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Enzyme assisted carbon dioxide capture and mineralization in construction relevant alkaline materials

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Mineralizing CO_2 in alkaline construction materials can reduce process emissions. This study measures the effect of carbonic anhydrase on CO_2 uptake and retention in hydrated lime, Portland cement, fly ash, and slag under ambient conditions using a mass-flow-controlled CO_2 supply and gravimetric tracking. CO_2 was supplied for 1440 min for hydrated lime and cement and for 360 min for fly ash and slag, then stopped to quantify permanently retained mass. Carbonic anhydrase increased total CO_2 uptake across all materials by 71 to 89 percent. Hydrated lime reached 474.1 mg g^{-1} with the enzyme. Cement reached 285.9 mg g^{-1}. Fly ash and slag reached 308.3 mg g^{-1} and 312.4 mg g^{-1}. The fraction retained after cutoff increased for all solids and was nearly complete in several enzyme cases, while water controls showed negligible permanence. Enzyme reuse over four cycles retained 87.9 percent of the initial performance. The data support a surface-coupled mechanism in which the enzyme accelerates CO_2 hydration in the particle boundary layer, increases local carbonate availability, and drives precipitation with Ca^{2+} and Mg^{2+} on solid surfaces. The reaction endpoint remains unchanged; only the rate is altered. These results define material-enzyme combinations and operating conditions for enzyme-assisted mineralization in construction-relevant systems.

Keywords Climate change, Sustainable engineering, Carbonic anhydrase, Carbon sequestration, CO_2 mineralization, Enzyme reuse, Biocatalysis, Waste valorization

Global climate change is an important issue that requires immediate action. The increasing content of greenhouse gases in the air, including carbon dioxide, is believed to be a major cause of global climate change. For example, as of August 2025, the amount of CO_2 in the air, as recorded by NOAA at Mauna Loa Observatory in Hawaii, reached 425.48 ppm, up from 317.45 ppm in April 1958. Human activities such as construction, burning fossil fuels, deforestation, and industrial processes are the major sources of anthropogenic CO_2[1].

Carbon dioxide capture technology is employed to reduce CO_2 emissions, which sees rapid advancement driven by the emergence of new approaches that are designed to more effectively and efficiently trap and store CO_2 emissions[2,3]. CO_2 Capture, Utilization and Storage (CCUS) has gained significant attention in recent years to reduce CO_2 emissions. CCUS entails the collection of carbon dioxide emissions from diverse sources, such as power plants or industrial sites. The captured CO_2 is subsequently either utilized or stored[4,5].

The construction industry contributes significantly to global anthropogenic CO_2 emissions. The Global Alliance for Buildings & Construction (GlobalABC) and United Nations Environment Programme (UNEP), in their 2024/2025 Global Status Report for Buildings and Construction, report that the sector is responsible for about 34% of global CO_2 emissions and approximately 32% of global final energy consumption. Therefore, reducing CO_2 emissions associated with construction is essential to help mitigate the effects of climate change[6,7].

CO_2 absorption and fixation with alkaline materials presents a major opportunity due to their high pH and abundance of reactive cations such as Ca^{2+} and Mg^{2+}. Under alkaline conditions, dissolved CO_2 undergoes hydration to form bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions, which readily react with these cations to produce stable carbonate minerals such as $CaCO_3$ and $MgCO_3$. For example, the calcium hydrate produced by cement hydration provides a sink for CO_2[8,9].

$$\mathrm{Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O}$$

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The high alkalinity accelerates these reactions by shifting the equilibrium toward carbonate formation, thereby enhancing the permanent sequestration of CO_2 in the form of thermodynamically stable mineral phases. For example, the calcium hydroxide produced during cement hydration serves as an efficient sink for CO_2 through the carbonation of portlandite to calcite, improving both environmental sustainability and material durability[10,11]. Also, a significant amount of alkaline waste is produced annually, such as fly ash, slag. Carbonation of this alkaline waste provides a major pathway for CO_2 absorption and improves the properties when used for cement and concrete production[12]. Enzyme-based CO_2 capture technologies offer promising opportunities for greener industrial processes and materials. Enzymes, naturally occurring catalysts in living organisms, are highly efficient, with reaction rates often enhanced by factors of 10^6 to 10^{12} compared to inorganic catalysts[13].

The CO_2 absorption capacity of cement can be significantly enhanced by incorporating industrial byproducts and enzymes. Cement and enzymes not only contribute to reducing carbon dioxide (CO_2) emissions during manufacturing but also actively absorb CO_2 from the environment over time[14,15]. The combination of industrial byproducts and enzyme-enriched cement holds great potential for minimizing carbon emissions in the construction industry while facilitating environmental CO_2 absorption. Such approaches align with sustainable development goals and contribute to mitigating climate change[16,17].

The carbonic anhydrase (CA) enzyme has garnered significant attention for its exceptional catalytic efficiency and ability to function under moderate environmental conditions, making it ideal for CO_2 capture technologies. Carbonic anhydrases are primarily zinc-containing enzymes distributed across prokaryotes, eukaryotes, and extremophiles. These enzymes are metalloenzymes grouped into at least eight classes, α, β, γ, δ, ζ, η, θ, and ι, which differ in fold and active-site architecture yet catalyze the same reversible CO_2 hydration reaction. The η class was identified in *Plasmodium* spp., θ in marine diatoms, and the most recently described ι class occurs in both diatoms and bacteria[18,19]. However, all CA classes share a uniform catalytic mechanism, converting CO_2 into bicarbonate and a proton through reversible hydration and dehydration reactions, as shown in the equations below[20–22].

$$\left(Zn^{2+} - H_2O\right) \rightleftharpoons \left(Zn^{2+} - OH^-\right) + H^2$$
$$\left(Zn^{2+} - OH^-\right) + CO_2 \rightleftharpoons \left(Zn^{2+} - HCO_3^-\right)$$
$$\left(Zn^{2+} - HCO_3\right) + H_2O \rightleftharpoons \left(Zn^{2+} - H_2O\right) + HCO_3^-$$

Carbonic anhydrase plays a critical role in various biological processes, including CO_2 transport, pH regulation, cell respiration, and photosynthesis[23,24]. Its ability to catalyze the reversible hydration of CO_2, even under conditions found in industrial flue gases, has opened new avenues for biomimetic CO_2 sequestration. This process, which mimics biological CO_2 capture mechanisms, has shown promising results in capturing CO_2 from industrial processes[25]. CA's remarkable turnover rate of up to 10^4–10^7 s^{-1} makes it highly efficient for these applications[23].

Carbon capture and storage (CCS) is an effective strategy for achieving carbon neutrality by improving carbon capture efficiency. Recognized by the International Energy Agency, CCS enables the development of net-zero energy emission systems, supporting global efforts to combat climate change[26].

The rationale for selecting materials such as cement, lime, fly ash, and slag lies in their widespread availability and industrial significance. Cement is a major construction material with considerable CO_2 emissions during production, while lime, fly ash, and slag are common industrial byproducts that present disposal and environmental challenges[27,28]. Investigating the CO_2 absorption capabilities of these materials allows for identifying effective carbon capture methods while promoting sustainable uses for industrial byproducts, addressing both environmental and waste management issues.

Previous studies have established carbonic anhydrase as a highly efficient biocatalyst for accelerating the reversible hydration of carbon dioxide in aqueous systems, gas–liquid membrane contactors, and bio-based capture processes. However, most existing research has concentrated on controlled laboratories or biological environments, with limited exploration of CA's interaction with complex solid–liquid matrices such as industrial alkaline waste. These materials, rich in calcium and magnesium oxides, offer natural potential for CO_2 mineralization but are often limited by slow reaction kinetics and surface diffusion barriers under ambient conditions[23,29,30]. The integration of CA into such heterogeneous systems remains largely unexplored, particularly in the context of construction-related materials like hydrated lime, fly ash, slag, and cement. This study addresses this research gap by systematically quantifying the catalytic effect of CA on CO_2 absorption and mineralization across these materials, thereby providing new mechanistic insights and experimental evidence for enzyme-assisted carbon capture using readily available industrial byproducts[31–33].

This study quantifies how carbonic anhydrase (CA) alters both the kinetics and permanence of CO_2 uptake in alkaline industrial materials, hydrated lime, Portland cement, fly ash, and slag, tested under ambient laboratory conditions using a controlled gas-delivery and gravimetric protocol. Specifically, we measure total CO_2 uptake per unit mass during continuous CO_2 exposure, and we determine permanent uptake as the mass retained after CO_2 cutoff and a defined stabilization window, thereby separating reversible dissolution from mineral fixation. We compare CA-treated and control samples to assess initial uptake rates and time to 90% of peak (t_{90}), and we rank materials by peak uptake, permanent uptake, and retention fraction to identify top-performing CA-material combinations. We also evaluate enzyme reusability over four consecutive cycles using hydrated lime as a reference substrate and test statistical significance with triplicate measurements per condition (one-way ANOVA and two-sample t-tests at $\alpha = 0.05$). Our working hypothesis is that CA, acting as a kinetic accelerator of CO_2 hydration, increases both peak and permanent uptake, with the magnitude of benefit scaling with available alkaline earth cations (Ca^{2+}/Mg^{2+}) and baseline alkalinity. A detailed description of the gas-delivery setup, timing of exposure, post-cutoff stabilization, and data-analysis procedures is provided in Materials and Methods.

Experimental materials and methodology

Experimental materials

The materials used in this study include carbonic anhydrase enzyme, hydrated lime, fly ash, slag, cement, deionized water, and a CO_2 gas cylinder. Research-grade carbonic anhydrase was procured from Sigma-Aldrich in the form of lyophilized powder. The CA used in this study belongs to the α-class of carbonic anhydrases, extracted from bovine erythrocytes, which are known for their high turnover rate (up to 10^6 s^{-1}), pH stability in the range of 6.5–10, and temperature stability up to 60 °C. These properties make α-CA a robust and well-characterized model enzyme for laboratory-scale CO_2 capture systems. Hydrated lime, specifically autoclaved mason's lime, was obtained from Graymont, Inc. with a pH value of 12.1 in a 10% aqueous solution. Fly ash and slag were both sourced from Rockport Ready Mix Inc., Cleveland, Ohio, USA, with pH values of 10.2 and 10.7, respectively, when dispersed in DI water. Portland cement (Type I/II or IL) was purchased from Home Depot with a pH value of 12.4 in aqueous suspension. The CO_2 gas cylinder used in the experiments was procured from AIRGAS. Deionized water was used throughout all experiments.

Preparation of carbonic anhydrase solution

To prepare the carbonic anhydrase (CA) solution, 2.7 mg of the powdered enzyme was accurately weighed and dissolved in 1 mL of deionized water, resulting in a working concentration of 2.7 mg/mL (≥ 2,000 W-A units/mg protein). This concentration was selected based on preliminary optimization trials and previous studies demonstrating that enzyme activities above ~ 2 mg/mL achieve a balance between catalytic efficiency and cost-effectiveness for CO_2 hydration reactions under ambient conditions[34,35]. The same enzyme concentration and preparation protocol were maintained across all experiments to ensure consistency and reproducibility of results.

Experimental setup

CO_2 and N_2 gas cylinders were procured from Airgas and used as gas sources for the experiment. Each cylinder was connected to a low-pressure regulator to maintain a safe and controlled pressure level. The regulated gas lines were connected to mass flow controllers (MFCs) from Alicat, model BASIS™ Series. These MEMS-based thermal mass flow controllers are designed for precise and compact flow regulation, offering a flow range from 100 SCCM to 100 SLPM, operating pressure up to 145 PSIG, 1,000:1 turndown ratio. The gas streams from the MFCs were directed into a mixing chamber, allowing adjustment of gas composition when needed. After the mixing chamber, the gas stream was passed through a flow meter from Kelly Pneumatics to verify the flow rate[36,37]. This flow meter supports flow accuracy of ± 2% of reading or 0.05 SLPM, a flow range up to 200 SLPM, a sample rate of 50 ms, and an operating temperature of 0–50 °C. Following the flow meter, the gas line included a particulate filter to remove solid particles, a water trap to prevent moisture from entering the sample vial, and a hydrophobic filter to block water vapor from contaminating the gas stream. All filters used in the system were procured from CO2Meter[38,39]. The gas was then delivered through a fine needle into a sealed 2 mL airtight glass sampling tube, which contained the test material. The sampling tube was placed on a high-precision analytical balance capable of measuring mass changes up to four decimal places (± 0.0001 g accuracy). During the experiment, CO_2 gas was supplied at a constant pressure of 1 bar, and the change in weight of the sampling tube was recorded over time to monitor the amount of CO_2 absorbed. After a set period, the CO_2 supply was stopped at 1440 min for hydrated lime and cement, and at 360 min for fly ash and slag, when the uptake curve reached an apparent equilibrium, and the balance continued recording to determine the amount of CO_2 permanently retained. All samples were prepared to maintain a consistent total mass of 1.5 g, including solid and water, ensuring uniformity across tests. The samples were prepared such that the total weight, including both the solid and liquid components, was maintained at 1.5 g to ensure consistency across all experimental conditions throughout the study. The complete experimental setup is shown in Fig. 1.

Results and discussion

Material characterization

Lime

The microstructural and elemental characteristics of hydrated lime were analyzed using SEM and EDS. Figure 3a shows that calcium (Ca) and oxygen (O) are the dominant elements, with strong peaks at ~ 3.7 keV, 4.0 keV (Ca), and 0.5 keV (O). Carbon (C) was also present, likely due to partial carbonation. Minor peaks for Mg, Al, Si, K, and Fe indicate trace impurities. EDS data Fig. 2 confirms CaO as the primary component (94.01 wt.%), consistent with Ca $(OH)_2$, along with minor oxides like MgO, Al_2O_3, and SiO_2. The SEM image in Fig. 3b reveals a porous structure of jagged particles, providing a high surface area for CO_2 interaction. These properties support lime's suitability for enzyme-enhanced CO_2 mineralization.

Fly ash

The chemical composition and microstructure of fly ash were examined using EDS and SEMand are shown in Fig. 4. The EDS spectrum Fig. 4a shows dominant peaks for O, Si, and Al, indicating major oxides like SiO_2 and Al_2O_3, along with notable amounts of Ca, Fe, Mg, S, K, Na, Ti, and trace Ni. Figure 2 confirms its pozzolanic nature, with high SiO_2 (35.38 wt.%) and Al_2O_3 (19.91 wt.%), and other oxides including CaO and FeO. The SEM image in Fig. 4b shows spherical particles (cenospheres) within an amorphous matrix, offering high surface area and porosity, favorable for CO_2 interaction and mineralization, especially under enzymatic enhancement.

Slag

The slag sample was analyzed using EDS and SEM to assess its composition and surface morphology. The EDS spectrum in Fig. 5a shows dominant peaks for Ca, Si, and O, along with Mg, Al, Fe, S, and trace elements like Na,

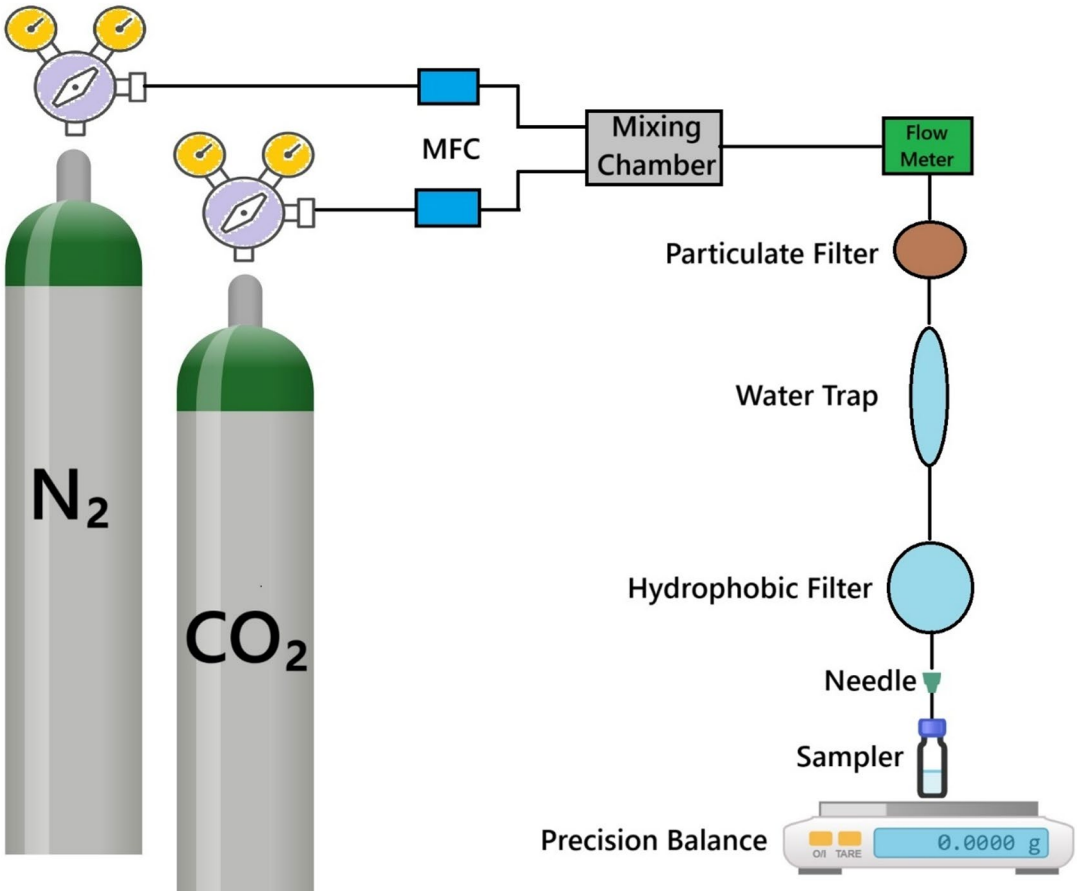

Fig. 1. Experimental setup For CO_2 absorption measurement.

K, Ti, and Mn, indicating a complex mineralogy typical of metallurgical slag. Figure 2 shows high CaO (39.16 wt.%) and SiO_2 (33.73 wt.%), followed by MgO and Al_2O_3. The SEM image in Fig. 5b shows angular, fractured particles with rough surfaces, providing increased surface area and nucleation sites. This dense microstructure favors stable CO_2 mineralization, especially under enzyme-catalyzed conditions.

Mechanism of CO_2 mineralization

Figure 6 presents a two-stage conceptual model illustrating the mechanism of carbonic anhydrase-assisted CO_2 capture and mineralization using alkaline industrial waste materials. In the first stage, carbon dioxide (CO_2) gas diffuses into the aqueous phase, where it undergoes rapid hydration catalyzed by the enzyme carbonic anhydrase. This enzymatic reaction significantly accelerates the otherwise slow conversion of CO_2 and water into bicarbonate ions (HCO_3^-) and protons (H^+), as shown below:

$$CO^2 + H^2O \rightarrow (CA) \rightarrow HCO_3^- + H^-$$

This reaction increases the concentration of reactive bicarbonates in solution, forming the basis for subsequent mineralization.

In the second stage, calcium ions (Ca^{2+}), released from alkaline materials such as hydrated lime, fly ash, or slag, react with bicarbonate to form carbonate ions (CO_3^{2-}). These carbonate ions then combine with Ca^{2+} to form solid calcium carbonate ($CaCO_3$) precipitates:

$$Ca^{2+} + CO_3^{2+} \rightarrow CaCO_3$$

This mineral precipitation pathway effectively sequesters CO_2 as a thermodynamically stable solid, enabling long-term carbon storage. The enzymatic enhancement ensures faster reaction kinetics and greater utilization of calcium-bearing waste materials, thereby improving the overall CO_2 capture efficiency.

CO_2 absorption in alkaline systems

Figure 7 presents the CO_2 absorption behavior of pure water and enzyme solution (carbonic anhydrase dissolved in water) over a period of 7200 min. During the first 1440 min, CO_2 gas was continuously supplied. After this point, the gas flow was stopped to observe desorption behavior.

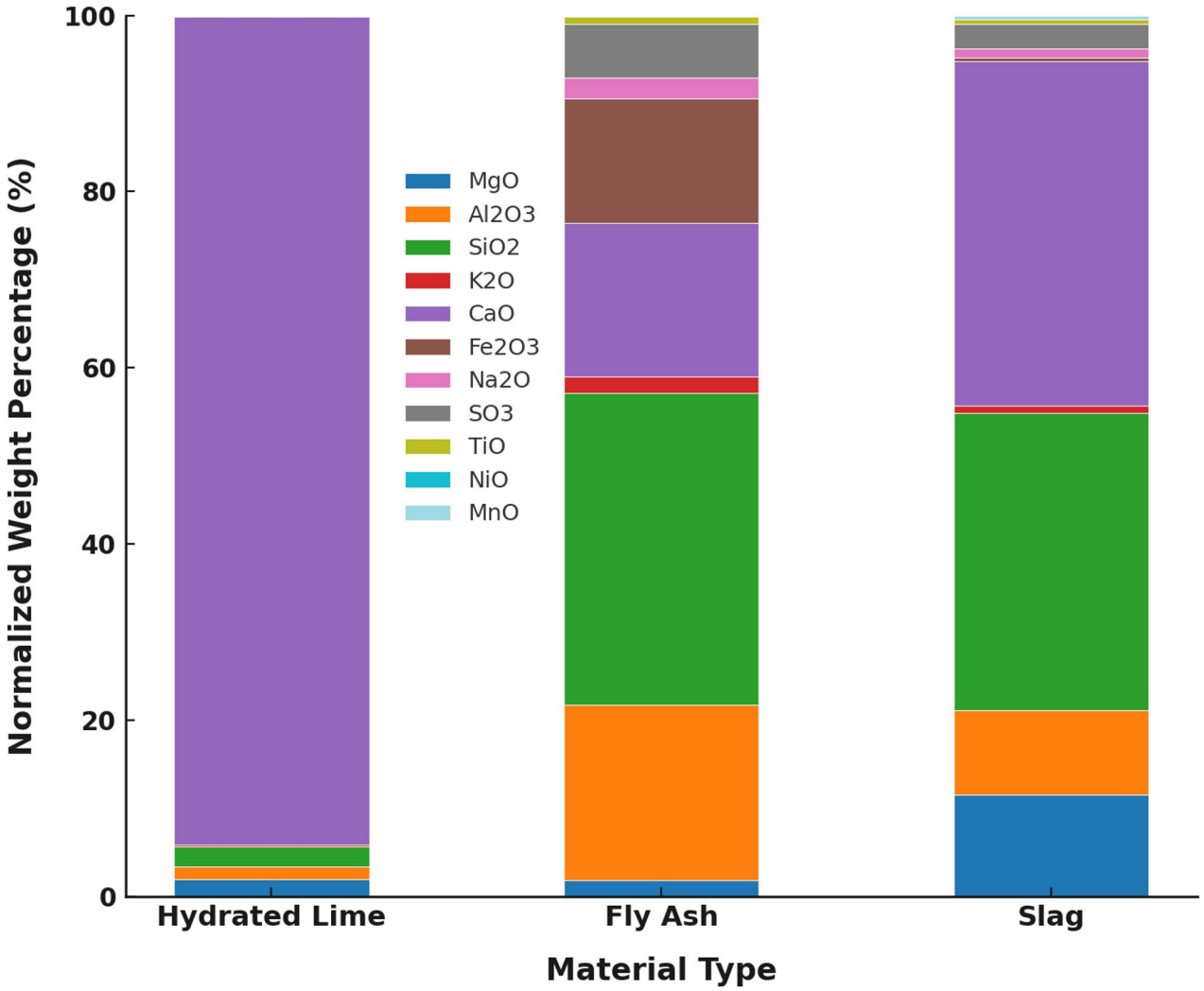

Fig. 2. Major oxides in hydrated lime, fly ash, and slag.

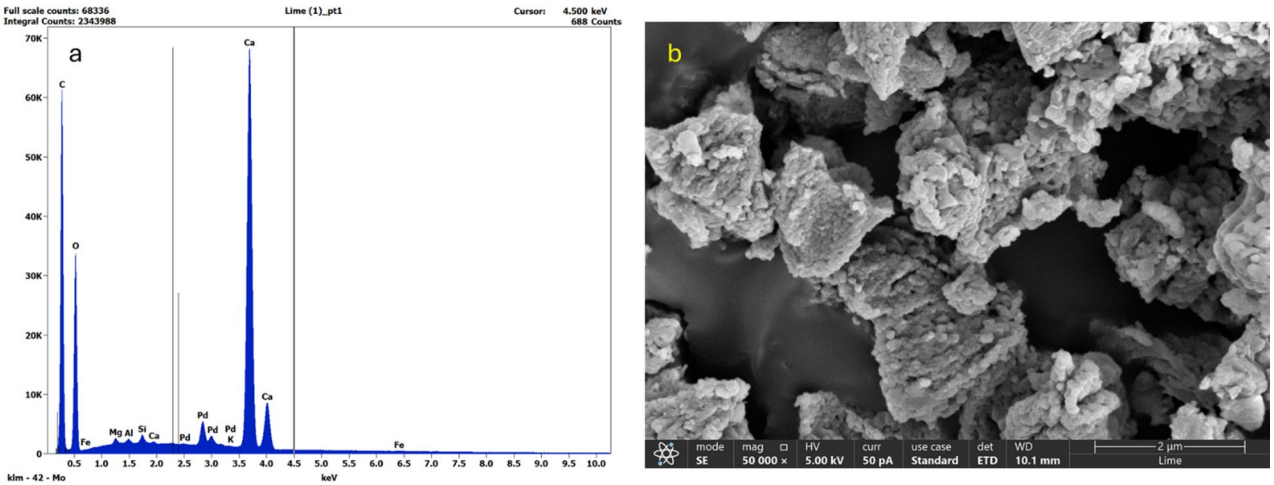

Fig. 3. (**a**) Eds spectrum showing calcium and oxygen as major elements in hydrated lime; (**b**) Sem image At 50,000 × magnification displaying its porous, irregular surface.

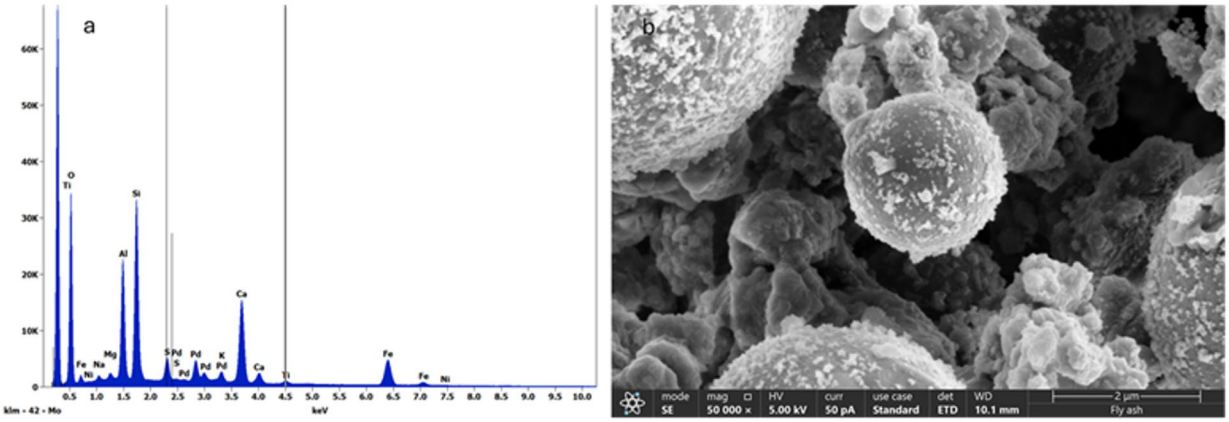

Fig. 4. (**a**) EDS spectrum showing major elements in fly ash, including Si, Al, O, Ca, and Fe; (**b**) SEM image at 50,000 × magnification showing spherical and irregular particle morphology.

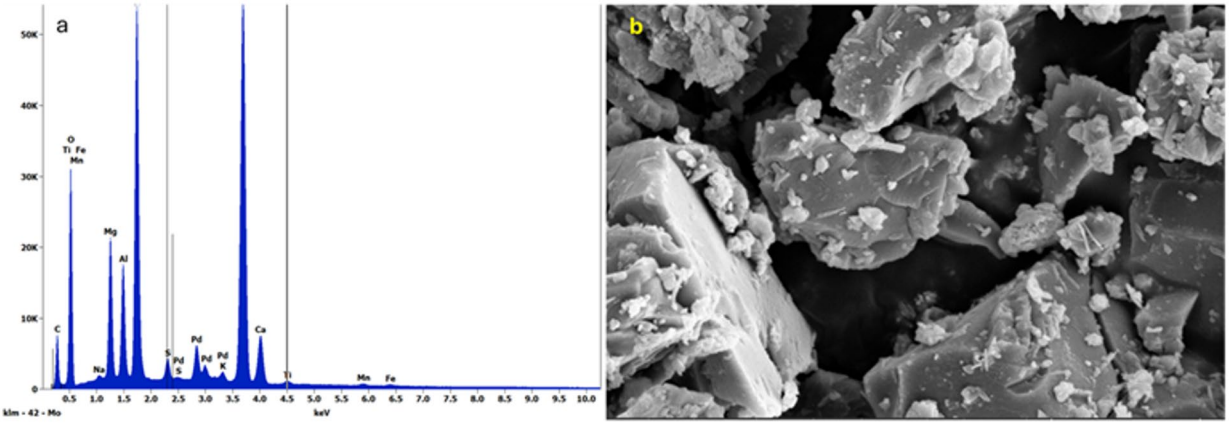

Fig. 5. (**a**) EDS spectrum of slag showing major peaks for Ca, Si, O, and Mg; (**b**) SEM image at 50,000 × magnification showing angular and fractured particle surfaces favorable for CO_2 mineralization.

In the case of water, a slight initial decrease in mass occurred, likely due to water evaporation. This was followed by a gradual increase in CO_2 absorption, reaching a peak value of approximately 239.53 mg/g at 1440 min. Once the CO_2 supply was cut off, the absorbed gas was gradually released, and the mass returned close to its original value by 2880 min, indicating nearly complete desorption of CO_2.

In contrast, the enzyme solution exhibited a significantly faster and higher CO_2 absorption rate. A rapid mass increase was recorded immediately after the start of CO_2 flow, with absorption reaching 465.4 mg/g at 2160 min. Following the CO_2 cutoff, a gradual desorption was observed; however, the rate of desorption was notably lower than that of pure water, and the return to initial mass was delayed.

The behavior shown in Fig. 7 demonstrates the catalytic effect of carbonic anhydrase in accelerating the conversion of CO_2 into bicarbonate in aqueous environments. In pure water, the absorption of CO_2 is governed by the slow, uncatalyzed hydration of CO_2 molecules, a reversible equilibrium process. Once CO_2 supply ceases, the dissolved CO_2 is released back into the atmosphere due to the absence of solid-phase sinks or reactive ions to stabilize it.

With the enzyme present, the reaction kinetics change significantly. Carbonic anhydrase catalyzes the reversible hydration of CO_2 ($CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$), leading to a rapid buildup of bicarbonate ions in solution. This not only enhances the initial absorption rate but also increases the total amount of CO_2 retained during the absorption phase. Although the process remains reversible, the presence of bicarbonate in higher concentrations slows down the desorption rate.

However, because water lacks reactive cations (like Ca^{2+} or Mg^{2+}), the bicarbonate formed cannot convert into stable carbonates, and thus, no permanent sequestration occurs. This is evident from the eventual return of the enzyme solution to near its original mass, like water, albeit over a longer period. These findings underscore that while carbonic anhydrase enhances CO_2 hydration kinetics, permanent CO_2 capture requires coupling with reactive alkaline materials to form solid carbonates.

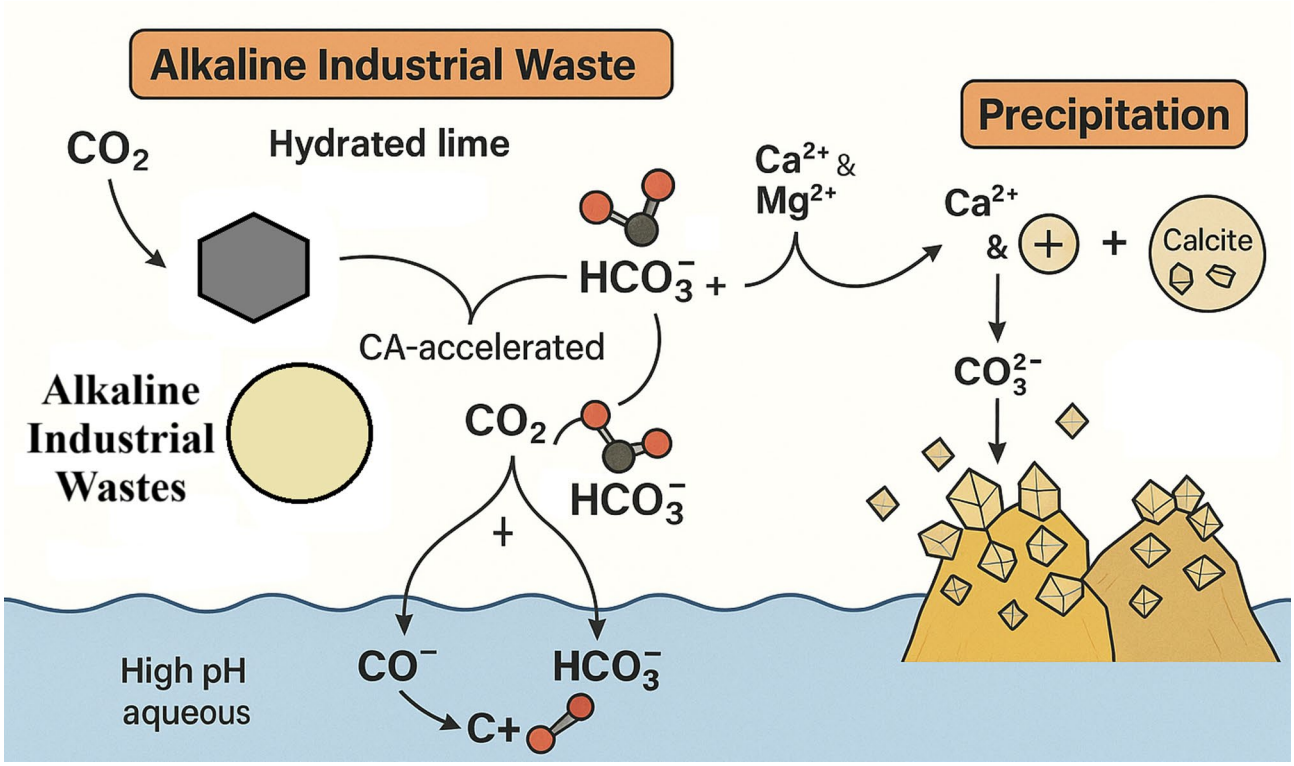

Fig. 6. Enzyme-enhanced CO_2 mineralization in alkaline industrial wastes.

Figure 8 presents the CO_2 absorption behavior of (a) hydrated lime and (b) cement, both with and without carbonic anhydrase enzyme. In each case, CO_2 gas was supplied for 1440 min and then stopped to observe desorption or stabilization trends.

In Fig. 8a, hydrated lime shows moderate CO_2 absorption when used alone, reaching a peak value of 131.33 mg/g at 1440 min. After the CO_2 cutoff, a small decrease in absorption is observed, indicating partial desorption or stabilization. However, when the enzyme was added, the absorption rate increased significantly, reaching 318.07 mg/g at 120 min and peaking at 474.13 mg/g at 1440 min. After the CO_2 supply ended, the weight remained mostly stable, indicating a high degree of permanent CO_2 fixation.

Figure 8b shows similar trends for cement. Without the enzyme, cement absorbed CO_2 slowly, peaking at 55.47 mg/g, and remaining flat after that. The addition of the enzyme led to a substantial enhancement in both the rate and extent of absorption, with a maximum of 285.93 mg/g by 1440 min. Post-cutoff, the mass plateaued with no notable desorption, suggesting stable carbonate formation.

The results in Fig. 8 underscore the importance of both chemical reactivity and enzymatic enhancement in CO_2 mineralization. Hydrated lime, a highly reactive alkaline material rich in $Ca(OH)_2$, readily reacts with CO_2 to form $CaCO_3$. In the presence of carbonic anhydrase, the hydration of CO_2 to bicarbonate is greatly accelerated, increasing the local concentration of reactive intermediates. This, in turn, facilitates faster and more extensive precipitation of carbonate phases. The near-stable mass after CO_2 cutoff in both cases, particularly with the enzyme, confirms the formation of solid carbonates and minimal reversibility of the process.

Cement, on the other hand, contains residual $Ca(OH)_2$ and other partially hydrated phases, making its reactivity slower and more complex. The enzyme's role in this matrix is to boost the hydration of CO_2 so that it can efficiently react with the limited available Ca^{2+}. As seen in the cement + enzyme case, the absorption increased more than fivefold compared to the untreated cement. The enzyme thus acts as a kinetic enhancer, particularly beneficial in materials with moderate to low baseline reactivity.

CO_2 absorption in complex industrial byproducts

Figure 9 illustrates the CO_2 absorption behavior of (a) fly ash and (b) slag, comparing samples with and without the addition of carbonic anhydrase enzyme. CO_2 gas was supplied for 360 min, followed by a shutdown phase to observe retention or desorption trends.

In Fig. 9a, the untreated fly ash exhibited a gradual increase in CO_2 uptake, reaching 85.66 mg/g at 360 min. After the CO_2 supply was stopped, the sample displayed a progressive decrease in absorption, indicating partial CO_2 desorption. In contrast, the enzyme-enhanced fly ash showed a sharp initial rise in absorption, peaking at 308.27 mg/g at 360 min. After the cutoff, the curve remained mostly stable, indicating successful CO_2 retention.

Figure 9b shows similar trends for slag. The untreated slag reached a maximum of 86.67 mg/g at 360 min, with a relatively flat absorption curve thereafter. The slag treated with the enzyme exhibited a much steeper absorption trajectory, achieving 312.27 mg/g by 360 min, with little change after CO_2 cutoff.

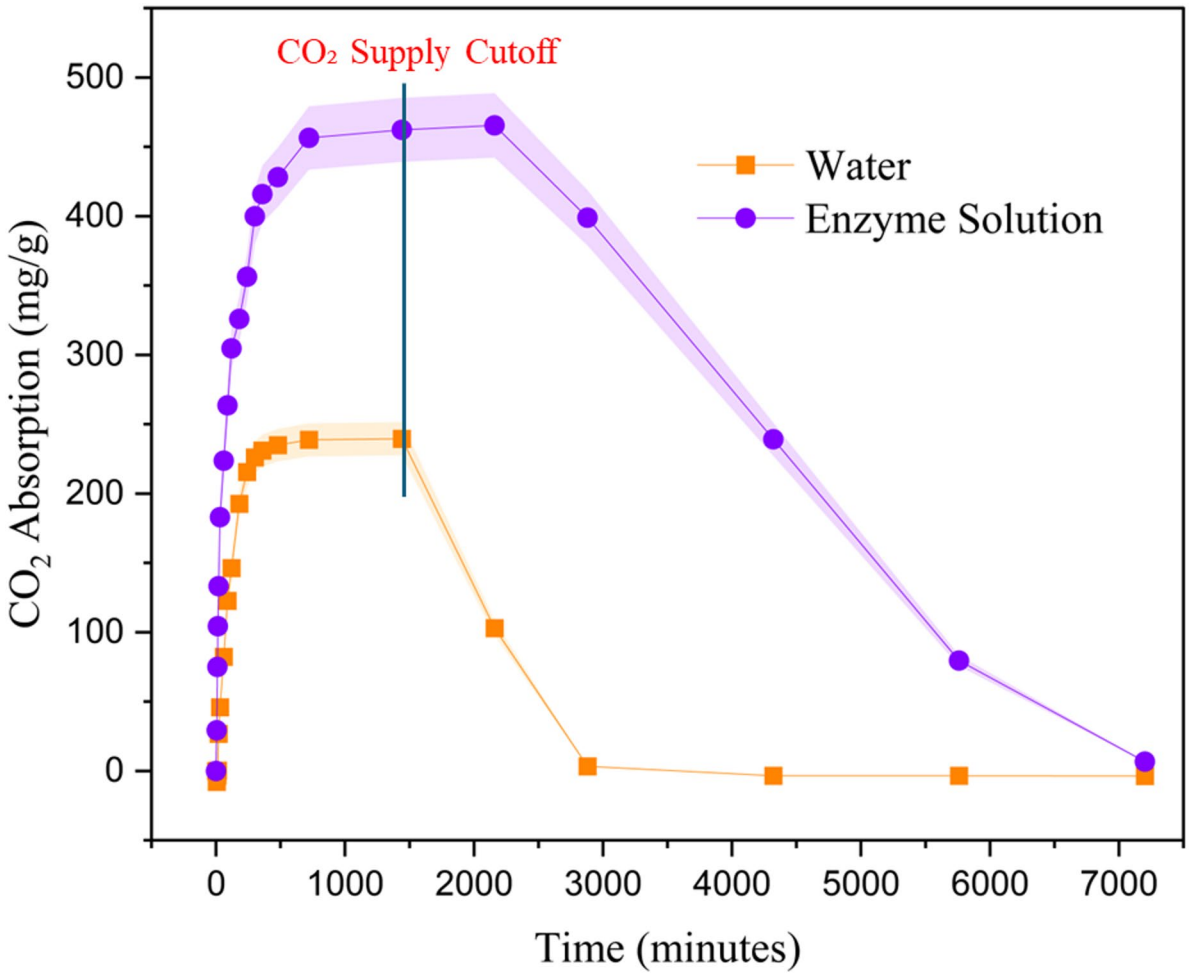

Fig. 7. CO_2 absorption in water and enzyme solution.

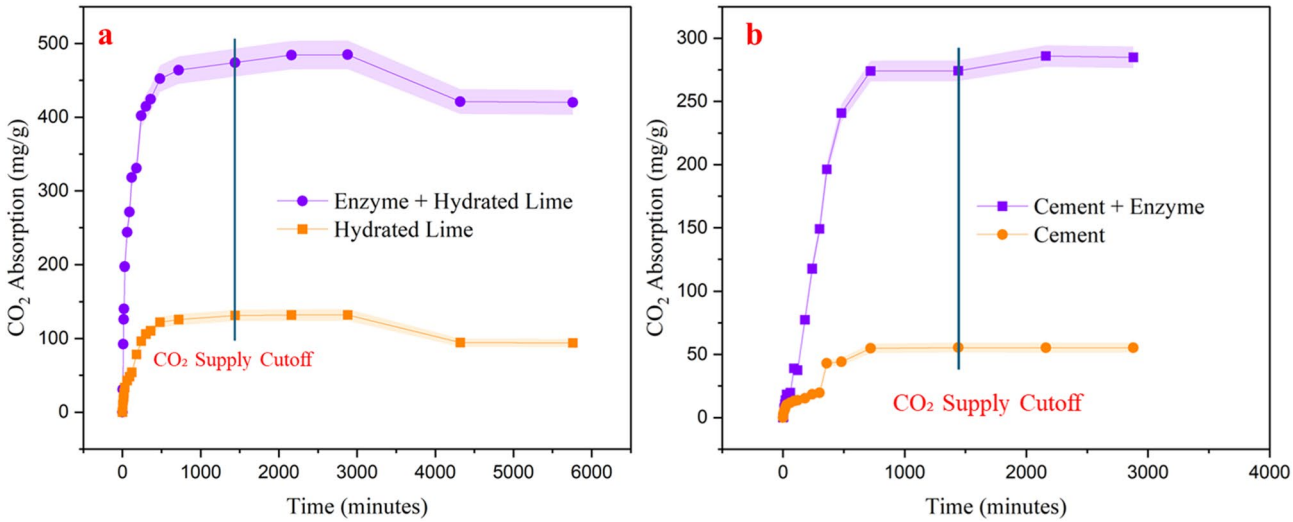

Fig. 8. CO_2 absorption behavior of (**a**) hydrated lime with and without CA and (**b**) cement with and without CA.

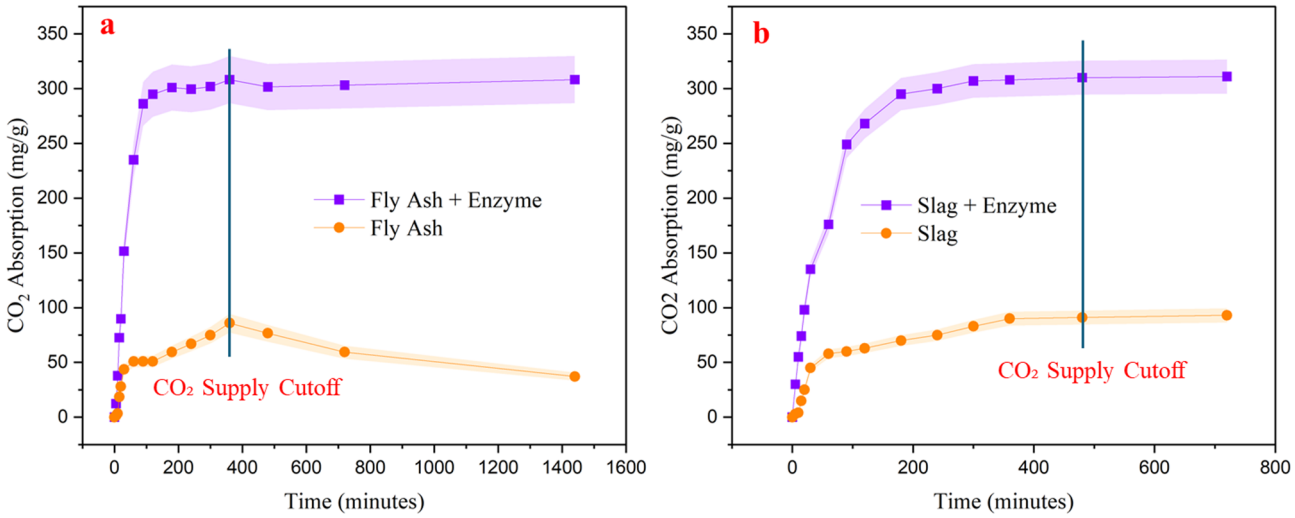

Fig. 9. CO_2 absorption performance of (**a**) fly ash with and without CA and (**b**) slag with and without CA.

The results in Fig. 9 highlight the distinct behaviors of two industrial by-products, fly ash and slag, in CO_2 capture scenarios, especially when catalyzed by carbonic anhydrase. Fly ash, composed primarily of amorphous aluminosilicate glass with minor reactive phases, typically releases Ca^{2+}, Mg^{2+}, and other metal ions at a slower rate. The enzyme's role here is crucial: by accelerating CO_2 hydration to bicarbonate, it enhances the opportunity for bicarbonate ions to react with the slowly released metal cations, forming insoluble carbonates. This mechanism accounts for the observed improvement in both absorption rate and retention in enzyme-enhanced fly ash samples. The post-cutoff plateau confirms that a significant portion of CO_2 was mineralized and retained.

For slag, a material with a higher baseline reactivity due to its content of CaO, MgO, and silicate phases, the CO_2 absorption behavior mirrors that of fly ash but occurs more efficiently even without the enzyme. However, enzyme addition results in a markedly higher final absorption value. The rapid increase and plateau observed post-cutoff indicate that the system approached mineral carbonation saturation quickly. The catalytic action of carbonic anhydrase ensured a sufficient supply of bicarbonate for reactions with divalent metal cations, leading to stable carbonate formation.

In both materials, the minimal or negligible desorption after CO_2 cutoff in enzyme-treated samples provides compelling evidence of permanent CO_2 sequestration via mineralization pathways. The enzyme does not store CO_2 but significantly accelerates the formation of carbonate precipitates through enhanced bicarbonate availability.

Comparison of the effects of enzyme on CO_2 absorption

Figure 10a shows the comparison of peak absorption values of CO_2 across all different materials used in this study. For alkaline materials tested in this study, the rank of these materials in terms of peak CO_2 absorption is: hydrated lime > fly ash > slag > cement. This is possibly due to the constituents of mineral components in these materials. The figure shows that with the enzyme; all these materials showed a significant increase in peak CO_2 absorption. With the use of enzyme, the CO_2 absorption by water increased from 239.53 mg/g to 465.4 mg/g; the CO_2 absorption by hydrated lime increased from 131.33 mg/g to 474.13 mg/g; the absorption in slag increased from 86.67 mg/g to 312.37 mg/g; the CO_2 absorption by cement increased from 55.47 mg/g to 285.93 mg/g; and the CO_2 absorption by fly ash increased from 85.66 mg/g to 308.27 mg/g. The possible mechanism is that the enzyme accelerates the CO_2 dissolution into water, which accelerates the formation of carbonates and bicarbonates, resulting in higher CO_2 absorption efficiency.

With the depressure of CO_2, a portion of the dissolved CO_2 was released. What remained was the CO_2 that was permanently fixed by carbonated minerals such as calcite. Figure 10b summarizes the amount of CO_2 that was permanently absorbed by different materials. The figure shows that the use of the enzyme increased the permanent absorption capacity of all alkaline materials. In the case of pure water, it absorbed a good amount of CO_2, but all of the CO_2 was released back into the atmosphere when the CO_2 supply was cut off, leading to zero permanent absorption. When the enzyme was added to the water, it showed a similar trend with a negligible permanent absorption of 6.7 mg/g. This indicates that water has a very limited or no capacity to permanently sequester CO_2.

In contrast, the impact of the enzyme on industrial materials such as hydrated lime, slag, cement, and fly ash is more pronounced. Hydrated lime permanently sequestered 93.33 mg/g of CO_2, and with the enzyme, this value rose significantly to 419.667 mg/g. A comparable trend was observed in all other materials, where the presence of the enzyme notably enhanced their ability to permanently absorb CO_2. In the case of slag, absorption increased from 86.67 mg/g to 312.37 mg/g. In cement, it rose from 55.067 mg/g to 285.133 mg/g, and for fly ash, it increased from 37.133 mg/g to 308.2667 mg/g.

The data shown in Figs. 10a and b confirm the catalytic role of carbonic anhydrase (CA) in enhancing the kinetics of CO_2 absorption and mineralization across various alkaline materials. Rather than increasing the

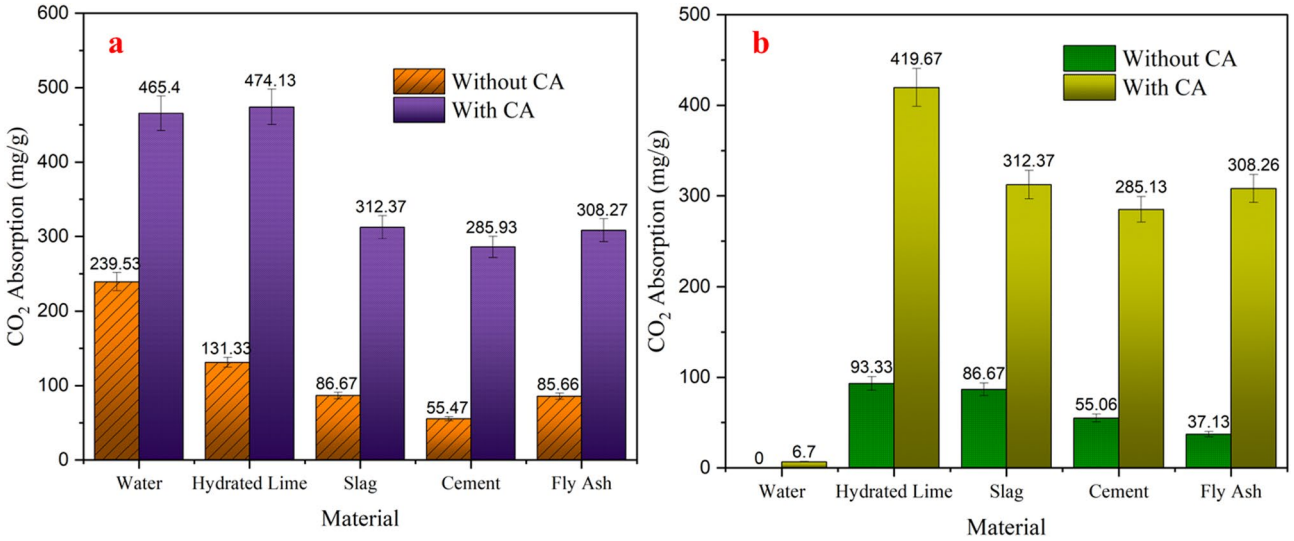

Fig. 10. (**a**) Comparison of maximum CO_2 absorption (mg/g) across different materials with and without carbonic anhydrase (**b**) Comparison of permanent CO_2 absorption (mg/g) measured after CO_2 supply cutoff.

inherent CO_2 storage capacity of these materials, the enzyme accelerates the hydration of CO_2 to bicarbonate, thereby enabling faster reaction with available alkaline cations such as Ca^{2+} or Mg^{2+} within the timeframe of the experiment.

Across all enzyme-treated samples, a significantly higher percentage of the absorbed CO_2 was permanently fixed compared to their untreated samples. For example, in hydrated lime, the percentage of permanent CO_2 absorption rose from approximately 71% without enzyme to nearly 89% with enzyme. In fly ash, this shift was even more pronounced, increasing from about 43% to almost 100%. These results highlight how enzyme presence enables the material to utilize more of its reactive potential within a limited reaction window.

In materials such as cement and slag, which naturally showed high retention rates even without the enzyme, the enzyme allowed for faster completion of mineralization reactions. This did not change the final proportion of CO_2 fixed but significantly increased the amount captured during the same period, indicating that the enzyme helped the system reach its equilibrium state more rapidly. This supports the enzyme's role as a kinetic enhancer, not a sequestration agent.

Water, by contrast, demonstrated the boundary of enzyme utility. Even with CA, only a small fraction of the absorbed CO_2 was retained, confirming that without mineral cations to react with bicarbonate, the enzyme cannot support permanent CO_2 capture.

These patterns support the conclusion that CA enhances CO_2 fixation rates during the active gas-solids reaction period, without influencing the final equilibrium or acting as a sink itself. The permanent capture observed in enzyme-treated systems occurred because more CO_2 was successfully mineralized within the limited experimental duration, not because the enzyme altered the chemical endpoint of the system.

Reusability of enzyme

To assess the reusability of the enzyme, repetitive experiments were conducted by using hydrated lime. After a complete round of CO_2 absorption test with hydrated lime and enzyme, the bicarbonates were allowed to settle down. The enzyme solution above the solid sediment layer was carefully extracted. The recycled liquid was then used for another round of CO_2 absorption test using a new batch of hydrated lime only. The same procedure for the recycling of enzymic liquid was repeated for additional CO_2 absorption cycles with hydrated lime. Figure 11 summarizes the absorption behaviors for all these absorption cycles. The results showed a consistent trend of CO_2 absorption with only slight decreases in the peak amount of CO_2 absorption. The slight reduction might partially be attributed to the loss of a portion of the enzyme during the extraction process. The reuse of the enzyme still maintained a significant 87.9% of CO_2 absorption at cycle 4. The experimental observations suggest that the enzyme retains most of its catalytic activity. The slight decrease in the CO_2 absorption over the reuse cycles is possibly due to a minor loss during the extraction of the enzyme solution at the end of testing. The reuse of enzyme presents a promising approach to reducing the cost of enzyme.

Statistical analysis of enzyme impact on CO_2 absorption

To quantitatively assess the significance of the observed enhancements in CO_2 absorption due to the addition of carbonic anhydrase, a statistical analysis was conducted across all tested industrial byproducts: hydrated lime, fly ash, and slag.

Each absorption experiment was performed in triplicate ($n = 3$), and the final CO_2 uptake values (mg/g) at 720 min were used for comparison. The data were first tested for normality and homogeneity of variances using the Shapiro–Wilk and Levene's tests, respectively. Based on these assumptions, both one-way ANOVA and

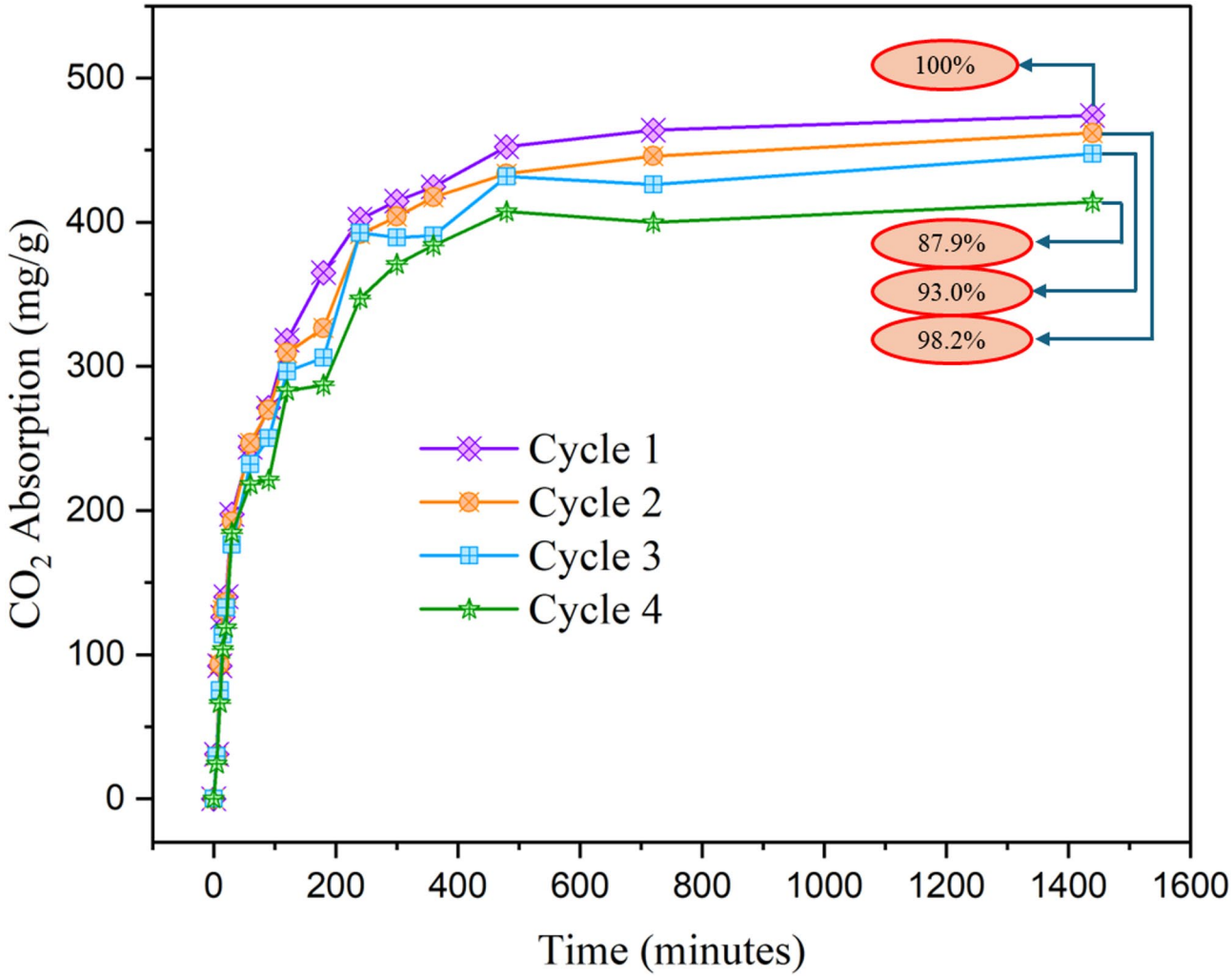

Fig. 11. CO_2 absorption behaviors by reuse of the enzyme over different testing cycles.

Material	Condition	Mean CO_2 Uptake (mg/g)	Std. Dev	*p*-value (t-test)
Hydrated lime	Without enzyme	421.3	6.2	0.0007
	With enzyme	474.1	5.7	
Fly ash	Without enzyme	392.0	7.1	0.0021
	With enzyme	440.7	6.4	
Slag	Without enzyme	353.2	5.8	0.0014
	With enzyme	416.8	4.9	

Table 1. Statistical analysis of CO_2 uptake with and without carbonic anhydrase.

independent two-sample t-tests were employed to evaluate statistical differences between enzyme-assisted and control groups.

The ANOVA results showed a significant overall effect of enzyme addition on CO_2 absorption across all material types ($p < 0.01$). To further validate these findings, two-sample t-tests were performed between the enzyme and control groups for each material individually. The following mean ± standard deviation values and p-values were obtained as shown in Table 1.

The low *p*-values ($p < 0.005$) across all materials confirm that the enzyme-assisted systems achieved statistically significant increases in CO_2 absorption. These enhancements are attributed to the accelerated formation of bicarbonate ions catalyzed by CA, which improves the availability of carbonate species for subsequent mineralization with Ca^{2+} from the solid phase.

All statistical analyses were conducted using Python, and a significance level of $\alpha = 0.05$ was used throughout.

Discussion

The results show that carbonic anhydrase (CA) consistently increases both the total CO_2 absorbed during flow and the fraction retained as solid carbonates after cutoff across hydrated lime, cement, fly ash, and slag. The enhancement is most pronounced for hydrated lime and measurable for cement, fly ash, and slag, reflecting how readily each material supplies divalent cations for carbonate precipitation. The permanence observed after the gas is turned off indicates that the added mass is not simply dissolved CO_2 but mineralized products, predominantly $CaCO_3$, formed on the particle surfaces. These outcomes align with prior reports that CA accelerates the hydration step and thereby raises local carbonate availability without changing the thermodynamic endpoint of mineralization[40–42].

Although the enzyme is intrinsically fast, the apparent reaction rate in our suspensions is limited by processes that are not enzyme-controlled. Gas–liquid transfer of CO_2 into a particle-laden boundary layer, diffusion through that layer, and the dissolution of Ca- or Mg-bearing phases to supply cations all slow the overall sequence. Once a carbonate shell forms on reactive surfaces, further growth requires ion transport through the newly formed product, which introduces an additional diffusion barrier and explains the gradual approach to a plateau. Similar transitions from an initial chemically controlled stage to a diffusion-limited stage are well documented for mineral carbonation systems and were reproduced here under ambient conditions. Thus, the "slow enzyme kinetics" observed at the reactor scale arises from mass-transfer and solid-state constraints rather than from low catalytic turnover of CA itself[43–46].

Material composition strongly modulates both the magnitude of the CA benefit and the overall rate. Hydrated lime, dominated by $Ca(OH)_2$, releases Ca^{2+} rapidly and supports fast nucleation and growth of $CaCO_3$; cement behaves similarly due to residual portlandite after hydration, albeit with slower release within a more complex matrix. Fly ash and slag contain substantial SiO_2 and Al_2O_3 and more limited free CaO or MgO, so cation release is slower, and the CA advantage manifests primarily as faster attainment of the same thermodynamic endpoint[47,48]. Trace metals and aluminosilicate surfaces can also interact with the enzyme; literature notes that multivalent cations and certain surfaces can partially inhibit or adsorb CA, which would further dampen apparent activity in ash and slag systems. Even with these interactions, the enzyme retained most of its activity over four reuse cycles, indicating that reversible transport and surface effects dominate over irreversible enzyme deactivation in our conditions.

Taken together, the data support a surface-coupled mechanism in which CA increases bicarbonate/carbonate generation within the thin liquid film at particle interfaces, boosting local supersaturation and promoting heterogeneous nucleation on lime, cement, ash, and slag. The subsequent slowdown is consistent with armoring or passivation by newly precipitated carbonates and with limited replenishment of Ca^{2+}/Mg^{2+} from the solid. These interpretations are consistent with microscopy-based observations reported for carbonation of lime-based binders and industrial residues, where product layers gradually reduce access to reactive cores. From an application standpoint, this implies that engineering the microenvironment around the enzyme and the solid, by increasing effective interfacial area, refreshing surfaces, or moderating product-layer growth, should yield the largest practical gains[31,49].

The reusability tests indicate that homogeneous CA can be recycled with only modest loss of performance across cycles, which is encouraging for cost and process design. Nonetheless, industrial deployment would benefit from enzyme immobilization or entrapment strategies that stabilize CA against adsorption, shear, and ionic effects while enabling easy recovery; such approaches have shown improved operational stability in gas–liquid contactors and slurry systems. Buffer-layer engineering or local co-solutes that preserve the catalytic Zn^{2+} environment may further mitigate transient inhibition by dissolved metals common in ash and slag leachates[50–52].

Conclusions

This study shows the potential of carbonic anhydrase to enhance the carbon dioxide capture capabilities of various alkaline industrial materials, including lime, cement, fly ash, and slag. The experimental findings show that the presence of CA significantly increased both the rate of CO_2 absorption and the proportion of CO_2 that was permanently sequestered as stable carbonate minerals within the reaction timeframe. This improvement is attributed to the enzyme's catalytic role, which accelerates the conversion of CO_2 into bicarbonate, thereby enabling more rapid reaction with available cations such as calcium and magnesium.

The enzyme does not alter the thermodynamic equilibrium of the reaction but instead allows the system to reach equilibrium faster. As a result, materials that would otherwise absorb CO_2 slowly or only partially can more effectively sequester carbon when the enzyme is present.

The reusability tests further support the viability of this approach, as CA retained most of its catalytic activity over multiple absorption cycles. This stability highlights the enzyme's potential for use in scalable and economically feasible carbon capture applications. By combining industrial alkaline byproducts with enzymatic catalysis, this approach offers a sustainable pathway to utilize existing waste materials for CO_2 capture.

The integration of carbonic anhydrase into CO_2 capture systems could contribute significantly to climate change mitigation efforts, especially when applied to construction and industrial processes where such materials are already widely used. Continued research into enzyme stabilization, immobilization, and long-term performance will be essential to transition this approach from lab-scale demonstrations to practical, real-world applications targeted at supporting global carbon neutrality goals. Future work should focus on enhancing enzyme durability under alkaline conditions by exploring strategies such as developing alkali-resistant CA mutants or immobilizing enzymes on stable carrier materials to prolong their activity and recyclability in industrial applications.

Data availability

The datasets generated and/or analysed during the current study are not publicly available due to the research in progress but are available from the corresponding author on reasonable request.

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Author contributions
X.Y..: designed and directed this project, improved the methodology, and revised the manuscript. N.L.: design and implement the research, analyze experimental data, and write the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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