Kinetic effect of carbonic anhydrase enzyme on the carbonation reaction of lime mortar

Özlem Cizer, Encarnación Ruiz-Agudo, and Carlos Rodriguez-Navarro

Civil Engineering Department, KU Leuven, Leuven, Belgium; Department of Mineralogy and Petrology, University of Granada, Granada, Spain

ABSTRACT

The effect of carbonic anhydrase enzyme on the precipitation kinetics and phase transformations of calcium carbonate, and on the strength development of lime mortars has been investigated with saturated lime solutions, lime pastes and lime mortars under atmospheric conditions. The results clearly show that carbonic anhydrase catalyzes the reaction between carbon dioxide and aqueous lime, and increases the rate of calcium carbonate crystallization, the yield of the carbonation reaction and mortar strength at early ages. This is most likely a kinetic effect associated with the increased rate of carbonate ions supply to the solution by the enzyme. In addition, this enzyme favors the formation of stable calcite and significantly modifies its morphology by developing new crystal faces. These results suggest a novel approach for accelerating the hardening of lime mortars using carbonic anhydrase enzyme, which may offer a potentially novel approach with significant benefits on the applications of lime mortars in architectural heritage conservation as well as in construction.

1. Introduction

Carbonation reaction is an essential process in the setting and hardening of traditional lime mortars successfully practiced successfully for building purposes since the advent of pyrotechnology ca. 12,000 years ago (Elert et al. 2002; Von Landsberg 1992). When a fresh lime mortar mixture is exposed to atmospheric conditions, it hardens by carbonation reaction between carbon dioxide (CO₂) in the atmosphere and calcium hydroxide in the aqueous medium, following the overall reaction Ca(OH)₂ + CO₂ + H₂O → CaCO₃ + 2H₂O. The chemical reaction of carbonation starts only after the setting period involving plastic shrinkage and subsequent initial strength gain upon drying. Once open space is formed upon drying, the carbonation reaction is allowed to take place via the diffusion of CO₂ through the open capillary pores and its dissolution in the capillary water (Cizer et al. 2012a; Van Balen 2005; Van Balen and Van Gemert 1994). The resulting calcium carbonate fine crystallites act as the binding agent due to the dense interconnected microstructure formed during the precipitation process (Beruto, Barberis, and Botter 2005; Cazalla et al. 2000; Cizer et al. 2012b; Rodriguez-Navarro et al. 2002). This causes further hardening of the mortar and strengthens its matrix towards an overall mechanical resistance that contributes uniquely to the overall deformability of the masonry (Hansen, Rodríguez-Navarro, and Van Balen 2008). The carbonated microstructure will also determine the pore structure of the mortar, which will be crucial in terms of moisture transport, diffusivity of gases (carbon dioxide and water vapor), mechanical properties, and durability.

Due to the low liquid-to-solid ratio (< 1), the carbonation reaction in lime mortars follows a complicated process which is different from, for example, industrial carbonation performed as a liquid-film or suspension process by bubbling CO₂ gas through an aqueous suspension of calcium hydroxide particles (liquid-to-solid ratio > 5). The reaction proceeds stepwise from the surface into the core of the mortar due to the CO₂-diffusion process according to the following steps (Burns and Jachuck 2005; Juvekar and Sharma 1973; Kern 1960): continuous dissolution of calcium hydroxide in the pore water in liquid phase with the dissociation of Ca²⁺ and OH⁻ ions (reaction 1); dissolution of the gaseous carbon dioxide into the alkaline pore solution to form a loosely hydrated aqueous form (reaction 2); hydration of carbon dioxide with OH⁻ ions to form carbonic acid (H₂CO₃) followed by its dissociation to bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) ions (reactions 3 and 4); and, finally, the reaction between...
Ca$^{2+}$ and CO$_3^{2-}$ ions forms calcium carbonate crystals through nucleation and subsequent crystal growth resulting in an interconnected microstructure (reaction 5). All these reaction steps are interrelated and altering the kinetics of one of them will influence the others. At pH > 10, which represents the situation of the pore solution in carbonating lime mortar, HCO$_3^-$ ions can be formed by reaction 3, more rapidly than by the standard reaction (CO$_2$(aq) + H$_2$O(aq) = HCO$_3^-$(aq) + H$^+$aq)), which occurs at a lower pH; subsequently, reaction 4 occurs, which is instantaneous (Kern 1960). Finally, reaction 5 takes place, resulting in the precipitation of CaCO$_3$. Reaction 3 is the slowest one, becoming the rate-controlling step in the overall chemical reaction of carbonation in this pH range:

$$\text{Ca(OH)}_2(\text{s}) \leftrightarrow \text{Ca}^{2+}(\text{aq}) + 2\text{OH}^-(\text{aq}) \quad (1)$$

$$\text{CO}_2(\text{g}) \leftrightarrow \text{CO}_2(\text{aq}) \quad (2)$$

$$\text{CO}_2(\text{aq}) + \text{OH}^-(\text{aq}) \leftrightarrow \text{HCO}_3^-(\text{aq}) \quad (3)$$

$$\text{HCO}_3^-(\text{aq}) + \text{OH}^-(\text{aq}) \leftrightarrow \text{CO}_3^{2-}(\text{aq}) + \text{H}_2\text{O}(\text{l}) \quad (4)$$

$$\text{Ca}^{2+}(\text{aq}) + \text{CO}_3^{2-}(\text{aq}) \leftrightarrow \text{CaCO}_3(\text{s}) \quad (5)$$

Carbonation reaction in lime mortars is a complex mechanism controlled by the diffusion-dissolution of carbon dioxide in the air/pore system and in the pore water (Cizer 2016; Cizer et al. 2012a; Van Balen 2005; Van Balen and Van Gemert 1994). Diffusion is slow relative to the chemical reaction because the degree of water saturation of the porous space influences (hammers) the diffusion process. Water is initially present in the fresh mortar paste and is self-supplied during the reaction. Therefore, the carbonation reaction in lime mortars is largely controlled by the pore structure and the pore water content. The final crystal polymorph is calcite with habit and morphology being similar at the exposed surface but different within the sample’s bulk volume, indicating carbon dioxide diffusion/dissolution as the controlling factor for the selection of calcium carbonate habit (Cizer et al. 2012b). Evolution of habit and morphology of calcium carbonate may help explaining why mechanical strength of lime mortars typically continues to increase beyond full (or nearly full) carbonation. Why these weak mortars show such an excellent long-term performance in ancient masonry structures existing for centuries, and why these mortars should be the choice as compatible materials in the conservation of the cultural-built heritage (Cizer 2016; Elert et al. 2002; Hansen, Rodriguez-Navarro, and Van Balen 2008).

Slow kinetics of carbonation reaction in lime mortars implies slow kinetics of setting and hardening which significantly hinders their application in restoration and construction practices. Various approaches have been investigated to accelerate the kinetics of carbonation reaction. Particularly, the use of organic molecules such as citric acid, sucrose, calcium lignosulfonate, and polyamphophenolic derivatives to foster the precipitation rate of calcium carbonate has been a commonly employed strategy in the last two decades (Agnihotri et al. 1999; Manoli and Dalas 2000; Dickinson & McGrath 2003; Seo et al. 2005; Xu et al. 2008; García-Carmona et al. 2003; Medici and Rinaldi 2002, Xiang et al. 2004). Nevertheless, the achieved increase in reaction rate was generally poor. It is well known that related molecules such as polysaccharides, proteins (animal glue and casein), fatty acids (olive oil), and Nopal juice as plant extract were used in traditional lime mortars to improve material properties (Rodriguez-Navarro et al. 2017), but they have negligible impact on the kinetics carbonation reaction (Chandra, Eklund, and Villarreal 1998; Ventolá et al. 2011). This is because such additives do not alter the kinetics of conversion of hydrated CO$_2$ into HCO$_3^-$ ions, which is the rate-controlling step in carbonation reaction.

Bioinspired approaches can provide a novel solution to master the kinetics of carbonation reaction in lime mortars. In the last decade, precipitation of calcium carbonate in the presence of biological factors (microbial and bovine) such as urease and carbonic anhydrase enzymes has been documented to enhance calcite crystal nucleation and growth (Li et al. 2010; Meldrum 2003; Sondi and Matijevic 2001). The carbonic anhydrase enzyme (CA) is a particularly efficient biocatalyst that takes part in many processes in living organisms such as respiration, CO$_2$ transport and photosynthesis, promoting the hydration of CO$_2$ and the production of HCO$_3^-$ ions (Lindskog 1997; Smith and Ferry 2000). This enzyme plays an important role in microbially induced calcium carbonate precipitation processes and is known to be the fastest catalyst for carbon dioxide hydration reaction (Achal and Pan. 2011; Khalifah 1971). CA belongs to a large group of zinc-based metalloenzymes that are known to catalyse the reversible hydration of carbon dioxide (CO$_2$ + H$_2$O $\leftrightarrow$ HCO$_3^-$ + H$^+$) without changing the solution chemistry (Lindskog and Coleman 1973; Lindskog 1997). The fastest human CA enzyme (HCA II) can hydrate at least 1.4 x 10$^6$ molecules of CO$_2$ per second (Khalifah 1971). Dreybrodt et al. (1996) reported the enhancement of carbon dioxide hydration with 0.6 μM CA enzyme by a factor of 1,500. At neutral pH and at slightly alkaline pH (ca. 8–9), this enzyme yields enzyme-bound Zn-OH$^-$ (reaction 6) that is readily available to react with CO$_2$ (reaction 7). As a result, HCO$_3^-$ ions formation is promoted (reaction 8) and the overall hydration rate of CO$_2$ is enhanced:
\[
E \times ZnH_2O \leftrightarrow E \times ZnO^- + H^+ \tag{6}
\]
\[
E \times ZnO^- + \ CO_2 \leftrightarrow E \times ZnHCO_3^- \tag{7}
\]
\[
E \times ZnHCO_3^- + H_2O \leftrightarrow E \times ZnH_2O + HCO_3^- . \tag{8}
\]

In this study, we have investigated the effect of CA enzyme on the reaction kinetics and phase transformations during carbonation of lime in solution and paste (i.e., Ca(OH)\(_2\)-H\(_2\)O-CO\(_2\) system) where the pH of the system continuously decreases from \(\approx 12.5\) down to \(\approx 8.2\) as the carbonation reaction advances. Here it is demonstrated that CA enzyme remarkably enhances the rate of lime carbonation, promotes the formation of stable calcite, and significantly modifies the morphology of calcite crystals. The addition of this enzyme to lime putty mortars results in a remarkable increase in strength within 28 days. These results provide insights into the potential use of this enzyme as an additive in lime mortars to enhance their hardening and to foster the application of lime in architectural and cultural heritage conservation.

**Materials and methods**

Saturated lime (Ca(OH)\(_2\)) solutions and lime putty pastes were used for carbonation experiments. The use of aqueous Ca(OH)\(_2\) solutions enables to study the kinetics of carbonation reaction and calcium carbonate precipitation in a system without any interference from pore space-controlled by CO\(_2\) diffusion/dissolution, as commonly occurs during carbonation in porous lime pastes and mortars (Rodríguez-Navarro et al. 2015). The use of thinly spread lime putty pastes allows a minimized diffusion path of CO\(_2\) through the sample depth profile to insure the 1D advancement of the carbonation reaction front, i.e., from the exposed upper surface down through the sample depth (Cizer et al. 2012b; Cizer et al. 2012a).

Saturated lime solutions were prepared by dissolving lime putty in distilled water under vigorous stirring during 24 hr in a sealed bottle with no empty space. CA enzyme from bovine erythrocytes (purchased from Sigma Aldrich) was dosed at a 0.6 µM concentration to the saturated lime solution and lime putty that were subjected to carbonation in air (pCO\(_2\) = \(10^{-3.5}\) atm at 20°C). The evolution of the carbonation reaction was followed by continuously monitoring pH and electrical conductivity of the lime solutions (150 ml) under constant stirring rate (600 rpm). Precipitates obtained at the end of carbonation (pH = 8.2–8.3) were collected and analyzed using powder X-ray diffraction (Philips X’Pert Pro X-ray diffractometer with Cu Kα radiation, \(\lambda = 1.5405\) Å) at \(\theta\)20 range between 10 and 60°. The morphology of calcium carbonate particles was investigated using a LEO Carl Zeiss GEMINI-1530 Field Emission Scanning Electron Microscope (FESEM) after sample coating with carbon.

In-situ phase transformations of calcium carbonate were investigated using a Jasco NRS - 5100 micro-Raman spectrometer equipped with a CCD detector and an integrated optical microscope (532.14 nm laser wavelength and 4 mW laser power). Optical microscopy images and micro-Raman spectra were collected at the air/solution interface in the frequency range 100–1200 cm\(^{-1}\) at a spectral resolution 4 cm\(^{-1}\). Carbonation was performed in air using a small volume of saturated lime solution (=200 µL). In addition, in-situ precipitation of CaCO\(_3\) at the air/solution interface was monitored with continuous acquisition of XRD patterns with Cu Kα radiation at \(\phi\)20 range between 10 and 60° and at a scanning rate of 0.017°/s, allowing the collection of a full diffractogram every ~2 min.

Besides lime solutions, lime putty with and without CA enzyme was also subjected to carbonation in air. The lime paste was thinly (<1 mm) smeared with a spatula over a sample holder to form a disk of 2 cm in diameter to obtain a flat geometry so that carbon dioxide diffusion path through the sample depth was maximized. Real-time precipitation of calcium carbonate at the surface of the lime putties was monitored using in-situ XRD analysis. During atmospheric carbonation up to 180 days, ex-situ analyses were performed to determine the amount of precipitated calcium carbonate via thermogravimetric analysis (TGA) using a Netzsch STA 409 PC DSC-TGA system in static N\(_2\) atmosphere at a T range of 20–1,000°C and at a controlled heating rate of 10°C/min.

Lime putty mortars were prepared in a 1:3 lime putty to siliceous sand (0–2 mm) volumetric ratio using standard mortar specimens (40 × 40 × 160 mm) prepared in accordance with the European standards EN 1015–2. Since lime putty contained 53 wt% water, no extra water was added because the fresh mortar mixes performed well in terms of workability. The mortar specimens were cured under moist atmospheric conditions (RH ≥ 92%, 20°C) to assure the presence of moisture for enzyme activity in the capillary pore solution. Compressive and flexural strength tests were performed in accordance with the European standards EN 1015–11.

**Results and discussion**

**Carbonation reaction of saturated lime solutions**

Figure 1 gives the evolution of pH vs. time during carbonation under atmospheric conditions of pure and 0.6 µM CA-containing lime solutions. The overall
trend of pH evolution clearly shows that carbonation takes place gradually. The starting pH values of pure solution and CA-containing solution drop respectively from 12.4 and 12.6 to the final values of 8.2 and 8.3 when no further decrease is recorded, indicating the end of the carbonation reaction. This pH profile has been typically reported in earlier works but for shorter reaction periods and much lower pH values (ca. 6) at the completion of the reaction for solutions enriched with carbon dioxide (Agnihotri et al. 1999).

Considering reactions 3 and 4, the pH profiles in Figure 1 represent the evolution in OH− ions concentration in the course of the carbonation reaction and thereby can be linked to the rate of calcium carbonate precipitation taking place in the solution. Two distinct stages are observed: (i) an initial constant rate period with a change in the slope which is different for both solutions, and (ii) a subsequent accelerating rate period with an inverse S-shaped profile. Agnihotri et al. (1999) reported that the major conversion to calcium carbonate (> 75%) occurs during the initial constant rate period. This implies that a large fraction of calcium carbonate precipitates during the constant rate period, and the accelerating rate period starting at pH ca. 11.83 corresponds to a nearly complete precipitation of calcium carbonate.

The pH profiles clearly show that the carbonation reaction proceeds much faster in the presence of CA enzyme. This can be attributed to the presence of enzyme-bound Zn-OH− which promotes a faster supply of HCO3− and CO32− ions to the solution as indicated in reactions 6–8. This is a strong evidence that CA enzyme promotes calcium carbonate precipitation in an alkaline solution where the pH of the system is continuously decreasing. This kinetic effect results in a shorter constant rate period where the major precipitation of calcium carbonate occurs. It has been established that the effect of CA enzyme on the carbonation kinetics is concentration dependent (Mirjafari, Asghari, and Mahinpey 2007). However, our study shows that for enzyme concentrations beyond 0.6–0.8 μM a remarkable kinetic effect on the carbonation reaction rate is not achieved. The amount of CA concentration used here is within the range of optimum enzyme concentration at which the catalytic effect on carbon dioxide hydration can be created under the experimental conditions studied.

XRD patterns in Figure 2 show that precipitates obtained at the end of the carbonation of pure lime solution are composed of a mixture of aragonite as the dominant crystalline phase (62%), and calcite (38%). Interestingly, in the presence of CA enzyme calcite precipitates exclusively as the final calcium carbonate phase. FESEM investigations support these findings. In pure solution needle-like crystals of aragonite of nanometer and micrometer size, and {1014} rhombohedral and prismatic calcite crystals of nanometer size precipitated (Figure 3). In the presence of the enzyme, much larger rhombohedral calcite crystals precipitated. Formation of additional crystal faces suggests the modification of crystal habit from rhombohedron to “football” shape due to an interaction between CA molecules and specific calcite faces.

Solution pH plays an important role in the formation of calcium carbonate polymorphs. Highly alkaline pH (> 11.5) favors the precipitation of calcite while at pH 10.5–11.5 the precipitation of aragonite is favoured (Tai and Chen 1998). The reason why only calcite exists as the final precipitate in the enzyme-aided system can be explained considering that the faster hydration of carbon dioxide and its rapid transformation into CO32− ions can lead to higher supersaturation and faster precipitation rates which favour calcite formation. Alternatively, it could be argued that the enzyme promotes the nucleation of calcite because it inhibits the crystallization of metastable phases such as vaterite or aragonite, or because it directly templates the heterogeneous nucleation of calcite.

In order to understand the kinetic effect that CA enzyme has on phase transformations and final polymorph selection of calcium carbonate, in-situ carbonation experiments were performed on the saturated lime solutions using XRD and micro-Raman analysis. Figure 4 shows the evolution of time-resolved XRD patterns collected at the air–solution interface of the carbonating saturated lime solutions without and with
Figure 2. XRD patterns of calcium carbonate precipitates collected at the end of carbonation of the pure lime solution and the solution containing CA enzyme.

Figure 3. FESEM images of aragonite and calcite crystals precipitated in the carbonated pure lime solution (left), and calcite crystals precipitated in the presence of CA enzyme (right). Scale bar: 500 nm.

Figure 4. Time-resolved XRD patterns recorded during in-situ carbonation in air of pure lime solution (left) and the solution with 0.6 µM CA enzyme (right).
0.6 μM CA. The first diffraction patterns represent the start of the carbonation reaction and indicate diffuse scattering with two broadened maxima between 20 and 50°2θ, the second band at a lower intensity towards 50°. Such broad maxima are derived from the combined scattering from the aqueous solution as well as from poorly ordered amorphous calcium carbonate (ACC) (Faatz, Grohn, and Wagner 2004), which almost instantly precipitated as shown by micro-Raman analyses (Figure 5). The first detectable reflection in both solutions belongs to 104 calcite Bragg peak which started to emerge weakly embedded within the diffuse maximum at around 30°2θ. This corresponds to the onset of detectable crystallisation at ca. 144 minutes in pure solution, while the waiting time decreases to 120 min in the presence of enzyme. This confirms the catalytic effect of CA enzyme on the calcium carbonate precipitation. In this case, calcite is the only final crystalline phase precipitated irrespectively of the presence or absence of the enzyme. The absence of detectable metastable CaCO3 polymorphs (e.g., aragonite and/or vaterite) is likely due to the fast kinetics of precipitation in this system.

In-situ micro-Raman investigations support these findings on the nearly instant phase formations of ACC and its transformation into crystalline CaCO3. Raman spectra and photomicrographs of the observed phases in carbonating pure lime solution are presented in Figure 5. Note that the silicon wafer, serving as the sample holder for the droplet of lime solution, is responsible for the diffuse Raman band at 994–922 cm⁻¹ and the sharp peak at 520.5 cm⁻¹ is associated with Si-Si bonds. Upon air exposure, a mesh of spherical colloidal particles ≤ 1 μm in diameter form nearly instantaneously. Raman spectra of these spherulites typically show a major broad peak at around 1,077 cm⁻¹ corresponding to the carbonate symmetric stretching (ν1) and a broad featureless hump appearing as a continuous baseline rise in the low-frequency lattice region at 100–350 cm⁻¹. These spectral features imply lattice disorder (i.e., amorphous) of the carbonate group to which water molecules are H-bonded (Addadi, Raz, and Weiner 2003). This typically suggests that calcium carbonate is in the hydrated amorphous state as indicated by its spherulitic morphology which is characteristic of ACC (Faatz, Grohn, and Wagner 2004). After ca. 30 min massive dissolution of ACC particles was observed, leading to the subsequent precipitation at the air-solution interface of abundant calcite rhombohedra and trace amounts of vaterite (Rodríguez-Navarro et al. 2015). These calcite crystals were continuously growing towards single crystals or locally developing clusters of coalesced or chained particles. Raman spectra of these crystals reveal two sharp peaks corresponding to internal vibration modes ν1 (1082 cm⁻¹) and ν4 (709 cm⁻¹), and two lattice vibration modes (280 and 155 cm⁻¹) that are typical for calcite (Rutt and Nicola 1974). Micro-Raman images indicate rhombohedral and prismatic morphology of calcite as the final stable polymorph formed during solution carbonation. The formation of aragonite was not detected during in-situ carbonation of small droplets. This is due to the fact that the higher
shows the evolution of time-resolved XRD and CO$_2$ instantaneously but its ions to the pore solution. This peak and lattice vibration modes. This Raman spectra show a single 2012a− and OH ions to the solution by means of which enhances the dissolution and led to a higher evaporation rate which ultimately induced crystallization to calcite crystals is significantly modified, and their size is remarkably increased due to the increased supply of HCO$_3$−. Upon ACC dissolution occurs at a faster rate in the presence of the enzyme. This implies that CA enzyme catalyses the chemical reaction of carbonation mechanism as confirmed also by in-situ atomic force microscopy (Rodriguez-Navarro et al. 2016). In the CA-aided solutions, the transformation to calcite and aragonite (Addadi, Raz, and Weiner 2003, 2012b) serves as a transient precursor in the formation of biogenic calcite and aragonite (Addadi, Raz, and Weiner 2003, 2012b). This anhydrous ACC is somehow stabilized and transformed to calcite via a dissolution/precipitation mechanism. Interestingly, another type of ACC formed nearly large round particles (5−10 μm) appear in the form of isolated particles as well as chain-like aggregates which has little or no structurally bound water and that has less calcium carbonate is precipitated, hereby carbonation at the surface of the pastes exposed to air is that the former is saturated with respect to portlandite (i.e., Ca(OH)$_2$). To verify these findings, lime putty pastes with and without the enzyme that the pure lime paste. Despite a lower value content, up to 180 days of carbonation is presented in Figure 7. The other calcite peaks appear in time as carbonation occurs much faster, the morphology of calcite protostructure), has been found to take place in pure lime systems and is associated with the dissolution of more hydrated stable intermediate ACC phase(s) which show medium-range order (calcite protostructure), has been found to take place in pure lime systems and is associated with the dissolution of more hydrated and thermodynamically more stable (more soluble, and therefore less stable) ACC associated with the dissolution of more hydrated or less hydrated and thermodynamically more stable intermediate ACC phase(s) which show medium-range order (calcite protostructure), has been found to take place in pure lime systems and is associated with the dissolution of more hydrated or less hydrated and thermodynamically more stable intermediate ACC phase(s) which show medium-range order (calcite protostructure), has been found to take place in pure lime systems and is associated with the dissolution of more hydrated or less hydrated and thermodynamically more stable ACC. This peak corresponds to another ACC phase which is more calcium carbonate is precipitated, hereby carbonation in the pure lime putty paste. This implies that CA enzyme catalyses the chemical reaction of carbonation mechanism. The other calcite peaks appear in time as carbonation occurs instantly as confirmed also by in-situ micro-Raman and XRD analysis, formation of calcite transformation to calcite occurred much faster than vaterite was transformation of vaterite was transformation to calcite occurred much faster than vaterite was transformation of vaterite was transformation to calcite occurred much faster than vaterite was transformation of vaterite was.
verifying the kinetic effect of the CA enzyme on lime carbonation.
The impact of enzyme-aided carbonation of lime on the mortar strength was tested. Compressive and flexural strength results revealed higher values for lime putty mortar with enzyme than the pure lime putty mortar at all ages of testing (Figures 8–9). A remarkable increase in strength is clearly observed at early ages (14 and 28 days) when carbonation is in progress. This evidences the enzymatic enhancement of carbonation reaction in the lime mortar. These findings are encouraging with respect to the effective role of CA enzyme in the enhancement of lime mortar strength.

Conclusions and implications
Carbonation reaction as the hardening process in lime mortars plays a crucial role in the ultimate performance of masonry lime mortars. The kinetics of lime mortar carbonation is generally slow due to the slow conversion of
hydrated carbon dioxide into $\text{HCO}_3^-$ ions. This generally creates a major hurdle for the practice of lime mortars in masonry despite their multiple beneficial properties. The use of CA enzyme to accelerate the kinetics of carbonation reaction of lime by mimicking the carbon dioxide transport mechanism in living organisms appears to be a promising solution to foster lime mortar practices in masonry in general. Here, it is demonstrated that the addition of this enzyme to saturated lime solution as well as in lime putty pastes and mortars results in an enhancement of carbonation reaction kinetics, and increases mortar strength. In addition, the morphology of the final stable $\text{CaCO}_3$ polymorph, which is calcite, is significantly modified while the particle size remarkably increases. This kinetic effect and the preferential modification of crystal habit and morphology of calcium carbonate are induced by enzyme-bound Zn-OH$^-$ which enhances the supply of $\text{HCO}_3^-$ and $\text{CO}_3^{2-}$ ions to the solution. These results provide crucial insights into the potential use of this enzyme as an additive in lime mortars to enhance their hardening and hereby to foster the application of lime in architectural and cultural heritage conservation not only in masonry mortars but also in stone conservation where nano-lime dispersions are used to consolidate degraded substrates by calcium carbonate precipitates.

**References**


Dreybrodt, W., J. Lauckner, L. Zaihua, U. Svensson, and D. Buhmann. 1996. The kinetics of the reaction CO$_2$ + H$_2$O $\rightleftharpoons$ H$^+$ + HCO$_3^-$ as one of the rate limiting steps for the dissolution of calcite in the system H$_2$O-CO$_2$-CaCO$_3$. *Geochimica Et Cosmochimica Acta* 60:3375–81. doi:10.1016/0016-7037(96)00181-0.


