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RESEARCH ARTICLE

CO_2 Storage Urease Enzyme-Mediated Calcite Precipitation: Implication for CO_2 Leakage Prevention

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ABSTRACT: The increase in anthropogenic CO_2 emissions has been acknowledged as an important issue arising within our environment. In response, the Korean government announced its goals to reduce CO_2 emissions to 40% of the current level by 2030. One of the possible techniques for reducing CO_2 is geologic CO_2 sequestration also termed carbon capture and storage. This is a process aimed at mitigating the release of CO2 into the atmosphere to reduce the contribution of fossil fuel emissions to global warming and ocean acidification. The storage formation ideally should have a structure with high porosity and permeability for effective CO_2 injection. However, a layer of low permeability (cap rock) with gas-tight installations and cementing materials must be used to seal the formation to prevent CO_2 leakage. Recently, the enzyme-induced carbonate precipitation (EICP) technique has been considered as one of the solutions: ureolysis-driven $CaCO_3$ precipitation was proposed and studied to be applied *in-situ* as a sealant for treating fractures and high permeability areas in cap rocks and bore cement. In this study, calcite precipitation experiments were conducted to observe the effect of enzyme amounts on the EICP. The results show that the amount of calcite formed by the EICP increased with the increase in enzyme amounts. Calcite precipitation was visible to the naked eye within a day and relatively more rapid calcite precipitation was observed as the enzyme amounts increased. This study shows that the amount and rate of calcite precipitation increased in proportion to the enzyme amounts.

Introduction

The increase in anthropogenic CO_2 emissions is acknowledged as an important issue arising within our environment. In the case of Korea, the government has announced its goal to reduce CO_2 emissions by 2030 to 40% of 2018 levels and achieve net-zero emissions by 2050.[1] Various technologies for reducing emissions of CO_2 to mitigate climate change have attracted the interest of scientists. One of the techniques is to capture and store carbon dioxide in a deep geologic formation.

The mineral carbonation of CO_2, converting atmospheric CO_2 into carbonate minerals, has been presented as a geologically stable and environmentally safe way to store carbon dioxide.[2] The major issue with spontaneous carbonate mineral formation is that this reaction tends to have slow reaction rates and is highly dependent on pH.[3] Biological carbonate mineral formation, called microbially-induced carbonate precipitation (MICP), has recently been suggested to participate in CO_2 sequestration.[4] This biological process is commonly involved in the hydrolysis of urea by the urease-producing bacteria.

On the other hand, the increasing demand for cement brings along environmental concerns because cement production is a large source of carbon dioxide emissions. There has been a large amount of research to find substitutes for cement that utilizes more eco-friendly sources. Biocementation is one method presented as a substitute, which includes the sustainable use of free urease enzymes. Enzyme-Induced *Carbonate Precipitation* (EICP) is the process of precipitating calcium carbonate utilizing a free urease enzyme, one of the innovative engineering uses of urease. Because no living organism is actively engaged in the precipitation process, EICP is considered a biomimetic method. The calcium carbonate precipitate formed from this process can effectively sequester and reduce the leakage of CO_2 from storage formations by plugging pores.

Urease-aided $CaCO_3$ mineralization, or urinalysis-driven $CaCO_3$ mineralization, depends on the activity of urease. The benefit of this process is that, by reproducing $CaCO_3$ bio-formation in nature, the process can be performed in an environmentally benign manner and, importantly, in field applications. Accordingly, the process offers exciting innovative potential in multiple engineering fields such as geotechnical engineering, bioremediation for heavy metals, plugging oil reservoir bedrocks for the enhancement of oil recovery, and reducing leakage in geologic CO_2 sequestration.[5,7] Two methods of ureolysis-driven calcite precipitation are summarized in Table 1.[8]

Table 1: The types of biomineralization of calcium carbonate

Type	Microbially-Induced Carbonate Precipitation (MICP)	Enzyme-Induced Carbonate Precipitation (EICP)
Condition	microbial organism	a free urease enzyme
Source	soil bacterium	extracted from plants (commonly jack beans)
Advantage	- widespread usage - unaffected by temperature	- commercial availability - smaller molecular size - efficient penetration of urease - natural degradation

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Disadvantages	- expensive (bacteria) - potential cause of bioplugging - lower durability of formed precipitate	restricted usage

Based on previous research on Enzyme-Induced Carbonate Precipitation (EICP), the objectives of this study are 1) examining the effect of the concentration of the enzyme in a solution with constant concentrations of urea and calcium chloride and 2) understanding calcite precipitation rate and morphology of the precipitates in a laboratory environment. These experimental data can be used as basic data for applying EICP to the reduction of CO_2 leakage in geologic CO_2 sequestration.

Theoretical Background (Urease-aided calcium carbonate mineralization):

The enzyme-induced carbonate precipitation (EICP) is triggered by the catalytic action of urease in the hydrolysis of urea. The products of the reaction are carbonic acid and ammonia (1):

$$H_2N\text{-}CO\text{-}NH_2 + 2H_2O \xrightarrow{\text{Urease}} H_2CO_3 + 2NH_3 \quad (1)$$

The products equilibrate in water to give bicarbonate, ammonium, and hydroxide ions, respectively:

$$H_2CO_3 \leftrightarrows HCO_3^- + H^+ \quad (2)$$

$$NH_3^+ H_2O \leftrightarrows NH_4^+ + OH^- \quad (3)$$

The production of hydroxide ions from reaction (3) brings about an increase in pH, which in turn leads to the formation of carbonate ions:

$$HCO_3^- + OH^- \leftrightarrows CO_3^{2-} + H_2O \quad (4)$$

In the presence of dissolved Ca^{2+}, the ions combine and calcium carbonate precipitates:

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3(s) \quad (5)$$

The overall process can thus be presented:

$$H_2N\text{-}CO\text{-}NH_2 + 2H_2O + Ca^{2+} \xrightarrow{\text{Urease}} 2NH_4^+ + CaCO_3(s) \quad (6)$$

As shown in the precipitation process, 1 mole of urea produces 1 mole of calcite.

Methods

For this study, two types of solutions were prepared:

1) a urease solution using a urease enzyme (U1500, Sigma Aldrich) and

2) an equimolar urea-$CaCl_2$ solution using urea (U5378, Sigma Aldrich, St. Louis, Mo, USA) and $CaCl_2$ (C3881, Sigma Aldrich).

The urease solutions were prepared at concentrations of 1000 µL, 2000 µL, and 3000 µL by dissolving the urease enzyme into distilled water, while the urea-$CaCl_2$ solutions were prepared at concentrations of 0.5 M. To identify the effect of concentrations of the enzyme on the production of calcite, each urease solution was mixed with 0.5 M urea-$CaCl_2$ solutions to precipitate calcite.

In this experiment, 20 mL of urea-$CaCl_2$ solution and 20 mL of urease solution were mixed in conical tubes to make a total solution volume of 40 ml. A total of 20 samples were prepared for the batch experiments and one blank sample without the enzyme was also tested. Those samples were reacted at 30 °C in a water bath without agitation. During the experiment, the mass of precipitated calcite was measured from a selected tube at 1 h, 3h, 5 h, 8 h, 15 h, 24 h, 48 h, and 72 h. The tubes were centrifuged at 3000 rpm for 10 minutes to separate the remaining liquid and precipitate. After removing the supernatant, the tubes were dried in an oven at 50°C for 48 hours, and the amount of precipitated calcium carbonate was determined by measuring the change in the mass of the tubes before and after the experiment. To identify the precipitated mineral, the precipitates were recovered and analyzed by scanning electron microscopy with energy-dispersive spectroscopy **(SEM-EDS)**.

The process of this experiment can be summarized in Figure 1.

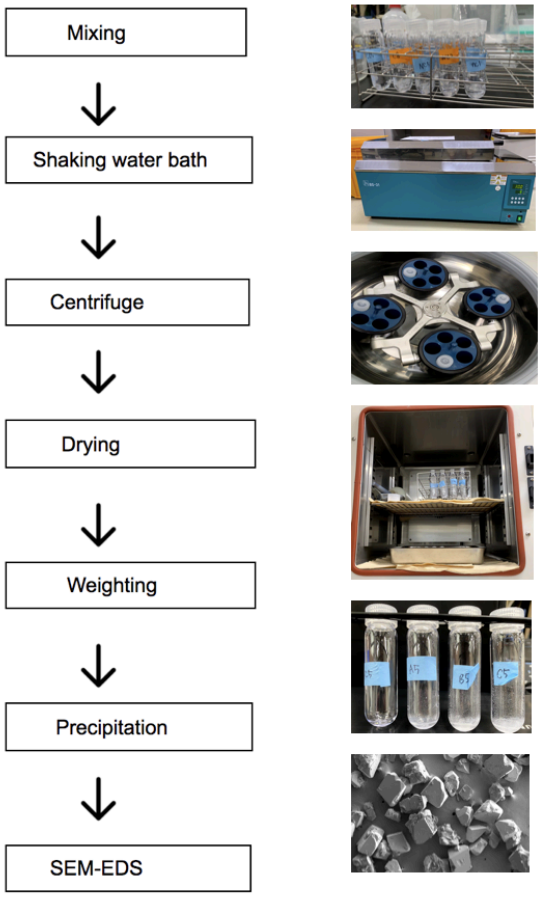

Figure 1: Schematic procedure and figures of EICP experiment. From these experiments, we determined the calcite amounts and observed morphology.

Results and Discussion

1) The change in the amount of calcite precipitation according to enzyme concentration:

Based on the method above, the results of measuring the mass of the blank bottle and calcite precipitated bottle according to three different enzyme concentrations are shown in Table 2. As the amount of enzyme increases, precipitation

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rates increase, the amount of calcite precipitated increases in proportion. One hour after the experiment, 0.0011 g was precipitated at 1000 μL enzyme concentration (hereinafter, concentration A), while 0.0151 g was precipitated at 3000 μL enzyme concentration (hereinafter, concentration C). This shows that the C concentration is about 1,372-fold higher than the A concentration. This tendency shows a similar trend in the 24 hours (546-fold) and 72 hours (386-fold) experimental results. The maximum difference is 1,728-fold, which is after 15 hours, and the minimum difference is 386-fold, which is after 72 hours (Figure 2).

Table 2: Experiment results of calcite precipitation depending on the amounts of enzyme concentrations (unit= g)

	A (1000 μL)			B (2000 μL)			C (3000 μL)		
	Blank*	B+P**	Net value	Blank	B+P	Net value	Blank	B+P	Net value
1hr	21.5658	21.5669	**0.0011**	21.6865	21.7001	**0.0136**	21.6855	21.7006	**0.0151**
3hr	21.8677	21.8708	**0.0031**	22.0082	22.0379	**0.0297**	21.891	21.9297	**0.0387**
5hr	21.6681	21.6834	**0.0153**	21.5147	21.5801	**0.0654**	21.8089	21.9001	**0.0912**
8hr	22.0029	22.0358	**0.0329**	21.5273	21.6884	**0.1611**	22.0003	22.1912	**0.1909**
15hr	22.0383	22.0490	**0.0107**	21.6929	21.8675	**0.1746**	21.6967	21.8817	**0.1850**
24hr	21.6924	21.7772	**0.0848**	21.8385	22.1581	**0.3196**	21.8771	22.3402	**0.4631**
48hr	21.5614	21.6999	**0.1385**	21.6905	22.1082	**0.4177**	21.6527	22.2484	**0.5957**
72hr	21.6790	21.8712	**0.1922**	22.0613	22.5812	**0.5199**	21.8932	22.6365	**0.7433**

*blank bottle
**calcite precipitation bottle

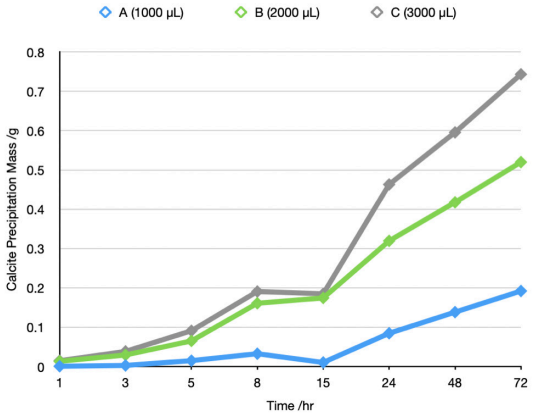

Figure 2: Changes in calcite precipitation according to three different enzyme concentrations.

The precipitate is white and exists as suspended particles in water. Calcite precipitation was visible to the naked eye within a day: 24 hours for concentration A, 15 hours for concentration B, and 8 hours for concentration C (Figure 3). Calcite precipitation occurred more rapidly as the enzyme concentration increased.

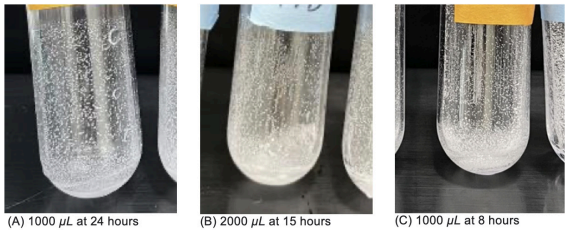

Figure 3: Calcite precipitation depending on the concentrations of enzymes.

2) The change in the amount of calcite precipitation according to reaction time:

According to reaction equation 6, 0.5M CaCl2 -urea solution must produce 0.5M calcite under equilibrium conditions. However, in the equilibrium state of the current experiment, the theoretical precipitation amount of 0.5M calcite is 2g. After 72 hours, the maximum precipitation at concentration C was 0.7433g, which is about 37% of the theoretical value.

Moreover, the three different enzyme concentrations show different trends over time. Based on the test results, concentration A showed the lowest precipitation rate whereas concentrations B and C showed relatively rare. At concentration A, the change in mass of precipitation is 0.01 g from 0 to 15 hours, 0.08 g from 15 to 24 hours, and 0.13 g from 24 to 48 hours. The difference between the change in precipitation mass in the 0 to 15 h range and the 24 to 48 h range is about 8 times. From the 15 to 24 h range to the 24 to 48 h range, the precipitation rate decreases rapidly by about 1.6 times. However, for concentration C, the precipitation mass increased by 0.18 g from 0 to 15 hours, 0.46 g from 15 to 24 hours, and 0.59 g from 24 to 48 hours. The change in mass during the 15 to 24 h increased 2.5 times faster than the 0 to 15hr range, while the mass change within the 24 to 48 hr. range was 1.3 times faster than the 15 to 24 hr. range. This shows a slight decrease in the rate of precipitation as the reaction proceeded. Nonetheless, the experimental results show that the amount and rate of calcite precipitation increases according to the increase in the enzyme concentration. This suggests that the concentration of the enzyme is not enough to completely hydrolyze the 0.5 M urea in this study.

In a previous study, it was also found that an increase in urease concentration enhanced the rate of $CaCO_3$ precipitation.[12]

3) Morphology of calcite:

Figure 4 shows the morphology of calcite after 72 hours of precipitation under the condition of concentrations A, B, and C respectively. Noticeable differences in the calcite morphology due to the change in concentration were not observed within this experiment. For further applications of calcite outside of the experiment setting, more experiments are needed to test the stability and persistence based on the size and morphology of calcite when filling the crack of subsurfaces.

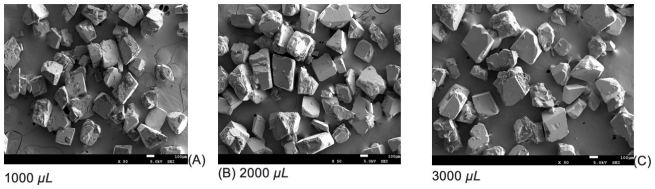

Figure 4: SEM-EDS Image of calcite after 72 hours of precipitation for different enzyme concentrations.

4) Implications for future experiments:

Factors that influence the precipitation process primarily include the concentration of urease and the concentrations of urea and Ca^{2+}, in addition to environmental factors such as temperature and salinity.[11,13]

In this regard, for a given concentration of urease, there would be optimal urea and Ca^{2+} concentrations. Yet, these

benefits are counterbalanced by the detrimental effects including the accumulation of salts and urea in the material. The temperature was also found to affect the rate of $CaCO_3$ precipitation in the enzymatic process, and practically not influence the bacterial process.[13]

Regardless of using MICP or EICP, the ureolysis-driven calcite precipitation produces the same by-products. One of which is the ammonium ion NH^{4+} resulting from the hydrolysis of urea (Equations. (3) and (6)). The ion poses an ecological risk that includes NH_3 toxicity to humans and acidification arising from NH_3 nitrification, in addition to stone discoloration.[14] These effects, however, can be minimized by using low and balanced urea concentrations or exchanged zeolite.[15]

Conclusion

In our experiment study, calcite precipitation experiments were conducted to observe the effect of enzyme amounts on the EICP.

1) The amount of calcite formed by the EICP increased with the increase in enzyme concentrations. Concentration C (3000 μL) resulted in a 550 times greater amount of calcite formation compared to concentration A (1000 μL) at 24 hours and about 387 times greater at 72 hours.

2) Calcite precipitation was white-colored, suspended particles, clearly visible to the naked eye within a day. Faster calcite precipitation was observed as the enzyme amounts increased.

3) There were no significant differences observed in the calcite morphology due to the change in enzyme concentration.

This study showed that the amount and rate of calcite precipitation increased in proportion to the enzyme amounts. Further studies need to identify the effects of other factors such as urea and calcium concentrations, temperature, and salinity on EICP to find optimal enzyme concentrations for practical applications of the technique.

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