶¹. This phenomenon arises from the attraction of free calcium ions to the negatively charged surfaces of bacteria, leading to their accumulation on bacterial surfaces, resulting in surface ion supersaturation, exceeding the solubility product constant, and subsequent carbonate ion crystallization. Supersaturation is a primary contributor to chemical potential at a given temperature and strongly influences nucleation behavior and subsequent crystal growth. A high supersaturation index represents a large chemical potential or high thermodynamic driving force, favoring rapid crystal growth⁶². In this study, the introduction of negatively charged shellfish powder leads to the adsorption of calcium ions on shellfish powder particles. Moreover, shellfish powder contains a significant amount of calcium carbonate, and partially ionized calcium ions may cause ion supersaturation near the shellfish powder, surpassing the solubility product constant and homogeneously nucleating precipitation. Additionally, calcium carbonate in shellfish powder may also serve as seed crystals, reducing the energy required for secondary nucleation, thus facilitating calcium carbonate precipitation on its surface.

Therefore, in the absence of shellfish powder addition, 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^{63–67}, 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 reveals the crystalline phases present in the samples to be silicon dioxide, vaterite and aragonite. Silicon dioxide is the main component of silica sand, while vaterite and aragonite represent crystalline polymorphs in calcium carbonate precipitation. It is notable that the crystalline structures of calcium carbonate among different groups exhibit similarities. However, the specific effects of these diverse crystal types on chemical bonding and ultimate mechanical strength properties remain unclear, warranting further investigation in future studies.

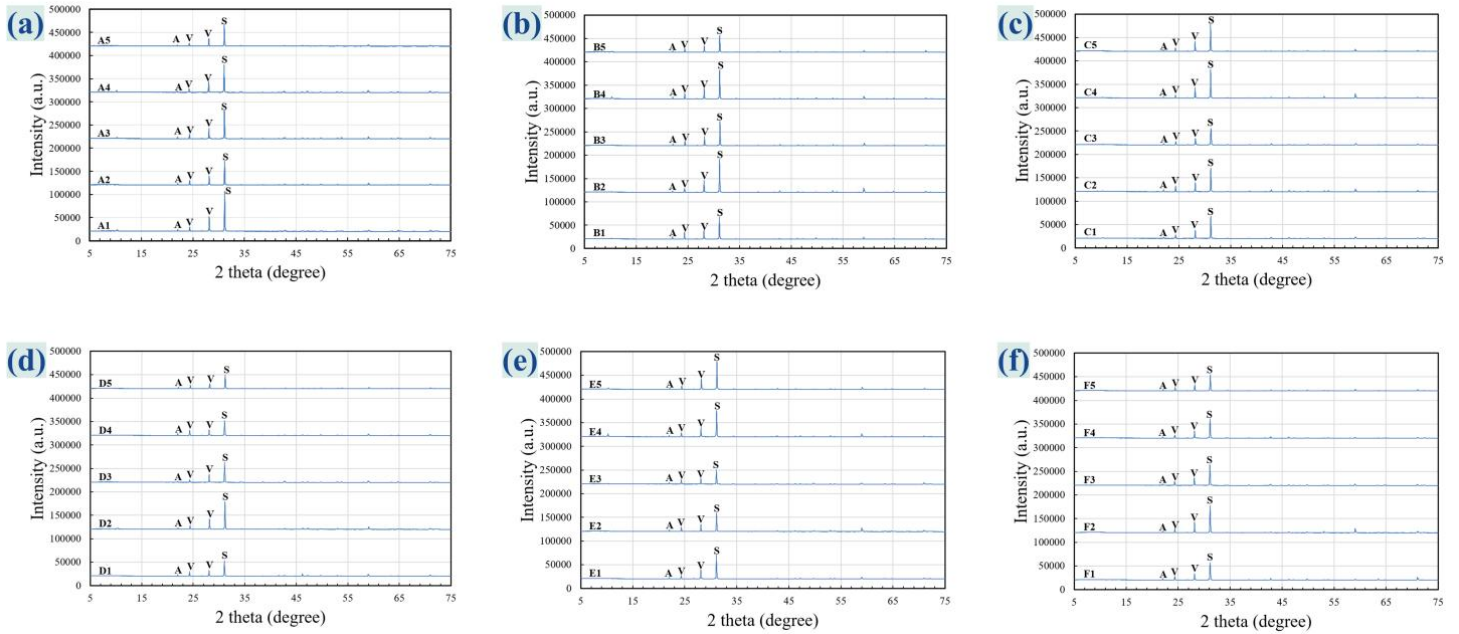


Figure 4.7 (a) to (f) represent XRD from Group A to Group F, where A represents aragonite, V represents vaterite, and S represents silica

4.3.3 Mineralogical and permeability analysis

Figure 8 illustrates the Compression wave velocity (V_p) of the samples post-curing alongside the total amount of precipitates within the samples, both of which exhibit an increase with treatment cycles. Considering Figure 5, wherein high calcium ion consumption rates across different groups imply that the majority of calcium ions are converted into precipitates, additional shellfish powder is only introduced once. Thus, the precipitate amounts and trends among different groups closely resemble each other.

Within the same group, V_p demonstrates an increasing trend with treatment cycles. This increase is attributed to the rise in calcium carbonate content under similar precipitation modes, enhancing the degree of cementation and gradually increasing the bridging sites at sand grain contact points, thereby facilitating better V_p transmission^{68–72}. However, among different groups, V_p decreases gradually with increasing shellfish powder addition. This phenomenon is largely influenced by the mode of calcium carbonate precipitation⁷³. When shellfish powder is absent, calcium carbonate primarily adopts a continuous coating pattern, effectively transmitting longitudinal waves and thus exhibiting the highest velocity. As shellfish powder content increases, calcium carbonate tends to aggregate around the shellfish powder core, diminishing the continuous coating pattern of calcium carbonate, hence resulting in a gradual decrease in V_p .

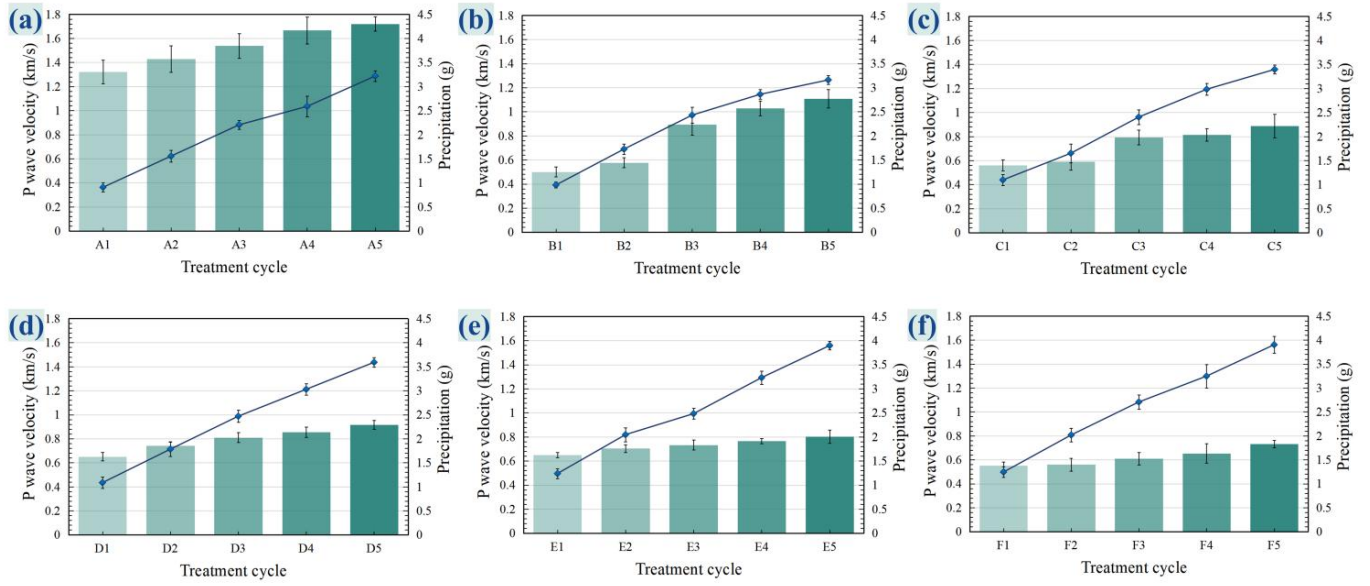


Figure 4.8 (a) to (f) represent Groups A to F, where bar charts depict P-wave velocity and line graphs represent precipitation quantity. Error bars represent the standard deviation after five independent experiments

Figure 9 depicts the dimensionless relative permeability (the ratio of measured permeability to initial permeability) and porosity of the samples. Both permeability and porosity exhibit a decreasing trend with increasing treatment cycles. Porosity is a crucial factor influencing permeability. Hence, to reduce the required treatment cycles, additional bentonite is introduced to decrease permeability by increasing the overall volume fraction of precipitates generated from biomineralization of calcium carbonate and additional bentonite. Within the same group, increasing treatment cycles lead to a reduction in porosity due to the increase in precipitate amount, resulting in a decrease in pore throat and effective pore radius, consequently reducing permeability. However, the rate of permeability decrease differs among different groups. Simple relationships between porosity and permeability cannot

fully explain the observed variations in permeability among different groups during this study. The changes in porosity among different groups are similar, yet with increasing shellfish powder content, the rate of permeability reduction initially increases, with Group C exhibiting the strongest decreasing trend in permeability. As shellfish powder continues to increase, the rate of permeability reduction slows down.

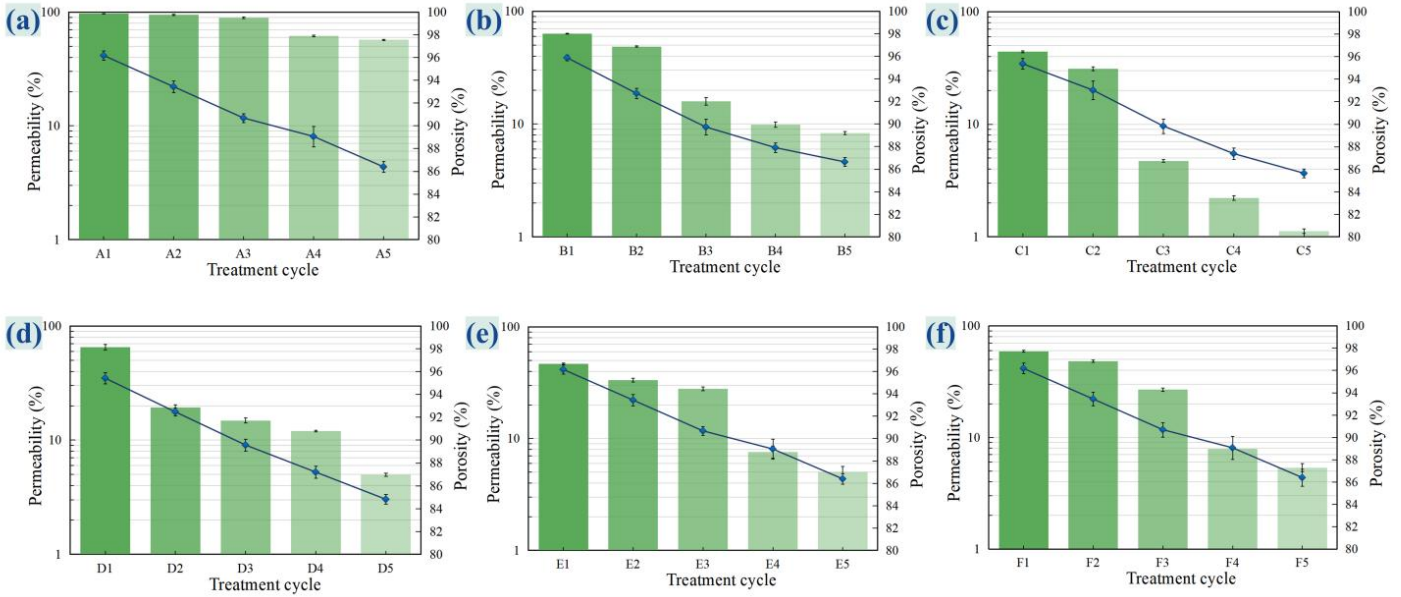


Figure 4.9 (a) to (f) represents Groups A to F, where the bar charts represent permeability, and the line graphs represent porosity. Error bars represent the standard deviation after five independent experiments

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 significant changes in micro-pore morphology. Microstructural changes directly influence macroscopic permeability alterations⁷⁴. 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.

4.3.4 Environmental impact and future application analysis

The expansion of aquaculture has led to an increase in bivalve production, with waste shellfish being a byproduct of this industry. Improper handling of waste shellfish can result in significant pollution issues^{75–79}. Consequently, oysters, scallops, conchs, among others, are currently being explored as potential raw materials for construction purposes^{80–84}. This study investigates the sustainable utilization of abalone shellfish, providing novel avenues for the waste management of shellfish. The addition of shellfish powder not only addresses

waste generated by aquaculture but also significantly reduces the permeability of samples treated with EICP, holding immense potential for in-situ hydraulic barrier formation as shown in Figure 10. In comparison to traditional enzyme-induced carbonate precipitation techniques, the proposed treatment approach in this experiment demonstrates rapid and efficient potential. This technological solution offers rapid deployment and the ability to respond urgently to pollutant migration, forming hydraulic barriers at critical moments to prevent further contamination of groundwater systems, thus minimizing environmental risks and safeguarding water sources. However, current single-phase injections, although facile and easy to scale, are susceptible to blockages at the injection site. Methods such as low temperature and low pH have been employed to mitigate blockages^{85–89}, requiring further investigation for future improvements. Additionally, considerations of environmental friendliness and sustainability are necessary for on-site scaling to ensure the viability and safety of this technology concerning groundwater resources and surrounding environments.

In forthcoming large-scale applications, the deliberation regarding the integration of lower-grade or technical-grade raw materials holds the potential to effectively alleviate costs^{90–94}. Animal urine could serve as a sustainable source of urea for biocement production^{95–99}, alleviating environmental impacts of animal waste and circumventing the energy-intensive and environmental concerns associated with synthetic urea production^{100–104}. Moreover, the utilization of soybean crude enzymes circumvents the resource expenditures entailed by commercial enzyme production, thereby substantially mitigating costs^{105–109}. Additionally, it offers advantages such as heightened biosafety compared to microorganisms^{110–112}. Solid protein-rich particles remaining from coarse

filtration processes can be utilized as livestock feed¹¹³, promoting resource recycling. Furthermore, future endeavors should conduct a comprehensive life cycle assessment of biocement, aiding in the evaluation of economic costs, carbon footprint, and environmental burdens^{114–118}.

Moreover, current research has demonstrated the potential applications of EICP in heavy metal remediation, as well as the significant environmental restoration potential of shellfish waste^{119–123}. Nevertheless, broader investigations are necessary to evaluate the feasibility of combining shellfish powder with EICP for heavy metal remediation. Additionally, as indicated in **Equation (1)**, ammonia emissions resulting from urea hydrolysis pose challenges to widespread implementation. Ammonia release triggers various environmental issues including haze pollution, nitrogen deposition, soil acidification, eutrophication, and biodiversity loss^{124–128}. Viable methods to mitigate this issue include pH regulation to convert ammonia into ionized states, such as the large-scale acidification of slurry with sulfuric acid in agricultural sectors, proven effective in reducing ammonia emissions^{129–133}. In biological solidification, the addition of extra acids similarly reduces ammonia emissions^{134–136}. This study maintained near-neutral pH values during columnar solidification tests, converting ammonia into ammonium ions, thus minimizing ammonia emissions and facilitating planned dissemination. Additionally, unreacted urea and reacted ammonium ions can be utilized as fertilizers for plants. The interaction between biocement and vegetation growth is also a focus of field experiments. Plant roots can interact with biocement to improve soil quality¹³⁷. The hardened surface formed on the soil layer after treatment reduces the rate of water evaporation, favoring plant growth¹³⁸. However, it is

noteworthy that despite the comprehensive consumption of most calcium ions in the treatment solution, implying no excess calcium ion pollution in groundwater and surrounding ecological environments, the residual salts in the soil increase soil osmotic pressure, potentially limiting plant growth¹³⁹. Given the complexity of field experiments, further exploration is required to understand the impact of biocemented soil on plant growth.

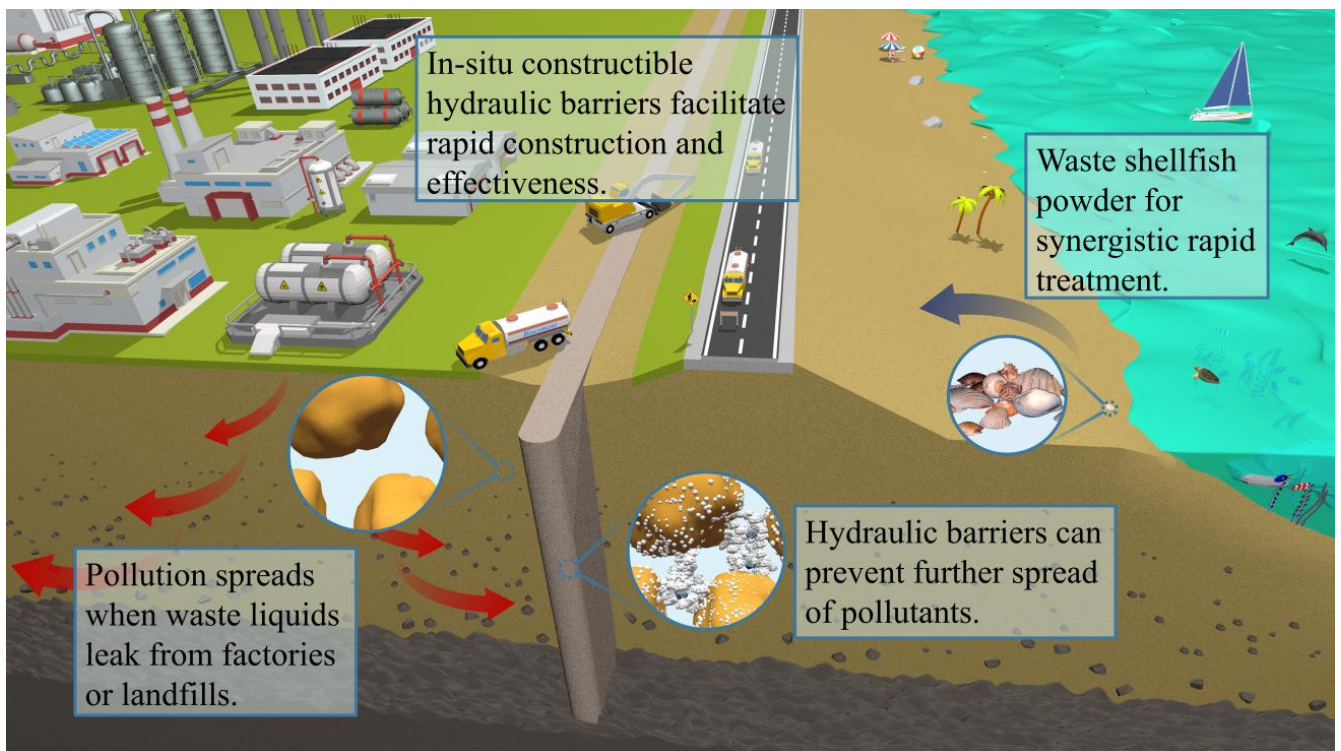


Figure 4.10 The vision of future solutions for in-situ hydraulic barrier construction using biocement to prevent pollutant migration

4.4 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.

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CHAPTER 5 Crystal transformation to enhance biocement from kitchen waste bones

5.1 Introduction

On a global scale, sustainable construction engineering practices are increasingly recognized as vital for combating climate change and achieving environmental objectives. The construction industry is a major contributor to global energy consumption and CO₂ emissions, with traditional portland cement production playing a significant role¹⁻⁵. This has led to a growing impetus to seek more environmentally friendly alternative materials. In recent years, biocement has emerged as an innovative technology. Due to its independence from the high-temperature calcination process typically associated with cement production, biocement offers significant sustainability advantages⁶⁻¹⁰, making it highly applicable in various construction engineering projects¹¹⁻¹⁵.

In practical engineering applications, there are usually specific requirements for treatment strength. In current biocement research, the factors influencing strength improvement often focus on the amount of sediment¹⁶⁻²⁰, which necessitates an extended treatment cycle. However, as the number of treatments increases, the soil's permeability decreases, hindering the penetration of the treatment solution into the soil, leading to a decline in treatment effectiveness over time, thus failing to meet expected outcomes. The inefficient use of solidification solution in the later stages of treatment may result in raw material waste. Additionally, prolonged treatment cycles extend construction time and overall costs. One method to achieve better treatment outcomes without blindly increasing the

number of treatments is through fiber blending^{21–25}. It is important to note that pre-mixing fibers into the soil may cause disturbances to in-situ soils, increase construction steps, and raise costs. Moreover, the combination of biopolymers with biocement offers a new perspective^{26–30}. However, as organic compounds, biopolymers may be degraded by indigenous bacteria in actual engineering applications, and their long-term treatment effects require further investigation.

This study uses temperature and pH to induce different crystalline forms of biocement, resulting in more than a fivefold increase in strength at the same sediment content level for samples with different crystalline structures. Following the procedure illustrated in **Figure 1**, various ratios of sustainable source solidification solutions were applied at different temperatures, and the reactions were monitored during the solidification tests. After completing the solidification tests, the samples were evaluated for strength and settlement, followed by Scanning electron microscopy (SEM) analysis to examine the microstructure, and XRD and EDS analyses to identify phase composition and elemental distribution. This research not only proposes a novel approach to enhancing the strength of biotreated samples but also offers more effective and sustainable solutions for future practical applications. These findings have the potential to advance the application of biocement in construction engineering and promote the development of sustainable building materials.

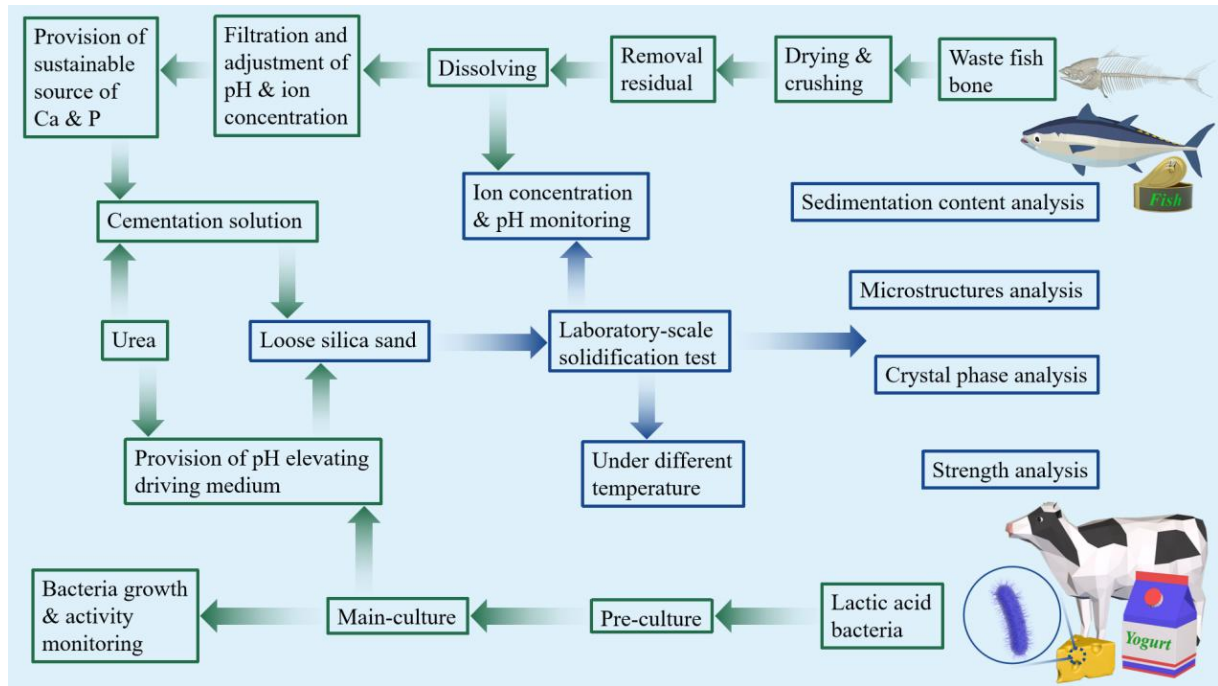


Figure 5.1 The main process of the experiment. The blue arrows represent the solidification test and related test processes, and the green arrows represent the preparation of sustainable kitchen waste bone-derived solidification solution and the cultivation of lactic acid bacteria with urease activity

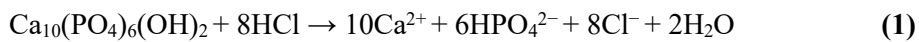
5.2 Materials and methods

5.2.1 Used sand and preparation of solidification solution

The initial characteristics of the soil to be treated, such as grain size and pore size distribution, relative density, and particle surface properties, determine permeability and fluid flow patterns. These characteristics can affect the distribution of bacteria and solidification solutions during the biocementation process, thereby potentially influencing treatment effectiveness. For this study, Toyoura sand, as shown in Figure 2c, was selected due to its physical uniformity, which minimizes the potential complex effects on the biocementation process. Toyoura sand has a soil particle density

of 2.64 g/cm³, a maximum void ratio of 0.973, and a minimum void ratio of 0.609. The uniformity coefficient is 1.30, and the curvature coefficient is 0.96. The sieve analysis depicted in Figure 2b confirms that this sand is poorly graded, with fairly uniform particle sizes and low fine content. The SEM image in Figure 2f reveals that the particle shapes range from angular to sub-angular, and the XRD analysis in Figure 2h indicates that its crystalline phase is primarily quartz.

In this study, kitchen waste fish bones obtained from the local market were used as a green source of calcium and phosphorus for the solidification solution. As shown in Figure 2e, their main component is hydroxyapatite. The preparation process is illustrated in the flowchart in Figure 1. Specifically, the bones were first dried at room temperature and then crushed into fragments smaller than 1 cm for the subsequent experiments. The fragments were then boiled in a 2% NaOH solution for 30 minutes to thoroughly remove attached organic matter such as grease. After filtering, the clean fish bones were dissolved in a 1% HCl solution as Equation (1), stirred at 1000 rpm to extract calcium and phosphorus ions from the bones, as shown in Figure 2g. The solution was then filtered, and its pH and calcium ion concentration were adjusted to approximately pH 3.0 (± 0.1) and 10,000 (± 100) ppm, respectively. Finally, urea was added to the solidification solution according to the different groups listed in Table 1.



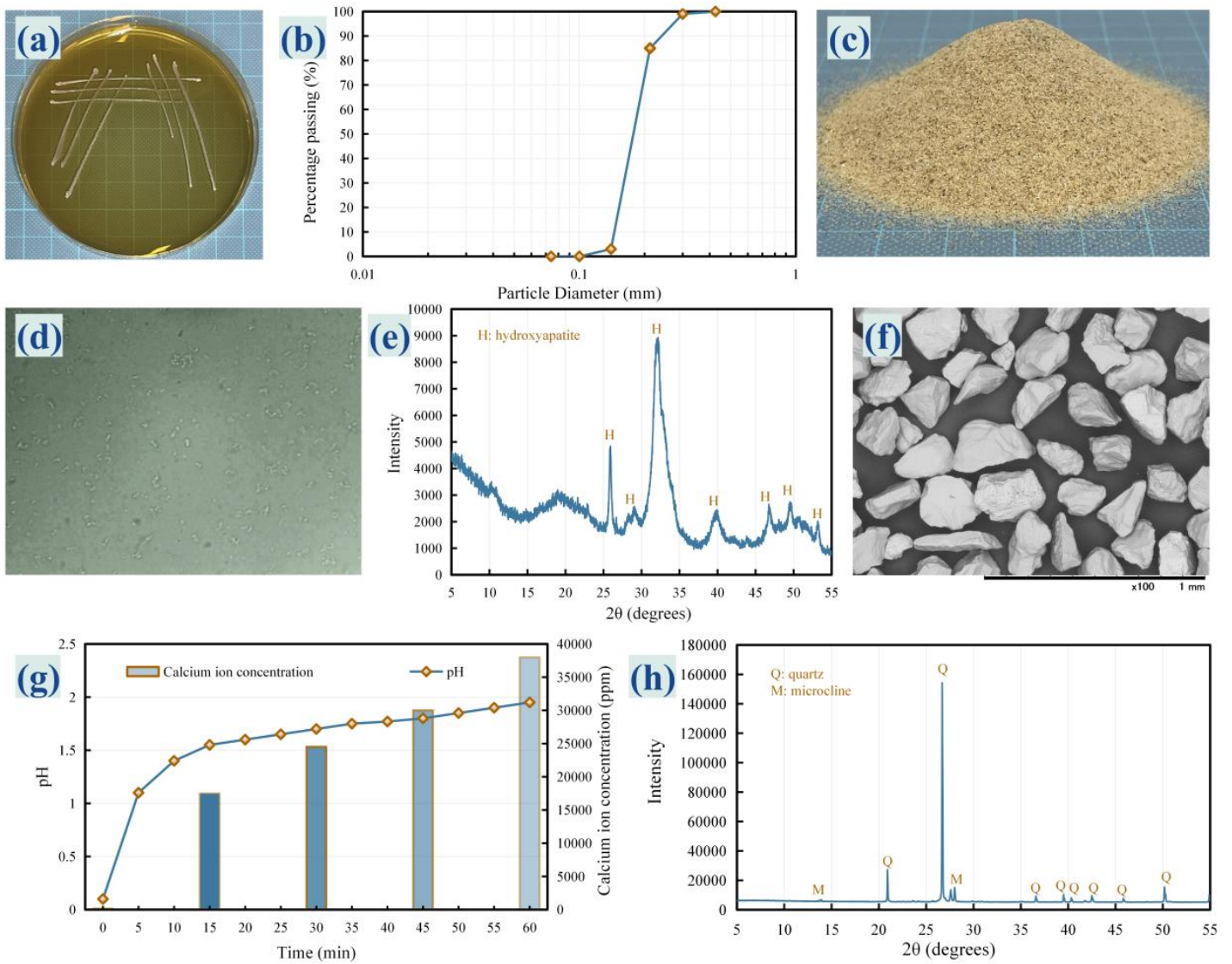
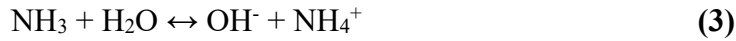
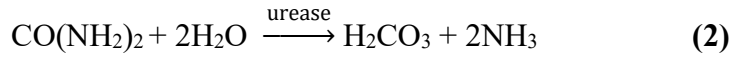


Figure 5.2 (a) shows the appearance of the cultured bacteria used, (b) displays the particle size distribution of the sand used, (c) illustrates the appearance of the sand used, (d) provides a microscopic magnification of the bacteria used, (e) presents the XRD analysis of the bones used, (f) depicts the SEM image of the sand used, (g) details the pH and calcium ion monitoring during the preparation of the solidification solution, (h) exhibits the XRD image of the sand used

5.2.2 Bacterial cultivation and monitoring

In this study, the bacterium illustrated in Figure 2a, which exhibits urease activity under acidic conditions, was provided by the Biological Resource Center at the National Institute of Technology and Evaluation in Tokyo, Japan. The strain, NBRC 14511, belongs to the *Limosilactobacillus* species. After rehydration, the bacteria were pre-cultured and then cultured at 35 °C in Man-Rogosa-Sharpe medium, a standard nutrient medium widely used for cultivating lactic acid bacteria^{31–35}. Daily measurements were taken during the main cultivation process to monitor bacterial growth and urease activity. The 1000x magnification of bacteria photographed using a Keyence VHX-500 digital microscope is shown in Figure 2d. Bacterial growth was measured at an optical density of 600 nm using a Jasco V-730 UV-Vis spectrophotometer. Urease activity was assessed using an AS ONE Thermal Robo TR-1 α water bath, maintaining a temperature of 35 °C. The activity was evaluated by measuring the amount of ammonia/ammonium produced from the hydrolysis of urea by the bacterial solution, as described in Equations (2) and (3), over a specified reaction period. Ammonia/ammonium generated from the hydrolysis of urea reacts with hypochlorite to form chloramine. Chloramine then reacts with phenol under alkaline conditions to produce indole blue dye^{36–40}. The intensity of the indole blue dye was measured at 630 nm. A calibration curve was established using ammonium chloride solutions of known concentrations and their corresponding absorbance values, allowing for the determination of ammonia/ammonium concentrations and the quantification of bacterial urease activity.



5.2.3 Laboratory-scale sand solidification tests

Laboratory-scale solidification studies utilized syringes as simplified molds. Pre-washed and oven-dried sand was loaded into the molds and compacted. Filter paper was placed at the bottom of the sand to prevent loss of sand during the experiments. The experimental procedure was shown in Table 1 for three replicates for 14 days. Each day, 10 mL of solidification solution, mixed with varying amounts of urea according to the experimental design, was injected. Bacterial solution of 10 mL was introduced on Day 1 and Day 8, two hours prior to the injection of the solidification solution. After injecting the solidification and bacterial solution from the top of the mold, gravity and capillary action alone were relied upon to infiltrate the sand. The bottom clamps were fully opened to allow the solidification solution to flow out, thereby simulating the unsaturated conditions that may occur in actual treatment processes. Samples were collected every 24 hours to monitor the pH value using a Horiba LAQUA F-71 benchtop pH meter and the calcium ion concentration using a Horiba LAQUAtwin Ca-11 compact calcium ion meter in the effluent. By comparing the initial calcium ion concentration of the solidification solution with the effluent measurements, the calcium ion consumption rate was determined. At the end of the solidification experiment, 100 mL of distilled water was added to each sample to wash out any unreacted chemicals. The samples were then dried in an oven at 60 °C for 48 hours to ensure complete dryness. After drying, the molds

were carefully cut open, and subsequent tests were conducted on the samples.

Table 5.1 Formulations of the samples in the solidification tests

Case name	Incubation temperature (°C)	Treatment duration (day)	Urea/ Ca ²⁺ ratio	Concentration of urea (mol/L)	Concentration of Ca ²⁺ (mol/L)	Amount of bacteria solution (mL)	Amount of cementation solution (mL)
1A	25	14	0	0	0.25	10*2	10*14
1B			0.5	0.125	0.25	10*2	10*14
1C			1	0.25	0.25	10*2	10*14
1D			2	0.5	0.25	10*2	10*14
2A	30	14	0	0	0.25	10*2	10*14
2B			0.5	0.125	0.25	10*2	10*14
2C			1	0.25	0.25	10*2	10*14
2D			2	0.5	0.25	10*2	10*14
3A	35	14	0	0	0.25	10*2	10*14
3B			0.5	0.125	0.25	10*2	10*14
3C			1	0.25	0.25	10*2	10*14
3D			2	0.5	0.25	10*2	10*14

5.2.4 Characterization

The strength of samples treated with biological cement is a critical parameter for evaluating the efficacy of the solidification process. Due to the irregular shape of the samples, the UCS was assessed in the central section after removing the upper and lower portions. This was accomplished using a penetration test with the SH-70 permeameter a widely employed instrument for evaluating the strength of soft rocks and stabilized soils in both field and laboratory settings. Each sample was tested at five different locations, and the average UCS value was recorded.

The sediment content was quantified using an acid digestion method. Specifically, a sufficient amount of 1 % hydrochloric acid was mixed with a known quantity of the sample to dissolve the sediment. After 24 hours, the sample was rinsed with distilled water and dried in an oven at 60 °C. The sediment amount was calculated based on the mass difference of the sample before and after the acid treatment.

The microstructural features of the samples were examined through SEM using Hitachi TM3000 microscope. To further elucidate the crystalline structure of the solidified samples, phase analysis was performed with a MultiFlex X-ray Diffractometer utilizing a Cu K-alpha source and a scanning rate of 6.5 °/min. Additionally, EDS was conducted with a JEOL JSM-IT200 to provide detailed elemental distribution.

5.3 Results and discussion

5.3.1. Monitoring of bacterial activity and solidification process

Figure 3a illustrates the monitoring results of urease activity in the cultured bacteria. The results indicate that the urease activity reached its peak platform from about the 7th day. Consequently, this cultivation period was selected for subsequent solidification tests to achieve optimal efficiency.

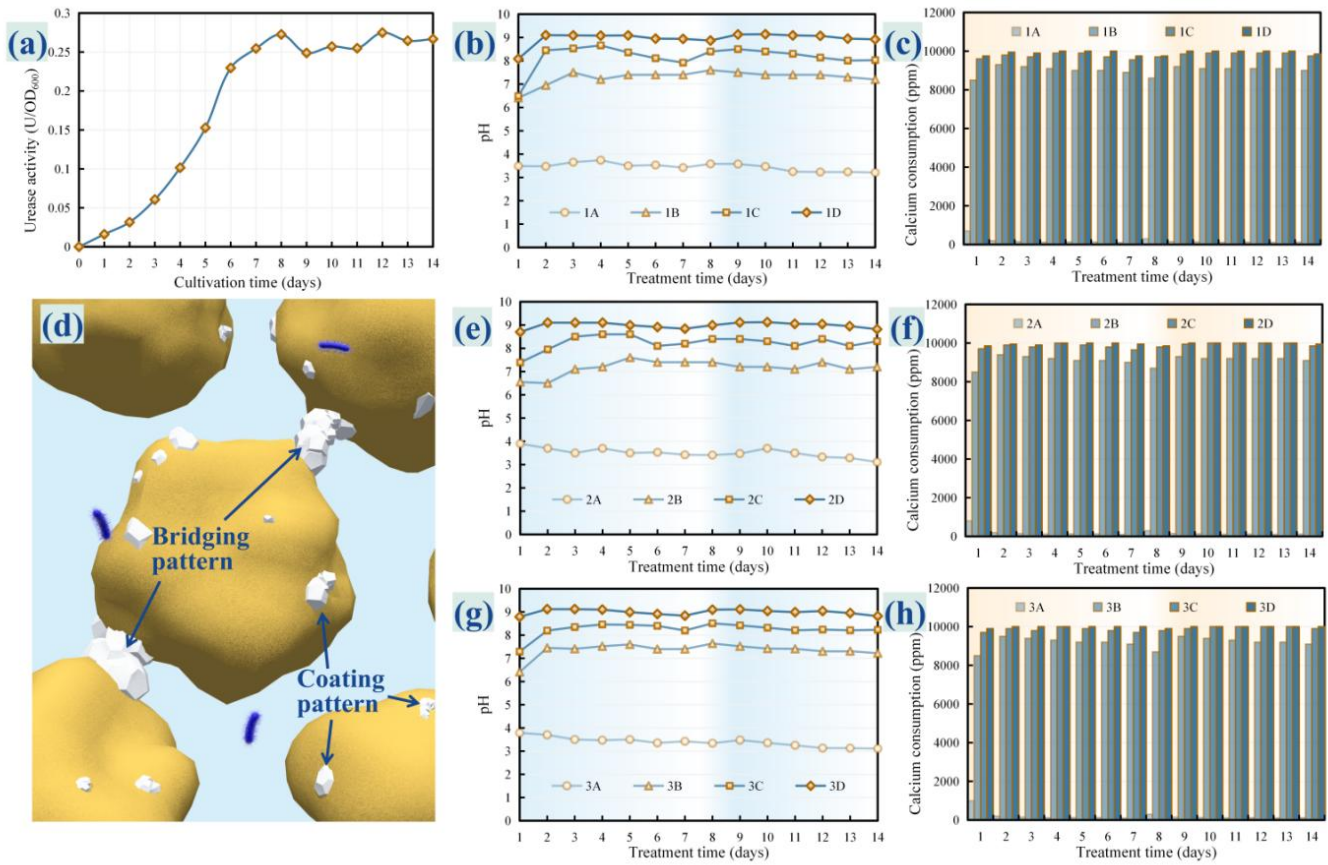
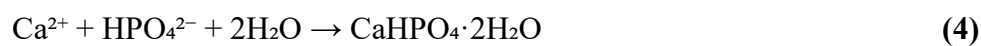


Figure 5.3 (a) shows the activity monitoring of the bacteria during cultivation, (b), (e), and (g) present the pH monitoring during the solidification tests, (c), (f), and (h) illustrate the calcium ion consumption during the solidification tests, and (d) depicts a schematic diagram of the solidification mechanism

Figure 3b, e, g depict the pH monitoring during the solidification tests. The initial pH of the solidification solution was 3.0, and the rise in the pH of the effluent is attributed to bacterial urea hydrolysis, as outlined in Equation (2) and (3). The magnitude of this increase is related to the amount of urea added. At a ratio of 2.0, the pH reached its maximum; as the ratio decreased, so did the pH. Notably, there were slight fluctuations in pH at the beginning and on the 8th day, likely due to the introduction of new bacteria. Moreover, the pH did not significantly vary across different temperatures during the solidification tests.

Figures 3c, f, and h show the variation in Ca^{2+} consumption over time during the solidification process. Following urea hydrolysis, the pH of the solidification solution increases, reducing the solubility of calcium phosphate and leading to precipitation[41–45], as described by the mechanism in Equation (4). Therefore, the consumption of calcium ions is also related to the urea/ Ca^{2+} ratio. At a ratio of 2.0 and 1.0, calcium ions are almost entirely consumed; at ratios of 0.5, the consumption is slightly lower. As illustrated in the mechanism diagram in Figure 3d, the consumed calcium ions form sediment that bind the sand particles, thus achieving solidification.



5.3.2 Evaluation of solidification effects

Figures 4a, d, and g provide a visual representation of the appearance of the specimens after solidification. Figures 4b illustrates the results of the sediment content in the samples. At the same temperature, the sediment content trends align with the previous calcium ion concentration data, showing an increasing trend with varying urea concentrations. There were no significant differences in sediment content among the different groups. The control group, which did not contain urea, showed no notable increase in sediment content, whereas the 0.5, 1, and 2 groups had substantial amounts of sediment that were comparable.

Figures 4e also presents the UCS results of the samples. The strength of the 0.5, 1 and 2 groups group reached above 1 Mpa. Notably, at 35 °C, the strength of the 2 group was significantly higher than that of the other specimens with similar amounts of sediment. Previous studies have indicated that, with the same aggregate particle size, the primary factor influencing the UCS of biocemented specimens is the amount of sediment. This suggests that other factors affecting UCS strength may be present, which will be analyzed in the subsequent section.

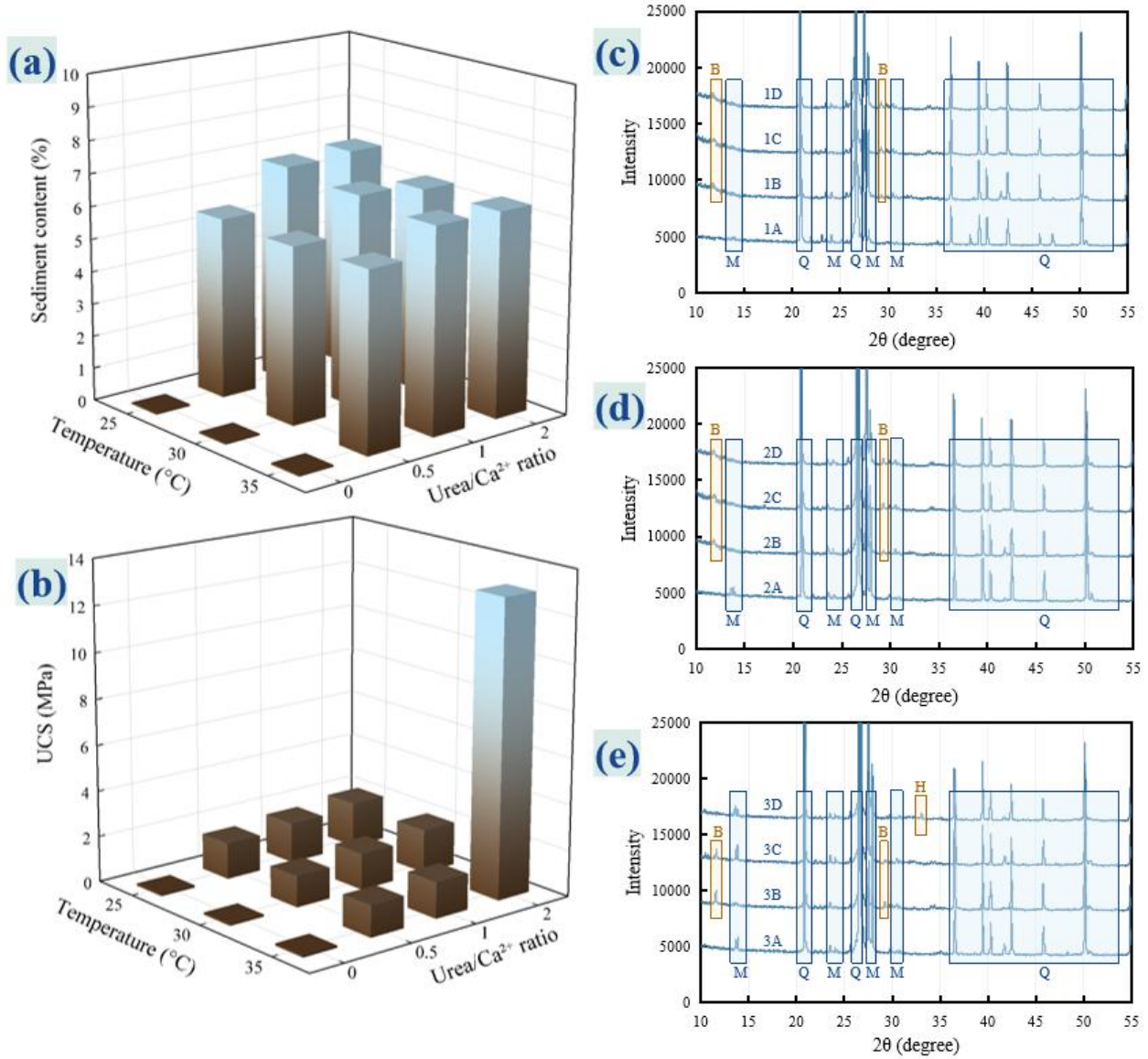


Figure 5.4 (a) and (b) display the distribution of Sediment content and UCS, and (c), (d), and (e) present the XRD analysis of the samples after solidification tests, where Q stands for quartz, M stands for microcline, B stands for brushite, and H stands for hydroxyapatite

5.3.3 Microstructural and crystal phase inspection

Changes in the microstructure of specimens often influence macroscopic properties, including strength. Figure 5 displays SEM images of all specimens to assess microstructural features and sediment morphology. The sediment patterns in biocement typically affect its strength^{46–50}, with bridging patterns contributing significantly to strength enhancement, and coating patterns also providing some improvement. It can be observed that, except for the control group, the remaining specimens exhibit both bridging patterns between soil particles and coating patterns on the particle surfaces. Therefore, sediment morphology does not appear to be the primary reason for the high strength observed in specimen 3D.

Combining the XRD results presented in Figures 4c, f, and h, it is apparent that the calcium phosphate sediment shown in the other samples are brushite, whereas those depicted in sample 3D are hydroxyapatite. The formation of calcium phosphate sediment in the samples is often influenced by temperature and pH adjustments^{51–55}, leading to the transformation of metastable brushite into the more stable hydroxyapatite over time. Hydroxyapatite possesses superior mechanical strength compared to brushite⁵⁶, which may account for the higher strength observed in specimen 3D, despite it containing the same amount of sediment as the other specimens.

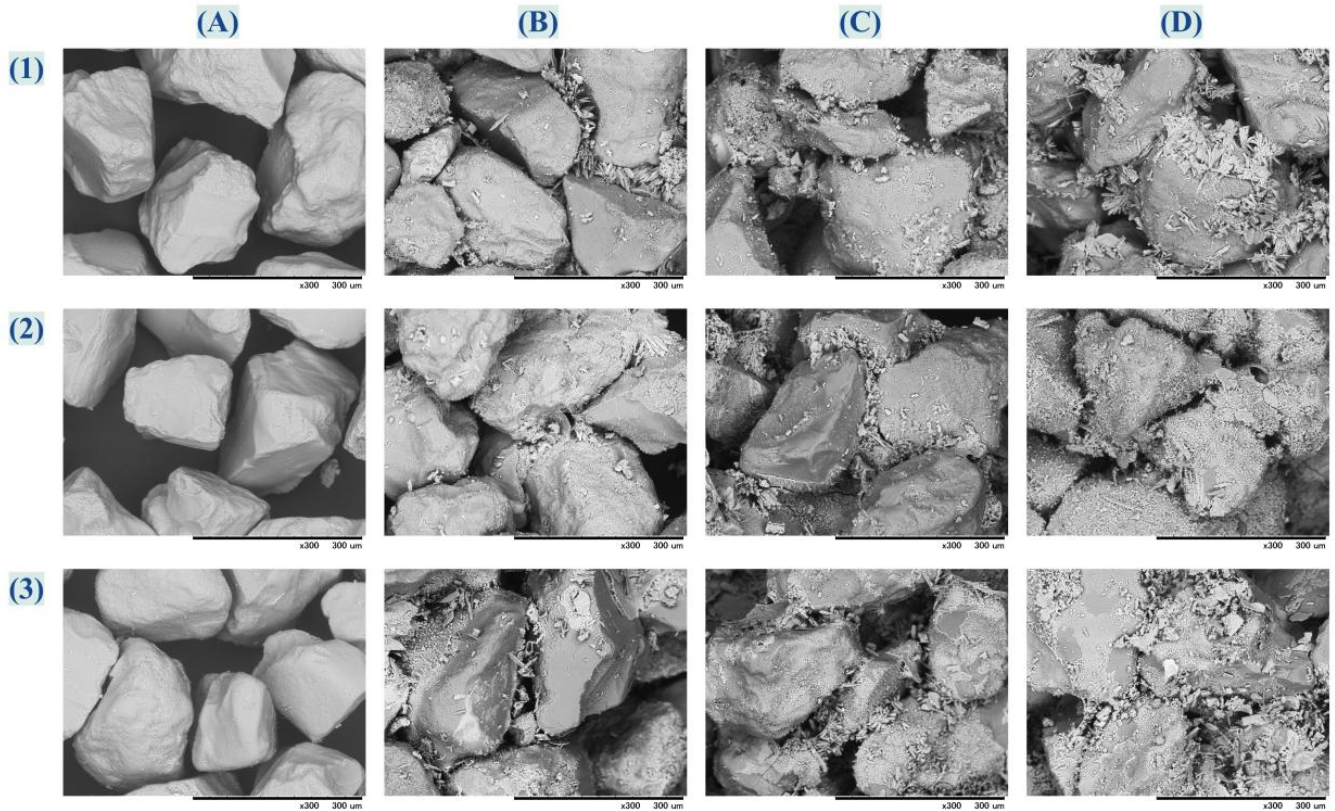


Figure 5.5 Displays the SEM analysis of the samples after the solidification tests. The numbers on the left and letters at the top correspond to the sample identifiers

Figure 6 shows the elemental distribution of phosphorus, calcium, and silicon as analyzed by EDS. During the analysis, Si was used to differentiate sand grains, while Ca and P represent the distribution of biocement components. Figure 6a shows the untreated quartz sand, which only shows intensity for Si with no detectable Ca or P. In Figure 6b and c, the distribution areas for Ca and P are comparably bright, while in Figure 6d, the brightness of the P region is significantly lower than that of the Ca region. This suggests that the abundance of phosphorus in the sediment in Figure 6d is

lower compared to other figures, the chemical composition of the sediment in Figure 6d may differ from those in Figure 6b and c. Given that the ideal P/Ca ratio for hydroxyapatite is 0.6, which is lower than the ideal P/Ca ratio of 1 for brushite, this further confirms the presence of hydroxyapatite in sample 3D.

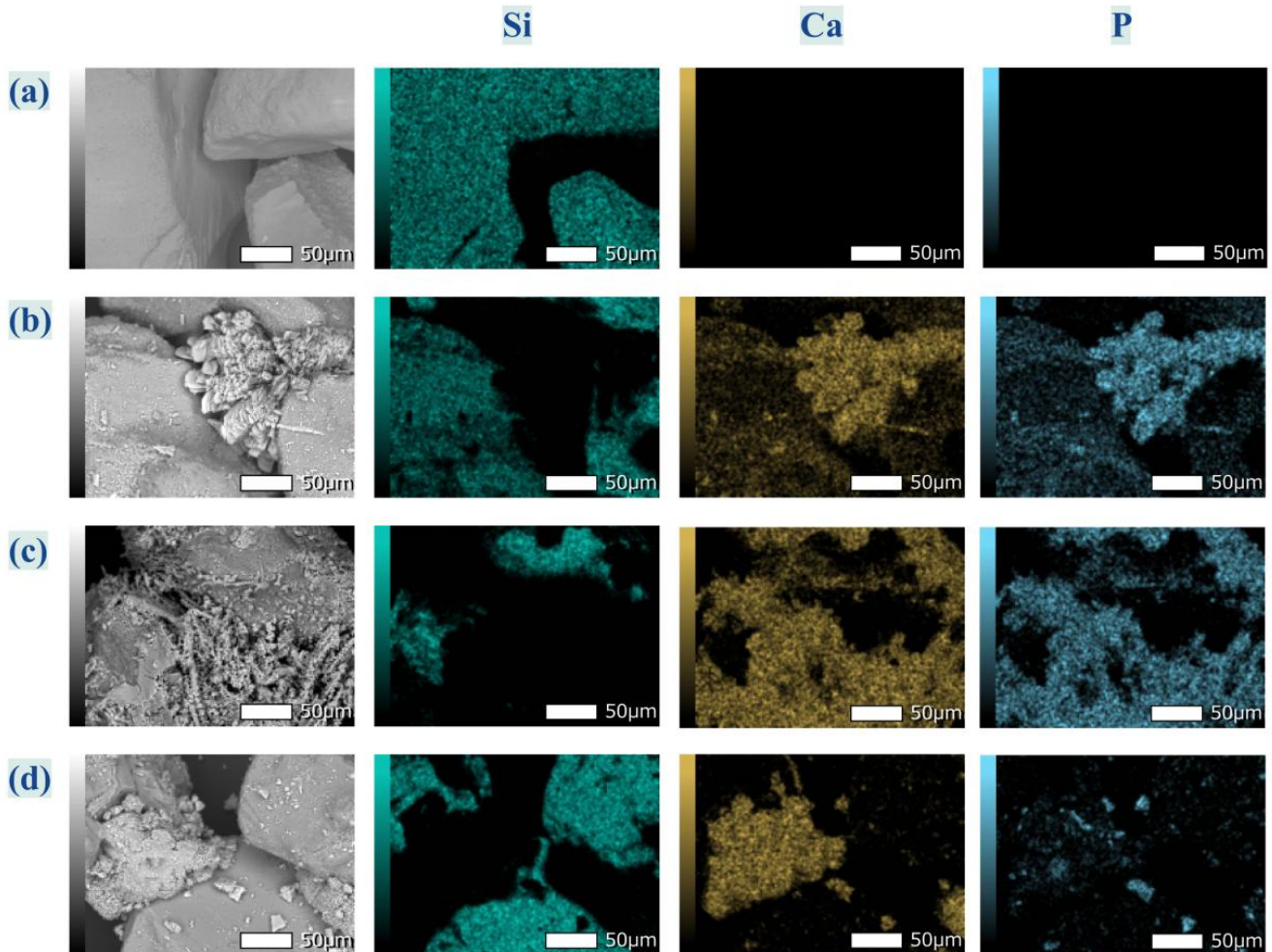


Figure 5.6 (a) shows the EDS analysis of the sand used. (b), (c), and (d) display the EDS analysis of the 1D, 2D, and 3D samples after solidification tests, respectively. The labels Si, Ca, and P at the top indicate the distribution of the corresponding elements

5.3.4 Environmental impact and future application analysis

When scaling up the application on site as shown in Figure 7, it is essential to consider their environmental friendliness and sustainability. The sustainable calcium sources for biocement, including construction waste, eggshells, and shellfish waste^{57–61}, have been investigated and applied. In this study, biocement based on calcium phosphate utilizes both calcium and phosphorus sources derived from fish bones from kitchen waste. With societal development and the expansion of the food industry, a significant quantity of bones has become an important waste in the food sector^{62–66}. Therefore, this study represents a novel approach to the sustainable utilization of bone waste. Utilizing waste bones not only addresses the waste problem generated by the food industry but also provides a cost-effective raw material for biocement production. This option reduces waste management costs, reflects the principles of a circular economy, helps lower costs, and promotes the large-scale application of biocement.

Moreover, despite the induced crystal phase change achieving a strength in 3D samples that was more than five times higher than that of other samples with similar sediment content, further research is needed to quantitatively evaluate crystal configuration factors and develop appropriate fitting functions. This study induced the formation of hydroxyapatite at high pH and 35 °C, conditions that may not accurately represent typical environmental scenarios. Therefore, this method may not always be suitable for field applications. Future research should explore additional methods for altering crystal phases, such as adjusting the calcium/phosphorus ratio in the solidification solution, to improve the practicality and efficiency of this method under varying environmental

conditions.

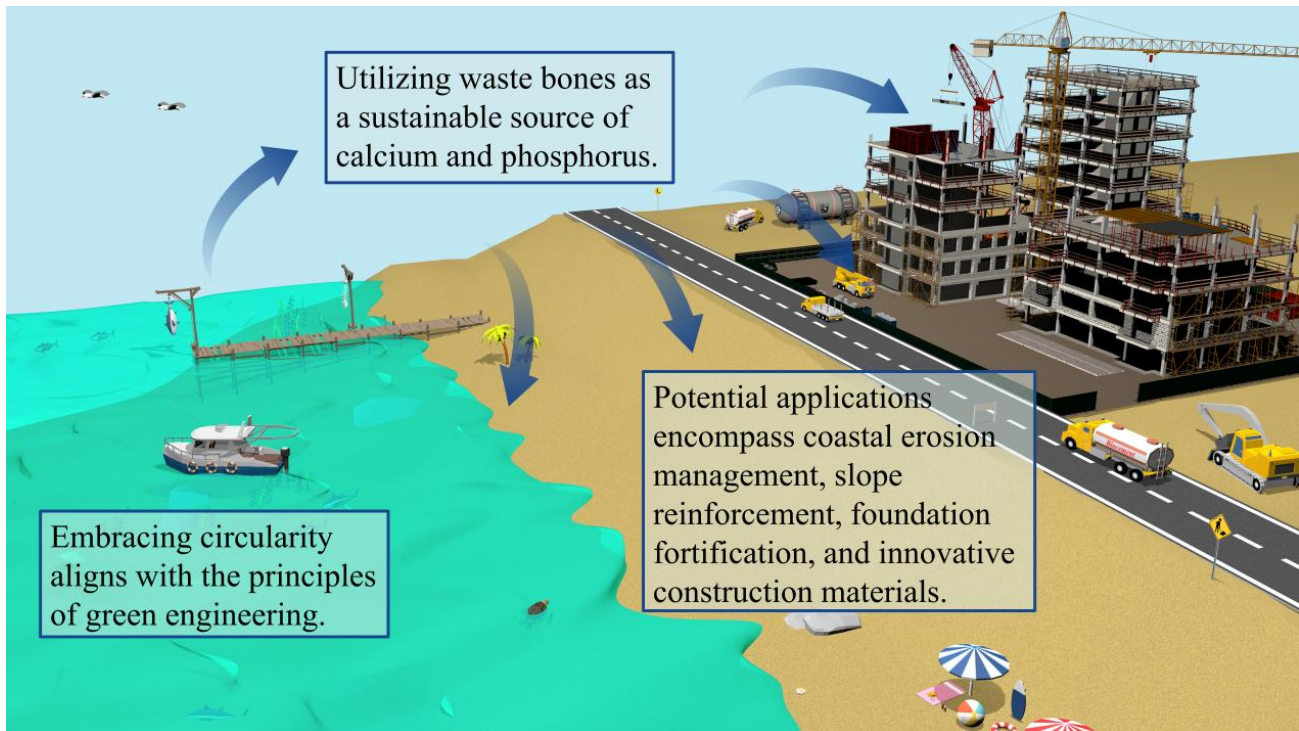


Figure 5.7 Illustrates potential future applications

Furthermore, animal urine produced in livestock farming can serve as a sustainable source of urea for biocement production^{67–71}. Utilizing animal urine effectively mitigates the environmental impact of animal manure, and avoids the energy-intensive and environmentally harmful issues associated with synthetic urea production^{72–76}. This practice contributes to resource recycling and reduces the environmental burden of the production process. Future research should focus on comprehensive lifecycle assessments and sustainability studies to systematically evaluate the economic costs, carbon footprint, and environmental impacts of this approach^{77–81}, providing a scientific basis for the promotion of biocement in various application scenarios.

5.4 Conclusion

This study aimed to enhance the strength of biocement derived from kitchen waste bones by inducing crystallographic changes through temperature variation. The key findings are as follows:

1. The use of food waste bones as a sustainable source for biocement has been validated, which helps in reducing both the costs of food waste disposal and the costs of biocement production.
2. Evidence provided by XRD and EDS has demonstrated crystallographic changes, resulting in more than a fivefold increase in strength at the same sediment levels.
3. Future research should continue to explore this study to gain a deeper understanding. This includes investigating and quantitatively considering the contribution of crystal structure factors to strength, establishing corresponding fitting functions, and exploring other methods for altering the crystalline phase, such as adjusting the calcium-to-phosphorus ratio in the solidification solution to enhance the practicality and efficiency of this method under different environmental conditions.

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CHAPTER 6 Preliminary exploration of the future kitchen waste bone-driven enzyme-induced calcium phosphate precipitation under microgravity for space biocementation

6.1 Introduction

With the advancement of human exploration into outer space, the objectives have gradually shifted from initial exploration and experimentation to the establishment of enduring bases or colonies on the Moon, Mars, and other celestial bodies. However, achieving this grand goal presents numerous challenges, one of which is the prohibitively high cost of transporting materials for construction and maintenance¹⁻⁵. This makes the task of transporting resources from Earth to space a formidable challenge. Additionally, frequent rocket launches impose an extra burden on the environment⁶⁻¹⁰. Consequently, finding alternative solutions to reduce the need for material transport from Earth has become an urgent issue for scientists. In response to this challenge, In-Situ Resource Utilization (ISRU) technology has emerged and gained increasing attention. The core concept of ISRU technology is to utilize local resources on the target celestial body to reduce dependence on Earth's resources¹¹⁻¹⁵. This encompasses not only the production of construction materials from local resources but also the acquisition of essential resources such as water, oxygen, and fuel¹⁶⁻²⁰. By effectively utilizing in-situ resources, the self-sufficiency of extraterrestrial bases can be enhanced, significantly lowering the economic costs of space construction.

As part of ISRU technology, biocementation is being extensively studied^{21–25}. This technique utilizes microorganisms or enzymes to induce mineral precipitation in a substrate, thereby enhancing the geotechnical properties of the soil. Biocementation has already demonstrated its broad application potential in terrestrial infrastructure projects^{26–30}. Among various biocementation methods, calcium phosphate biocement is notable for its low pH system, which helps to reduce ammonia emissions^{31–33}, making it potentially suitable for space construction where safety is paramount. However, the need for acid-resistant bacteria or enzymes somewhat limits its development. This study explores an innovative approach by using inexpensive and readily available soybean crude enzymes^{34–38}, to utilize kitchen waste bones as sources of calcium and phosphorus in the enzyme-induced calcium phosphate precipitation (EICPP) technique, with the aim of evaluating its potential application in microgravity environments.

In this study, as shown in the **Figure 1**, calcium and phosphorus were extracted from kitchen waste bones using acid. In the presence of soybean crude enzymes, which hydrolyze urea and induce a rise in pH, calcium and phosphorus in the cementation solution effectively precipitated as calcium phosphate. The experimental results indicate that under both terrestrial and microgravity conditions, the degree of EICPP reaction, the uniformity of calcium phosphate precipitation, and the microstructural characteristics of the precipitates did not exhibit significant differences. These results provide support for the application of EICPP technology in extraterrestrial construction. This technology holds significant promise for the expansion phase of extraterrestrial settlements. As extraterrestrial colonies expand and populations increase, the generation of organic waste such as animal bones will rise substantially. Utilizing these waste materials as sources of calcium and phosphorus in EICPP can not only help reduce the cost of construction materials in space but also

effectively address waste management challenges, thereby enhancing the sustainability of resource utilization in space environments.

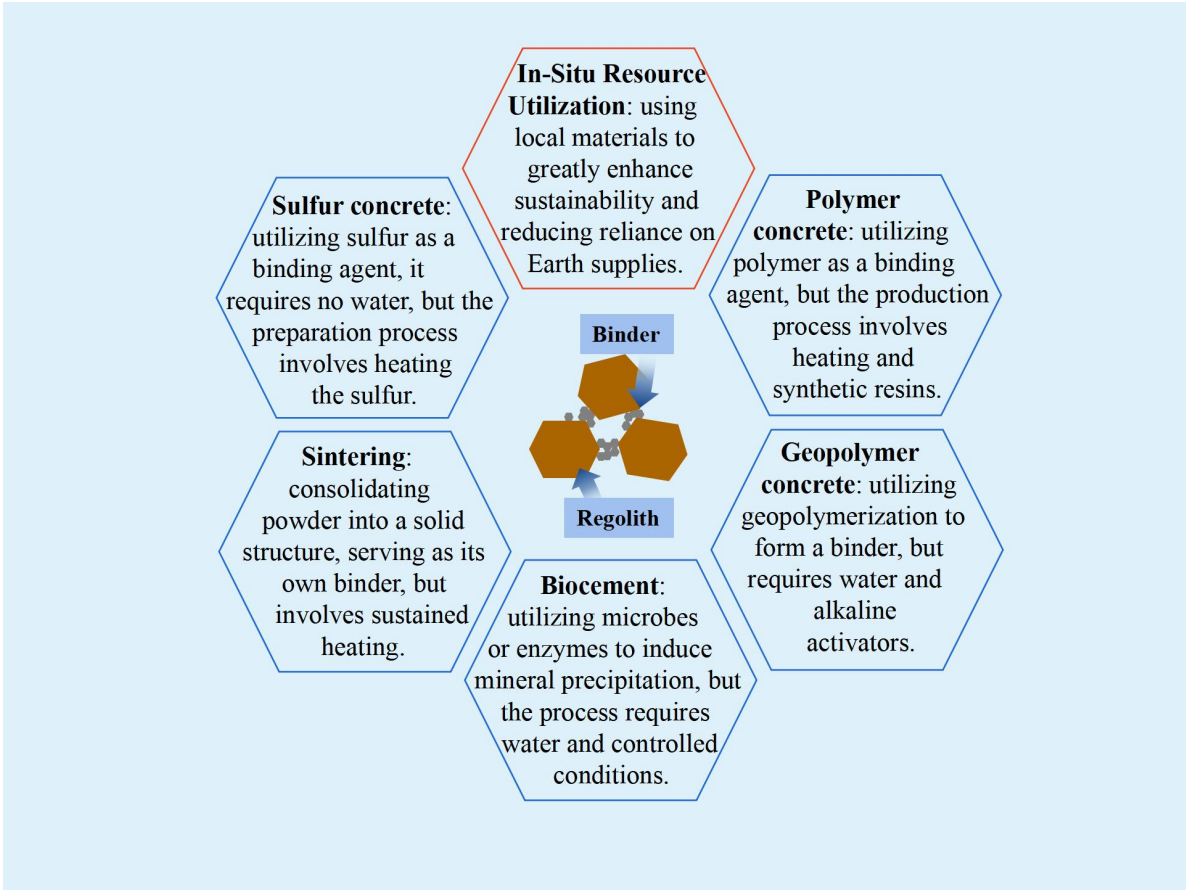


Figure 6.1 Current Space Geotechnical Construction Technology

6.2 LITERATURE REVIEW

Overview of current construction technologies based on in-situ resource utilization

6.2.1 Sulfur concrete

Sulfur concrete can be produced using only sulfur and regolith as aggregate¹, offering a distinct advantage in extraterrestrial environments where water is scarce². Unlike the hydration hardening process of traditional concrete, sulfur concrete hardens through a unique mechanism: sulfur melts and becomes liquid at 120°C, then transitions to a monoclinic crystalline form upon cooling to 114°C, and finally adopts an orthorhombic state. This crystallization enhances its bonding with the aggregate, resulting in high strength³. The production of sulfur concrete on the Moon and Mars depends fundamentally on the availability of sulfur itself. Fortunately, sulfur is abundant on both these celestial bodies—especially on Mars, which is considered to be a sulfur-rich planet where sulfur cycling is a significant geochemical process⁴⁻⁸. The Martian surface contains large amounts of sulfates and sulfites, with sulfur concentrations that greatly exceed those found on Earth, the Moon, or various asteroids. Sulfur can be extracted from sulfides or sulfates through diverse chemical and physical processes; for instance, heating lunar regolith to 1100°C can yield sulfur with a purity of 85–95%⁹. Sulfur may also be obtained from pyrite¹⁰. Additionally, sulfur is produced as a byproduct in processes that use Martian calcium sulfate dihydrate and gypsum to generate oxygen and water, with the resulting sulfur comprising up to 10% of the oxygen's mass output¹¹.

The typical production process of sulfur concrete involves heating sulfur until it liquefies, mixing it with aggregate, pouring the mixture into molds, and then allowing it to harden. Maintaining a controlled heating temperature throughout this process is essential to keep the sulfur in its liquid state. Studies using the Martian soil simulant JSC Mars-1A indicate that the optimal mixture for

producing Martian concrete is 50% sulfur and 50% simulant, with a maximum aggregate size of 1 mm. This Martian concrete formulation achieves compressive strengths exceeding 50 MPa, and its mechanical behavior under various loading conditions has been effectively simulated using the Lattice Discrete Particle Model. Additionally, the presence of metals in the Martian soil simulant contributes to concrete strength by reacting with sulfur to form sulfates and polysulfates¹². Research has also investigated the effects of extreme space conditions, such as impacts and radiation, on sulfur concrete performance. Samples composed of 65% JSC-1 lunar regolith simulant and 35% sulfur were subjected to hypervelocity impact tests and space radiation exposure analyses. Detailed assessments of damage caused by such events can inform the design of future structures to minimize harmful impacts¹³. When magnetite is used as an aggregate, its rapid heating under microwave energy makes it an efficient heating source for casting Martian concrete. A lattice Boltzmann model was developed to determine the optimal microwave power and heating duration for casting Martian concrete. Experiments have measured the compressive strength and microstructure of microwave-cast Martian concrete, with the compressive strength of concrete produced using magnetite lunar aggregate simulant reaching 17.44 MPa¹⁴.

While sulfur concrete offers high strength and does not require water for production, its durability, especially in space environments¹⁵, warrants further investigation. Under vacuum and elevated temperatures, the sublimation of sulfur in sulfur concrete becomes a significant concern. Research shows that in vacuum conditions, the sublimation rate of sulfur is 8,000 times slower at $-60\text{ }^{\circ}\text{C}$ compared to $20\text{ }^{\circ}\text{C}$ ¹⁶. However, after exposure to vacuum at approximately $20\text{ }^{\circ}\text{C}$ for 58 days, sulfur sublimates extensively, leading to a mass loss of about 30% from the initial weight. This sulfur loss creates voids and defects within the concrete matrix, which compromises its structural integrity. Sublimation rates increase drastically with temperature; for instance, it takes less than two hours to

form a 1 cm sulfur layer at 120 °C, while at 15 °C, this process spans 3.7 years¹⁷. Furthermore, temperature cycling also significantly impacts the durability of sulfur concrete. During hardening, sulfur cools and crystallizes: at 114 °C, it crystallizes into monoclinic sulfur with a 7% volume contraction, and at 96 °C, monoclinic sulfur transforms into orthorhombic sulfur, leading to an additional 6% volume reduction. This shrinkage induces internal stresses, which negatively affect the material's durability^{18–22}. Samples exposed to temperature cycles ranging from room temperature to –191 °C exhibit strength reductions of at least fivefold compared to non-cycled samples. Microscopic analysis of fracture surfaces indicates significant debonding between SiO₂ aggregate particles and sulfur, attributed to the substantial difference in thermal expansion coefficients. This differential expansion and contraction produce internal defects and cracks, compromising the macro-mechanical properties of sulfur concrete²³. To address these durability challenges, research is focusing on optimizing combinations of sulfur, aggregate, and various additives. The interaction of these additives with sulfur and aggregate holds promise for enhancing the longevity of sulfur-based concrete in harsh environments^{24–28}.

6.2.2 Polymer concrete

Another form of space concrete that eliminates the need for water is polymer concrete, which uses polymers instead of cement paste and water to bond the aggregates. The general preparation method for polymer concrete involves heating the concrete mixture to melt the polymer, thereby bonding the aggregates and fillers. Thus, the method of heating and the source of the polymer become especially critical.

By applying a 230°C heating plate to the top of the sample, polyethylene is melted and bonded with lunar soil to form lunar concrete composed of 10% polymer and 90% lunar soil, achieving compressive strengths between 12.6 and 12.9 MPa²⁹. Additionally, an investigation into bottom-up heating, performed at 200°C instead of 230°C within a vacuum chamber simulating lunar conditions, revealed that while the compressive strength was lower than that of the top-down heating method (maximum strength reaching 5.7 MPa), the bottom-up heating method allowed for curing at double the speed³⁰. Through the thermal treatment of soil mixed with recycled polyethylene powder using solar energy, bricks with sufficient compressive strength can be produced. Bricks with a polymer content of 5% achieved strengths between 1 and 2 MPa, while those with 10% polymer reached between 3 and 8 MPa³¹.

Since polymers are generally transported from Earth, researchers have studied key processing parameters in polymer concrete preparation to minimize the adhesive content and, consequently, the burden on space transportation. Pre-mixed JSC-1A lunar regolith simulant particles and a small amount of polymer binder are compacted quasi-statically, achieving a closely packed structure where large binder droplets fragment. As compaction pressure increases further, larger capillary forces encourage the binder to spread around the contact points of filler particles, forming polymer micro-agglomerates. With a binder content as low as 2 wt% to 5 wt%, this process can produce flexural strengths comparable to, or even surpassing, typical reinforced concrete³². Moreover, studies have proposed that hydrogen obtained by electrolyzing water from Martian soil could catalytically reduce atmospheric CO₂ to produce CH₄. The conversion of CH₄ to C₂H₄, based on catalytic oxidation at temperatures between 800 and 900°C, represents the next step in producing polyethylene from Martian resources, with the final step involving the catalytic linkage of C₂H₄ monomers. Samples of synthesized polyethylene mixed with JSC Mars-1 simulated Martian regolith

and exposed to a 500 MeV/u ^{56}Fe beam demonstrated their effectiveness in attenuating incident radiation, achieving the basic requirements for passive radiation shielding. These samples also withstood impact loads equivalent to 7 km/s micrometeoroid impacts. Simulations and experiments have shown that ballistic impact resistance under high-velocity conditions is proportional to the polyethylene binder content in the composite material. JSC Mars-1 simulant bricks shattered completely upon impact, with compressive strains generated by shockwave propagation exceeding the failure strain of Martian simulant bricks. However, composite bricks containing 40 wt% polyethylene sustained only minimal damage under similar impact conditions³³.

One of the primary limitations of polymer concrete in extraterrestrial applications may be its durability under extreme temperature conditions. Some studies have examined these composites printed at -30°C and subjected to weekly lunar thermal cycling, ranging from 127°C during the lunar day to -190°C at night. Findings confirmed that thermal stresses lead to physical and chemical degradation. Therefore, further research into the long-term durability of polymer concrete in extreme environments is essential for future applications³⁴.

6.2.3 Geopolymer concrete

Geopolymers are a class of inorganic polymers that serve as cementitious binders and can be combined with aggregates from regolith to produce geopolymer concrete. Their production involves a geosynthesis process with three main stages: alkali activation, where monomers are generated through the dissolution of amorphous aluminosilicates; reorientation, in which these monomers are transported, oriented, and condensed; and polycondensation, through which the material hardens into an inorganic polymer structure³⁵. Lunar regolith contains abundant alumina and silica oxides with a chemical composition similar to the requirements for geopolymers³⁶, suggesting its potential

for in-situ production. Additionally, the presence of alkali metals on the Moon allows for the local production of alkaline activators³⁷. Given the challenges in water extraction on the Moon, despite evidence of lunar water resources, minimizing water use or developing water recycling techniques within the production process is still essential.

The Si/Al ratio influences the properties of geopolymers, making the choice of a suitable simulant important³⁸⁻⁴². Volcanic ash, due to its comparable chemical and mineralogical composition, suggests that lunar regolith's alkali activation could be similar. Potential lunar cement materials have been prepared by using volcanic ash and sodium hydroxide solution as activators. Geopolymer samples made from volcanic ash exhibited compressive strengths of 45.53 MPa after 24 hours under vacuum conditions and 44.95 MPa after 30 freeze-thaw cycles in liquid nitrogen. Notably, 98.61% of the water used in the process could be recovered⁴³. Additional studies have shown that the simulant DNA-1, which mimics the lunar mare regolith, can act as an effective water-reducing agent and retarder when urea is added, by breaking hydrogen bonds and delaying setting time, reducing the water requirement by up to 32% for achieving similar workability⁴⁴. Other studies have explored using glass meteoritic powder and sodium hydroxide as activators to create geopolymers and proposed a process for geopolymer concrete production on the Moon, which incorporates a water recycling system. In this process, prepared geopolymer concrete is placed in a vacuum curing chamber, where a condensation recovery system collects and reuses evaporated water⁴⁵.

However, current research has found that the Martian environment may impose certain limitations on geopolymer concrete. Lunar regolith simulants generally have smaller particle sizes and higher degrees of geopolymerization⁴⁶. In contrast, the high iron content in Martian soil results in geopolymers with lower compressive strengths than those produced from lunar soil simulants⁴⁷. Additionally, the high concentration of carbon dioxide in the Martian atmosphere has been shown to

reduce the strength of geopolymers due to carbonation^{48–52}, which could hinder the application of geopolymer concrete on Mars.

6.2.4 Sintering

Sintering can be employed to manufacture structural components in dry environments, involving the compaction of regolith into the desired shape of the structural element. This compaction results in reduced porosity and limits shrinkage, thereby decreasing the energy (and duration) required for the sintering process. The compacted regolith is subsequently heated using an energy source, such as amplified (concentrated) sunlight, microwaves, or lasers. Through sintering, the particles bond together while simultaneously decreasing porosity until a dense medium is formed. The efficiency of sintering for producing construction materials is higher than that of melting, as it can be achieved at temperatures significantly below the material's melting point (approximately 50–70%), yet it still demands substantial energy. This factor is particularly critical for applications on the Moon and Mars, where resources and conditions are constrained. Due to the thin atmospheres of the Moon and Mars, which do not reflect and absorb significant amounts of energy, solar sintering emerges as the primary viable processing method in these environments. To enhance the collection of solar energy, solar concentrators/collectors/furnaces can be utilized to gather and focus sunlight.

Utilizing a high-flux solar furnace, light can be focused and layered sintering of lunar regolith can be achieved. By depositing layers of 0.1 mm thickness, brick-shaped components can be sintered within approximately 5 hours⁵³. The use of a solar simulator allows for additive manufacturing of interlocking components from JSC-2A lunar regolith simulant. Experimental data is further integrated into finite element calculations, and structural analyses indicate that specific geometries can be constructed without the need for additional binding materials. These interlocking components

hold the potential to form structures that protect pressurized inflatable habitats from radiation and micro-meteorite impacts or to create non-pressurized shelters for robotic machinery⁵⁴. Furthermore, the study investigates the impact of simulant composition on sintering by examining the sintering or melting behaviors of four different lunar simulants at varying temperatures. It was discovered that the glass transition temperature of the lunar simulant was the lowest, thus melting first and playing a critical role in the simulated sintering process⁵⁵.

However, challenges remain in the current sintering techniques. The use of lasers or microwaves for sintering consumes a particularly high amount of energy, while solar sintering systems require additional complex maintenance, including cleaning lunar dust from mirrors and lenses to maintain concentrator efficiency and managing positioning controls to maintain the necessary focal point relative to the sun and the solar concentrator's movement⁵⁶.

6.2.5. Biocement

Biocement technology primarily encompasses two methods: MICP and EICP. MICP utilizes specific microorganisms, whereas EICP employs free enzymes. The reaction is typically achieved through the urease pathway, wherein urea is decomposed to produce ammonia and carbon dioxide, thereby increasing the alkalinity of the solution and facilitating the combination of calcium ions and carbonate ions to form calcium carbonate precipitates at the microscale. These precipitates can serve as binding materials, thus enhancing the geotechnical properties of soil in a controlled manner to achieve the desired response. The effectiveness and feasibility of this technology have been validated on Earth, with applications including foundation reinforcement^{57–61}, brick manufacturing^{62–66}, and erosion control^{67–71}, while also demonstrating potential for space applications⁷².

Current research into the application of biocement in space has shown promise. Studies indicate that MICP technology mediated by *Sporosarcina pasteurii* can effectively consolidate lunar and Martian regolith simulants into brick-like structures with satisfactory structural strength. The potential effects of supplementing nickel to enhance urease activity may further improve the entire MICP process. The addition of the natural polymer guar gum facilitates a strong binding with soil particles through electrostatic interactions and additional hydrogen bonds, while the introduction of larger molecules may increase bacterial metabolic activity, thereby synergizing MICP. Samples augmented with 1% guar gum and 10 mM nickel exhibited the highest average compressive strength. However, results suggest that the treatment effects of Martian simulant soil are inferior compared to those of Lunar simulant soil. Fe and Mg are essential metal ions needed for bacterial growth, yet their concentrations are limited. The significantly higher presence of metals like Fe and Mg in Martian simulant soil may create a less than ideal environment for bacterial growth⁷³. Building upon the addition of guar gum, incorporating short-cut glass fibers into the samples, while somewhat reducing compressive strength—by nearly 30% in some cases—significantly increased strain under maximum load. This toughening effect may be attributed to the formation of a network by the chopped glass fibers, which delays the occurrence of sample fracture. This preliminary observation warrants further careful consideration and may provide a pathway to enhance the toughness of space bricks. Moreover, the primary advantage of glass fibers lies in improving the workability of the final samples. In contrast to samples without glass fibers, which were found to be difficult to process and prone to breakage, glass fiber-reinforced samples maintained their integrity during machining, with material being removed in powder form rather than as continuous fragments, allowing for the production of free shapes without the need for custom casting molds⁷⁴.

Research has also investigated the biocementation of Martian regolith simulants using the microalga *Thraustochytrium striatum*. This study examined the effects of various biogrout methods and calcium sources on the unconfined compressive strength and hydraulic conductivity of biocemented Martian regolith simulants columns. In the presence of urea and CaCl_2 , *Thraustochytrium striatum* can induce biocementation. The research further found that *Thraustochytrium striatum* could generate CaCO_3 precipitate from calcium acetate by degrading it into CO_3^{2-} and forming CaCO_3 with Ca^{2+} , even in the absence of urea. In this process, calcium acetate serves a dual role as both an organic carbon source and calcium source. Therefore, utilizing acetate from simulated anaerobic digestion of kitchen waste can avoid the ammonia emissions associated with traditional MICP, contributing to a cleaner cementation process⁷⁵. Additionally, response surface methodology was employed to optimize the influence of three factors—cell biomass loading, nutrient loading, and particle size—on the unconfined compressive strength of Martian regolith simulants columns. Although nutrient loading did not show a consistent trend regarding its effect on unconfined compressive strength, the overall unconfined compressive strength of the columns increased with rising cell biomass loading, with medium-sized Martian regolith simulants achieving the strongest biocemented columns. The maximum unconfined compressive strength reached 603.83 kPa when the particle size was 0.25 to 0.425 mm, cell biomass loading was 3.8 g/100 mL, and nutrient loading was 5.83/14.18 (CaCl_2 /urea) g/100 mL⁷⁶.

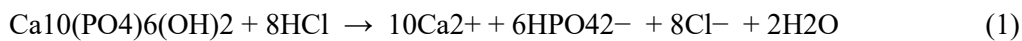
Currently, research on biocement technology for space applications is extremely limited, with many critical issues remaining unresolved. Although previous studies have attempted to verify the feasibility of biocement under simulated lunar and Martian regolith conditions, truly adaptable technological solutions for extreme space environments are still scarce. The impacts of low gravity, high radiation, and extreme temperature fluctuations on microbial activity, enzyme stability, and

reaction efficiency, as well as the durability of samples after solidification, are all important research directions that urgently need to be addressed.

6.3 Materials and methods

6.3.1 Waste bone and crude enzyme and extraction

In this study, kitchen waste pig bones, as shown in Figure 2a, were utilized as a sustainable source for the extraction of the curing solution. X-ray fluorescence (XRF) analysis obtained using the Hitachi SEA1200VX X-ray fluorescence analyzer, depicted in Figure 2b, revealed that the bones are rich in calcium and phosphorus, while X-ray diffraction (XRD) analysis, shown in Figure 2c, further identified the primary component as hydroxyapatite. The process of calcium and phosphorus extraction from the bones involved several steps. First, the cleaned bones were dried at room temperature and then ground into particles smaller than 150 μm , as shown in Figure 3a, to prepare them for the subsequent steps. A 40g sample of bone powder was then dissolved in 100mL of 2M HCl solution, as illustrated in Figure 3b, with stirring at 1500 rpm. The dissolution process was monitored through pH and calcium ion concentration, as indicated in Figure 3d, before the solution was filtered, as shown in Figure 3c. The primary hydroxyapatite in the bones was acid hydrolyzed according to Equation 1. The solution was diluted and adjusted to reach a pH of approximately 3.6 (± 0.2) and a calcium ion concentration of around 8500 (± 200) ppm. Finally, urea was added to the bone extract solution according to the different groups listed in Table 1.



The extraction of crude soybean (*Glycine max*) urease in this experiment followed a simple procedure: commercially available dry soybeans were ground and passed through a 150 μ m sieve. The soybean powder was added to distilled water at a concentration of 50 g/L, stirred for 30 minutes, and then centrifuged to collect the supernatant. The crude soybean urease solution, which required no further purification, was obtained through this cost-effective and practical method of extraction from soybeans. In this study, the cementation solution was prepared by mixing equal volumes of the crude enzyme solution and the bone extract. The crude enzyme and bone extract were only combined immediately before use to prevent premature reactions.

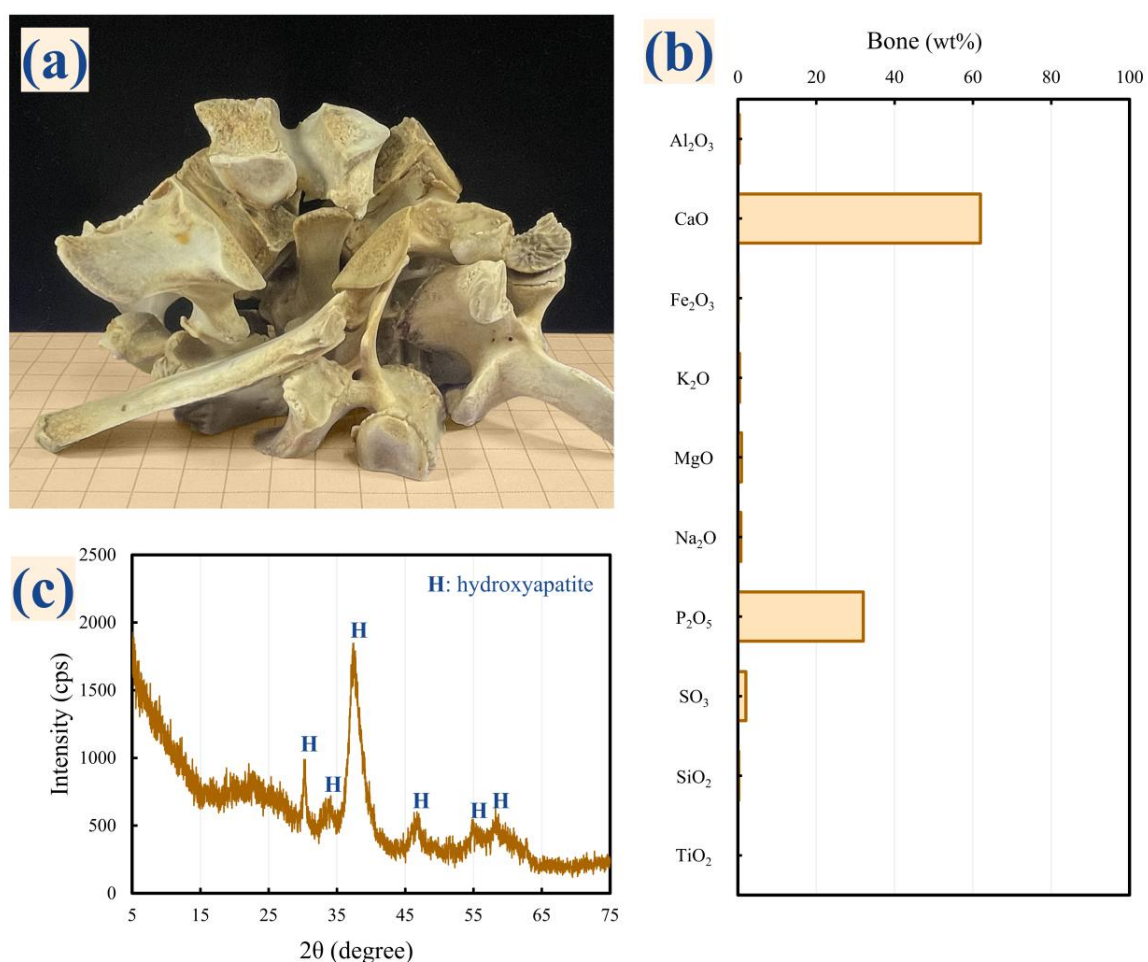


Figure 6.2 (a) depicts the appearance of the kitchen waste bone used, (b) depicts the XRF analysis of the bone powder, while (c) corresponds to XRD

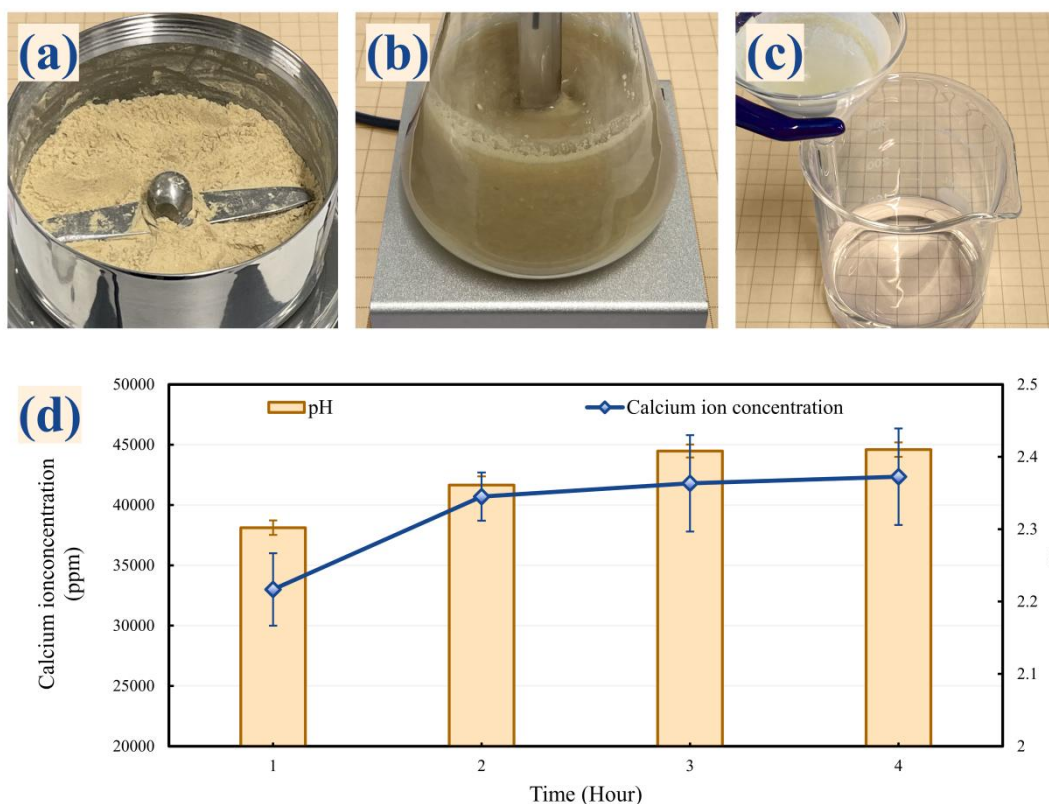


Figure 6.3 (a), (b), and (c) respectively show the processes of grinding, dissolving, and filtering during the preparation of bone extracts, while (d) monitors the pH and calcium ions during bone dissolution

6.3.2 Liquid phase experiment

To monitor the reaction progress in the liquid-phase environment and to understand the effects of microgravity on crude soybean enzyme-induced calcium phosphate precipitation, experiments were conducted under both microgravity and normal gravity conditions, as shown in Table 1. Prior to the

experiments, the crude enzyme and bone extract solutions, prepared as described above, were stored in a refrigerator at 4 °C to pre-cool the solutions. The reason for cooling process is that low temperature can delay the start of the experiment by inhibiting the activity of the enzyme, and as much as possible, the reaction can be temporarily delayed during the operation steps such as the injection of the cementation solution before the microgravity test machine provides microgravity to the sample, thereby ensuring that the experimental process can be carried out in a microgravity environment to the greatest extent possible.

Once cooled, the solutions were quickly mixed and transferred into the appropriate containers. For the samples subjected to microgravity, they were placed in a microgravity simulator, which generated a gravitational force of 10^{-3} g through three-dimensional movement. The microgravity simulator was housed in a temperature-controlled chamber set to 25 °C. The samples under normal gravity conditions followed the same protocol to ensure that all experimental variables, aside from gravity, were consistent. After 24 hours of reaction, the samples were collected and analyzed. In this study, calcium ion concentration was monitored using the Horiba LAQUAtwin Ca-11 compact calcium ion meter, and pH was measured using the Horiba LAQUA F-71 benchtop pH meter. Additionally, ammonia/ammonium concentration was determined using the indophenol spectrophotometric method based on the Berthelot reaction^{125–129}.

6.3.3 Precipitation characterization

After 24 hours, the samples were centrifuged to remove the liquid, and the precipitates were rinsed with distilled water, followed by drying in an oven at 60°C for 48 hours in preparation for further analysis. The morphology of the precipitates was examined via scanning electron microscopy (SEM) using a JEOL JSM-IT200, and energy-dispersive spectroscopy (EDS) was conducted for elemental analysis. Additionally, the crystalline structure of the precipitates and the bone was characterized using the Rigaku MiniFlex benchtop X-ray diffractometer equipped with a Co K α radiation source, with a scanning rate of 6.5°/min.

Table 6.1 *Formulations of the samples*

Case name	Gravity condition	Urea/ Ca ²⁺ ratio
MA	Microgravity	0
MB		0.25
MC		0.5
MD		0.75
ME		1
NA	Normal gravity	0
NB		0.25
NC		0.5
ND		0.75
NE		1

6.4 Results and discussion

6.4.1 Reaction Monitoring and Analysis

Figure 4 shows the pH values in the liquid-phase environment after 24 hours of reaction. It is evident that the pH increases with the rising urea content in the samples. Since the amount of added enzyme remains constant, an increase in urea implies a greater degree of hydrolysis, as described by Equation 2 and Equation 3, leading to more hydrolysis products and consequently a larger rise in pH. Notably, even in sample ME and NE, which exhibited the highest reaction extent, the pH remained close to neutral. This near-neutral pH facilitates the conversion of ammonia into its ionic form, favoring ammonia control and ammonium recovery. This phenomenon can be attributed to the residual acid from bone dissolution, which contributes to the relatively low initial pH, as well as the buffering effect of the significant protein content in the crude enzyme¹³⁰.

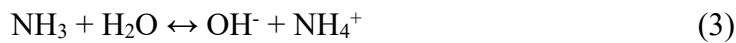
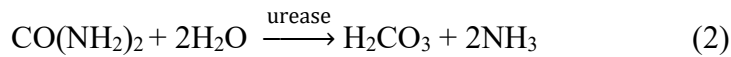


Figure 5 illustrates the calcium ion concentration in the cementation solution after 24 hours of reaction. As shown, the calcium ion concentration decreases as the urea content increases. The rise in pH resulting from increased urea reduces calcium solubility, which, in turn, promotes precipitation as outlined in the corresponding equations, consuming calcium ions. It is noteworthy that even in the sample MA and NA, where no urea was added, the pH slightly increased compared to the bone extract solution due to the addition of the crude enzyme, leading to a certain degree of precipitation and calcium ion consumption.

Figure 6 shows the concentration of ammonia/ammonium in the consolidation solution after 24 hours. Since ammonia/ammonium is a product of urea hydrolysis, its concentration increases proportionally with the urea content. Throughout the experiments, no significant differences were observed between the microgravity and normal gravity conditions in terms of pH, calcium ion concentration, or ammonia levels, suggesting the potential applicability of this method in future space applications.

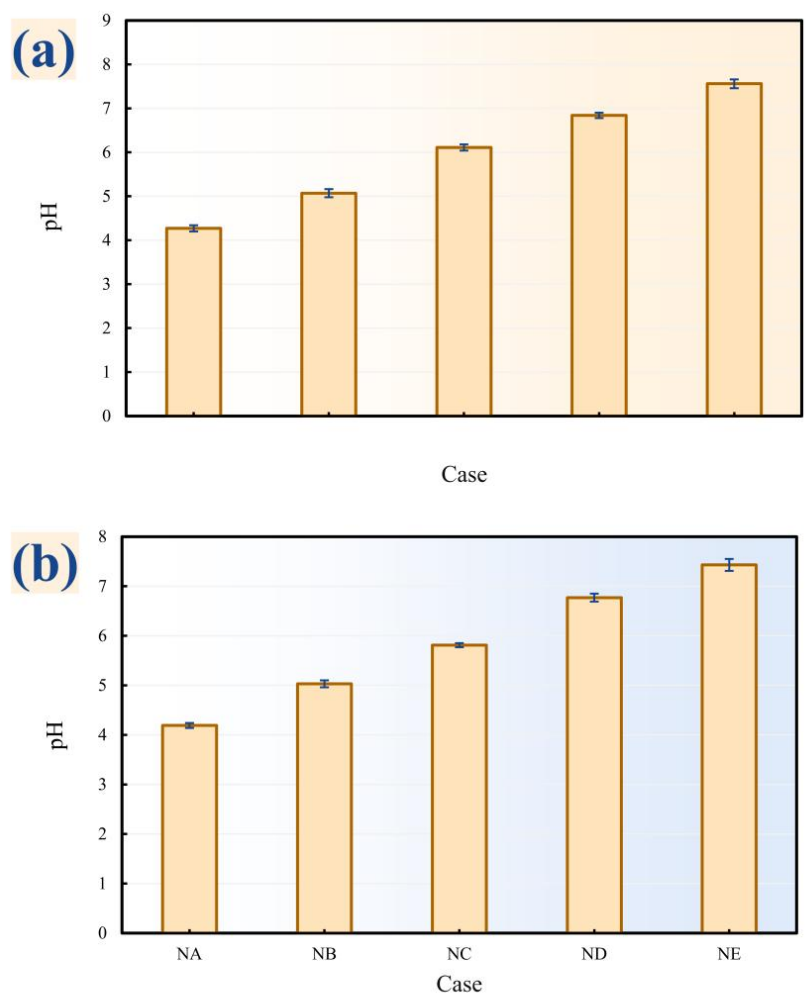


Figure 6.4 (a) and (b) show the pH monitoring of the liquid phase experiment under microgravity and normal gravity conditions, respectively.

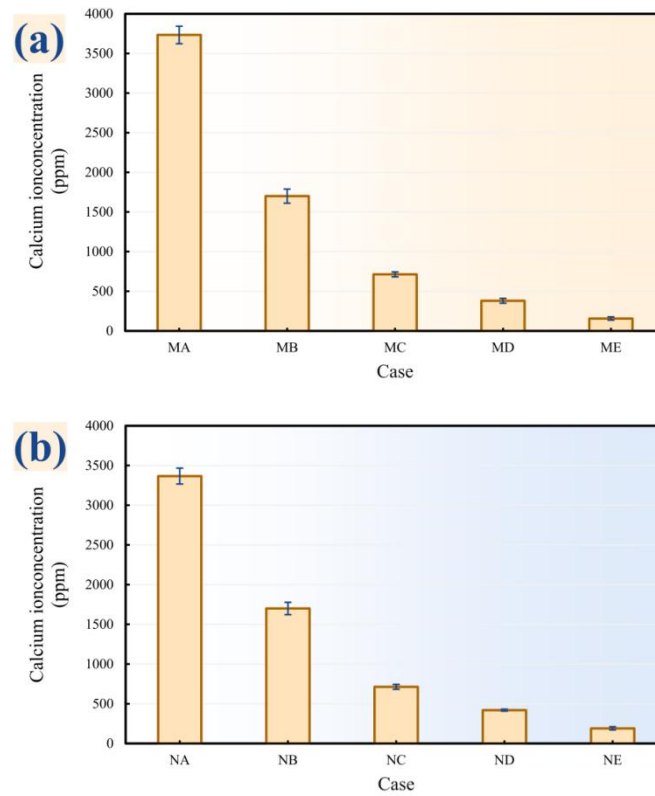


Figure 6.5 (a) and (b) show the calcium ion concentration monitoring of the liquid phase experiment under microgravity and normal gravity conditions, respectively

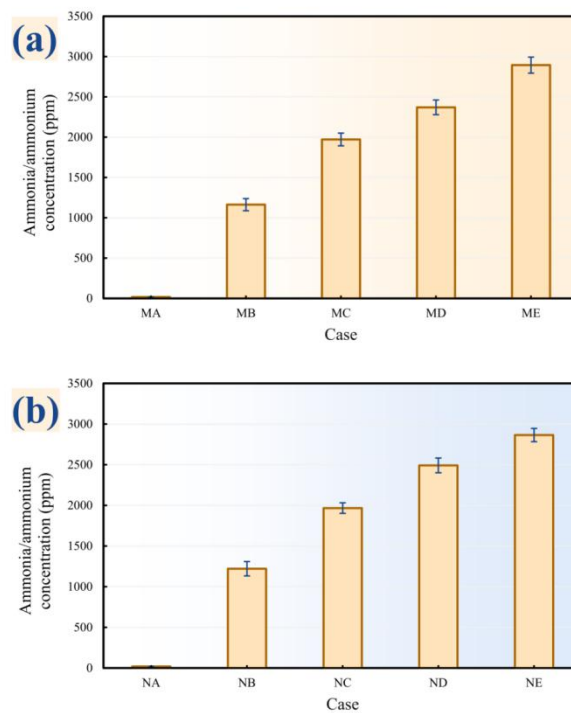


Figure 6.6 (a) and (b) show the ammonia/ammonium concentration monitoring of the liquid phase experiment under microgravity and normal gravity conditions, respectively

6.4.2 Precipitation analysis

As illustrated in Figure 7, the SEM images further confirm that the morphology of precipitates in both microgravity and normal gravity environments showed no significant differences, with the precipitates being generally small in size. When precipitate particles are small, gravity-driven convection is minimal, which explains why the reaction rates under both gravity conditions were quite similar.

The EDS and XRD analyses also revealed no significant variations. The EDS results in Figure 7 indicated that the precipitates primarily consisted of calcium, phosphorus, oxygen, and carbon. The presence of chlorine in the EDS data stems from the hydrochloric acid used during bone dissolution, and despite thorough washing, a small amount of chloride ions remained in the precipitates. XRD analysis in Figure 8 identified hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) as the dominant crystalline phase, corroborating the EDS results. Previous studies have demonstrated that hydroxyapatite, as the thermodynamically most stable crystalline phase^{131–135}, possesses higher mechanical strength than brushite¹³⁶. The transformation of brushite into hydroxyapatite typically requires high pH and elevated temperatures^{137–141}. In this study, however, the precipitated crystalline phase was directly hydroxyapatite, which may be partly due to the high concentration of soybean proteins in the crude enzyme extract, promoting hydroxyapatite formation, and partly due to the drying process in a 60°C oven, which likely favored its crystallization. Further investigation is needed to explore these factors, along with solidification experiments incorporating regolith or its simulants.

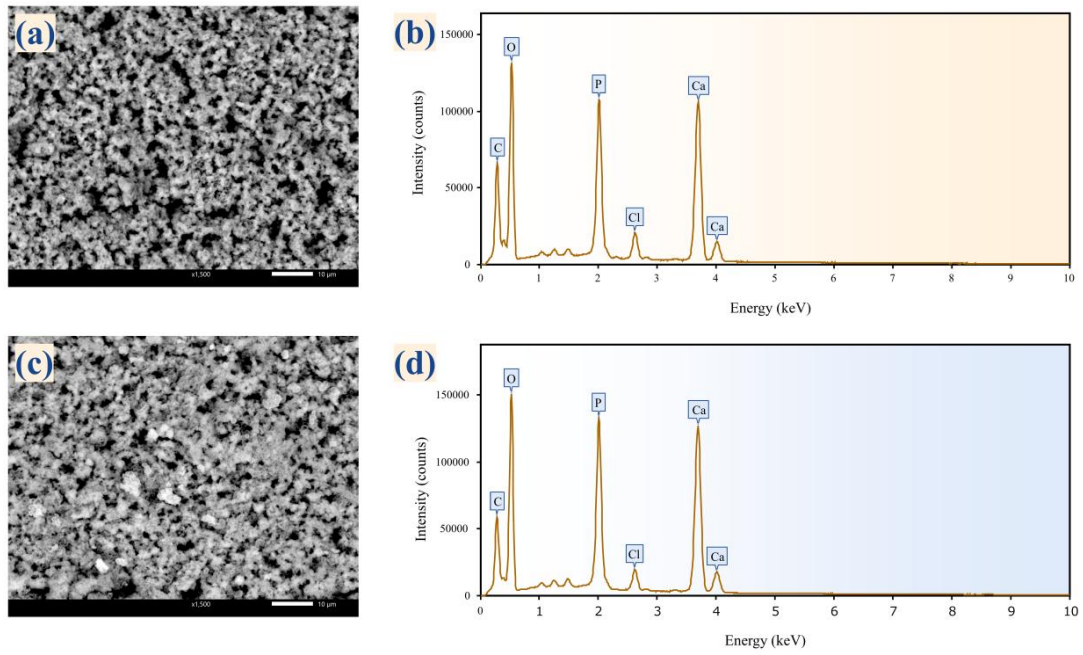


Figure 6.7 (a) and (b) show SEM and EDS analysis of the precipitate under microgravity conditions, and (c) and (d) show SEM and EDS analysis of the precipitate under normal gravity conditions

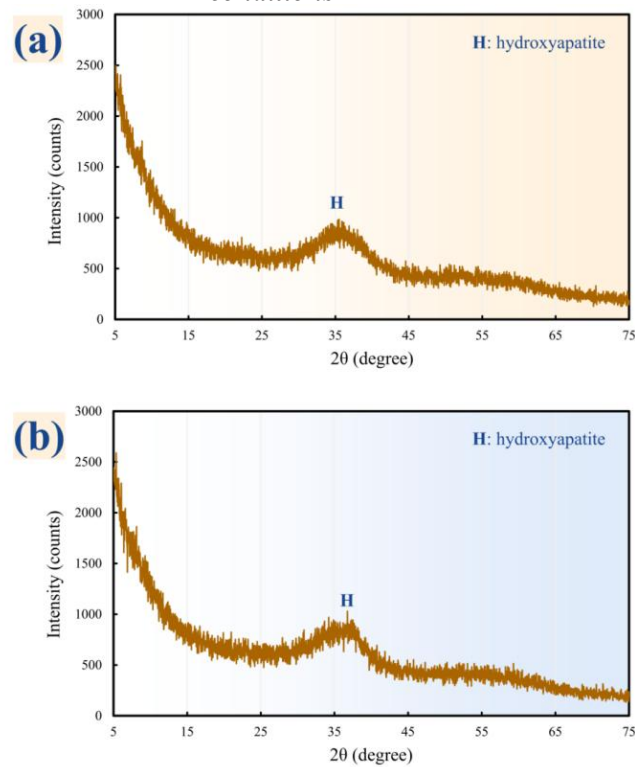


Figure 6.8 (a) and (b) show the XRD analysis of the precipitate under microgravity and normal gravity conditions, respectively

6.4.3 Future applications analysis

As humanity ventures deeper into outer space and gradually begins establishing permanent bases and colonies on the Moon and Mars, the significance of certain technologies becomes more pronounced, especially during the expansion phase following long-term extraterrestrial settlement. With the gradual growth of extraterrestrial colonies and the increase in both human and animal populations, there will inevitably be a significant generation of waste, including animal excrement and bone waste. Effective waste management is critical to ensuring resource sustainability in space missions. Integrating EICPP technology into long-term extraterrestrial settlement plans allows for the utilization of such waste as sources of urea, calcium, and phosphorus in construction processes. This not only helps reduce the costs of interstellar construction but also addresses the challenge of waste disposal in space bases, thereby enhancing the sustainability of resource utilization. The neutral pH of the EICP process helps minimize ammonia emissions and promotes the recycling of ammonium ions. It also enables the use of recycled ammonium ions for plant growth, further advancing resource circularity¹⁴². This capability is of great importance to life support and resource management systems in extraterrestrial environments.

One promising application of EICP in space base construction is the utilization of lunar and Martian regolith for building and other extraterrestrial geotechnical applications as shown in Figure 9. The high levels of radiation on the Moon and Mars make EICP technology, combined with local regolith, a potential solution for creating radiation barriers that offer sustainable protection for interplanetary bases¹⁴³. Additionally, EICP can stabilize the regolith on the Moon and Mars, reducing dust accumulation and erosion. The surfaces of these celestial bodies are covered with fine dust and

particles, which not only pose challenges for construction but also threaten equipment and human health^{144–148}. This problem is exacerbated in Mars' frequent dust storms. The accumulation and erosion of dust on these surfaces can gradually degrade the functionality of solar panels, thermal control systems, optical equipment, spacesuits, and machinery, and in some cases, may even jeopardize astronaut safety. By employing EICP to stabilize the regolith, future space missions can enhance both safety and efficiency. Unlike biocements that rely on live microorganisms, EICP uses crude enzymes, significantly reducing the risk of microbial contamination in the confined ecosystems of extraterrestrial environments, thus offering significant biosecurity advantages.

Moreover, the plants used to extract crude enzymes, such as soybeans, could bring additional benefits to interstellar bases. In space, oxygen is a precious resource that must be meticulously managed and prioritized for life support systems. During plant growth, they can provide valuable oxygen for life support. Additionally, EICPP's biogeochemical process does not require an oxygen supply, making it an ideal choice for lunar and Martian construction. Furthermore, plants can serve as a food source, enhancing the sustainability of extraterrestrial bases. Even after enzyme extraction, the protein-rich soybean residue can be repurposed as a nutritional supplement¹⁴⁹, further closing the loop on resource utilization. Beyond their functional contributions, plants can also provide psychological and emotional support. Research has shown that in closed environments, the presence of plants helps alleviate astronaut stress, mitigates the psychological challenges of isolation and harsh conditions in long-duration missions, and improves overall mental health, contributing to team morale^{150–154}. However, further research is necessary to better understand the physical and chemical properties of lunar and Martian regolith^{155–159}, and how these characteristics might influence the EICPP process. Consolidation experiments should also be conducted under microgravity conditions,

as regolith composition and behavior could impact the efficiency of biochemical processes. Understanding these variables is crucial for optimizing this technology. Additionally, larger-scale experiments simulating lunar and Martian conditions, alongside LCA analyses, are needed to validate the feasibility and cost-effectiveness of EICP for actual space missions, paving the way for its implementation in future extraterrestrial colonization efforts.

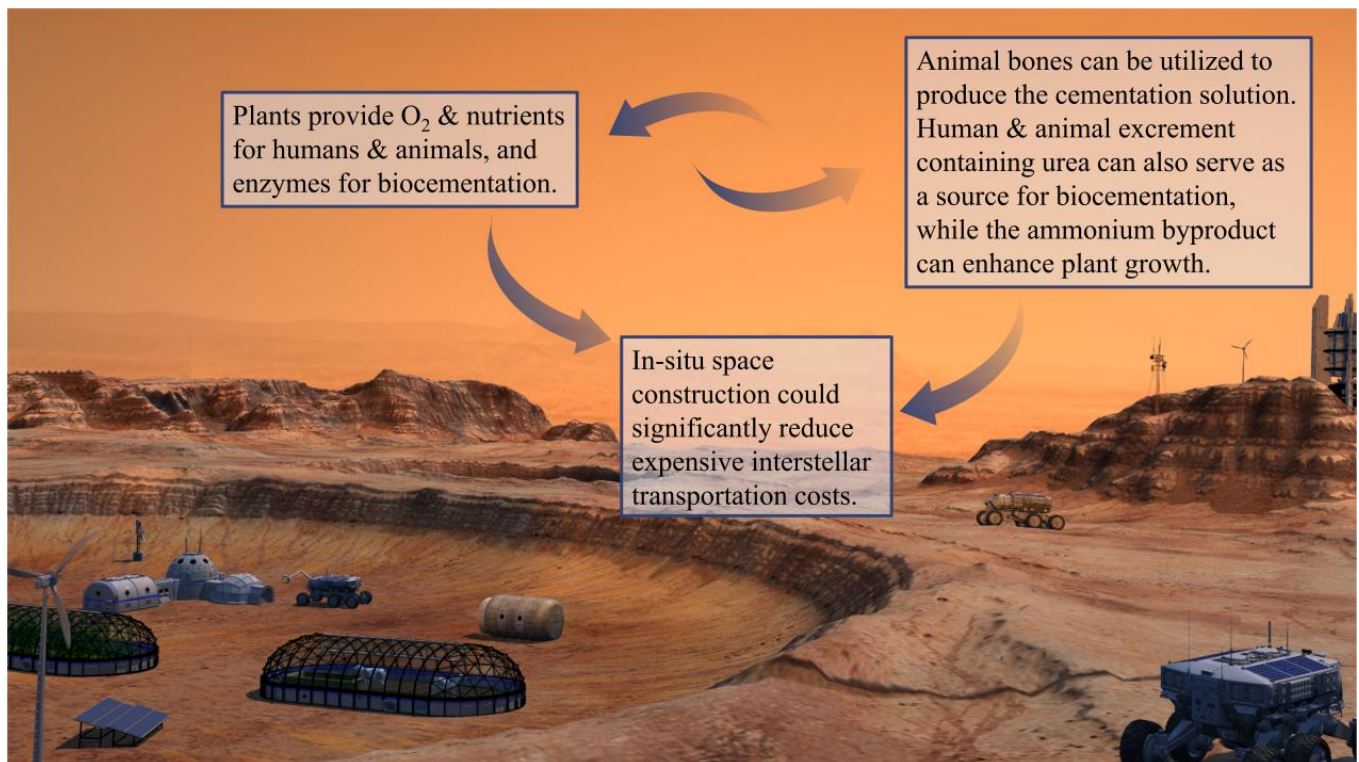


Figure 6.9 Vision of using EICPP biocement for extraterrestrial construction

6.5 Conclusion

This study demonstrates the feasibility of kitchen waste bone-driven crude enzyme-induced calcium phosphate precipitation under both normal gravity and microgravity conditions, offering promising insights for space biocementation applications. The experimental results showed no significant differences in the degree of precipitation, reaction uniformity, or microstructural characteristics between normal and microgravity environments. This suggests that EICPP is a viable solution for extraterrestrial construction, particularly during the expansion phase of human settlement in space. The utilization of waste materials, such as animal bones, for the extraction of calcium and phosphorus further highlights the potential for this technology to support sustainable resource management in extraterrestrial environments. By integrating local resources and waste materials, EICPP can reduce the need for material transport from Earth, lowering costs and minimizing environmental impacts associated with frequent space missions. Moreover, the use of a neutral pH system reduces the risk of ammonia emissions, enhancing the biosecurity of enclosed space habitats. This study underscores the potential of EICPP not only for space base construction but also for addressing waste disposal challenges, contributing to the overall sustainability and self-sufficiency of future extraterrestrial colonies.

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CHAPTER 7 Conclusion

In *Chapter 2*, a comprehensive analysis of cost-reduction strategies for biocementation was presented, marking the first integrated review of measures to lower production costs from multiple perspectives. By addressing MICP's nutrient medium, EICP's plant-based enzyme sources, and alternative urea and calcium sources, this chapter provides a holistic approach to cost-efficiency. These innovations are pivotal in advancing biocement as a commercially viable material, ensuring its competitiveness against traditional cement while reducing environmental impact.

Chapter 3 introduced a breakthrough in enhancing biocement strength by altering precipitation modes. Using waste eggshells as nucleation sites for calcium carbonate in a bridging mode enabled significant improvements in material strength within the same processing duration, marking a pioneering approach to strength enhancement without altering reaction conditions. The neutral reaction conditions, achieved without acidic additives, underscore the process's environmental friendliness and support sustainable reuse of waste materials.

In *Chapter 4*, the research explored methods to reduce biocement permeability by modifying precipitation patterns. Shell waste was used to produce pore-filling calcium carbonate, effectively reducing permeability in an acid-free, neutral reaction environment. This chapter thus demonstrates a sustainable approach to improving biocement's structural integrity and durability, while advancing waste shell recycling in construction applications.

Chapter 5 focused on improving biocement strength by transforming the crystal phase of the precipitates. Using bone waste and adjusting temperature and pH levels, this work achieved the conversion of calcium phosphate to hydroxyapatite, significantly boosting material strength at

equivalent precipitation levels. This approach presents a groundbreaking method for achieving higher strength in biocement, paving the way for broader geotechnical applications requiring high load-bearing capacity.

Finally, *Chapter 6* explored the potential of biocementation under microgravity, a first in the field. This chapter demonstrated the feasibility of crude enzyme-induced calcium phosphate precipitation in space-like conditions, laying a foundational framework for in-situ resource utilization (ISRU) in extraterrestrial construction. This advance positions biocement as a feasible and sustainable building material for future space missions, supporting long-term habitat construction and resource conservation in space.