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Utilization of Bio-Mineral Carbonation for Enhancing CO_2 Sequestration and Mechanical Properties in Cementitious Materials

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Abstract: Microorganisms can perform mineral carbonation in various metabolic pathways, and this process can be utilized in the field of construction materials. The present study investigated the role of bio-mediated mineral carbonation in carbon sequestration performance and mechanical properties of cementitious materials. Bacterial-mediated ureolysis and CO_2 hydration metabolism were selected as the main mechanisms for the mineral carbonation, and a microorganism, generating both urease and carbonic anhydrase, was incorporated into cementitious materials in the form of a bacterial culture solution. Four paste specimens were cured in water or carbonation conditions for 28 days, and a compressive strength test and a mercury intrusion porosimetry analysis were performed to investigate the changes in mechanical properties and microstructures. The obtained results showed that the pore size of the specimens incorporating bacteria was reduced by the precipitation of $CaCO_3$ through the mineral carbonation process, thereby improving the mechanical properties of the paste specimens, regardless of the curing conditions. In addition, in the case of the paste specimens cured in carbonation conditions, more amorphous $CaCO_3$ was observed and a larger amount of $CaCO_3$ in the specimens incorporating the bacteria was measured than in the specimens without bacteria. This is attributed to promotion of the inflow and diffusion of CO_2 via mineral carbonation through bacterial CO_2 hydration metabolism.

Keywords: mineral carbonation; carbonic anhydrase; CO_2 sequestration; CO_2 hydration; ureolysis process

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1. Introduction

With the advent of the industrial revolution, carbon dioxide emissions have increased due to the use of fossil fuels, which is a major cause of global warming. To reduce the carbon dioxide (CO_2) emission, numerous studies on carbon capture, utilization, and storage (CCUS) technology is being conducted. In particular, development of the technologies for mineral carbonation process using biological reaction has been attracted, since conventional chemical catalysis reaction for carbonation requires high energy and heat. The mineral carbonation accelerates the natural process of storing carbon in a carbonate mineral form, and various minerals, such as dolomite, calcite, magnesite, aragonite, and vaterite, can be formed through chemical bonding with various metal ions [1,2]. In particular, in the field of construction materials, carbonate minerals can be used to improve mechanical properties and secure long-term durability by sealing voids or cracks. The mineral carbonation mechanism in cementitious materials can proceed by carbonation curing, which introduces CO_2 gas into the interior of cementitious materials in the hydration and hardening period, thereby causing changes in the type of hydrates and microstructure [3,4]. Carbonation of concrete at an early age can generate calcium-silicate-hydrate (C–S–H) gel or calcium carbonate ($CaCO_3$) by reacting cement clinkers with CO_2, providing sequestration of CO_2 and improving the strength at the early age [4–7]. In addition, the carbonation of concrete

can further generate $CaCO_3$ by decalcifying the hydrates (i.e., $Ca(OH)_2$, C–S–H gel), which can lower the alkalinity of the pore solution, causing corrosion of rebars in reinforced concrete [8–10].

Meanwhile, the mineral carbonation mechanism can be realized through various bacterial metabolisms generating carbonate ions, such as ureolysis, denitrification, CO_2 hydration, and a sulfate reducing mechanism [11]. These metabolisms can seal cracks by precipitating the carbonate minerals on the bacterial cell surface, which act as nucleation sites, by combining the carbonate ions with Ca^{2+} [11]. In particular, the ureolysis process generates carbonate ions (CO_3^{2-}) from urea using the enzyme of ureolytic bacteria, and the CO_3^{2-} can be sequestrated in a form of $CaCO_3$ in cementitious materials. The ureolytic bacteria are activated by the inflow of air and moisture into the cracks in the cementitious materials [12], and can perform the metabolism in an aerobic environment, and have relatively rapid growth rate and metabolic activity [12]. Accordingly, they have been widely used in studies aimed at improving the durability of concrete structures through bacterial mineral carbonation. Nevertheless, this mechanism may raise environmental concerns as a result of producing an excessive amount of ammonium ions (NH_4^+). As an alternative method for mineral carbonation, CO_2 hydration through bacterial induced carbonic anhydrase (CA) has recently garnered attention.

The bacterial induced CA is a metalloenzyme that contains zinc as a cofactor for binding CO_2 in active sites, and facilitates the process of CO_2 hydration for generating the carbonate ions [13]. The incorporation of bacteria, expressing the CA, into cementitious materials can facilitate the diffusion of CO_2 in carbonation-curing condition and can sequestrate the CO_2 by converting CO_2 into carbonate minerals [14]. In addition, this phenomenon may secure improved mechanical properties by generating a stable carbonate mineral in cementitious materials. Qian et al. (2015) [15] verified that 0.3 mm size cracks were completely filled after five days of curing by incorporating CA of *Bacillus mucilaginous* L3 into cement-based materials, which decreased the water permeability coefficient. Nevertheless, little research on the effect of CO_2 hydration by bacteria CA on CO_2 sequestration performance, the changes in the microstructure, and the mechanical properties of cementitious materials has been conducted.

In the present study, the mineral carbonation mechanism based on bacterial CO_2 hydration metabolism was proposed to improve the carbon sequestration performance and mechanical properties of cementitious materials. A microorganism capable of both ureolysis and the CO_2 hydration process was used. The mineral carbonation processes were introduced, and the performance of each mechanism in cementitious materials was investigated. To evaluate the effect of the bio-mediated ureolytic process and CO_2 hydration on the microstructure and mechanical properties of cementitious materials. Specimens incorporated with and without a bacterial culture solution were cured in water or carbonation conditions, and subsequently pH evolution, a compressive strength test, a mercury intrusion porosimetry (MIP) analysis, and a thermogravimetric analysis were performed.

2. Experiment Procedures

2.1. Characterization of Bio-Mediated Ureolysis and CO_2 Hydration Metabolism

Urease and CA, which are bacterial enzymes, could offer CO_3^{2-} for precipitating carbonate minerals [11]. The CO_3^{2-} could be generated from urea and CO_2 source through a bio-mediated ureolysis and CO_2 hydration process, respectively. In this study, bacterium SAML2018, which has both enzymes and was isolated in river sediment, belongs to *Bacillus* sp. and can form an endospore. This bacterium was isolated in a previous study [16], and was used for simultaneously estimating the effects of ureolysis and the bio-mediated CO_2 sequestration process on hydration and mechanical properties of cementitious materials. To investigate the ureolytic activity, SAML2018 was incubated in a TSB-urea medium containing 3% tryptic soy broth (TSB, Becton Dickinson, Franklin Lakes, NJ, USA) and 2% urea, and the changes of NH_4^+ concentration in the culture solution overtime were

measured by means of ion chromatography using a Metrohm 930 Compact IC Flex (IC, Metrohm, Riverview, Herisau, Switzerland).

Meanwhile, the activity of bio-mediated CO_2 hydration could be determined by measuring the amounts of produced p-nitrophenol through esterase activity estimation. This was accomplished by measuring the increase of absorbance at 405 nm via a microplate reader (Tecan Ltd., Manneedorf, Switzerland), since 1 mole p-nitrophenyl acetate could be decomposed to 1 mole p-nitrophenol (yellow color) and 1 mole acetate by enzymatic catalysis of CA. The esterase activity of CA was estimated by using a colorimetric carbonic anhydrase activity assay kit (BioVision Inc., Milpitas, CA, USA). SAML2018 was incubated in a TSB-$ZnSO_4$ medium containing 3% TSB and 1mM zinc sulfate ($ZnSO_4$, Sigma-Aldrich, St. Louis, MO, USA) for activating the CA, since the CA is a metalloenzyme requiring Zn as a cofactor. To extract the CA from SAML2018, cell wall lysis of the bacterial pellet and a purification process were conducted via homogenization through the addition of 0.1-mm glass beads and Tris-EDTA buffer to a BioSpec 3110BX Mini-BeadBeater (Biospec Products, Bartlesville, OK, USA). In order to identify the conditions for obtaining the highest enzyme effects, the specific activity of CA (unit/mL) was calculated from the amount of p-nitrophenol obtained by carbonic anhydrase activity assay protocol.

2.2. Specimen Preparation and Characrization Test

In order to investigate the impacts of bio-mediated ureolysis and CO_2 hydration metabolism on hydration kinetics and mechanical properties of cement-based materials, the Portland cement paste specimens incorporating SAML2018 culture solutions for ureolytic and CO_2 hydration metabolism were prepared. Details of the mix proportion of the paste specimens are tabulated in Table 1. All paste specimens were cast into $50 \times 50 \times 50$ mm^3 molds, and demolded after 2 days of air-curing at 25 °C. Thereafter, A and B paste specimens were immediately immersed in water at 25 °C, since bacterial metabolic performance could be more activated in environment with adequate moisture and air. On the other hand, C and D paste specimens were placed in a carbonation chamber with atmospheric CO_2 concentration of 3%, at 25 °C, RH 60%. The accelerated carbonation condition was set at 3% CO_2, known as the CO_2 concentration leading to mineralogical changes of the cementitious materials during cement hydration [17]. A and C specimens are used as a control sample under water curing and carbonation conditions, respectively. In addition, the specimen for identifying physicochemical properties was cured in the carbonation chamber after being crushed to about 3–5 mm for smooth CO_2 supply to the inside of the cementitious materials. Carbonation coefficients were calculated by Equation (1) [18].

$$\text{Carbonation coefficieint} = \frac{D_t - D_0}{\sqrt{t}} \quad (1)$$

where D_t and D_0 display the carbonation depth (mm) after t times of carbonation-curing and initial carbonation depth, respectively.

Table 1. Mix proportions of paste specimens by mass ratio.

Specimen ID	Cement	Water	Ureolysis Solution *	CO_2 Sequestration Solution **	AE Agent ***	Curing Condition
A	1	0.4			0.001	Water
B	1		0.4		0.001	curing
C	1	0.4			0.001	Carbonation
D	1			0.4	0.001	curing

* Ureolysis solution is cultural medium of bacteria incubated in 30 °C for 24 h. The solution consisted of 3% TSB, 2% urea, 0.3% bacterial paste. ** CO_2 hydration solution is cultural medium of bacteria incubated in 30 °C for 24 h. The solution consisted of 3% TSB, 1mM $ZnSO_4$, 0.3% bacterial paste. *** AE Agent: Air entraining agent.

The mechanical and physical properties of the paste specimens were investigated by a compressive strength test and MIP analysis. A universal testing machine (Dongwoo DNS, Gwangmyeong, Korea) was used for the compressive strength test after 7, 14, and 28 days of curing in accordance with ASTM C109 [19]. The compressive strength test was conducted in triplicate of all specimens. The MIP analysis was performed after 28 days of curing for measuring the pore size diameter and porosity of all specimens, by using the AutoPore IV 9500 (Micromeritics Corp., Norcross, GA, USA) installed at Korea Basic Science Institute Jeonju Center. All samples for the MIP analysis were prepared at sizes of 3–5 mm, and the pressure range of 1–60,000 psia was used for MIP analysis. For investigating pH evolution, thermal behavior, and hydration products of the paste specimens, all specimens were crushed to have particle sizes smaller than 76 μm after 7, 14, and 28 days of curing. The hydration was arrested at designated testing days by the solvent exchange method [20] in accordance with RILEM TC-238 SCM recommendation. The pH evolution was measured via using a portable pH meter (Mettler Tolredo, Columbus, OH, USA), and a mineralogical analysis of hydration products was conducted by using an Empyrean X-ray diffractometer (XRD, Malvern Panalytical B.V., Almelo, The Netherlands) upon being loaded on a Cu-Kα radiation with a tube current of 30 mA, installed at Smart Open Lab in Korea Basic Science Institute Daejeon Center. The thermal decomposition of hydration products was observed through a Thermogravimetry/Differential thermogravimetry (TG/DTG), which was performed via using a thermogravimetric analyzer TGA/DSC1/1600 LF (Mettler-Toledo, Columbus, OH, USA) with a temperature range of 25–1000 °C. In addition, quantitative estimation of hydration products after 7, 14, and 28 days of curing were determined by calculating the weight loss value corresponding to a particular product.

3. Results and Discussions

3.1. Evaluation of Ureolysis and CO_2 Hydration Performances

Bio-mediated mineral carbonation is based on various mechanisms for generating the CO_3^{2-}. The most actively used mechanisms are hydrolysis of urea and CO_2. The urea hydrolysis process is a mechanism by which 1 mol of urea is decomposed into 1 mol of NH_4^+ and CO_3^{2-} by urease, as a bacterial inherent enzyme; hence, the ureolytic activity can be determined by measuring the NH_4^+ concentration [21]. On the other hand, CO_2 hydrolysis is a mechanism by which CO_2 becomes HCO_3^- by CA, as a bio-induced enzyme, but the CA can decompose p-nitrophenyl acetate into yellow colored p-nitrophenol; the capacity of CA thus can be inferred by measuring the amount of p-nitrophenol [22].

Figure 1 displays the changes in optical density, and the amounts of produced NH_4^+ and p-nitrophenol in the bacterial culture solution over time. The bacterial growth rate over time can be determined by the measuring optical density from the turbidity of the bacterial culture solution. The bacteria reached the exponential growth phase in 24 h after a lag period of first 12 h (Figure 1a). It can be inferred that the bacteria began active production of urease and CA after 12 h. Therefore, the amounts of NH_4^+ and p-nitrophenol produced by urease and CA were the highest at 14 mmoles and 700 nmoles after 48 h of incubation, respectively (Figure 1b).

In order to investigate the amount of CA produced according to bacterial growth time and catalytic reaction rate, the CA was extracted at each time and the amount of p-nitrophenol produced according to the contact time between the CA and p-nitrophenyl acetate was measured. Figure 2 shows the enzyme reaction rate and the specific activity of CA extracted from SAML2018 after 12, 24, 36, and 48 h of incubation. The reaction between CA extracted at each incubation time and p-nitrophenyl acetate reached an equilibrium state after 60 min, and the catalytic reaction rate was highest in the CA extracted at 24 h of incubation (Figure 2a).

Meanwhile, the specific activity of CA was the highest at 79.09 unit/mL when extracted at 24 h, although the amount of p-nitrophenol produced gradually increased with incubation time (Figure 2b). This means that the production rate and activity of CA are highest at the exponential phase, which is the time when the bacterial growth rate rapidly

increases. Although the highest value of OD was observed at 48 h, the specific activity of CA was highest at 24 h. This indicates that the CA production and its catalytic metabolic activity are closely related not to the OD value, but to the bacterial growth rate. Meanwhile, it is necessary to secure the maximum survival rate and metabolic activity in aan extreme environment of concrete, since the bacterial growth rate and catalytic reaction rate can be influenced by environmental condition. Therefore, SAML2018 cultured for 24 h was used to obtain the highest activity of the mineral carbonation in the cementitious materials.

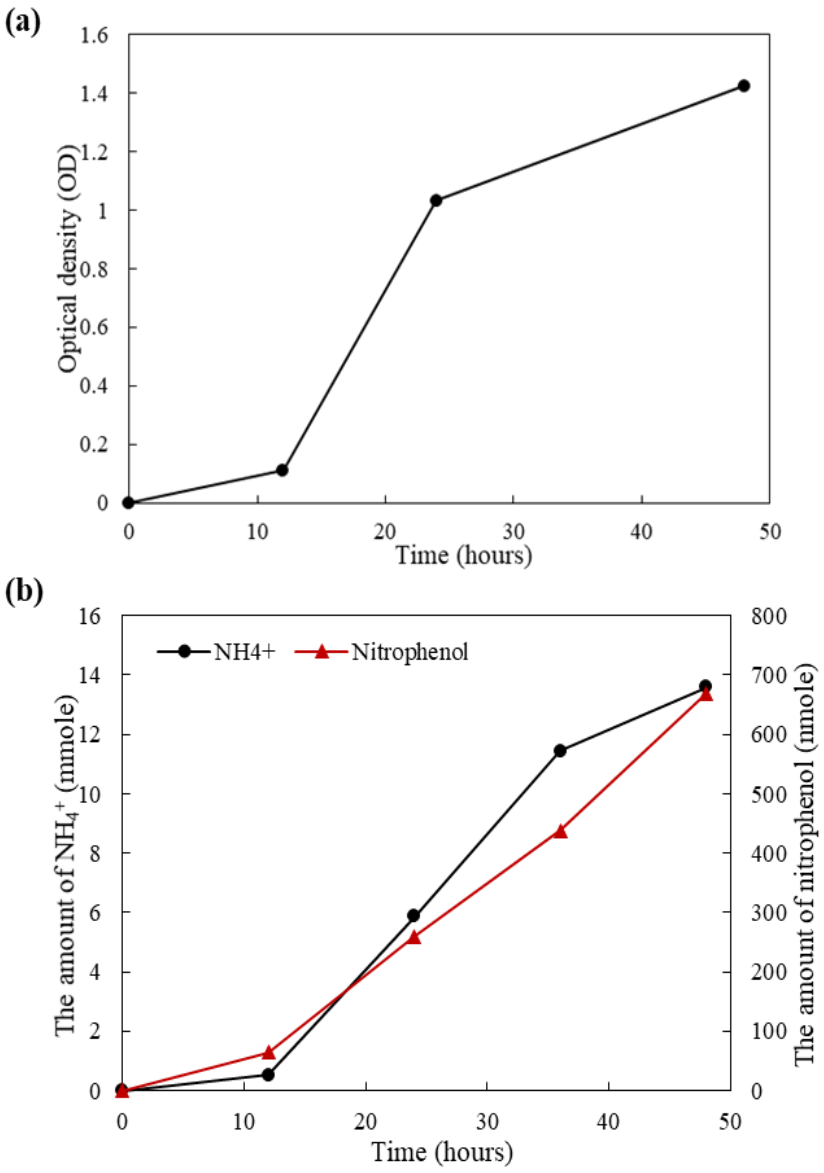

Figure 1. Changes in (**a**) optical density and (**b**) the amounts of produced NH_4^+ and nitrophenol of bacterial culture solution over time.

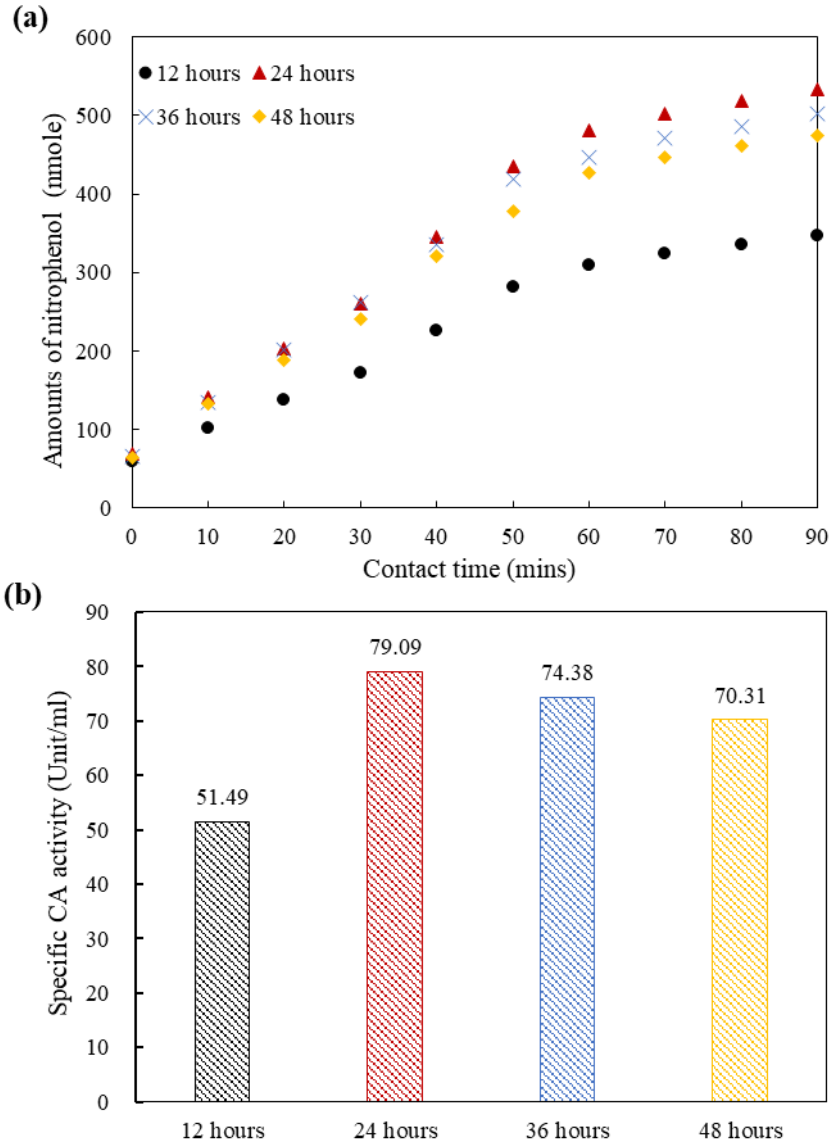

Figure 2. (**a**) Enzyme reaction rate and (**b**) specific activity of carbonic anhydrase extracted from SAML2018 after 12, 24, 36, and 48 h of incubation.

3.2. Changes in the Microstructure and Mechanical Properties by Bacterial Mineral Carbonation

To evaluate the effect of bacterial mineral carbonation metabolism on the mechanical properties in cementitious materials, the compressive strength test of all specimens was conducted after 7, 14, and 28 days of water or carbonation curing. Figure 3 shows the compressive strength values of paste specimens after 7, 14, and 28 days of curing. The strength values of the specimen B to which the mineral carbonation effect by ureolysis process was applied were higher by 12.45, 30.56, and 3.24% than that of control specimen A, at 7, 14, and 28 days, respectively. In general, the nutrients necessary for the metabolic activity of bacteria include various carbohydrates and proteins, and excessive incorporation of these nutrients can retard the contact between water and the clinker of cement grains at the initial stage of hydration [23]. In addition, since the urea decomposition metabolism essentially uses water, the ureolysis process may have a relatively small amount of water molecules compared to control specimen A. Hence, these phenomena can retard the hydration of cementitious materials. Nevertheless, it can be seen that carbonate minerals produced by microbial ureolysis metabolism have a stable state and can fill the cracks and voids, thereby improving the mechanical properties.

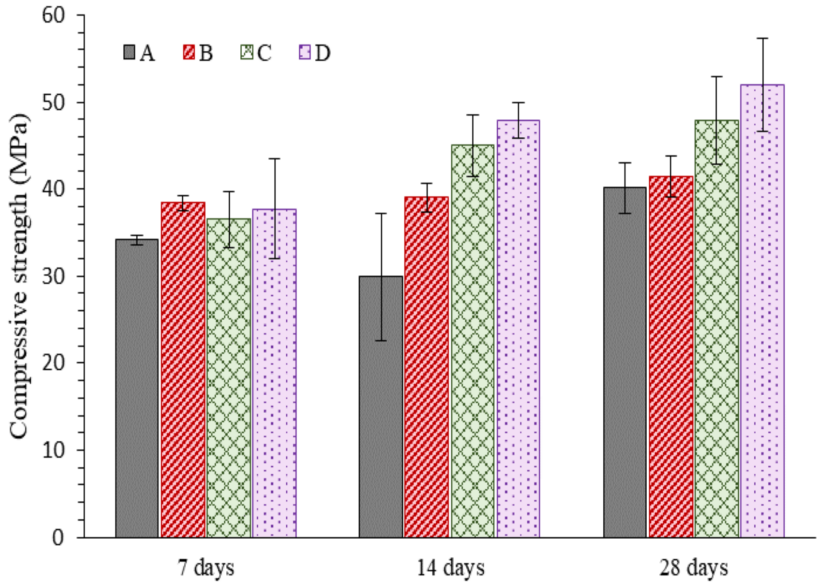

Figure 3. Compressive strength values of paste specimens after 7, 14, and 28 days of curing (A and B specimens: without and with bacterial culture solution in water curing, respectively. C and D specimens: without and with bacterial culture solution in carbonation condition, respectively).

Meanwhile, under carbonation curing conditions, $CaCO_3$ is produced by carbonation of unhydrated clinker in the pore phase, and simultaneously, by the decalcification of C–S–H gel and $Ca(OH)_2$ [24]. In particular, the hydration rate of belite (C_2S), which is a stable state of the clinker, is slower than that of alite (C_3S), but carbonation conditions accelerate the hydration of C_2S and cause a dense microstructure [25]. Indeed, the slow hydration of C_2S causes the formation of a wide interfacial transition zone, but $CaCO_3$, which is produced from carbonation curing, can fill the internal voids, thereby improving the strength and reducing the pore volume. Therefore, it appears that the carbonation-cured specimens C and D had higher strength values than the water-cured specimens A and B after 14 days of curing. In addition, the strength value of specimen D, to which the CO_2 hydration process was applied by the incorporation of bacteria, was 3.37, 6.43, and 8.39% higher than that of control specimen C, after 7, 14, and 28 days of carbonation curing, respectively. It can be inferred that the hydration process of CO_2 by bacterial CA promotes the inflow of CO_2 into cementitious materials and accelerates the conversion of CO_2 into CO_3^{2-} at room temperature and pressure relative to other chemical conversion methods.

The effect of bacterial mineral carbonation on changes in the microstructure can be evaluated by investigating the pore size diameter and porosity. Figure 4 shows the log differential and cumulative intrusion/extrusion curve by the pore size diameter of paste specimens after 28 days of curing. In addition, the porosity of paste specimens after 28 days of curing is tabulated in Table 2. The cumulative intrusion/extrusion values were relatively lower in the carbonation-cured specimens C and D than those of the water-cured specimens A and B. In particular, although specimen A had a low pore size diameter as a result of the pores being filled through ureolysis metabolism, the porosity and median pore diameter of specimen D were the lowest, as 22.03% and 5.22 nm, respectively. It can be implied that the carbonation curing yields a denser microstructure by improving the additional hydration of cement and the generation of $CaCO_3$. In addition, the cumulative intrusion/extrusion value of specimen D was much lower than that of control specimen C, and the porosity and average pore diameter of specimen D were 4.17% and 1.2 nm lower than that of specimen C, respectively. It is speculated that the bio-catalytic reaction by the bacterial CAs may accelerate the mineral carbonation process, and has a synergistic effect on the carbonation mechanism of cement in terms of forming a dense microstructure.

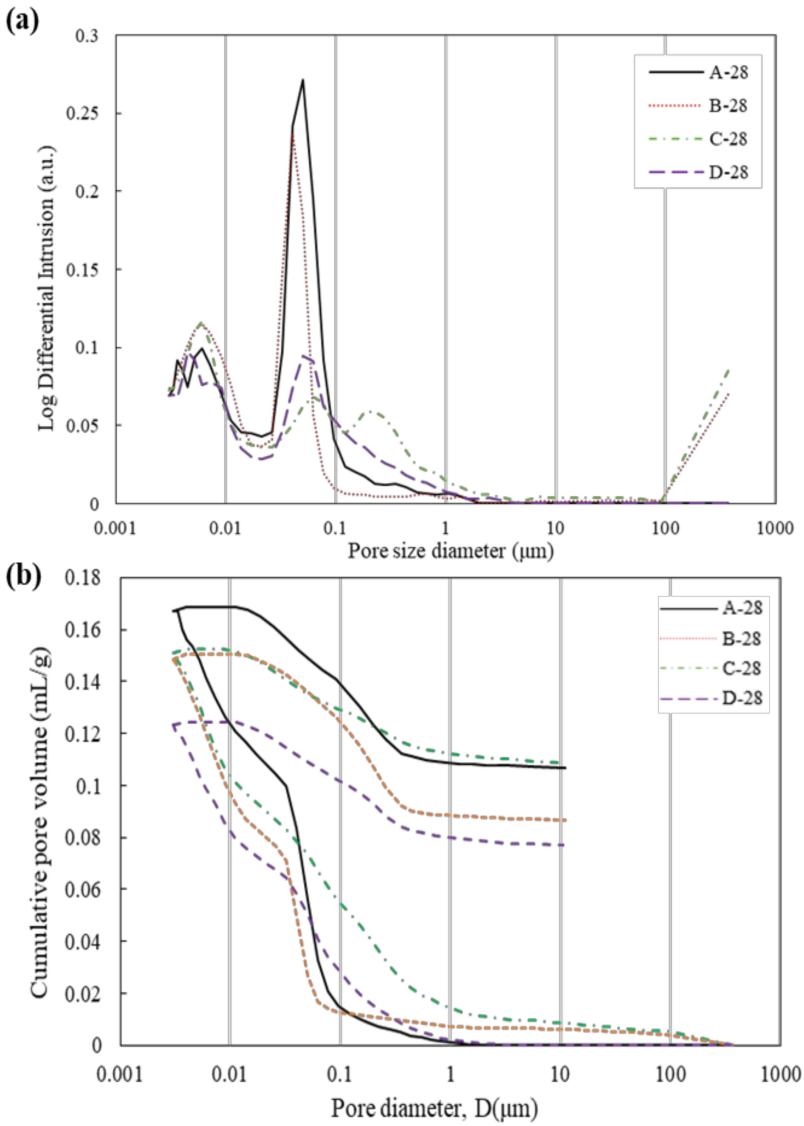

Figure 4. (**a**) Log differential and (**b**) cumulative intrusion/extrusion curve by pore size diameter of paste specimens after 28 days of curing.

Table 2. Porosity of paste specimens after 28 days of curing.

Specimen ID	Porosity	Average Pore Diameter	Median Pore Diameter (Area) *
A	27.54%	14.50 nm	5.86 nm
B	28.51%	11.83 nm	5.78 nm
C	26.20%	13.78 nm	5.44 nm
D	22.03%	12.58 nm	5.22 nm

* Median pore diameter (volume) at 4487.04 psia and 0.084 mL/g.

3.3. Phase Identification and Quantitative Analysis of Hydrates of Paste Specimens

In order to investigate the effect of bacterial ureolysis and the CO_2 hydration process on the amount and types of hydrates, XRD and TG/DTG analyses of the paste specimens after 7 and 28 days of curing were performed. Figure 5 presents the results of the XRD analysis of the paste specimens after 7 and 28 days of curing. In addition, Figures 6 and 7 display the TG and DTG analysis results, and the mass loss (%) corresponding decarbonation of $CaCO_3$ in paste specimens after 7 and 28 days of curing.

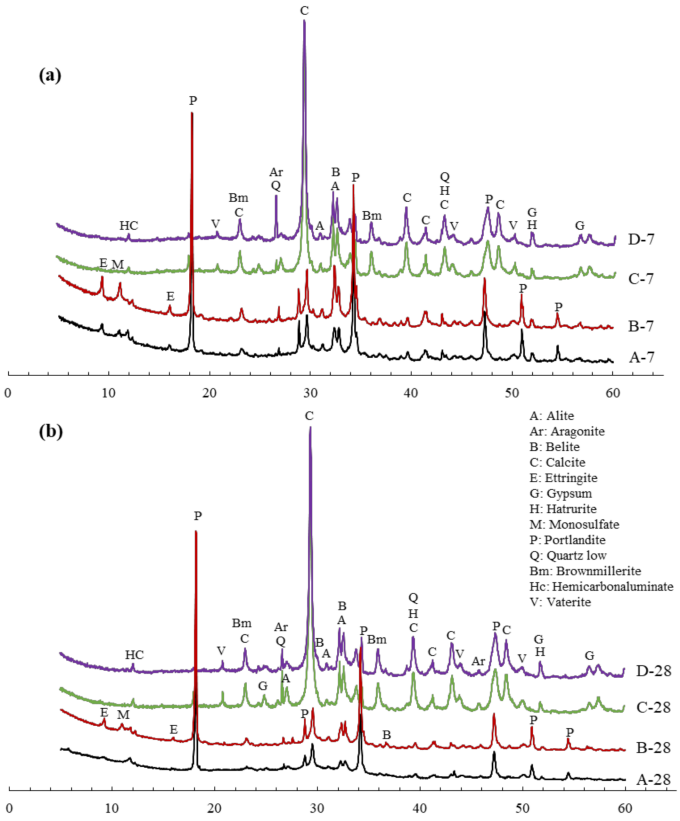

Figure 5. X-ray diffractometer (XRD) analysis of paste specimens after (**a**) 7 and (**b**) 28 days.

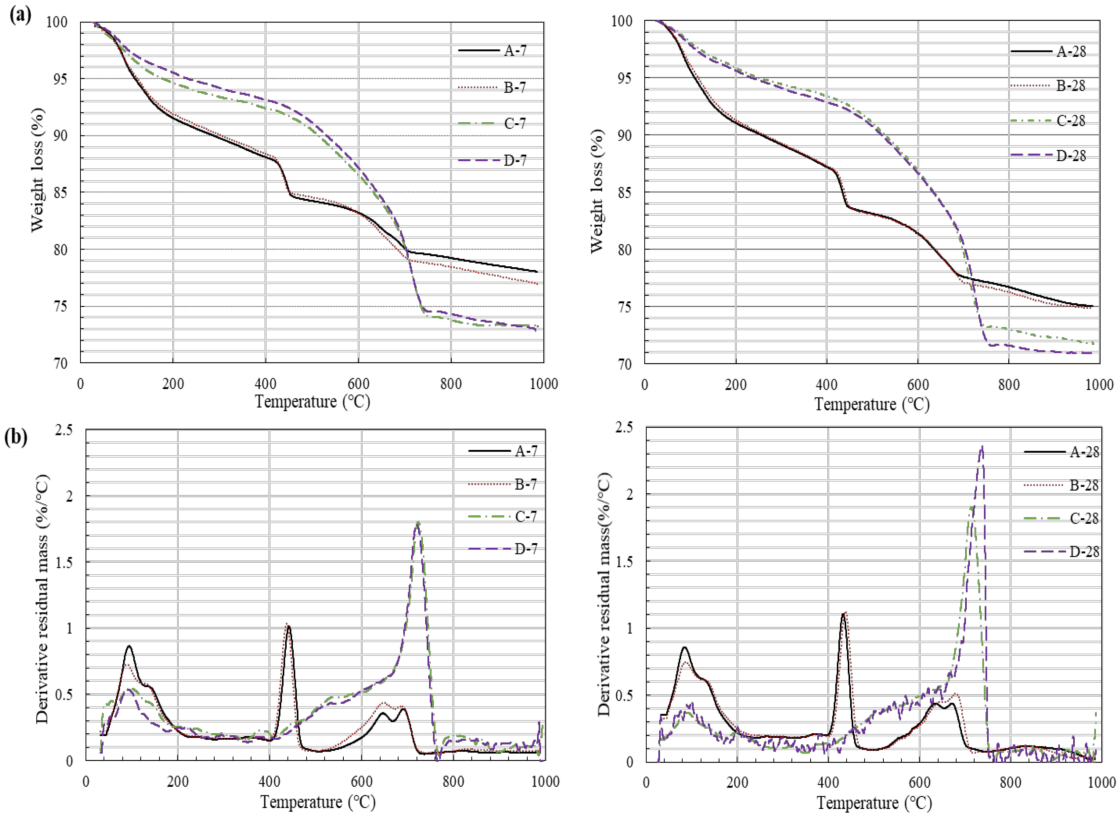

Figure 6. (**a**) Thermogravimetry (TG) and (**b**) Differential thermogravimetry (DTG) analysis results after 7 and 28 days.

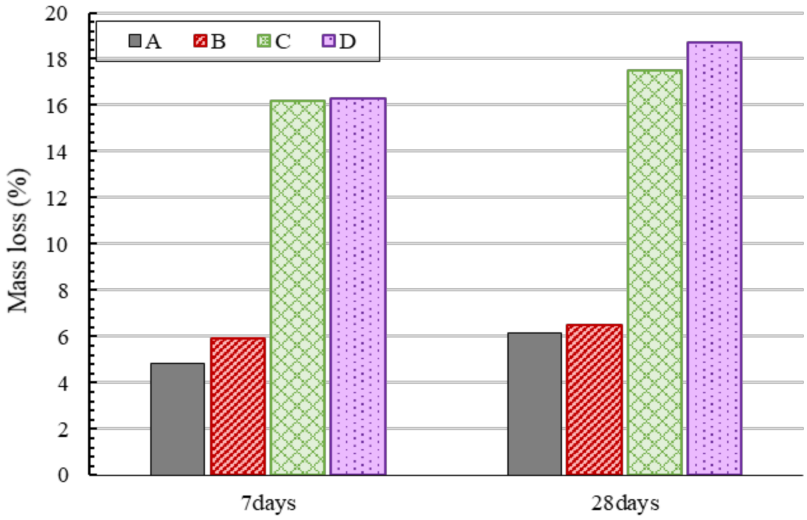

Figure 7. Mass loss (%) of $CaCO_3$ calculated from TG curves in paste specimens after 7 and 28 days of curing.

There were no prominent differences in the types of hydrates in the specimens containing the bacteria and pure nutrients compared to the control specimens. It can be seen that the incorporation of the bacteria culture solution can retard of hydration [16], but does not have a significant effect on the phase assemblage of hydrates. Meanwhile, a peak indicating gypsum ($CaSO_4$. $2H_2O$, PDF# 99-001-0216) was observed at 25° (2θ) in the carbonation-cured specimens after 28 days of curing, but the amount of ettringite ($Ca_6Al_2(SO_4)_3(OH)_{12}$, PDF# 99-002-0006) decreased, compared to the results with the 7 days of curing. It is suggested that the ettringite can react with CO_2 and produce the $CaCO_3$, gypsum, and aluminate gel. Under carbonation conditions, hydrates can be decalcified, and especially $Ca(OH)_2$ can be converted into calcite or aragonite. Indeed, calcite is the main product of decalcification of hydrates in natural carbonation conditions (CO_2 concentration < 0.04%), while accelerated carbonation conditions promote the conversion of $Ca(OH)_2$ and C-S-H gel to vaterite or aragonite [17]. Hence, in carbonated specimens C and D, vaterite ($CaCO_3$, PDF# 99-001-0197) was observed at around 21° (2θ) and 34° (2θ), and also aragonite ($CaCO_3$, PDF# 99-002-0015) was observed at around 23° (2θ) and 48° (2θ), compared to water-cured specimens.

When cement clinkers C_3S and C_2S react with water, Ca^{2+} is eluted and C–S–H gel and $Ca(OH)_2$ are formed as the main hydrates. Indeed, weight loss by the decomposition of C–S–H gel, AFm and $Ca(OH)_2$, the main hydration products, was observed at around 100 °C, 150 °C and 400–500 °C, respectively (Figure 6). In general, $Ca(OH)_2$ is dissolved in the pores and the $CaCO_3$ can be produced by decalcification of $Ca(OH)_2$ from the inflow of CO_2, which is referred to as natural carbonation [26]. Meanwhile, as C_3S and C_2S clinker are hydrated, CSH gel and $Ca(OH)_2$ are produced, which become $CaCO_3$ by uptake of CO_2. In addition, under carbonation conditions, the reaction of clinker is promoted, so that a large amount of CSH gel is produced and a relatively small amount of $Ca(OH)_2$ is produced [27]. For this reason, $Ca(OH)_2$ was hardly observed in specimens C and D cured in carbonation conditions, while a greater amount of $Ca(OH)_2$ was observed after 7 and 28 days of water-curing. In addition, in the water-cured specimens, the amount of $Ca(OH)_2$ and C–S–H gel in the specimen A without bacterial solution was more than that of the specimen B with bacterial solution, since the components of bacterial solution could retard the early cement hydration.

Meanwhile, weight loss resulting from $CaCO_3$ decomposition was observed between 600 and 800 °C, and, in particular, the carbonation-cured specimens showed broad peaks between 500 and 600 °C. $CaCO_3$ decomposition is shown by Equation (2):

$$CaCO_3 \rightarrow CaO + CO_2 \quad (2)$$

This is attributed to the fact that carbonation of $Ca(OH)_2$ generates crystalline $CaCO_3$, whereas the carbonation of C–S–H gel can produce amorphous (poorly crystalline) $CaCO_3$ [2]. In addition, the carbonation-cured specimen C showed 11.38% higher $CaCO_3$ contents than the water-cured specimen A, after 28 days of curing. This is explained by the consumption of $Ca(OH)_2$ and C–S–H gel to produce the $CaCO_3$ during carbonation curing. After carbonation curing for 7 to 28 days, the mass percentage increase in $CaCO_3$ of the specimen D was higher than that of specimen C. A possible explanation is that the bacteria produce $CaCO_3$ by reacting CO_3^{2-} with free Ca^{2+} eluted from the unhydrated clinker, unlike the control specimen that generate $CaCO_3$ through decalcification of hydrates in the carbonation curing condition.

Figure 8 shows the pH evolution of paste specimens after 7, 14, and 28 days of curing. In the initial hydration phase, an alkaline environment (pH > 13) is created by formation of free alkali metal ions (i.e., Na, K) and portlandite ($Ca(OH)_2$) [28]. However, as the curing time passes, the pH decreases due to the dissolution of C–S–H gel (10 < pH < 12.5) and formation of $CaCO_3$ (pH < 10) [28]. Meanwhile, in the case of the carbonation curing condition, the hydration of C_2S, which has a stable state, is promoted, and C–S–H gel is mainly produced. However, at the same time, the pH can be decreased by the decalcification of $Ca(OH)_2$ and C–S–H gel. Therefore, it appears that the carbonation-cured specimens C and D had a lower pH than the water-cured specimens A and B. In particular, the pH value of specimen D, to which metabolism by CA was applied, was the lowest at 9 at 28 days of curing, since, as shown in Figure 6a,b, the smallest amount of $Ca(OH)_2$ and the highest amount of $CaCO_3$ were present.

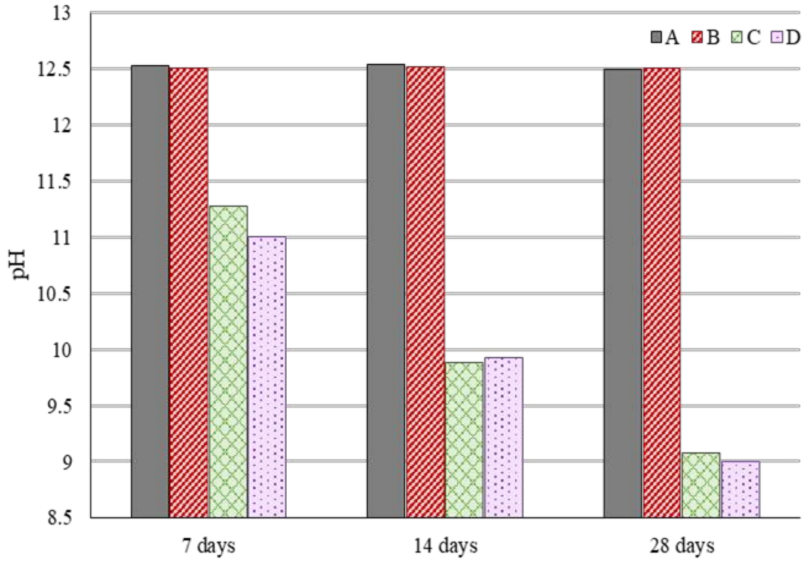

Figure 8. pH evolution of paste specimens after 7, 14, and 28 days of curing.

In addition, the pH of specimens B and D with ureolytic bacterial paste solution and CO_2 hydration bacterial paste solution, respectively, was lower than control specimens A and C without bacterial solution. It can be said that the initial pH of the specimens with a bacterial paste solution may be low, since bacterial nutrient components can retard the contact with clinker and water, thereby reducing the formation of alkali metal ions and portlandite. Furthermore, as curing time progresses, pH may be lowered by the formation of carbonate minerals through bacterial carbonation metabolism of the ureolytic process and CO_2 hydration.

Meanwhile, the carbonation degree of the specimens could be measured by spraying a 1% phenolphthalein solution into the specimens after 7, 14, and 28 days of curing. Table 3 displays the carbonation degree (%) and carbonation coefficient value of paste specimens. Carbonation coefficient is a value that can quantify the degree of carbonation. However, it may be slightly different from TG result, a quantitative analysis of $CaCO_3$ measured by

crushing the specimen, since the degree of carbonation is visually checked by spraying phenolphthalein on the surface of the specimen. Nevertheless, as a similar result of pH, the highest carbonation degree and coefficient value were observed in the specimen D with CO_2 hydration bacterial solution. In the early hydration phase of the specimen D, the hydration of cement could be delayed by the incorporation of bacterial paste and nutrients. This phenomenon might accelerate the CO_2 uptake through voids or pores, which was relatively more produced by low strength development and hydration rate in the specimens with bacteria. In addition, the carbonation rate of the specimen D was higher than that of specimen C, due to additional bacterial CO_2 hydration and mineralization metabolism through bacterial catalytic reaction. In this regard, the bacterial carbonation metabolism could be expected as a promising method for enhancing CO_2 sequestration into cementitious materials.

Table 3. Carbonation degree (%) and carbonation coefficient value of paste specimens.

	Time	Specimen C	Specimen D
Carbonation degree (%)	7 days	15.36%	44%
	14 days	22.56%	59.04%
	28 days	44%	72.96%
Carbonation coefficient $\left(\mathrm{mm}/\sqrt{yr}\right)$		18.05	43.32

4. Conclusions

This study investigated the impact of microbial-mediated ureolysis and CO_2 hydration metabolism on the microstructures and mechanical properties of cementitious materials. The microorganism used in this study had both urease and carbonic anhydrase for performing urease and CO_2 hydration, respectively. Four specimens, which were prepared with and without the bacteria, were cured in water and carbonation conditions. The obtained results showed that the bacterial metabolism mediated mineral carbonation could play a positive role in reducing the pore size and improving the mechanical properties of cementitious materials. Major findings of this study are as follows:

(1) The bacterial mineral carbonation metabolism proceeds by bacterial inherent enzyme-based catalytic reactions, and the metabolism activity by enzymes is closely related to the growth rate. In the exponential growth phase, the largest amounts of urease and carbonic anhydrase are produced, which resulted in rapid metabolic activity.

(2) The compressive strength value of carbonation-cured specimens was higher than that of water-cured specimens. This is attributed to the promotion of the hydration of stable C_2S under carbonation conditions, and a large amount of $CaCO_3$ in a stable state is produced by decalcification of hydrates.

(3) The mineral carbonation based on ureolysis and the CO_2 hydration process can produce carbonate minerals from urea and CO_2, respectively. The carbonate minerals reduced the pore size and porosity by filling the pores of cementitious materials, thereby improving the mechanical properties.

(4) In the carbonation-cured specimens without bacteria, the hydrates (i.e., $Ca(OH)_2$, C–S–H gel) were decalcified by inflow of CO_2, which lowered the pH due to a decrease in $Ca(OH)_2$ in the pore solution. In addition, in the carbonation-cured specimens incorporating bacteria forming carbonic anhydrase, a significant decrease of pH and high carbonation degree value were observed. It is postulated that the carbonation process can be accelerated by incorporation of the bacterial paste solution, due to relatively more CO_2 uptake by hydration retardation and the bacterial mineral carbonation metabolism.

Nevertheless, additional studies are needed, such as the effect of the amount and types of nutrients on the carbonation rate. Moreover, studies on the optimal carbonation process and CO_2 sequestration performance of bacteria should be implemented.

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