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Biomineralization using Enzyme-induced carbonate precipitation under vacuum environment for bio-rocks formation on lunar surface

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Abstract

The utilization of lunar regolith as a construction material is a key strategy for in situ resource utilization (ISRU) in future lunar exploration. Enzyme-induced calcium carbonate precipitation (EICP) has been widely studied for soil improvement on Earth, but its performance under vacuum conditions remains unclear. In this study, soybean urease was used to investigate the feasibility of EICP under atmospheric and vacuum conditions. Urease activity, $CaCO_3$ precipitation, and the morphology of the precipitation were systematically evaluated. The results show that soybean urease maintained effective catalytic activity under both conditions, and higher soybean concentration resulted in higher urease activity. In particular, the urease activity under vacuum conditions was generally higher than that under atmospheric conditions, indicating that reduced pressure can enhance urease-catalyzed urea hydrolysis. Ca^{2+} consumption occurred more rapidly under vacuum conditions, suggesting accelerated mineralization and more efficient $CaCO_3$ precipitation. SEM observations revealed that $CaCO_3$ crystals nucleated on particle surfaces and gradually developed into aggregated clusters, confirming that enzymatic mineralization and crystal growth can proceed effectively under vacuum conditions. These findings indicate that EICP under vacuum can generate sufficient mineral binders to produce bio-rocks from lunar regolith, offering a practical foundation for future lunar infrastructure and habitat construction.

Keywords

Lunar regolith, In situ resource utilization (ISRU), Enzymeinduced calcium carbonate precipitation (EICP), Vacuum, Bio-rocks.

1 Introduction

The construction of future lunar bases will rely heavily on in situ resource utilization (ISRU), particularly the use of local lunar regolith as a building material. Lunar regolith is a loose and highly angular granular material without natural cementation, which makes it mechanically unstable in its untreated state (Cheng et al. 2023). In the lunar environment, characterized by high vacuum, low gravity, and extreme temperature variations (Wang et al. 2025), conventional cement-based binders face practical limitations due to material transport costs (Lee et al. 2026), water demand (Suhaizan et al. 2024), and energy consumption. Therefore, alternative solidification strategies that require minimal resources and operate under extreme conditions are essential for sustainable lunar infrastructure development.

The biological method has emerged as a promising soil improvement technique on Earth. Microbially induced calcium carbonate precipitation (MICP) relies on ureolytic bacteria to hydrolyze urea and generate carbonate ions, which subsequently react with calcium ions to form calcium carbonate (Tian et al. 2024). However, the use of living bacteria under extraterrestrial conditions raises concerns regarding survival, metabolic stability, and environmental adaptability (Keaney et al. 2024). Enzyme-induced calcium carbonate precipitation (EICP), which employs plant urease instead of bacteria, provides a simplified and potentially more robust alternative. By directly utilizing urease to catalyze urea hydrolysis, EICP avoids the constraints associated with microbial growth and may be more suitable for application in extreme environments (Chen et al. 2023). Among available enzyme sources, soybean urease is widely used due to its availability and relatively stable catalytic performance.

Although the lunar environment involves multiple extreme, high vacuum is the most fundamental and continuously acting condition on the lunar surface (Lyu et al. 2025). For EICP, which is inherently a solution-based reaction process, vacuum is expected to exert a direct influence on liquid stability, mass transfer, and enzyme catalytic behavior. Rapid evaporation and altered physicochemical conditions (Ma et al. 2023) may significantly affect urea hydrolysis kinetics and subsequent calcium carbonate precipitation. However, most existing EICP studies have been conducted under atmospheric conditions, and the behavior of urease under vacuum remains largely unexplored. For lunar applications, it is therefore necessary to clarify whether urease retains sufficient catalytic activity in vacuum and whether effective mineralization can still occur. These aspects are critical for evaluating the feasibility of applying EICP to lunar regolith solidification.

This study investigates the performance of soybean urease under both atmospheric and vacuum conditions. Urease activity is evaluated to determine the influence of vacuum on its catalytic behavior. The mineralization process is subsequently assessed by monitoring calcium ion consumption during EICP treatment. The morphology and elemental composition of the precipitated calcium carbonate are further characterized using scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS). Through these analyses, the feasibility of lunar regolith solidification under vacuum by EICP is evaluated, contributing to the development of EICP mineralization approaches for ISRU in future lunar infrastructure.

2 Materials and methods

2.1 Soybean urease

Urease is the key catalyst in EICP, and it can be obtained from various sources, including bacteria, plants, and purified commercial enzymes (Cui et al. 2024). Among these, plant-derived urease has received increasing attention in EICP. Soybean urease is widely used due to its natural abundance (Shu et al. 2022) relatively high enzymatic activity, and low cost compared with purified enzymes.

Soybeans used in this study were sourced from Hokkaido, Japan. The soybeans were ground using a grinder to produce soybean powder. The prepared powder was then mixed with deionized water to prepare suspensions with concentrations of 50 g/L and 100 g/L. These two concentrations were selected to evaluate the influence of soybean dosage on the extracted urease activity. The suspensions were magnetically stirred for 10 min, followed by centrifugation at 3000 r/min for 15 min to remove insoluble residues (Liu et al. 2024). After centrifugation, the supernatant containing dissolved urease was collected and used as the soybean urease solution in the EICP experiments. The extracted urease solution was stored at 4 °C prior to use to preserve its enzymatic activity.

2.2 Experimental setup under vacuum conditions

A laboratory vacuum system was used to perform experiments under reduced-pressure conditions. The system consisted of a sealed vacuum chamber (VK-ALN, ULVAC, Japan), a built-in vacuum pump, a pressure gauge, and an internal sample platform. The vacuum chamber was used to provide a stable reduced-pressure environment for both urease activity tests and $CaCO_3$ precipitation tests. The internal pressure was monitored using the pressure gauge installed on the device during the experiments.

Before each test, the prepared reaction solutions were placed in culture dishes and then positioned on the sample platform inside the vacuum chamber. After the chamber lid was sealed, the vacuum pump was started to continuously remove air from the chamber until the target reduced-pressure condition was reached. The minimum pressure attainable by the system was approximately 1300 Pa, and this pressure was used as the vacuum condition in this study. During the reaction period, the samples were maintained inside the sealed chamber under reduced pressure. For comparison, control samples were prepared using the same solution composition and reaction time but were kept under atmospheric pressure.A schematic photograph of the laboratory vacuum system is shown in Fig. 1. The system allowed the EICP reaction to be conducted under controlled reduced-pressure conditions, enabling the effects of vacuum on urease activity, Ca^{2+} consumption, and $CaCO_3$ precipitation to be evaluated.



Fig. 1 Photograph of the vacuum chamber.

2.3 Urease activity test

Urease activity was measured to evaluate the catalytic ability of soybean urease for urea hydrolysis. The activity was determined using electrical conductivity method (EC) because the hydrolysis of urea produces NH_4^+ and CO_3^{2-}, leading to a measurable increase in electrical conductivity that is proportional to the reaction rate.

In the test, 9 mL of a 1.11 mol/L urea solution was used as the reaction substrate. Subsequently, 1 mL of the urease solution was added to initiate the reaction at 24 °C. The electrical conductivity of the solution was continuously monitored using a conductivity meter under constant stirring. The change in conductivity over time was recorded, and the initial rate of conductivity increase was used to determine the urease activity.

2.4 Precipitation test

The precipitation test was conducted to evaluate the $CaCO_3$ formation during the EICP process under atmospheric and vacuum conditions. The precipitation efficiency was assessed by monitoring the variation of Ca^{2+} concentration in the solution, as the consumption of Ca^{2+} directly reflects the formation of $CaCO_3$.

In the test, the cementation solution consisted of an equimolar mixture of 0.5 mol/L urea and $CaCl_2$. A total of 9 mL of the cementation solution was mixed with 1 mL of the urease solution in the culture dishes to initiate the EICP reaction at 24 °C. The experiments were conducted under both atmospheric and vacuum conditions and maintained for 3 d. During the reaction period, the Ca^{2+} concentration in the solution was periodically measured using a Ca^{2+} meter to monitor the precipitation process.

2.5 SEM characterization test

After the precipitation tests were completed, the obtained solid precipitates were collected for microstructural characterization. Scanning electron microscopy (SEM) was used to observe the morphology and distribution of the precipitated $CaCO_3$ formed during the EICP process.

3 Results and discussions

3.1 Urease activity

The urease activity of soybean urease under different concentrations and environmental conditions is shown in Fig. 2. Overall, the urease activity exhibited a decreasing trend with increasing reaction time for both concentrations under atmospheric and vacuum conditions. However, the activity at the early stage remained relatively high, indicating that the extracted soybean urease maintained considerable catalytic capability during the initial reaction period.

Comparing the two concentrations, the urease solution prepared from 100 g/L soybean powder consistently exhibited higher enzymatic activity than 50 g/L. At the initial stage, the urease activity of the 100 g/L group was approximately 12 mM/min, whereas the activity of the 50 g/L group was around 7 mM/min, indicating that increasing the soybean powder dosage significantly enhanced the extracted urease activity.

A clear difference between atmospheric (A) and vacuum (V) conditions was also observed. For both soybean concentrations, the urease activity under vacuum conditions was consistently higher than that under atmospheric conditions. The maximum urease activity appeared at 1 h, where the 100 g/L vacuum group reached the highest value of approximately 14.5 mM/min, while the corresponding atmospheric condition showed a lower value. A similar trend was observed for the 50 g/L group, although the absolute activity values were lower. The higher activity under vacuum conditions may be attributed to the liquid rapid evaporation and the altered mass transfer environment, which may facilitate substrate interactions during the reaction.

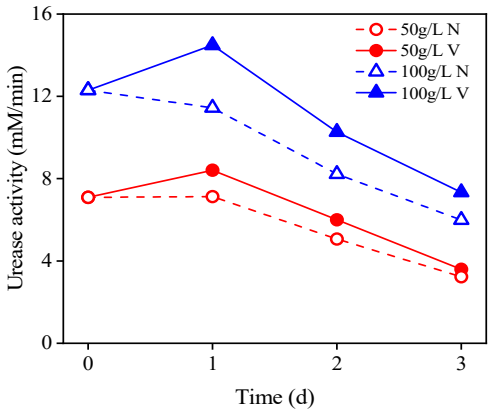


Fig. 2 Urease activity of soybean urease under different conditions.

3.2 $CaCO_3$ precipitation

The variation of Ca^{2+} concentration during the precipitation tests under different conditions is shown in Fig. 3. Overall, the Ca^{2+} concentration decreased continuously with reaction time in all experimental groups, indicating the progressive consumption of Ca^{2+} during the EICP process and the formation of $CaCO_3$ precipitates.

Comparing the two concentrations, the 100 g/L groups exhibited a significantly faster decrease in Ca^{2+} concentration than the 50 g/L groups. Within the first day, the Ca^{2+} concentration in the 100 g/L groups decreased sharply from approximately 0.38 mol/L to around 0.075 mol/L, while the decrease in the 50 g/L groups was relatively moderate. This behavior is consistent with the urease activity results discussed in Section 3.1 (Urease activity), where higher concentration resulted in higher enzymatic activity, thereby accelerating urea hydrolysis and carbonate production, which promoted the precipitation of $CaCO_3$.

For both soybean concentrations, the vacuum groups generally showed a faster reduction in Ca^{2+} concentration compared with the atmospheric groups, particularly during the early reaction stage. In the 100 g/L vacuum condition, the Ca^{2+} concentration dropped rapidly to below 0.1 mol/L within the first day, whereas under atmospheric conditions the decrease was slower. This trend suggests that the enhanced urease activity observed under vacuum conditions may have accelerated the EICP reaction and the subsequent $CaCO_3$ formation. By day 3, the gradual stabilization of Ca^{2+} concentration suggests that the system approached a reaction equilibrium as the substrates were consumed and the precipitation process became limited by the remaining reactants and urease activity.

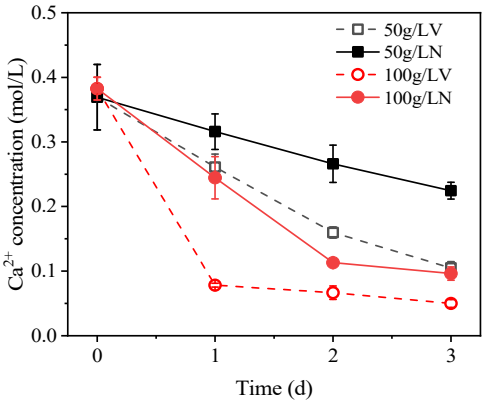
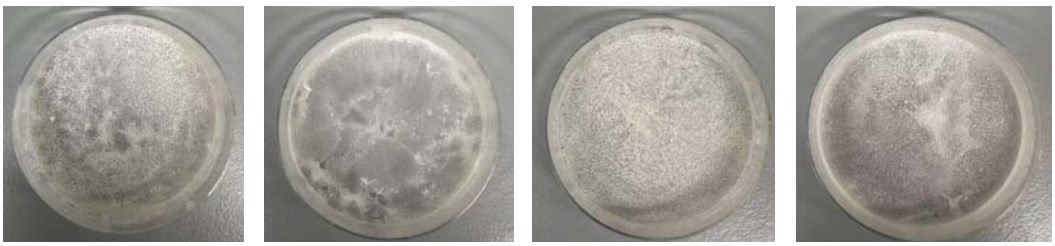


Fig. 3 Variation of Ca^{2+} concentration during precipitation tests under different conditions.

The macroscopic morphology of the precipitation products under different conditions is shown in Fig. 4. The precipitates were formed in culture dishes, indicating successful $CaCO_3$ formation through the EICP process. Compared with the 50 g/L groups, the 100 g/L samples exhibited a more uniform and denser distribution of precipitates, suggesting that higher soybean concentration promoted $CaCO_3$ formation due to higher urease activity. In addition, the vacuum samples showed relatively more continuous precipitation layers than those under atmospheric conditions, which is consistent with the higher urease activity and faster Ca^{2+} consumption observed in the previous results.



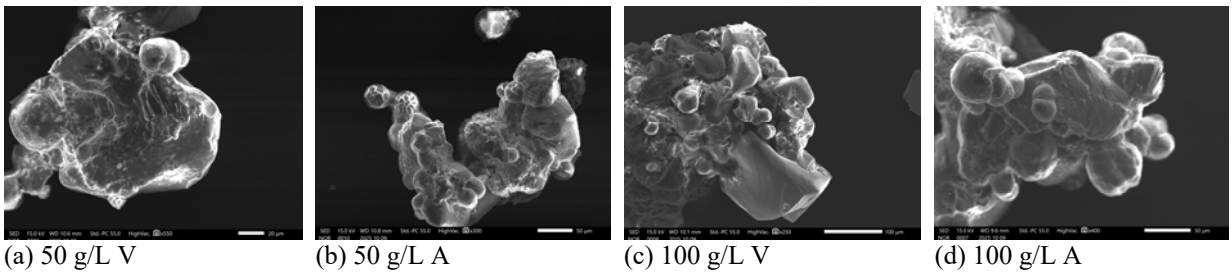
(a) 50 g/L V (b) 50 g/L A (c) 100 g/L V (d) 100 g/L A

Fig. 4 Macroscopic morphology of $CaCO_3$ precipitation formed by EICP under different conditions.

3.3 Microstructural characteristics of precipitation

The SEM images of the precipitation products under different conditions are shown in Fig. 5. In all samples, $CaCO_3$ precipitates can be observed, forming aggregated crystal clusters. The precipitates exhibit irregular and granular morphologies.

Under both concentrations, the $CaCO_3$ crystals tend to nucleate on the particle surfaces and gradually grow into clustered structures. In the vacuum samples, the crystals appear more aggregated and tend to form compact clusters. In contrast, the precipitates formed under atmospheric conditions show relatively more isolated crystal particles.



(a) 50 g/L V (b) 50 g/L A (c) 100 g/L V (d) 100 g/L A

Fig. 5 Microscopic morphology of $CaCO_3$ precipitation formed by EICP under different conditions.

4 Future perspectives for lunar bio-rock formation

Based on the findings of this study, the future work will focus on extending the EICP mineralization mechanism to the in situ solidification of lunar regolith simulant under vacuum conditions, with the ultimate goal of producing structural materials known as bio-rocks. In this approach, EICP will be employed to transform loose regolith particles into consolidated mineralized structures through controlled biomineralization processes.

Specifically, EICP solutions can be introduced into the regolith layer through infiltration or mixing. The urease catalyzes the hydrolysis of urea, generating carbonate ions that subsequently react with Ca^{2+} to precipitate calcium carbonate. These precipitates nucleate preferentially on the surfaces of regolith particles and progressively develop into mineral bridges that interconnect adjacent grains. Through repeated mineralization cycles, the continuous growth and accumulation of $CaCO_3$ can gradually establish a stable cementation network, effectively binding individual particles together and forming consolidated bio-rock structures.

The biomineralized materials could significantly enhance the mechanical integrity and stability of the regolith layer, enabling the development of functional surfaces and structural elements on the lunar terrain. Therefore, the formation of EICP-derived bio-rocks under vacuum conditions represents a promising pathway for regolith stabilization, surface hardening, and the construction of infrastructure components within future ISRU lunar engineering systems.

5 Conclusions

This study investigated the performance of soybean urease and the EICP mineralization process under atmospheric and vacuum conditions. The effects of environmental pressure on urease activity, Ca^{2+} consumption, and $CaCO_3$ precipitation were systematically evaluated. The main conclusions are summarized as follows:

(1) Soybean urease maintained effective catalytic activity under both atmospheric and vacuum conditions. The urease activity increased with soybean concentration, and the highest activity was observed under the 100 g/L vacuum condition, indicating that the vacuum environment promoted the catalytic hydrolysis of urea by soybean urease.

(2) The precipitation tests showed that Ca^{2+} concentration decreased continuously during the reaction process. Compared with atmospheric conditions, the vacuum condition generally promoted faster Ca^{2+} consumption, suggesting that the EICP reaction can proceed effectively in a reduced-pressure environment.

(3) Macroscopic observations and SEM analysis revealed that $CaCO_3$ crystals were successfully formed under all experimental conditions. The precipitates under vacuum conditions tended to form more aggregated crystal structures, demonstrating that EICP mineralization using soybean urease can occur under vacuum conditions and has potential for lunar regolith solidification in ISRU applications.

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