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Review

CO_2 Mineralized Sequestration and Assistance by Microorganisms in Reservoirs: Development and Outlook

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Abstract: The goals of carbon neutrality and peak carbon have officially been proposed; consequently, carbon dioxide utilization and sequestration technology are now in the limelight. Injecting carbon dioxide into reservoirs and solidifying and sequestering it in the form of carbonates after a series of geochemical reactions not only reduces carbon emissions but also prevents carbon dioxide from leaking out of the formation. Carbon dioxide mineralization sequestration, which has good stability, has been considered the best choice for large-scale underground CO_2 sequestration. To provide a comprehensive exploration of the research and prospective advancements in CO_2 mineralization sequestration within Chinese oil and gas reservoirs, this paper undertakes a thorough review of the mechanisms involved in CO_2 mineralization and sequestration. Special attention is given to the advancing front of carbon dioxide mineralization, which is driven by microbial metabolic activities and the presence of carbonic anhydrase within oil and gas reservoirs. The paper presents an in-depth analysis of the catalytic mechanisms, site locations, and structural attributes of carbonic anhydrase that are crucial to the mineralization processes of carbon dioxide. Particular emphasis is placed on delineating the pivotal role of this enzyme in the catalysis of carbon dioxide hydration and the promotion of carbonate mineralization and, ultimately, in the facilitation of efficient, stable sequestration.

Keywords: carbon dioxide sequestration; mechanism of mineralization; microbial-assisted mineralization; carbonic anhydrase

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1. Introduction

Excessive emissions of greenhouse gases such as carbon dioxide have caused global temperatures to rise [1]. According to six major international datasets integrated by the World Meteorological Organization (WMO), the world's average temperature in 2021 is about 1.11 (±0.13) °C above pre-industrial levels (1850–1900) [2–4]. During the meeting of the United Nations General Assembly in September 2020, the Chinese government outlined its 2030 "carbon peak" and 2060 "carbon neutrality" goals in response to the global climate crisis [5]. Carbon dioxide capture, sequestration, and utilization (CCUS) are recognized as critical and viable means of achieving carbon neutrality and mitigating the greenhouse effect [6–8], as illustrated in Figure 1. Carbon dioxide sequestration represents a pivotal aspect of CCUS technology; primarily, it involves the injection of captured carbon dioxide into deep geological formations and storing it in geological bodies such as saline aquifers [9], depleted oil and gas reservoirs, and unexploited coal seams [10,11] through geological structures, capillary force binding, dissolution, and mineralization [12–14]. Among the four main sequestration methods, mineralization sequestration involves reacting carbon dioxide with minerals in the rock layer to create a stable secondary mineral and then permanently

storing it in the form of solid carbonate. Mineralized sequestration is considered the most secure and stable method for carbon sequestration due to its durability and the safe and stable storage of the products. Achieving mineralized sequestration through natural forces alone typically requires at least a century [15], as shown in Figure 2. Consequently, how to accelerate the mining process with the assistance of microorganisms has become a research hotspot. This paper comprehensively reviews the metabolic mechanism of microbial-induced mineralization, the historical research, the action sites, and the mechanisms of the key enzyme, carbonic anhydrase, in mineralization induction. Additionally, it addresses the existing challenges and prospects.

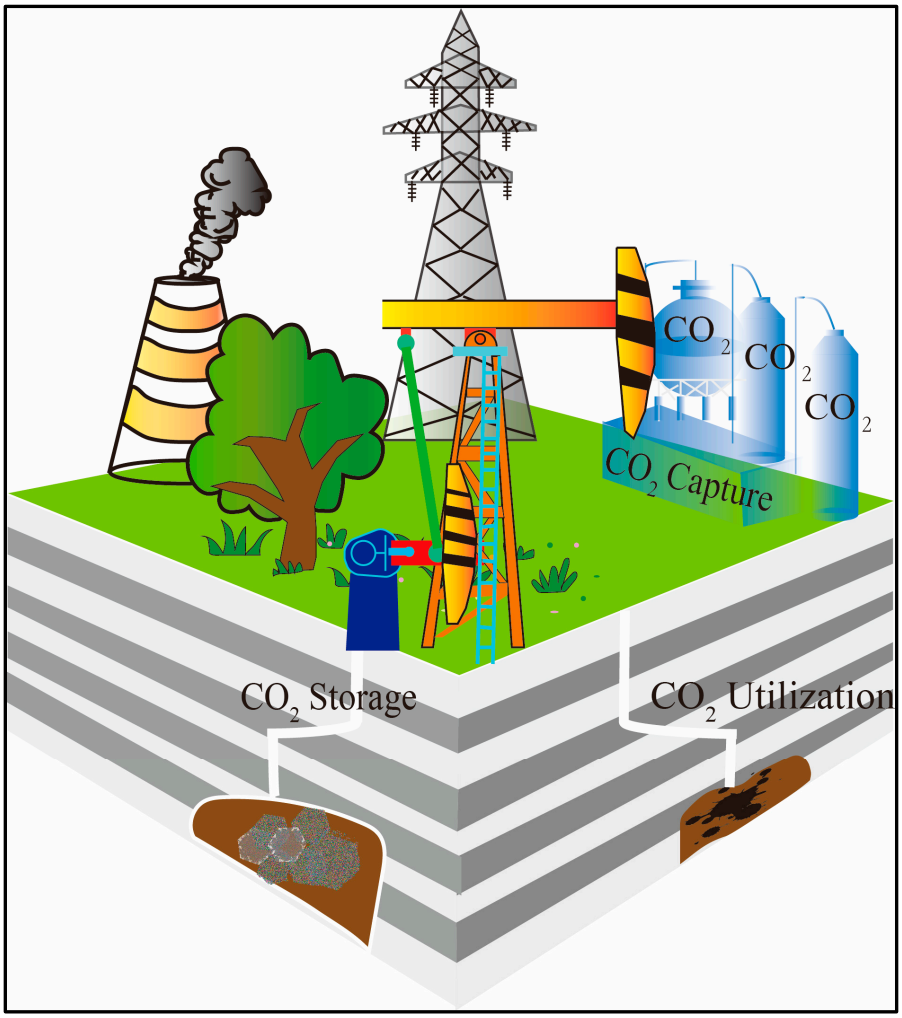


Figure 1. CCUS industrial concept diagram.

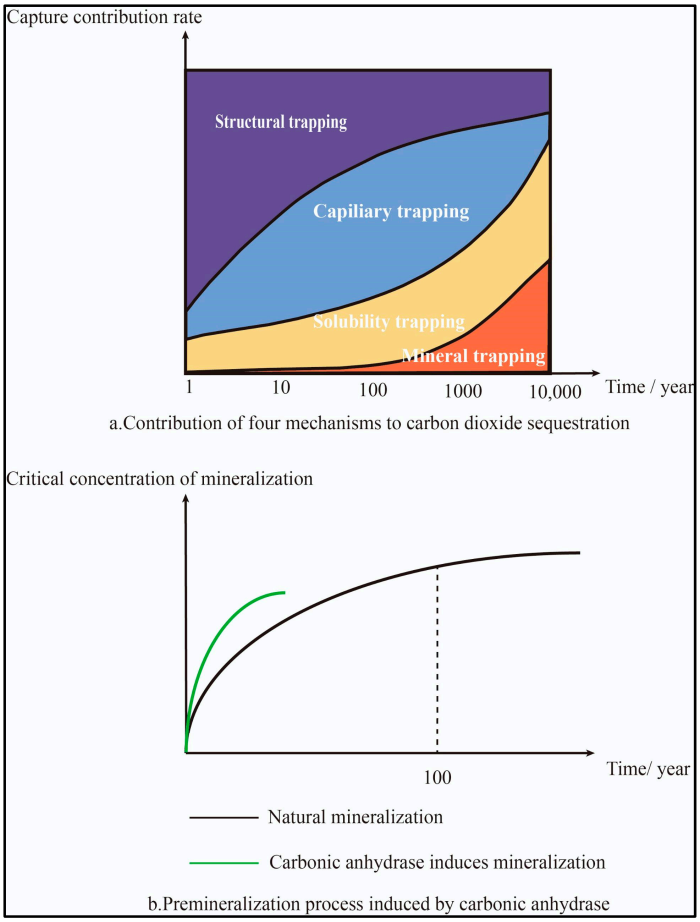


Figure 2. Carbonic anhydrase accelerates mineralization [16].

2. Sequestration Mechanism of Carbon Dioxide Mineralization

Currently, oil fields such as Daqing and Shengli are in the developmental phase; they are characterized by low permeability and a high water cut and present challenges in their operation [17–19]. Injecting carbon dioxide into oil reservoirs has been shown to enhance the recovery rate while securely sequestering carbon dioxide [20–22]. Figure 3 illustrates the schematic diagram of carbon dioxide geological sequestration. This technology has a double effect in fostering economic development and contributing to environmental protection. Therefore, CO_2 mineralization in an underground reservoir environment is considered to be one of the most promising and safe methods for reducing CO_2 emissions.

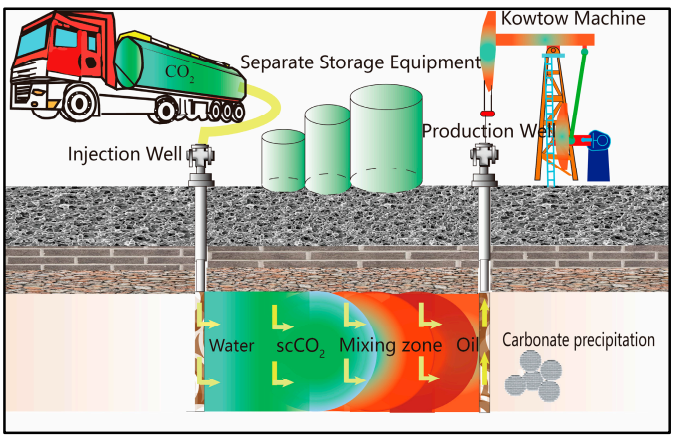


Figure 3. Schematic diagram of carbon dioxide geological sequestration.

The concept of mineralized utilization of carbon dioxide emerged in the 1990s; it primarily involves the carbonation of natural silicate minerals [23,24]. Carbonation can be broadly categorized into dry carbonation and wet carbonation [25]. In wet carbonation, water primarily serves as a medium to facilitate mineral dissolution and carbon dioxide dissolution.

Carbon dioxide mineralization in the reservoir belongs to in situ wet carbonation. During the process of carbon dioxide injection into the target layer (usually at a depth of 800–3000 m), both the temperature and the pressure exceed the critical point of the carbon dioxide phase diagram (31.1 °C and 73.9 bars), as shown in Figure 4, and the carbon dioxide is in a supercritical state [26].

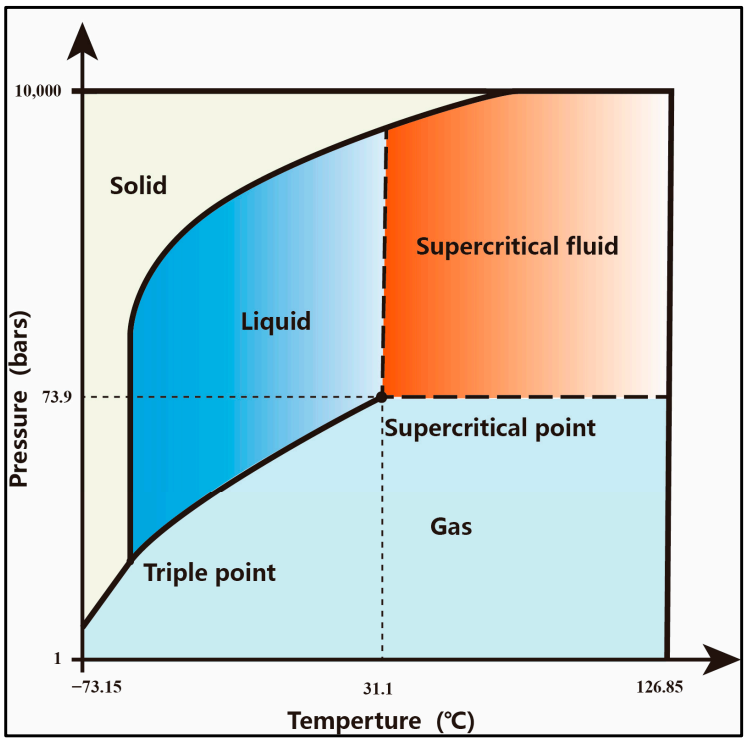


Figure 4. Carbon dioxide phase diagram (Copyright © 1999 Chemical Logic Corporation, 99 South Bedford Street, Suite 207, Burlington, MA 01803 USA. All rights reserved).

Supercritical carbon dioxide is introduced into the deep reservoir, following the principle of similar phase dissolution. During this process, a portion of the carbon dioxide dissolves in the crude oil while another portion dissolves in the formation water. Notably, the solubility of carbon dioxide in crude oil is significantly higher than in water. A fraction of the CO_2 undergoes hydration within the formation, resulting in the formation of carbonic acid, which subsequently ionizes into CO_3^{2-} and HCO_3^{-}. Biogeochemical processes eventually convert some of the CO_2 into solid carbonate, while a minute amount remains in a free state. The different occurrence states of CO_2 within the reservoir are illustrated in Figure 5 below.

Supercritical carbon dioxide undergoes a reaction with water, resulting in the production of carbonic acid. This carbonic acid, in turn, engages in distinct dissolution reactions with various types of minerals [28–30]. Leaching cations (Ca^{2+}, Mg^{2+}, Fe^{2+}, etc.) combine with carbonic anions produced by carbonic acid ionization to produce carbonate precipitation. Under specific conditions, this combination leads to the precipitation of carbonates, facilitating the enduring sequestration of carbon dioxide in a solid state. The whole process can be roughly divided into three stages: carbon dioxide dissolution and ionization, mineral dissolution, and carbonate mineral deposition.

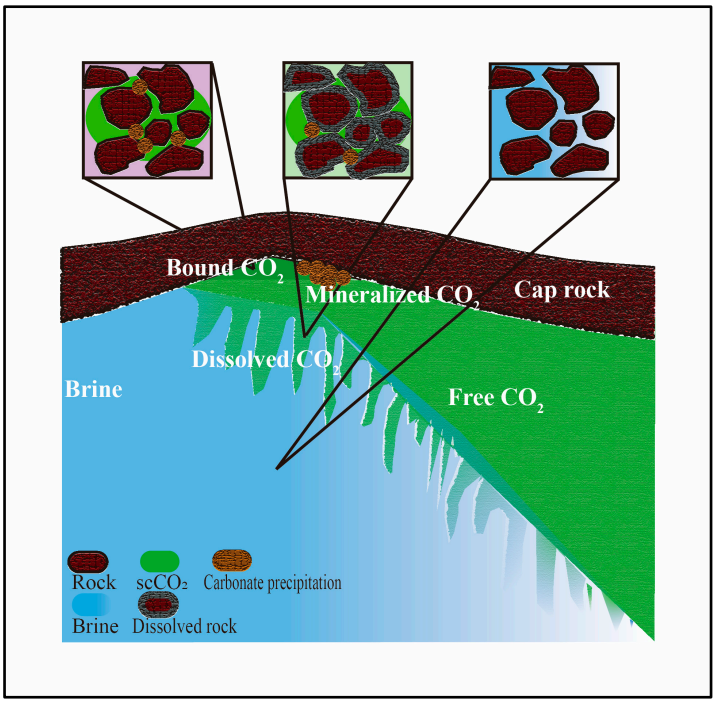


Figure 5. Carbon dioxide in the reservoir occurrence state [27].

(1) Dissolution and ionization of carbon dioxide

Upon the entry of supercritical carbon dioxide ($scCO_2$) into the formation, a density disparity between the $scCO_2$ and the formation water is evident. This prompts the initiation of a gravity-driven flow in the longitudinal direction, causing some $scCO_2$ to ascend and react with the cap rock. Due to the higher density of the $scCO_2$ flow compared to the formation of water, a significant portion of the $scCO_2$ settles, resulting in natural convection between the $scCO_2$ and the brine aquifer during this phase [31]. Subsequently, as the gravity flow reaches the interface of the $scCO_2$–brine phase, the $scCO_2$ undergoes molecular diffusion and dissolves into the brine aquifer due to concentration differences. Concurrently, at the phase interface, the CO_2 undergoes a hydration reaction, forming carbonic acid water, and continues to engage in ionization reactions, generating free protons. The hydration reaction of carbon dioxide exhibits a pH dependence, presenting roughly two reaction forms in different pH ranges. The reaction formulas are as follows:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3{}^-(pH < 8) \quad (1)$$

$$CO_2 + OH^- \rightarrow HCO_3{}^-(pH > 10) \quad (2)$$

Considering that the pH of reservoir groundwater typically remains within a neutral or weakly alkaline range within the oil–gas reservoir environment, the carbon dioxide hydration reaction aligns with Equation (1). The kinetic constant of the reaction ranges from 0.026 to 0.044 s^{-1} at a room temperature of 25 °C [32,33]. The hydration rate of carbon dioxide is relatively slow. To expedite the hydration reaction of carbon dioxide, domestic and international researchers primarily employ catalysts such as phosphate buffers, metal nanoparticles (e.g., nickel nanoparticles or NiNPs), and carbonic anhydrase [34–36].

(2) Mineral corrosion

The surfaces of clay minerals, carbonate minerals, and feldspar mineral particles are enveloped by a carbonate water film. This film leads to the leaching of alkaline earth metal ions such as calcium, magnesium, and iron, resulting in the acquisition of Ca^{2+}, Mg^{2+}, Fe^{2+},

OH^-, HCO_3^-, CO_3^{2-}, and Si-O groups [37]. The mineral dissolution process is depicted in Figure 6, and the reaction equations are presented below:

$$M_xSi_yO_{x+2y}(s) + 2H^+(aq) \rightarrow M^{2+}(aq) + SiO_2(s) + H_2O(l) \quad (3)$$

$$\text{Calcite} + H^+ \leftrightarrow Ca^{2+} + HCO_3^- \quad (4)$$

$$\text{Dolomite} + H^+ \leftrightarrow Ca^{2+} + Mg^{2+} + HCO_3^- \quad (5)$$

$$\text{Siderite} + H^+ \leftrightarrow Fe^{2+} + HCO_3^- \quad (6)$$

$$\text{Magnesite} + H^+ \leftrightarrow Mg^{2+} + HCO_3^- \quad (7)$$

$$Ca - \text{felspar} + 2H^+ + H_2O \leftrightarrow Ca^{2+} + Al_2Si_2O_5(OH)_4 \quad (8)$$

$$Na - \text{felspar} + 2H^+ + H_2O \leftrightarrow 2Na^+ + Al_2Si_2O_5(OH)_4 + 4SiO_2(aq) \quad (9)$$

$$K - \text{feldspar} + 2H^+ + H_2O \leftrightarrow 2K^+ + Al_2Si_2O_5(OH)_4 + 4SiO_2(aq) \quad (10)$$

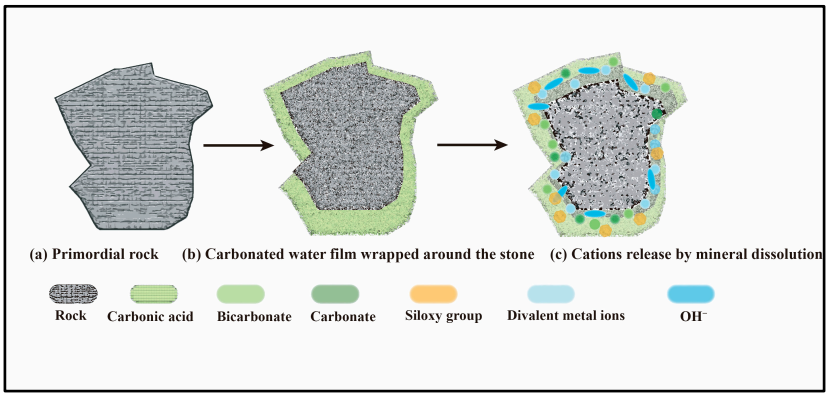


Figure 6. Mineral dissolution process.

Through laboratory static reaction experiments, domestic and foreign researchers have found that carbonate minerals represented by dolomite, feldspar minerals represented by potassium albite, and clay minerals such as chlorite show different degrees of dissolution in the early stage of the reaction, indicating that the degree of dissolution of minerals depends on their chemical structure [38–41]. Due to the limited presence of carbonate rocks and the comparatively low reactivity of feldspar minerals in domestic reservoir minerals, the dissolution of minerals is generally considered to be the rate-limiting step in the mineral carbonation process. Literature indicates that factors such as reservoir temperature, pH [42], and mineral surface area influence the dissolution rate [37,43]. In general, higher temperatures, lower pH values, and the larger specific surface areas of minerals lead to faster dissolution rates and higher degrees of mineral dissolution within the same time frame. Furthermore, research has shown that the addition of a salt solution (e.g., a mixed solution of sodium chloride and sodium bicarbonate or a mixed solution of potassium nitrate and sodium nitrate) during the reaction can act as a catalyst, accelerating the leaching of alkaline earth metal ions to a certain extent [44,45].

(3) Carbonate mineral deposition

As the minerals continue to dissolve and consume protons, the pH of the environment gradually rises, reaching a slightly alkaline level. In this alkaline environment, when carbon anions (carbonates and bicarbonates) within the environment reach the supersaturated state,

they combine with cations such as Ca^{2+}, Mg^{2+}, Fe^{2+}, and others and form the respective carbonate precipitates. The equations are listed as follows:

$$M^{2+}(aq) + CO_3{}^{2-}(aq) \rightarrow MCO_3(s) \quad (11)$$

$$Mg_2SiO_4 + 2CO_2 + 2H_2O \rightarrow 2MgCO_3 + H_4SiO_4 \quad (12)$$

$$Mg_3Si_2O_5(OH)_4 + 3CO_2 + 2H_2O \rightarrow 3MgCO_3 + 2H_4SiO_4 \quad (13)$$

$$CaSiO_3 + CO_2 + H_2O \rightarrow CaCO_3 + H_2SiO_3 \quad (14)$$

Indoor experiments involving carbon dioxide–water–rock interactions have been conducted to detect and analyze sedimentary minerals. These experiments have revealed that the sedimentary minerals mainly consist of carbonate rocks, predominantly calcite and dolomite. Additionally, a smaller proportion consists of silicate rocks, including kaolin, dolomite, and quartz [40,46,47].

Regarding the three phases of geochemical reactions, the literature has consolidated the key factors influencing mineral deposition efficiency, encompassing the concentration of carbon anions in the solution, the concentration of divalent metal cations like calcium and magnesium, and the availability of mineral nucleation sites [48]. From a reaction kinetics perspective, the rate of carbonate deposition is directly influenced by the concentrations of carbon anions and divalent metal cations such as calcium, magnesium, and iron in the solution. Divalent metal cations primarily stem from groundwater composition and the dissolution and weathering of rock minerals [49]. The existing literature data demonstrate marked distinctions in the mineral composition of reservoir rocks between those of typical domestic regions and those of foreign regions, as outlined in Table 1. Among these minerals, the dissolution of both carbonate minerals and clay minerals can supply divalent metal ions. However, the dissolution and the re-precipitation of carbonate minerals do not significantly contribute to carbon dioxide sequestration. Therefore, the content of clay minerals in reservoir rock holds substantial importance in the context of carbon dioxide mineralization and sequestration.

Most of the reservoirs in China are terrestrial and are characterized by quartz clastic minerals and carbonate minerals, which are their principal components. In contrast, clay minerals constitute a relatively minor portion compared to foreign marine-phase reservoirs [50,51]. Among these minerals, the dissolution of both carbonate minerals and clay minerals can supply divalent metal ions. However, the dissolution and the re-precipitation of carbonate minerals do not significantly contribute to carbon dioxide sequestration. Therefore, the content of clay minerals in reservoir rock holds substantial importance in the context of carbon dioxide mineralization and sequestration.

To maximize the sequestration of CO_2 in the form of solid carbonates, it is essential for the reservoir rock to possess a relatively high clay mineral content, as this indicates richness in silicate minerals (e.g., chlorite, illite, montmorillonite, kaolinite, etc.). These silicate minerals neutralize acids and provide the necessary raw materials for precipitation by providing Ca^{2+}, Mg^{2+}, and Fe^{2+}. Consequently, achieving effective carbonation of natural silicate minerals is most feasible in porous minerals rich in divalent metal cations, such as basalts and mantle peridotites [61–63]. An exemplar of implementation is the Carbon Fix project in Iceland, where carbon dioxide was injected into the ground in the form of carbonated water. Remarkably, over 95% of the carbon dioxide mineralized into carbonate minerals within 2 years and completed in situ mineralization [64].

Constrained by reservoir conditions, mineralized carbon dioxide sequestration in China's oil reservoirs faces significant challenges, which are primarily characterized by a slow natural mineralization rate and a lengthy cycle. Furthermore, the indoor in situ static simulation experiments conducted thus far have predominantly remained at the dissolution stage of rock minerals. To expedite the mineralization process, both domestic and international researchers commonly opt for the introduction of microorganisms.

Table 1. Rock mineral composition of typical regions at home and abroad.

Region	Felsic Mineral Content/%	Carbonate Mineral Content/%	Clay Mineral Content/%	Reference
Cambrian Yurtus Formation, Tarim Basin, China	21.2–94.8 57.8 (Mean)	0.1–69.6 16.9 (Mean)	0.8–48.5 15.7 (Mean)	[52]
Cretaceous Qingshankou Formation, Songliao Basin, China	3.7–73.4 49.4 (Mean)	0.1–93.5 13.1 (Mean)	2.5–49.6 33.6 (Mean)	[53]
Permian Luchaogou Formation, Junggar Basin, China	0.1–73.5 49.4 (Mean)	0.1–91.5 32.8 (Mean)	0.8–48.5 11.9 (Mean)	[54]
Paleogene Kongdian Formation, Cangdong Sag, Bohai Bay Basin, China	0.1–56.0 36.7 (Mean)	0.1–95.0 32.8 (Mean)	0.8–48.5 15.7 (Mean)	[55]
Permian Longtan Formation, Southeast Xiang-tan Depression, China	9.0–43.0 27.0 (Mean)	23.0–50.0 38.0 (Mean)	0.8–48.5 15.7 (Mean)	[56]
Devonian–Mississippian of the Western Canadian Basin	21.2–94.8 57.8 (Mean)	0.1–85.4 4.7 (Mean)	0.8–48.5 15.7 (Mean)	[57]
Barnett Shale, Fort Worth Basin, USA	51.9	8.1	35.0	[58]
Upper Jurassic Haynesville Shale, Gulf of Mexico Basin, USA	31.8	22.7	45.5	[59]
West Philippine Sea	22.7–75.4 66.3 (Mean)	14.6–41.5 28.0 (Mean)	5.3–54.3 21.5 (Mean)	[60]

3. Mechanism of Microbial-Assisted Mineralization

Microorganisms play critical roles in elemental cycling, as well as in the transformation of metals and minerals, decomposition, and the formation of soil and sediment [65]. Reservoirs act as natural geological bioreactors [66], harboring a diverse array of microorganisms, which primarily include fermenting bacteria, sulfate-reducing bacteria, nitrate-reducing bacteria, iron-reducing bacteria, methanogens, etc. [67]. These microorganisms are directly or indirectly involved in the nucleation, crystallization, and growth processes of minerals during mineral formation. Academics classify the microbial involvement in mineralization into two main categories based on the mode of microbial action: microbial-induced mineralization and microbial-controlled mineralization. Microbial-controlled mineralization refers to the process in which microbial cells and metabolic products are directly involved in the carbonation process, resulting in microbial minerals with distinctive properties. On the other hand, microbial-induced mineralization involves microbes altering the local microenvironment through metabolic activities, creating physicochemical conditions that are conducive to mineral precipitation and thus lead to the formation of induced microbial minerals. The process of microbial-assisted carbon dioxide mineralization in the in situ environment of oil reservoirs is considered a microbial-induced process. As early as 1973, Boquest et al. isolated 210 strains of Proteus vulgaris from soil microorganisms, demonstrating the ability to induce calcium carbonate precipitation generation through metabolic activities [68]. Subsequently, numerous experiments have highlighted the fact that microbial-induced mineralization is mainly achieved through metabolic activities [69], such as urea hydrolysis [70,71], denitrification [72,73], trivalent iron reduction [74], sulfate reduction [75], and organic matter degradation. Table 2 lists the main microorganisms involved in mineralization.

Table 2. Major microorganisms that assist mineralization.

Types of Microorganisms that Assist Mineralization	Typical Microbial Representative	Main Mechanism	Environment	Reference
Sulfate-reducing bacteria	*Acinetobacter calcoaceticus* SRB4	Consumption of specific electron donors, forming a metal sulfide precipitate	Anaerobic environment rich in organic matter, calcium, and sulfate; can survive in oil reservoirs	[76]
Iron-reducing bacteria	*Shewanella oneidensis* MR-4	Consumption of specific electron donors, adjusting Eh value, promoting siderite precipitation	Anaerobic environment; most of them are thermophilic bacteria, and a few can survive in oil reservoirs	[77]
Urea-decomposing bacteria	*Thermoanaerobacterium*	Decomposition of urea	Aerobic environment	[78]
Denitrifying bacteria	*Pseudomonas stutzeri*	Consumption of specific electron donors	Anaerobic environment; can survive in oil reservoirs	[79]
Methanogenic bacteria	Methanococcales	Oxidization of methane, producing carbon anions	Anaerobic environment; can survive in oil reservoirs	[55,80]
Photosynthetic microorganisms	Cyanobacteria	Consumption of CO_2, promoting carbon anion generation	Aerobic environment, light conditions	[81]
Microorganisms producing carbonic anhydrase	*Sporosarcina Kluyver*	Accelerating CO_2 hydration, increasing carbon anion concentration	Aerobic environment	[82]

(1) Reservoir microbial function in carbon dioxide mineralization

Reservoir microorganisms play a pivotal role in CO_2 mineralization and sequestration; this role is primarily manifested in two key aspects: the regulation of environmental factors (e.g., pH, Eh value) and the adherence to minerals to offer nucleation sites.

Reservoir microorganisms engage in metabolic activities that utilize crude oil components in the environment, humic substances in minerals, and sulfates in the brine aquifer, resulting in the generation of organic acids (formic acid, acetic acid, citric acid, etc.) and H_2S, among other acidic substances. These acidic substances can regulate the environmental pH by hydrolyzing and producing protons. Concurrently, the generated protons can react with feldspar minerals (e.g., potassium feldspar and sodium feldspar) and carbonate rock minerals (e.g., calcite and dolomite) to liberate additional free metal ions (e.g., Ca^{2+}, Mg^{2+}, Fe^{2+}, etc.), providing raw materials for the formation of carbonate mineral precipitates. Moreover, microorganisms in oil reservoirs encompass a significant population of electron acceptor-reducing bacteria. These bacteria, which are categorized based on their electron acceptor redox potential from high to low, include nitrate-reducing bacteria, iron-reducing bacteria, and sulfate-reducing bacteria. Among them, nitrate-reducing bacteria are responsible for reducing nitrate to nitrogen or ammonium, and some have the ability to metabolize monosaccharides, polysaccharides, and volatile fatty acids into small organic acids and gases such as nitrogen and carbon dioxide. Iron-reducing bacteria utilize hydrogen, carbon dioxide, and certain small organic acids as electron donors. These bacteria utilize extracellular Fe(III) as the terminal electron acceptor, reducing Fe(III) to Fe(II) through the oxidation of organic matter. The presence of iron-reducing bacteria facilitates the reduction of Fe(III) to Fe(II), which regulates the environmental redox value (Eh) and creates a conducive environment for the generation of rhodochrosite precipitation. The potential metabolic pathways of reservoir mineralization and precipitation are depicted in Figure 7.

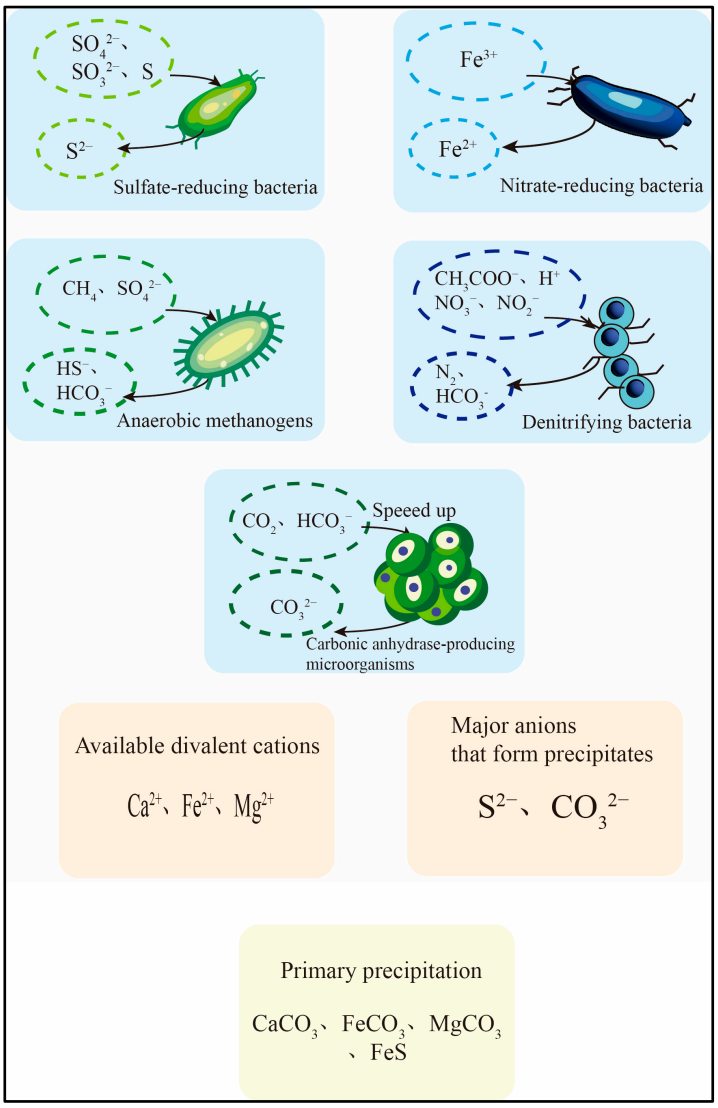


Figure 7. Possible metabolic pathways of reservoir mineralization and precipitation.

More importantly, microorganisms create a favorable microenvironment for precipitation, and their metabolism produces extracellular polymers (EPSs) that can provide the nucleation sites necessary for nucleation and accelerate the growth of carbonate crystals, as shown in Figure 8. Reservoir microorganisms predominantly exist in the form of microbial communities within the $scCO_2$–water–rock system, which can synergistically resist extreme environments and further develop into biofilms [65]. In the $scCO_2$–water–rock system, where sandstone serves as a porous medium, microbial retention and attachment are highly favorable. Microorganisms proliferate on the moist surface of the porous reservoir medium, secreting viscous extracellular polymeric substances (EPSs) that culminate in biofilm formation. Due to the presence of various organic functional groups, such as carboxyl and hydroxyl, on the biofilm surface, it exhibits a certain degree of hydrophobicity [83–85]. The interplay of the electrostatic force, the van der Waals force, and hydrophobicity leads to a robust and stable adhesion between microorganisms and porous media. Additionally, the extracellular polymers typically consist of polar amino acids such as metabolically produced glutamic acid and aspartic acid. These amino acids are highly prone to proton dissociation under alkaline conditions, which results in an overall negative charge on the biofilm surface. This charge facilitates the adsorption of cations such as calcium, magnesium, and iron, providing essential nucleation sites for the formation of minerals like dolomite, calcite, and ferro-dolomite [61,86,87]. In parallel with extracellular mineralization,

intracellular bio-mineralization occurs in certain prokaryotic bacteria, algae, etc. Benzerara et al. found that amorphous calcium carbonate can be formed by some cyanobacterial cells [88]. Yan Huaxiao and other researchers utilized Bacillus subtilis Daniel-1 to study biomineralization and found that intracellular and extracellular mineralization can occur simultaneously and that the two have a synergistic effect under certain conditions [89].

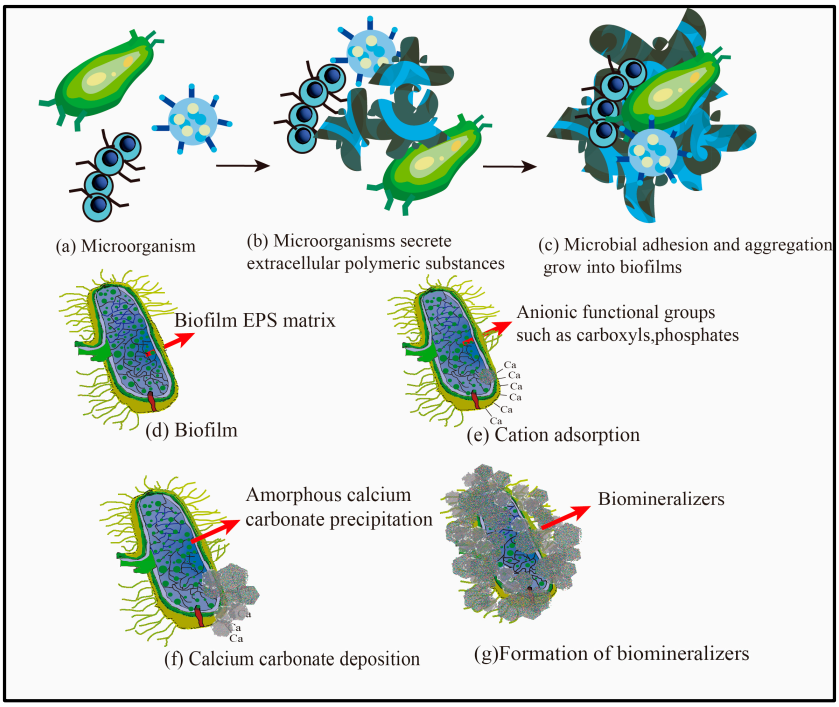


Figure 8. Microorganisms provide nucleation sites [90].

(2) Key microbial enzymes in CO_2 mineralization

Currently, the critical enzymes implicated in mineralization and sequestration primarily encompass urease and carbonic anhydrase, as demonstrated in Table 3. Urease chiefly elevates the environmental pH by decomposing urea, whereas carbonic anhydrase augments the CO_3^{2-} concentration by expediting CO_2 hydration. However, most urea-producing and urea-decomposing bacteria are aerobic bacteria, which do not exist in the oil reservoir environment. Furthermore, the ammonia generated during urea decomposition poses new environmental risks. Consequently, this paper places emphasis on carbonic anhydrase as the auxiliary enzyme for CO_2 mineralization in the reservoir. The core function of carbonic anhydrase in accelerating CO_2 mineralization in reservoirs is to achieve rapid and efficient conversion between CO_2 and bicarbonate and carbonate. Notably, CO_2 hydration stands as a pivotal component of this conversion and represents one of the critical rate-limiting steps in the mineralization process, alongside mineral dissolution. The reaction rates of both processes are somewhat dependent on the ambient pH value. The mineralization of carbonate deposition occurs in weakly alkaline environments, and the kinetics of CO_2 hydration in mildly alkaline solutions are notably sluggish, merely 0.026–0.044 s^{-1} at room temperature [91], during which the rate of carbonic anhydrase-catalyzed hydration is dramatically increased by the addition of carbonic anhydrase, with turnover numbers of up to 1.4 × 10^6 s^{-1} at 25 °C and pH 9, surpassing the natural rate by 10^8 times [92,93]. Thus, carbonic anhydrase plays an important role as a key enzyme catalyzing the CO_2 hydration reaction during CO_2 mineralization.

Table 3. Key enzymes involved in carbon dioxide mineralization.

Key Enzyme for Carbon Dioxide Mineralization	Main Role	Catalytic Rate	Maintain Active Environment	Application	Reference
Urease	Decomposes urea and increases the pH	-	pH 7.0, 40 °C catalyzed the conversion of urea to ammonium carbonate; the optimal pH is 7.4.	Ecological restoration, soil reinforcement	[78]
Carbonic anhydrase	Accelerates CO_2 hydration and increases CO_3^{2-} ion concentration	K_{cat} 10^4–10^6/s	The pH value between 4.0 and 9.0 and the temperature below 65 °C can maintain high activity and stability.	Fixed CO_2, biological monitoring	[94]

3.1. Catalytic Mechanism of Carbonic Anhydrase

Carbonic anhydrase (CA) constitutes a class of metalloenzymes that utilize metal ions such as Zn^{2+}, Co^{2+}, Fe^{2+}, Cd^{2+}($\zeta-$CAcontaining), Cu^{2+}, and other related metal ions; the active sites are responsible for the participation in the reversible hydration of carbon dioxide [91,95]. The discovery of carbonic anhydrase traces back to the 1930s, when scientists like Meldrum, in their investigations into carbon dioxide transport in the blood, identified a protein in the blood that was responsible for converting carbon dioxide into carbonic acid [96]. Since then, scientists have progressively detected carbonic anhydrase in various mammals, plants, and microorganisms [97–99]. Carbonic anhydrases can be broadly categorized into eight major groups based on amino acid sequence differences; these are α, β, γ, δ, ζ, η, θ, and ζ types [100–104], of which α, β, and γ are widely distributed in plants and animals, algae, bacteria, and other organisms, with α-carbonic anhydrase alone exhibiting the presence of at least a dozen isozymes.

Currently, the most detailed structural analysis of carbonic anhydrase is that of the isoenzyme CAII within α-CA. Due to its robust catalytic activity, the predominant carbonic anhydrase sequestered by carbon dioxide mineralization in reservoirs belongs to the α type. The structure of α-CA is depicted in Figure 9 [105]. The active region of carbonic anhydrase comprises multiple peptide chains shaped by β-folding, α-helix, and other structural elements within the cavity. The catalytic center of the cavity is anchored by a Zn^{2+} positioned approximately 15Å (1.5×10^{-9} m) from the bottom of the cavity. Simultaneously, four coordination bonds extend around the zinc ion, connecting three amino acid residues (His-94, 96, 119) and a water molecule or OH^-, which together form a nearly symmetric tetrahedral coordination geometry [106,107]. When the environmental pH becomes neutral or weakly basic, the ligand H_2O attached to the site at the time deprotonates, resulting in the formation of a hydroxide ligand (-OH^-). The genesis of this tetrahedral structure primarily hinges on the binding force between the zinc ions and the nitrogen atoms in the imidazole group of the side chain of the three histidine residues, as well as the oxygen atoms in the water molecule. Additionally, the cavity environment near the catalytic site of Zn^{2+} can be roughly divided into hydrophobic and hydrophilic regions. The hydrophobic side chain is composed of branched amino acids such as valine (Val143,121), tryptophan (Trp-209), and leucine (Leu-198), which surround the active site to form a hydrophobic pocket responsible for CO_2 fixation and act as a catalytic binding site for CO_2 [108]. On the other hand, the hydrophilic portion consists mainly of histidine (His-64), threonine (Thr-199), glutamic acid (Glu-106), and water molecules, forming a proton channel in which histidine plays the role of a proton shuttle, which provides a chain of water molecules to remotely control the E·ZnH_2O deprotonation process [109].

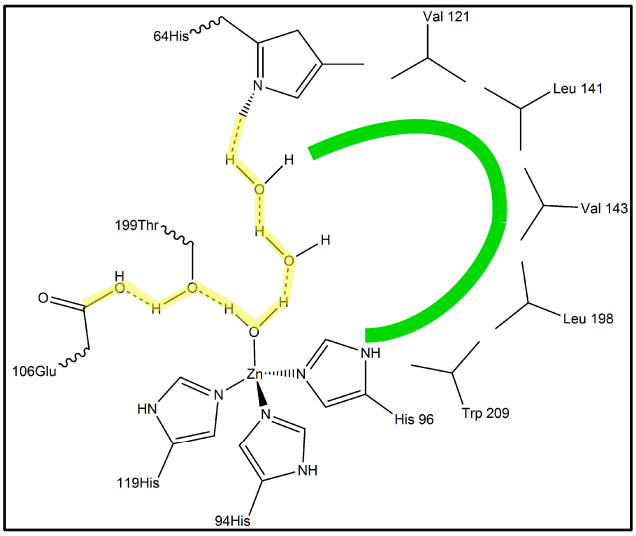


Figure 9. Active region structure of carbonic anhydrase [105]. Yellow represents the proton transport chain and green represents the hydrophobic pocket.

A large number of studies have revealed the structure of α-CA with zinc as the active center and have provided insights into its catalytic mechanism [95,97,99,110]. During its active state, carbonic anhydrase readily binds to OH^- to form $E \cdot ZnOH^-$. Subsequently, $E \cdot ZnOH^-$ launches a nucleophilic attack on the carbon atom of the carbon dioxide molecule surrounded by a hydrophobic pocket and combines to form $E \cdot ZnHCO_3{}^-$. Later, the water molecule reacts with the zinc bicarbonate, $E \cdot ZnHCO_3{}^-$. Once the bicarbonate is displaced into the solution, the enzyme is inactivated as it combines with water to produce $E \cdot ZnH_2O$. The catalytic process of carbonic anhydrase is illustrated in Figure 10.

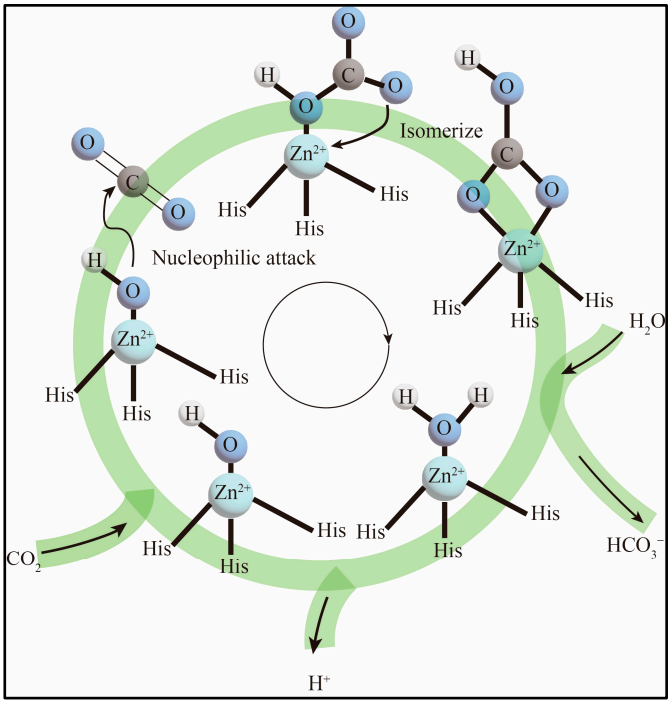


Figure 10. Catalytic process of carbonic anhydrase [111].

To restore carbonic anhydride enzyme activity, the deprotonation of $E \cdot ZnH_2O$ has to be completed [95,112,113]. The $E \cdot ZnH_2O$ deprotonation process, during which the proton transfer rate is only 10^6 s^{-1}, represents the key rate-limiting step in the carbonic

anhydrase catalytic process and is crucial for ensuring the sustained stability of the catalytic process. The proton is transferred to His64 in three steps along the hydrogen-bonded water molecule chain. This protonated His64 side chain undergoes rotation to maximize its exposure to the solvent outside the enzyme. Upon the release of H^+, His64 rotates back to its initial position. The literature suggests that the proton exchanger during deprotonation may be a buffer in the solution, in addition to water, as depicted in Equation (20). However, the contribution of carbonic anhydrase to the catalytic efficiency of the deprotonation process diminishes, resulting in a reduced catalytic effect [110]. The equations describing the carbonate precipitation process induced by carbonic anhydrase are as follows:

$$E \cdot ZnH_2O \leftrightarrow E \cdot ZnOH^- + H^+ \quad (15)$$

$$E \cdot ZnOH^- + CO_2 \leftrightarrow E \cdot ZnHCO_3{}^- \quad (16)$$

$$E \cdot ZnHCO_3{}^- + H_2O \leftrightarrow E \cdot ZnH_2O + HCO_3{}^- \quad (17)$$

$$HCO_3{}^- + OH^- \leftrightarrow CO_3{}^{2-} + H_2O \quad (18)$$

$$Cell + Ca^{2+} \leftrightarrow Cell - Ca^{2+} \quad (19)$$

$$E \cdot ZnH_2O + buffer \rightarrow E \cdot ZnOH^- + bufferH^+ \quad (20)$$

Overall, carbonic anhydrase-producing bacteria grow and multiply in the reservoir environment and secrete carbonic anhydrase to catalyze carbon dioxide hydration, thus promoting the conversion between carbon dioxide and bicarbonate and carbonate and increasing the $CO_3{}^{2-}$ concentration in the formation water. Meanwhile, carbonic acid formed by carbon dioxide hydration dissolves minerals such as silicates, releasing divalent cations, which are adhered to the surface of microbial membranes through electrostatic forces. Eventually, the cations combine with locally oversaturated carbonate ions, culminating in carbonate precipitation, and thereby achieve the solid-state mineralization of carbon dioxide. The process of carbonate deposition induced by carbonic anhydrase-producing microorganisms is shown in Figure 11.

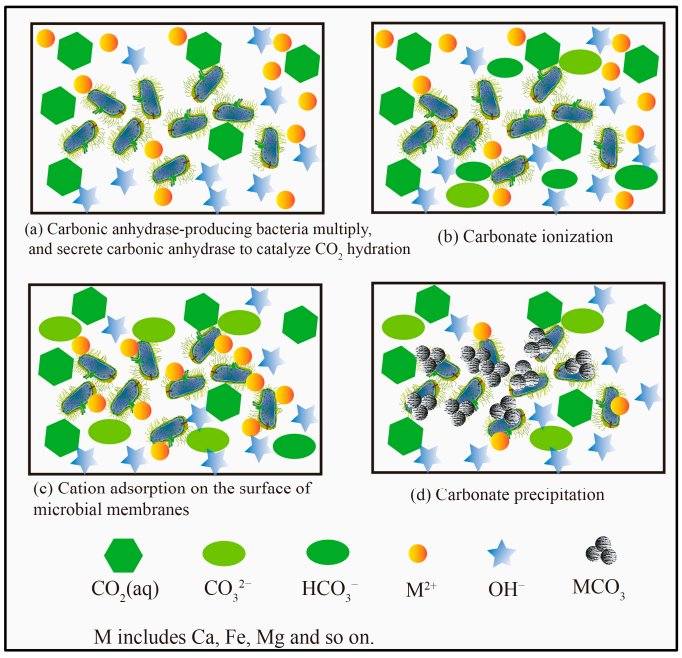


Figure 11. Mechanistic modeling of carbonate deposition induced by carbonic anhydrase-producing microorganisms.

3.2. Carbonic Anhydrase Catalytic Activity and Stability Factors

Carbonic anhydrase (CA), which is renowned as an environmentally friendly and highly efficient catalyst for carbon dioxide hydration, is widely used in various industrial processes for carbon dioxide abatement. To align with the technical requirements of industrial applications, CA must endure severe environments, including, but not limited to, those with high-temperature and high-salinity conditions. Thus, a key research focus is on the preservation of its catalytic activity and stability under extreme conditions [114,115]. Some studies have underscored that the catalytic activity and thermal stability of carbonic anhydrase are largely influenced by the enzyme structure [104,116].

The function and catalytic activity of proteases are fundamentally controlled by the sequence of amino acids comprising the protease (enzyme primary structure). The peptide chain adopts stable secondary structures such as α-helix and β-folding, forming a foundation upon which the peptide chain further folds to attain a three-dimensional or four-dimensional protease structure. Most carbonic anhydrases exist in forms like dimers, trimers, and tetramers. Upon the three-dimensional structure analysis of several isozymes (I, II, III, IV, V, XII, XIII, and XIV), a notable structural resemblance is observed. They all exhibit a tetrahedral-like structure, with the metal ions located within the interior of the tetrahedral cavity, the three conserved histidine residues, and a water molecule or hydroxyl as the four ligands of the tetrahedral structure. The tetrahedral cavity structure can be roughly divided into two parts according to its distribution of amino acid sequences; the hydrophobic part is mostly composed of nonpolar amino acids such as alanine (Ala121, 135), valine (Val 207), phenylalanine (Phe91), leucine (Leu131, 138, 146, 109), and proline (Pro201, 202), whereas the hydrophilic portion of the cavity is mainly composed of polar amino acids such as histidine (His64, 67, 200), aspartic acid (Asn69), glutamine (Gln92), threonine (Thr199), and tyrosine (Tyr7). The hydrophobicity of these residues plays a crucial role in catalysis. Consequently, some researchers have utilized amino acid modification to reinforce the hydrophobic pocket to minimize the exposure of hydrophobic groups in aqueous solutions, thus enhancing the structural stability and enzymatic activity. Furthermore, the arrangement of amino acids and the presence of disulfide bonding also influence the enhancement of stability and catalytic activity. In CAII, which is characterized by high catalytic activity, the histidine residue His64 primarily governs the conversion of Zn^{2+}-bound water molecules into hydroxide ions before catalysis. This histidine residue is a pivotal component of the proton shuttle process. The replacement of this histidine residue with other residues, such as Phe66, Tyr64, Glu207, etc., significantly impacts the catalytic activity of the CA enzyme. Additionally, the formation of a disulfide bond between the two conserved cysteine residues can reduce the degree of freedom for conformational changes in the protease, enhancing thermal stability. Consequently, researchers have explored methods to enhance protease surface densification by introducing disulfide bonds, among other approaches, to bolster rigidity and thermal stability.

3.3. Application of Carbonic Anhydrase Immobilization in CO_2 Mineralization

Carbon dioxide geological sequestration is a crucial strategy for meeting carbon emission reduction targets [8]. Carbon dioxide in geological formations exists in various states, including dissolved, free, bound, and mineralized states, among which the mineralized state in the form of carbonate is the most secure, stable, and sustainable. The mineralization process is facilitated by carbonic anhydrase (CA), an efficient and environmentally friendly catalyst that accelerates carbonate deposition by catalyzing CO_2 hydration. However, its protease activity is affected by a variety of factors, including temperature, pressure, pH, ionic strength, and the presence of metal ions [117,118]. Even under optimal conditions, the enzyme's catalytic activity gradually decreases as the reaction progresses [119]. To maintain the tolerance and catalytic activity of carbonic anhydrase in the anaerobic environment at the high temperature and pressure in oil and gas reservoirs [114], domestic and foreign researchers have pursued immobilization techniques, binding the enzyme in a specialized phase to enhance its suitability within oil reservoirs.

Common enzyme immobilization methods mainly include adsorption, embedding, covalent binding, and cross-linking [117,120–122], which are shown in Figure 12. Various types of carrier materials are used for immobilization and encompass organic and inorganic materials such as polyacrylamide gel [123], polyurethane foam, diatomaceous earth [124], and silica [125]. Additionally, newer materials like magnetic nanoparticles and hollow nanofiber membranes are being explored [126]. Scientists, such as B. Ray and others, have embedded and immobilized carbonic anhydrase on polyacrylamide gel to enhance the thermal stability; carbonic anhydrase (CA) immobilized on polyacrylamide gel improved the tolerance to sulfonamide inhibitors [123]. Another study immobilized carbonic anhydrase on surfactant-modified silylated chitosan (SMSC) to induce bionic-driven carbonate precipitation. The results demonstrated improved stability, prolonged enzyme activity, and increased carbonate production compared to the free enzyme under the same conditions [127]. Building upon this research, Vinoba et al. modified the porous nanomaterials and immobilized carbonic anhydrase on the modified materials, and the experimental results indicated that the cross-linking method for immobilizing the CA enzyme resulted in catalytic activity comparable to that of the free enzyme [128]. Utilizing biomimetic principles, the synthesis of biomimetic materials emulating the activity of natural carbonic anhydrase and the subsequent immobilization through material modification using chemical reagents has shown promise. This method can solve not only the problem of the slow rate of carbon dioxide absorption and hydration but also the problem of the poor stability of the natural carbonic anhydrase. Furthermore, it allows controllable adjustments in the molecular structure, catalytic rate, and other parameters, enhancing its potential for practical applications. Simultaneously, domestic and international researchers have conducted numerical simulations to predict carbon dioxide sequestration on a 10,000-year scale. The consensus is that natural conditions will facilitate carbon dioxide mineral sequestration within approximately one hundred years. Currently, by employing bionic carbonic anhydrase immobilization technology, the introduction of carbonic anhydrase coagulation nuclei has proven effective in reducing the critical concentration for induced carbonate precipitation. This advancement enables a mineralization process with controllable speed and the ability to regulate the size of carbonate precipitation particles.

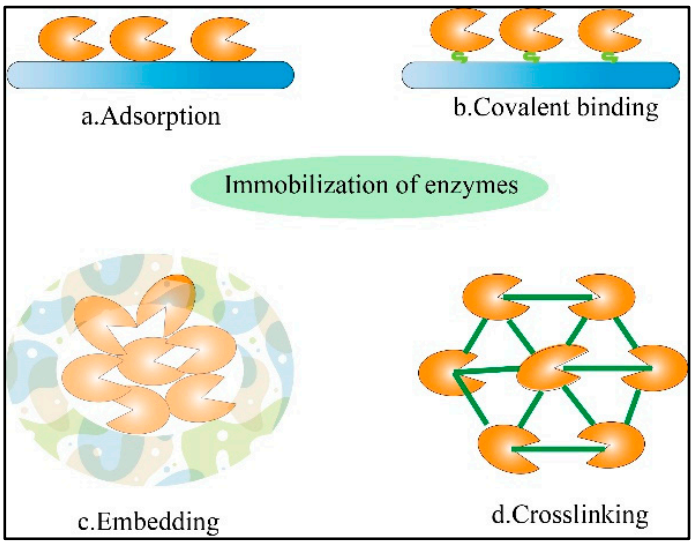


Figure 12. Common enzyme immobilization methods [120].

4. Comparisons of the Natural and Microbial-Catalyzed Sequestration Processes

Based on the natural mineralization mechanism, microbial-assisted mineralization integrates the characteristics of accelerated hydration by carbonic anhydrase and those of the surface nucleation site provided by microorganisms. Compared with the natural

mineralization process, it has more advantages in terms of storage time, storage cost, efficiency, and derivative application value.

According to previous studies [16,129–131], the storage processes of sedimentary basins within 1000 years primarily consist of structural storage, residual storage, and dissolution storage, as seen in Figure 2. Mineralization storage is limited due to the comparatively low reactivity of sedimentary rock minerals and the low content of bivalent metals producing carbonate minerals, and it may take thousands of years to accomplish in situ formation mineralization storage. Currently, most of the research on in situ mineralized carbon sequestration is conducted in laboratories, with only a few countries carrying out pilot test projects. Unlike the traditional carbon dioxide storage of sandstone reservoirs, dissolution storage and mineralization storage play a major role in the in situ carbon sequestration projects of basalt reservoirs; this, in a sense, is equivalent to advancing the mineralization process and achieving the mineralization effect in a short time, thus enhancing the project's safety. In the case of the supercritical CO_2 mineralization sequestration project in Wallula, USA, 60% of the injected CO_2 (293 tons/year) was fixed as a carbonate mineral within two years [132,133]. Similarly, the CarbFix dissolved CO_2 mineralization sequestration project in Iceland mineralized 95% of the injected CO_2 (57–75 tons/year) within two years [64,134]. The latter sequestration time is advanced in part because the injection of dissolved CO_2 reduces the time for CO_2 hydration. In the natural state, the hydration rate of CO_2 is slow; the microbial carbonic anhydrase catalyzes the hydration, and the conversion number is increased by about 10^8 times that of the natural hydration, thus significantly shortening the hydration time and accelerating the mineralization process. At present, the research on microbial-assisted mineralization mainly remains at the laboratory research stage, and relevant engineering test data are temporarily lacking. Combined with the results of the laboratory experiments, it can be seen that microbial-assisted mineralization can significantly improve the mineralization rate and is expected to advance the time scale to several decades.

Carbon dioxide is turned into stable carbonate minerals by injecting captured carbon dioxide into geological formations such as oil reservoirs; this results in long-term carbon fixation and emissions reduction while enhancing oil and gas recovery. In terms of environmental benefits, in situ mineral carbonation achieves storage by fixing carbon dioxide in the reservoir minerals as stable carbonates, successfully capturing carbon dioxide and lowering the danger of carbon dioxide leakage. Mineralization is accelerated by microbial-assisted in situ mineralization with no additional energy use. From the perspective of economic benefits, even though carbon dioxide storage accounts for the smallest proportion of investment in the entire CCUS industry chain, the cost of carbon dioxide storage is expected to be CNY 40–50/ton by 2030 [135]. However, due to the high capture and transport and operating costs of CCUS projects, oil companies must increase oil and gas production to break even. In recent years, China has carried out CO_2 flooding demonstration projects for low-permeability and ultra-low-permeability reservoirs, covering about 100 million tons of reserves and improving the recovery rate by 6–20% [21]. Carbon dioxide injection into oil and gas reservoirs has both the economic benefits of enhanced oil recovery and the environmental benefits of storage and emissions reduction. At present, microbial-assisted mineralization for the porous media of oil and gas reservoirs is still at the laboratory research stage, and the mineralization rate and effect are affected by many factors; therefore, the economic benefits and emission reduction efficiency of reservoir mineralization storage are difficult to predict.

In terms of mineralization storage derivative applications, microbial-induced mineralization is primarily employed in soil reinforcement [136,137], micro-crack repair [138], cement bonding [139], environmental remediation, and other domains [140]. Carbonate formed by carbon dioxide mineralization in reservoirs can be employed to plug dominant seepage pathways, thereby improving volumetric sweep efficiency and oil recovery. The activity and stability of microorganisms and their enzymes will continue to improve in the future as the fields of extreme-environment microorganism mining, protein transformation

engineering, and enzyme immobilization technology develop. This will broaden the scope of applications and increase the likelihood of industrial application.

5. Outlook

In the context of the carbon peak and the commitment to carbon neutrality, carbon capture, utilization, and storage (CCUS) technology holds significant potential for application and development. The Annual Report of China on Carbon Dioxide Capture, Utilization, and Storage (CCUS) in 2021 highlights that CCUS technology stands as the sole technological option for achieving the decarbonization of fossil energy. Additionally, it underscores that China possesses a substantial geological sequestration potential for CO_2, which is estimated to range from 12.1 to 41.3 million tons [141]. CO_2 mineralization and fixation represent effective technologies for mitigating the greenhouse effect and decelerating global warming. These approaches offer new avenues for the study of the multiphase reactions of CO_2 with water and rocks, as well as the exploration of the application of microbial carbonic anhydrase in multicomponent reactions.

Overall, CO_2 mineralization and sequestration are still primarily at the laboratory stage, with only a few pilot demonstration projects. Currently, several key challenges are being faced in the realm of CO_2 mineralization sequestration.

The storage potential of supercritical carbon dioxide in the subsurface is influenced by various complex environmental factors, including the stratum temperature, pressure, mineralization level of the formation water, and mineral composition of the stratum. However, there is currently a lack of established methods to measure and assess the specific impact of each factor. It is crucial to introduce evaluation indices to quantitatively measure the influence of each factor on the rate of mineralization and the amount of sequestered carbon dioxide.

In contrast to the extensive research conducted by foreign scholars on carbon dioxide sequestration mechanisms in typical marine sedimentary minerals, China boasts abundant deep saline aquifers with terrestrial sedimentation. Given the significant disparities in rock types and mineral compositions between marine and terrestrial sedimentary strata, the mechanisms of mineralization and sequestration differ. Currently, there is a dearth of research on the CO_2–water–rock interaction mechanisms specific to CO_2 mineralization and storage in the deep saline aquifers associated with terrestrial deposition. Future research should focus on investigating the intricate mechanisms of CO_2 mineralization and storage. Particular attention should be given to the reaction mechanisms between the typical terrestrial sedimentary minerals and CO_2 in saline solutions. This research will facilitate the evaluation of suitable geological conditions and storage technologies and ultimately enhance the efficiency and safety of CO_2 storage.

Investigation into accelerated mineralization induced by microorganisms is currently confined to indoor simulations and lacks on-site samples. Numerous studies have been conducted at ambient temperature and pressure and have primarily employed exogenously cultivated CA-producing bacteria instead of reservoir-originated microorganisms. The adaptability of microorganisms to the extreme environment of mineralization and sequestration in oil reservoirs and deep saline aquifers has not been thoroughly assessed. Future research should prioritize the targeted evolution, screening, and cultivation of microorganisms that are highly adaptable to extreme environments and should aim to obtain carbonic anhydrase with superior catalytic activity from native microorganisms. Furthermore, the research should delve into the catalytic mechanism and active sites of key enzymes like carbonic anhydrase in microbial-assisted mineralization. Structural alterations and immobilization should be explored for enzyme modifications, with the aim of enhancing enzyme activity and stability in extreme environments.

Additionally, most microorganisms are adversely affected by high temperature, high pressure, and $scCO_2$, resulting in a significant reduction in their activity and biomass within a specific time frame. There is a need to identify viable alternatives to microorganisms to assist with mineralization. Simultaneously, chemo-mimicry is vital to the enhancement of

the adaptability for engineering applications, given the impact of extreme environments on microbial activity. In ongoing studies related to the synthesis and preparation of biomimetic enzymes, broader focus should be placed on evaluating enzyme-induced mineralization performance in simulated in situ reservoir core replacement experiments. Moreover, it is critical to investigate the factors influencing the activity and stability of bionic CA enzymes. Establishing a comprehensive evaluation system for bionic carbonic anhydrase, including reference comparisons with biological enzymes, will optimize the function of bionic enzymes and provide a scientific basis for the subsequent engineering applications of bionic carbonic anhydride-induced mineralization.

6. Conclusions

Carbon dioxide mineralization sequestration is regarded as the safest and most effective method for CO_2 geological sequestration; it has a crucial role to play in the achievement of carbon neutrality goals. Through the utilization of the calcium- and magnesium-based resources present in reservoir rocks, captured industrial CO_2 is injected into reservoirs. Through the carbonation reaction between silicate minerals and CO_2 within the reservoir, a permanent fixation of CO_2 is achieved, which is in alignment with the objective of carbon neutrality.

Drawing upon the relevant literature on CO_2 mineralization sequestration and microbial-induced mineralization, several fundamental conclusions can be outlined:

(1) The geophysical–chemical process of carbon dioxide mineralization in reservoirs primarily encompasses three stages: carbon dioxide dissolution ionization, mineral dissolution, and carbonate mineral precipitation.
(2) The rate of CO_2 mineralization sequestration in alkaline environments is influenced by factors such as carbon anion concentrations; concentrations of divalent metal cations like calcium, magnesium, and iron; and the availability of mineral nucleation sites.
(3) Microbial induction can expedite the mineralization process by enhancing the precipitation environment and providing nucleation sites. Additionally, the carbonic anhydrase produced during microbial metabolism is a pivotal enzyme in the process of CO_2 mineralization induced by bacteria.
(4) Carbonic anhydrase primarily catalyzes the carbon dioxide hydration reaction, influencing the CO_2 mineralization process. Its enzyme activity and stability are impacted by factors like the structure of the active region, the environmental temperature, and the pressure. Utilizing covalent bonding and other methods to prepare immobilized carbonic anhydrase by chemo-mimicry can augment enzyme catalytic activity, thereby enhancing the rate of bio-induced mineralization.

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