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Review

Engineering Carbonic Anhydrase for Enhanced CO_2 Capture and Valorization: A Review

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Abstract

The continuous increase in atmospheric CO_2 concentration exacerbates global climate change, making carbon reduction an urgent global priority. Carbonic anhydrase (CA), a highly efficient biocatalyst that converts CO_2 into bicarbonate, demonstrates significant potential for carbon capture and resource utilization. However, the stability and catalytic efficiency of native CA in industrial environments are limited, particularly its poor thermal tolerance under flue gas conditions and its sensitivity to impurities, hindering its direct large-scale application. This review systematically summarizes recent advances in modifying microbial CA through protein engineering (e.g., directed evolution, rational design) and immobilization techniques, which have markedly enhanced its thermal stability, adaptability, and reusability. Among these, the integration of machine learning with high-throughput experimentation has emerged as a transformative strategy for CA engineering. Furthermore, we outline CA-driven pathways for CO_2 conversion into high-value chemicals and bioenergy. Finally, future prospects are discussed, including interdisciplinary integration, computational modeling coupled with experimental validation, and comprehensive life-cycle and techno-economic assessments, to facilitate the scaled application of engineered microbial CA in carbon neutrality pathways. Collectively, this review highlights the critical role of engineered CA in bridging biocatalysis with industrial carbon management, offering a viable and sustainable pathway toward carbon neutrality.

Keywords: carbonic anhydrase; CO_2 mitigation; enzyme engineering; immobilization; synthetic biology; carbon capture and utilization

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1. Introduction

Rising anthropogenic CO_2 emissions have become the primary driver of global climate change, making carbon reduction an urgent global priority [1]. In 2023, global CO_2 emissions reached a record high of 37.4 billion tonnes, marking a 1.1% increase from the previous year (Figure 1) [2,3]. The Intergovernmental Panel on Climate Change (IPCC) has underscored that limiting global temperature rise to 1.5 °Cabove pre-industrial levels necessitates a 45% reduction in global CO_2 emissions by 2030 relative to 2010 levels [4]. Therefore, effective CO_2 management strategies and innovative technologies are essential to achieve deep CO_2 emission reductions in high-carbon industries while supporting societal growth [5].

Such a strategy typically comprises three integrated steps: capture, utilization, and sequestration of CO_2, collectively known as Carbon Capture, Utilization and Storage (CCUS), which plays a crucial role in advancing the "carbon neutrality" agenda [6–8]. Conventional

CO_2 capture technologies, including pre-/post-combustion capture, physical adsorption, cryogenic separation and membrane separation, have achieved technical feasibility in industrial scenarios, but their high operating costs, severe medium degradation, equipment corrosion and secondary pollution risks have become critical barriers to large-scale commercial deployment in high-emission industries [9,10]. In this context, enzymes have demonstrated significant potential in CO_2 fixation processes and show great promise for converting CO_2 into commercially valuable organic compounds [11]. Among them, carbonic anhydrase (CA)-mediated enzymatic CO_2 capture technology has emerged as a promising green alternative to address these bottlenecks.

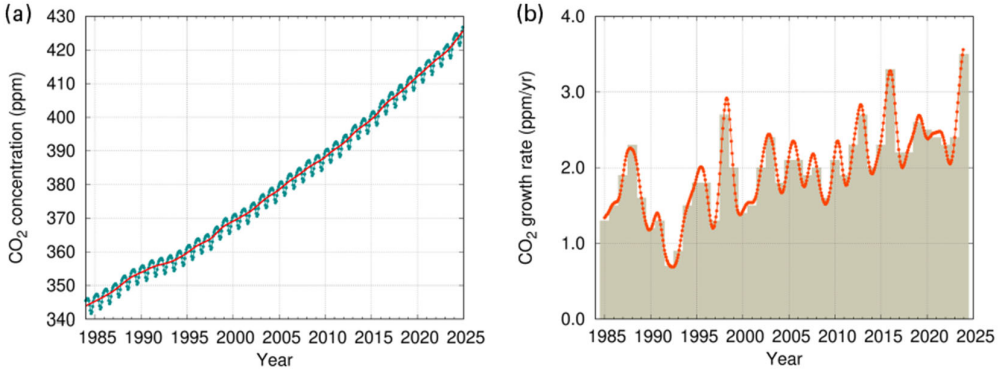

Figure 1. Globally averaged CO_2 concentration (**a**) and its growth rate (**b**) from 1984 to 2024 [12].

CA, one of the most active enzymes in nature, commonly contains a zinc ion in its active center and is widely present across living organisms [13]. CA efficiently catalyzes the conversion of CO_2 to bicarbonate ($CO_2 + H_2O \rightarrow HCO_3^- + H^+$), capable of hydrating 10^4-10^6 CO_2 molecules per second ($k_{cat} = 10^6\ s^{-1}$, $k_{cat}/K_m = 10^8\ M^{-1}\ s^{-1}$), which makes it highly efficient catalyst for CO_2 capture and storage [14,15]. Bond et al. (2001) [16] successfully demonstrated the application of CA in capturing CO_2 from flue gas. Further studies have shown that its catalytic efficiency increases significantly when dissolved in aqueous solvents. Compared with conventional technologies, CA-based systems feature mild operating conditions, low regeneration energy consumption, no toxic solvent emissions, and seamless coupling with subsequent CO_2 valorization pathways, making it a promising candidate for industrial CCUS applications [1,17,18]. However, native CA exhibits poor stability under harsh industrial conditions, including high temperature (e.g., flue gas environments), alkaline pH, high salinity, and flue gas impurities (heavy metals, sulfur oxides, and nitrogen oxides), which leads to rapid enzyme inactivation and hinders its direct large-scale industrial application [2,15,19,20]. In recent years, with rapid advances in protein engineering, enzyme immobilization, and synthetic biology, obtaining naturally stable CA from extremophilic microorganisms, engineering CA via protein design, and enhancing its robustness through immobilization have significantly improved its stability, catalytic efficiency, and environmental adaptability—thereby accelerating the transition of CA from laboratory research toward industrial application [15].

The scope of this review is focused on CA for industrial CO_2 capture and valorization. This paper first elaborates the classification, catalytic mechanism and thermal stability of CA; subsequently, it systematically summarizes the latest advances in CA performance enhancement via protein engineering (directed evolution, rational design) and multi-type carrier-based immobilization technologies; then, it details CA-driven CO_2 conversion pathways to high-value chemicals and bioenergy; finally, it concludes core industrial application challenges and proposes future research directions. This review aims to provide theoretical insights and technical perspectives for engineered CA's scaled application in CCUS and carbon neutrality pathways.

2. Catalytic Mechanism and Properties of CA

2.1. Origin and Catalytic Mechanism

CA is a zinc-dependent metalloenzyme first identified in 1933 [21,22], best known for its ultra-fast catalytic ability to mediate the reversible hydration of CO_2 to bicarbonate-with a turnover frequency of up to 10^6 s^{-1}, this property makes CA the key biocatalyst for engineering biological CO_2 capture, utilization and storage [23,24]. The catalytic performance of CA is dominated by its metal-containing active center, in which the essential Zn^{2+} was first identified in 1939 [25,26]. Typically, the catalytic triad consists of three amino acid residues and a water molecule/hydroxide molecule that form a coordinate bond with the central metal ion [27]. The active site of CA typically adopts a tetrahedral configuration, with the central metal ion (predominantly Zn^{2+}, occasionally Fe^{2+}, Cd^{2+} or other divalent cations) coordinated by the these three amino acid residues and a water molecule/hydroxide ion [20,24]; rarer octahedral or bipyramidal geometric configurations have also been reported [21]. This active structure is the primary target for rational enzyme engineering and immobilization design, to enhance CA stability under harsh industrial conditions. Based on amino acid sequence divergence, CAs are grouped into eight distinct classes: alpha (α), beta (β), gamma (γ), delta (δ), zeta (ζ), eta (η), theta (θ), and iota (ι) [22,23,28]. The α-CA family, including human CAs (HCAs) and bovine CAs (BCAs), is the most thoroughly studied since its discovery and provided the first published 3D structure (Figure 2).

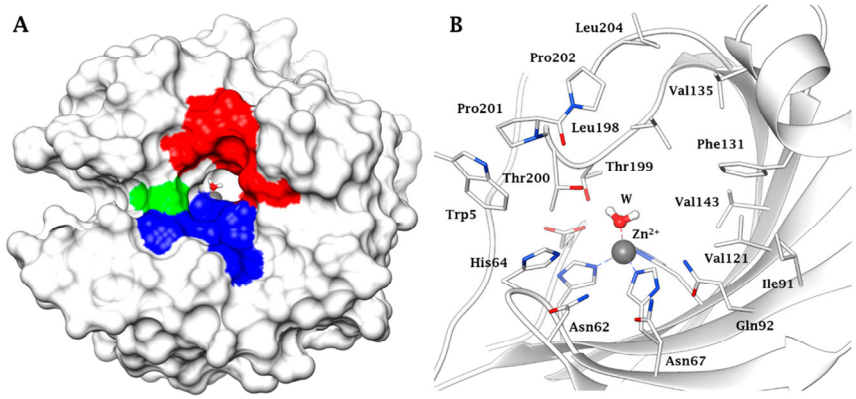

Figure 2. (**A**) Surface representation of human isoform carbonic anhydrase (hCA II) (pdb 3KKX). The hydrophobic half of the active site is colored in red (Ile91, Val-121, Phe-131, Val-135, Val-143, Leu-198, Pro-201, Pro-202, Leu-204), the hydrophilic one in blue (Asn62, Asn67, Glu69, Gln92, His94). His64, the proton shuttle residue, is in green. (**B**) Active site view of hCA II. The zinc ion, represented as gray sphere, is tetrahedrally coordinated to residues His94, His96 and His119 and to a water molecule/hydroxide ion as fourth ligand [29].

CA is essential for catalyzing the reversible hydration of CO_2 to HCO_3^- and H^+. This reaction proceeds too slowly in the absence of a catalyst to support key physiological processes. CA thus enables rapid interconversion, achieving a remarkable turnover number (k_{cat}) of approximately 10^6 s^{-1} [30]. Functionally, the CA active site contains two distinct regions: a hydrophobic pocket and an opposing hydrophilic region. Hydrophobic amino acids (Leu-198, Val-121, Val-143, Trp-209, Thr-199 and Val-207) are responsible for trapping CO_2 substrate, while hydrophilic amino acids (Asn-62, His-64, Tyr-7, Thr-199-Og1, Thr-200-Og1 and Asn-67) mediate the transfer of protons and bicarbonate product generated during the CO_2 hydration reaction. The catalytic cycle for CO_2 hydration mediated by wild CA proceeds via four sequential, well-defined steps (Figure 3): (1) Zn-bound hydroxide reacts with CO_2 to yield Zn-bound bicarbonate through a nucleophilic attack; (2) the bicarbonate then undergoes structural rearrangements to form a more favorable leaving group; (3) bicarbonate is displaced by a bulk solvent water molecule; and (4) depro-

tonation of the newly bound Zn^{2+}-water complex regenerates the initial catalytically active Zn^{2+}-hydroxide state of the enzyme. Notably, step 1 corresponds to bicarbonate formation, steps 2 and 3 mediate HCO_3^- release from the active site, and step 4 completes the catalytic cycle via active site regeneration [31,32].

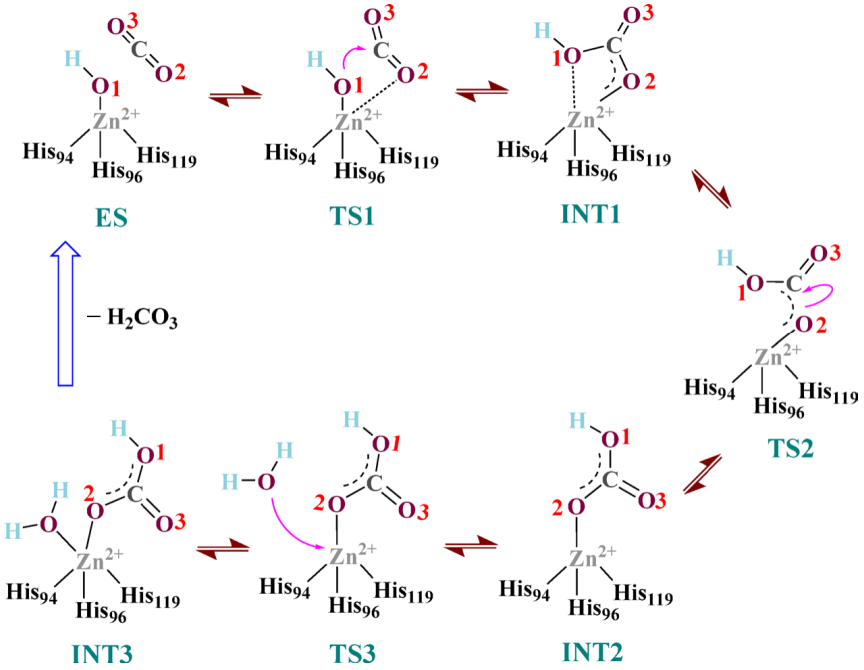

Figure 3. Representation of the catalytic mechanism for the hydration of CO_2 by the hCA II cluster model [31].

2.2. Thermal Stability of CA

To date, researchers have identified diverse CA from microorganisms and explored their potential for CO_2 sequestration [18]. However, the cost-effective industrial deployment of CA-based CO_2 capture technologies requires enzymes that can tolerate the harsh operating conditions of industrial processes. For post-combustion capture, flue gas is typically discharged at ~140 °C and needs to be cooled to around 60 °C for CA- based absorption. Furthermore, in absorption-based CO_2 capture systems where CA is dissolved in a solvent, the high temperatures required in the subsequent stripping column for CO_2 desorption can also lead to enzyme inactivation. Consequently, developing thermostable CAs that retain activity at elevated temperatures is a critical requirement for CA-based CO_2 capture technologies.

Extremophilic microorganisms are important natural reservoirs of thermostable CAs, with numerous robust CAs isolated from thermophilic, halophilic, and alkaliphilic microbes [18,33]. Most high-performance thermostable CAs are derived from deep-sea hydrothermal vent microorganisms, including *Thermosulfurimonas dismutans* (tdCA) [34], *Thermovibrio ammonificans* (TaCA) [35], *Sulfurihydrogenibium azorens* (SazCA) [36], and *Sulfurihydrogenibium yellowstonense* (SspCA) [37]. These isoforms exhibit exceptional thermal tolerance: SazCA and SspCA remain active after 3 h of incubation at 100 °C; TaCA retains 60% activity after 1 h at 70 °C, with half-lives of 77 days and 152 days at 60 °C and 40 °C, respectively. PmCA shows 57% and 27% residual activity at 40 °C and 60 °C even after 60 days [34]. Among these, TaCA and SazCA exhibit superior thermostability and more favorable kinetic profiles [38]. Beyond natural enzymes, engineered CA variants can further improve thermal stability: for example, PmCA SC, an engineered PmCA variant with modified surface electrostatic properties, retains more than 70% of its initial activity after 7 days

at 70 °C [39]. The synergistic optimization of CA thermal stability and chemical tolerance has become the core research direction for industrial flue gas treatment applications [2].

3. Engineering CA for CO_2 Capture Application

CA, with its high catalytic efficiency, is regarded as a promising biocatalyst for industrial carbon fixation, but its environmental sensitivity often causes inactivation under harsh conditions such as high temperatures and extreme pH [19]. Moreover, certain metal ions can inhibit CA activity, causing temporary or permanent functional impairment. Enhancing CA's thermal stability and chemical tolerance is thus critical for industrial deployment, with two core optimization strategies:protein engineering and enzyme immobilization. For industrial CO_2 capture, protein engineering enables precise molecular-level CA modification [14,40], and the two mainstream approached are directed evolution and rational design.

3.1. Directed Evolution

As a strategy that mimics natural evolutionary pathways, directed evolution relies on generating high genetic diversity coupled with appropriate selection or screening methods. The process typically involves: (i) creating a diverse gene library through random mutagenesis, error-prone PCR, or chemical mutagenesis; (ii) expressing the mutant variants and screening for those with desired properties; and (iii) amplifying the identified genes [41]. Numerous studies have demonstrated the effectiveness of directed evolution in enhancing enzymatic catalytic activity through mutation introduction and screening. For example, Alvizo et al. (2014) [42] subjected a mesophilic CA from *Desulfovibrio vulgaris* to nine rounds of directed evolution, progressively increasing its thermal stability, and obtained a mutant with a significantly elevated thermal denaturation midpoint (T_{50}), raised by 44.5 °C. Voyer et al. (2019) [43] engineered the N-terminal region of a CA from *Thermovibrio ammonificans* (TaCA)—a promising candidate for CO_2 capture—resulting in variants with extended half-lives and markedly improved stability under high-cycling conditions and in strongly alkaline media, clearly demonstrating their potential for industrial application. Systematic studies on various catalytic residues have shown that specific amino acids such as arginine, glutamate, aspartate, histidine, and lysine play key roles in maintaining catalytic activity. Understanding the mutational tolerance of these residues helps predict the impact of mutations on enzyme activity, thereby facilitating the development of enzymes with tailored properties [44]. Furthermore, integrating directed evolution with strategic amino acid modifications—such as incorporating non-canonical amino acids, leveraging conserved residue functions, and optimizing amino acid-metal interactions—can effectively enhance catalytic activity and generate efficient biocatalysts [13]. Despite its success in significantly improving CA performance, directed evolution has limitations, including the need to construct large gene libraries to increase the probability of success and its relatively low efficiency.

3.2. Rational Design

As a complementary strategy to directed evolution, rational design employs targeted, non-random molecular modifications based on an in-depth understanding of enzyme structure and catalytic mechanisms, to introduce precise point mutations at specific sites. For instance, Warden et al. (2015) [45] engineered the surface charge of bovine CA (bCA) by mimicking the high negative surface charge of halophilic enzymes, yielding a variant with significantly enhanced high-salt stability suitable for CO_2 capture using concentrated K_2CO_3 solutions. In another study, Parra-Cruz et al. (2018) [46] constructed α-CA variants by targeting highly flexible amino acids with high root-mean-square fluctuation; five of

eight designed mutants exhibited enhanced structural rigidity at 400 K, with improved thermostability, higher catalytic activity and a wider operational temperature range. Rational design for improved CA stability aims to enhance protein rigidity without disrupting substrate binding or catalysis. Structural studies show CO_2 binding causes only minor local conformational changes in the CA active site, while the zinc coordination geometry and core hydrogen-bond network remain fully stable (Figure 4) [47]. This validates the key design principle: modifying non-catalytic regions, flexible loops, and surface residues remote from the active site can improve rigidity without impairing catalytic function. Molecular dynamics (MD) simulation-based rational design has further advanced this field by linking local structural flexibility to global enzyme stability in nanoscale confined environments, such as membrane nanopores and metal–organic framework cavities [48]. The root-mean-square deviation (RMSD) analysis of the CA active site has revealed that the stability of the zine-coordinated histidine residues and the surrounding hydrogen bond networks is the primary determinant of catalytic activity retention at high temperatures, providing a precise target for rational mutagenesis [48]. This structure-guided design strategy has been successfully applied to develop TaCA mutants that retain 100% of initial activity after 1 h at 90 °C, representing a significant improvement over the wild-type enzyme, which retains only 30% activity under the same conditions [49].

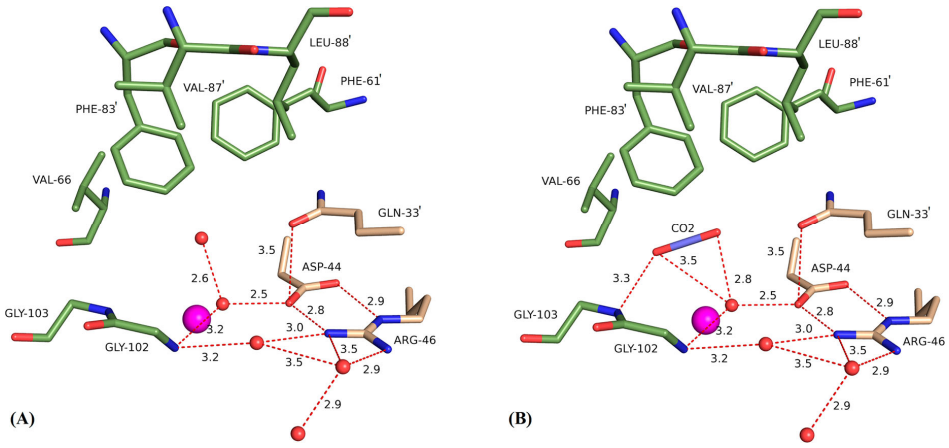

Figure 4. Structural comparison of the CA active site with and without CO_2 binding. (**A**) Open state of the psCA3 active site without CO_2, showing the zinc-bound water molecule (deep water) in the hydrophobic pocket; (**B**) CO_2-bound state of the psCA3 active site, showing the displacement of deep water by the CO_2 substrate, with the core zinc coordination geometry and hydrogen-bond network remaining stable. Adapted with permission from Ref. [47]. Copyright 2015 Biochemistry.

Stability enhancement often introduces conformational constraints that may impair active-site flexibility, thereby reducing catalytic turnover. Directed evolution addresses this trade-off by mimicking natural selection without requiring prior structural knowledge. It can enrich rare, synergistic mutations that globally enhance rigidity while preserving active-site dynamics. As demonstrated by the N-terminal engineering of TaCA [42], this strategy achieved extended half-lives under alkaline conditions while maintaining the catalytic performance required for industrial CO_2 capture. However, its effectiveness is limited by screening throughput; low-efficiency systems often fail to identify balanced variants from large libraries. Rational design, by contrast, offers precision through structure-guided modification. Successful cases consistently target non-catalytic regions remote from the zinc-coordinated active center. For instance, surface-charge engineering [43] and MD simulation-guided design [49] have both enhanced thermal and chemical stability without compromising catalytic efficiency. The main limitation is its dependence on high-resolution structural data; inappropriate target selection can directly impair catalytic dynamics. In

summary, directed evolution and rational design are complementary strategies for navigating the activity-stability trade-off, with selection dependent on structural information availability and application requirements.

3.3. Emerging Technology

Beyond directed evolution and rational design, emerging technologies offer new avenues for enhancing CA stability. Machine learning can analyze large-scale experimental data to efficiently predict the impacts of sequence mutations on CA stability and activity, while guiding the design of smart mutant libraries, significantly accelerating the enzyme engineering pipeline [50]. The integration of machine learning and high-throughput experimentation has become a transformative trend in CA engineering, as it reduces the reliance on trial-and-error mutagenesis and enables rapid identification of beneficial mutation combinations that simultaneously enhance thermal stability, catalytic activity, and solvent tolerance [2,38]. This is particularly critical for the development of CA variants suitable for nanoscale CO_2 capture devices, where enzyme performance is strongly influenced by surface interactions with the support material and confined mass transfer effects [51]. In addition, *de novo* protein design offers a radical alternative to generate CA with tailored stability and function via hyperstable scaffolds that reduce product inhibition, enhance activity, or avoid destabilizing mutations [13,52]. This strategy moves beyond evolving natural enzymes to constructing entirely novel, hyperstable protein scaffolds based on fundamental folding principles. Within these designed frameworks, CA activity can be "installed" by incorporating essential catalytic elements, such as metal-coordinating residues. Artificial CA mimics, constructed by embedding Zn^{2+}-coordination sites into designed protein folds like three-helix bundles [53], exhibit substantial catalytic rate enhancement (up to 10^4-fold), despite lower absolute efficiency than natural CA. These protein-based mimics offer superior kinetics, biosynthetic feasibility, and operational advantages in aqueous systems compared to small-molecule alternatives [15]. The simplicity of CA's active site makes it an excellent platform for accelerating the development of stable biocatalysts via these cutting-edge design technologies. The integration of machine learning, high-throughput experimentation, and *de novo* design holds great promise for rapidly advancing the development of high-performance, robust carbonic anhydrases, thereby overcoming the stability bottleneck for their application in harsh industrial environments [13].

4. Immobilization of CA for CO_2 Sequestration

CA is a small enzyme with average dimensions of $5 \times 4 \times 4$ nm^3, making its direct separation and recovery via conventional unit operations (filtration, centrifugation) challenging [20,54], which hinders its practical industrial application. Immobilizing CA onto solid supports significantly enhances its application potential by conferring recyclability, thermal stability, storage stability, and tolerance to harsh chemicals, while enabling stable operation under high pH and elevated temperatures [20,54–56]. This strategy also effectively addresses the demand for thermostable CA for CO_2 conversion to calcium carbonate in Ca^{2+} ions-containing systems, facilitates product separation, and delivers strong commercial viability. The selection of appropriate carrier materials and immobilization methods is crucial, as these factors directly determine the activity and stability of the immobilized enzyme. Enzyme-immobilized membrane systems are a typical industrial implementation of immobilized CA, which integrates CO_2 adsorption, catalytic conversion and separation in a single compact module (Figure 5) [48].

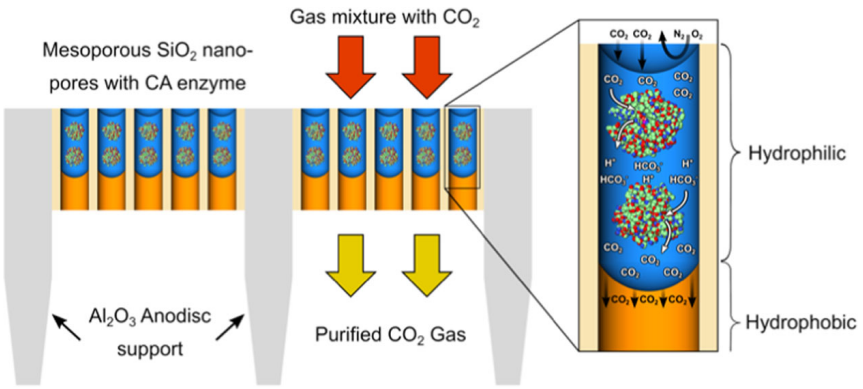

Figure 5. Schematic representation of the ultrathin mesoporous silica (SiO_2) membrane layer containing CA enzymes in aqueous solution, for integrated CO_2 capture and separation [48].

4.1. Carrier Materials for CA Immobilization

Solid support selection decisively impacts the catalytic performance, stability, and economic feasibility of immobilized CA. Ideal carriers are cost-effective, biocompatible, mechanically robust, and rich in modifiable surface functional groups [40,57]. Currently, the primary categories of carrier materials used for CA immobilization include:

4.1.1. Inorganic Oxide Materials

Silica is the most extensively used inorganic support for CA immobilization, thanks to its high surface area, tunable porous structure, excellent thermal/chemical stability, and abundant surface hydroxyl groups for facile functionalization. Mesoporous silica sieves including SBA-15, MCM-41 and KIT-6 have been systematically evaluated, with SBA-15 achieving superior CA loading and 96% initial activity retention after 6 days at 40 °C [58]. Mesoporous silica pore size directly impacts CA loading efficiency and conformational stability, with 5–10 nm pores (matching enzyme dimensions) delivering the optimal balance between high loading and low mass transfer resistance [59]. SBA-15 functionalization with gold or silver nanoparticles further boosts enzyme stability and activity, with silver-conjugated CA delivering 25-fold higher $CaCO_3$ mineralization activity than free enzyme. To mitigate silica dissolution under alkaline conditions, zirconia-doped SiO_2-ZrO_2 composite nanoparticles have been developed with markedly improved stability [38,59]. Recently, a scalable silica-immobilized CA system on antifouling epoxy-coated metal supports has shown excellent stability, with CA-coated nickel foams retaining 60% activity after 6 cycles over 28 days [60]. Beyond silica, titania, alumina and zirconia have also proven effective CA supports, with immobilized systems retaining >80% activity after long-term storage or repeated reuse [61]. Overall, inorganic oxide materials offer versatile, robust, tunable platforms to enhance CA stability, reusability, and CO_2 conversion efficiency.

4.1.2. Magnetic Materials

Magnetic nanoparticles, typified by Fe_3O_4, offer the core advantage of enabling rapid and efficient separation and recovery of immobilized CA using an external magnetic field, greatly simplifying downstream processing [62]. Typically, functional groups such as amines are introduced onto the particle surface via silanization (e.g., using APTES), followed by covalent conjugation of CA using cross-linkers like glutaraldehyde, resulting in highly stable magnetic biocatalysts [63]. This approach not only confers good operational stability to CA (e.g., maintaining high activity after 30 reuse cycles) but also effectively mitigates issues of aggregation and loss common to conventionally immobilized enzymes during separation. Combining magnetic particles with materials like mesoporous foam

silica can further synergistically increase enzyme loading and prevent leaching. Recent studies have further developed magnetic CA biocatalysts for application in packed bed absorption reactors, where the magnetic field can be used to control the distribution of immobilized CA, optimize gas–liquid–solid mass transfer, and achieve rapid catalyst recovery and regeneration [64]. Pilot-scale experiments have demonstrated that magnetic CA biocatalysts can increase the CO_2 absorption rate of methyldiethanolamine (MDEA) solution by more than 9 times compared to the blank solvent, with stable performance over 10 consecutive operation cycles [65].

4.1.3. Carbon-Based Materials

Carbon-based materials have attracted considerable research interest due to their high electrical conductivity, rich surface functional groups, and robust physicochemical stability [66,67]. Carbon nanotubes (CNTs) occupy a prominent position carbon-based nanomaterials for enzyme immobilization, owing to their exceptional mechanical strength and unique dual metallic, as well as semiconducting characteristics. These attributes render CNTs promising substrates for enzyme immobilization, enabling conjugation on either the outer walls or inner cavities of the nanotubes [68]. Additionally, graphene and graphene oxide have been extensively explored as supports for enzyme immobilization, owing to their large surface area, high electrical conductivity, and excellent biocompatibility [68,69]. Fu et al. (2018) [70] constructed multilayer biomimetic membranes for CO_2/N_2 separation, where the functionalized graphene oxide nanosheets provide a high-density binding platform for CA while faciliating rapid CO_2 diffusion. The resulting biocatalytic membranes have achieved CO_2 performance up to 2600 gas permeation units and excellent selectivity, with stable performance under long-term operation [70]. Heo et al. (2023) [71] assembled graphene oxide and CA into a nanoscale multilayer configuration designed for CO_2 capture. Emerging carbon materials hold promise to meet all essential requirements for CA immobilization with improved economic viability. Optimizing enzyme—material interactions will further enable the development of efficient and cost-effective nanobiocatalysts. As illustrated in Figure 6, diverse nanomaterials and immobilization strategies have been developed to construct stable CA nanobiocatalysts, which effectively improve stability, reusability, and ease of recovery for industrial CO_2 sequestration.

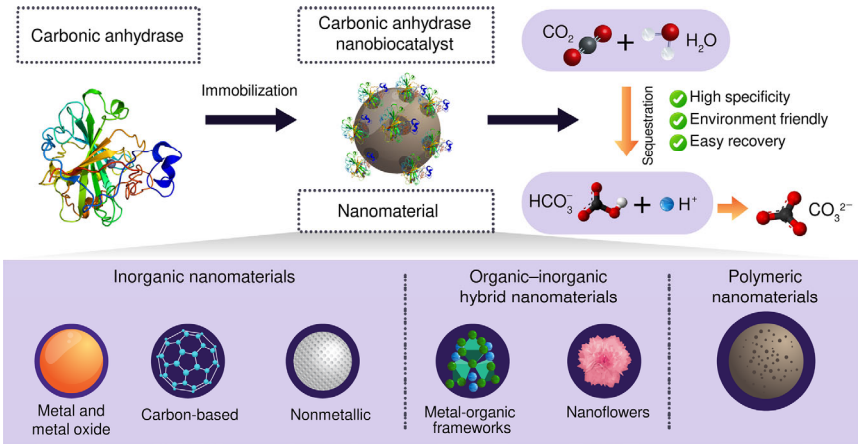

Figure 6. Schematic illustration of carbonic anhydrase (CA) immobilization onto nanomaterial supports to form robust nanobiocatalysts for CO_2 capture. Various immobilization strategies (adsorption, entrapment, covalent coupling, and cross-linking) and support categories (inorganic, carbon-based, MOFs, and polymeric nanomaterials) are summarized. Adapted with permission from Ref. [68]. Copyright 2024 Springer Nature.

4.1.4. Synthetic Polymers

Synthetic polymers are broadly categorized into hydrophilic and hydrophobic types. Hydrophilic polymers, rich in hydroxyl groups, are readily activated, whereas hydrophobic polymers such as PVDF and PE possess few reactive groups and thus require multi-step activation or post-modification [31,40]. Although polymers possess diverse functional groups (e.g., carbonyl, carboxyl, hydroxyl, epoxy, amine, diol, alkyl, and trialkylammonium) that enable robust enzyme immobilization and facile surface modification [72], achieving such tailored polymer architectures typically involves laborious and expensive synthetic procedures [66]. Polyurethane foam is commonly used as a carrier for CA immobilization; enzymes immobilized within this material can retain their total activity for over 45 days at room temperature. Meanwhile, Wen et al. (2020) [73] constructed a nanocomposite hydrogel based on polyvinyl alcohol (PVA) and chitosan (CS), which combined good mechanical strength, thermal stability, storage stability, pH stability, and reusability, demonstrating significantly superior CO_2 capture capacity compared to free CA. Studies show that optimized membrane-immobilized CA can maintain high activity over multiple reuses. Furthermore, surface modification layers (e.g., polydopamine/polyethyleneimine) not only enhance enzyme loading but also tune membrane hydrophilicity/hydrophobicity and pore size distribution, optimizing mass transfer [74].

4.1.5. Biopolymers

Biopolymers derived from natural sources, such as chitin, chitosan, and alginate, have emerged as attractive alternatives to synthetic polymers for CA immobilization due to their unique combination of biodegradability, non-toxicity, biocompatibility, and exceptional affinity for proteins. Their natural origin minimizes detrimental effects on enzyme structure and function, enabling immobilized CA to retain high catalytic activity [75], while the presence of reactive functional groups—primarily hydroxyl, amine, and carbonyl moieties—facilitates direct enzyme−facilitates direct enzyme—matrix interactions and surface modification [76]. Among these, chitosan has received the most attention and has been utilized in various forms including beads [18], composites with polyvinyl alcohol or mesoporous alumina, and chitosan-stabilized nanoparticles for CA immobilization via adsorption [77]. Alginate offers a cost-effective entrapment matrix; CA immobilized in alginate beads retained approximately 67% of its original activity after six cycles, demonstrating superior operational stability [78]. Beyond conventional biopolymers, innovative strategies such as dynamic polymer encapsulation and CA-conjugated liposomes have been developed, with the latter retaining 82% of initial activity after 126 days of storage at 4 °C while maintaining substrate affinity comparable to free CA [31,79]. CA immobilized in chitosan–alginate composite beads has been shown to double the rate of magnesium carbonate precipitation at low CO_2 partial pressures, a critical performance improvement for industrial mineralization carbon sequestration [38]. Collectively, these natural polymer-based systems offer renewable, affordable, and environmentally friendly platforms for CA immobilization, with performance characteristics well-suited for sustainable CO_2 capture applications.

4.1.6. Metal–Organic Frameworks (MOFs)

Metal–organic frameworks (MOFs), ordered crystalline materials assembled from metal ions and organic ligands, are excellent supports for CA immobilization, featuring ultrahigh specific surface area, tunable porosity, and modifiable surface functionality [68,79]. Among MOFs, zeolitic imidazolate frameworks (ZIFs, e.g., ZIF-8, ZIF-11, ZIF-90) -especially ZIF-8-are the most widely studied for CA immobilization, thanks to their mild synthesis conditions, biocompatibility, and enzyme structure-preserving hydrophilicity [66,80,81].

Common CA immobilization strategies on MOFs include in situ encapsulation during MOF synthesis, surface adsorption, and covalent attachment; encapsulation typically delivers superior stabilization via confinement- induced restriction of enzyme conformational changes. ZIF-8-encapsulated CA consistently shows enhanced catalytic performance, with reported CO_2 hydration/mineralization activity 1.5-fold [82] to 22-fold [83] higher than free enzyme. This is attributed to synergistic effects: imidazole groups actively participate in CO_2 catalysis, while the framework stabilizes the enzyme's secondary structure [84]. These nanobiocatalysts also deliver markedly improved thermal stability, up to 35 days of storage stability, and excellent reusability (>80% activity retained after multiple cycles) [68,85]. Recent work has further optimized CA spatial confinement within MOFs, leveraging the nanoscale cavity "confinement effect" to enhance enzyme stability. MD simulations confirm that MOF encapsulation restricts CA unfolding at high temperatures, preserving the active site hydrogen bond network and zinc coordination geometry-the core mechanism for enhanced thermal stability [48]. For functional expansion, multi-enzyme co-immobilization in MOFs has emerged as a key direction, enabling cascade conversion of captured CO_2 into high-value chemicals (e.g., formate, methanol) in a single system, with yields up to 13.1-fold higher than those of free enzyme systems [86,87]. To address limitations in MOF mechanical strength and processability, recent studies have integrated CA-MOF composites into membranes and hydrogels, delivering enhanced CO_2 permeation flux, selectivity, and operational stability for industrial deployment [88,89].

To facilitate the selection of suitable carriers for specific applications, a comparative assessment of the main immobilization materials is provided herein. Inorganic oxide materials (e.g., silica, titania) offer excellent mechanical strength, moderate cost, and good scalability, with immobilized CA retaining >80% activity after multiple cycles [59,61]. However, their efficiency is often limited by enzyme leaching under alkaline conditions unless surface modification or composite strategies are employed [59]. Magnetic nanoparticles enable facile enzyme recovery via external magnetic fields, significantly improving operational convenience and reducing downstream processing costs [63,90], yet their scalability is constrained by the complexity of uniform functionalization and potential aggregation in large-scale reactors [63]. Biopolymers such as chitosan and alginate are low-cost, renewable, and highly scalable, but their relatively low mechanical strength and susceptibility to enzyme leakage under continuous operation remain challenges [75,78]. MOFs, particularly ZIF-8, achieve exceptional efficiency with reported activity retention >90% after multiple cycles and superior thermal stability [82,83,85], but their current high synthesis cost and limited large-scale production capacity hinder widespread industrial adoption [66,87]. Collectively, consistent with the activity-stability trade-off in CA protein engineering, no single carrier can simultaneously optimize cost, efficiency, and scalability. The optimal carrier selection depends on the specific operating conditions of the target CO_2 sequestration process.

4.2. Techniques for CA Immobilization

4.2.1. Adsorption

This method relies on weak physical interactions such as van der Waals forces, hydrogen bonding, and electrostatic attractions between the enzyme and support materials (Figure 7) [31]. This technique offers several advantages, including mild operating conditions that preserve enzyme conformation, high loading capacity, and no requirement for complex surface modification or chemical additives [66]. However, the reversible nature of these weak interactions often leads to enzyme leakage over time, resulting in gradual activity loss that limits long-term operational stability [31,58]. Despite this limitation, adsorption has been successfully employed with various supports: CA immobilized on SBA-15 via

adsorption retained high initial activity [59], while CA adsorbed on ZIF-8 achieved 75% residual activity at 60 °C and demonstrated 22-fold higher CO_2 mineralization compared to free enzyme [83]. To address leaching issues, strategies such as selecting supports with complementary surface charges, optimizing pore size to match enzyme dimensions, or introducing additional functional groups for enhanced binding interactions have been explored [91].

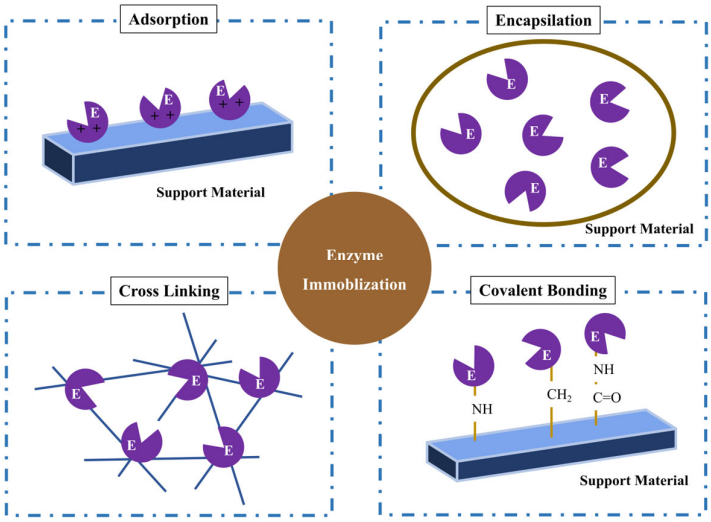

Figure 7. Various approaches for enzyme immobilization.

4.2.2. Entrapment

This technique involves the physical confinement of CA within a polymeric network where the enzyme remains unbound and retains its native conformation while substrates and products freely diffuse through the matrix [92]. This method utilizes various materials including biopolymers such as alginate and chitosan, as well as silica-based and synthetic polymer matrices, offering advantages such as enhanced thermostability and protection against harsh reaction environments [58,93]. For instance, CA entrapped in bioinspired silica maintained full activity at 50 °C while free enzyme was completely deactivated [94], and chitosan–alginate entrapped CA retained 95% residual activity after immobilization and preserved 45% activity at pH 11 where free enzyme was inactivated [95]. However, the technique faces limitations including reduced apparent activity due to restricted substrate access and enzyme leakage from matrices with large pore sizes, often resulting in diminished reusability [64,76]. To address these drawbacks, additional cross-linking steps or the use of composite materials have been explored to strengthen the polymer network and improve operational stability [96].

4.2.3. Covalent Coupling

Covalent coupling forms stable chemical bonds between support surface functional groups (amine, epoxy, hydroxyl, carboxyl) and amino acid residues of CA, typically using cross-linkers such as glutaraldehyde or carbodiimide [97,98]. As an irreversible immobilization method, it delivers key advantages: strong binding to prevent enzyme leakage, enhanced thermal/operational stability, and excellent reusability [55]. For example, CA covalently immobilized on amine-functionalized magnetic nanoparticles retained 50% initial activity after 22 reuse cycles [63], while CA coupled to functionalized SBA-15 showed better stability and reusability than adsorption-based systems [99]. However, this method requires precise control: covalent bonds may occasionally alter enzyme conformation or modify active site residues, potentially reducing catalytic activity, and it also involves relatively complex, time-consuming preparation processes. To mitigate these limitations, recent

optimization strategies focus on site-specific coupling targeting non-critical regions of CA. This rational design avoids unintended modification of the catalytic center, minimizing conformational disruption and activity loss [2]. In a key industrial-scale demonstration, CA covalently immobilized on polydopamine/polyethyleneimine-modified textile structured packing was deployed in full-size absorption towers, delivering stable CO_2 capture performance over 14 consecutive cycles with >71% initial activity retained [100], marking a critical breakthrough in immobilized CA scale-up. Despite remaining challenges, covalent coupling remains one of the most widely used immobilization strategies, enabling the fabrication of robust biocatalysts with extended operational lifespans well suited for industrial CO_2 capture [101,102].

4.2.4. Cross-Linked Enzyme Aggregates (CLEAs)

Cross-linked enzyme aggregates (CLEAs) represent a carrier-free immobilization approach where CA molecules are precipitated using salts or organic solvents, then covalently cross-linked with bifunctional reagents (e.g., glutaraldehyde) to form a stable three-dimensional network [18,90]. This method eliminates the need for solid supports, significantly reducing material costs, while achieving high enzyme loading and effective stabilization of the enzyme's quaternary structure [103]. Studies have verified its excellent performance: bovine CA immobilized via the CLEA strategy retained up to 84% of its initial activity after immobilization process, and maintained 95% activity after 10 consecutive cycles [90]. Compared to adsorption and covalent coupling on SBA-supports, CA-CLEA composites exhibited superior thermostability, reusability, and storage stability, retaining 95% activity after 30 days at 25 °C versus only 45% for free enzyme [104]. Despite these strengths, CLEA technology has two critical limitations: non-specific cross-linking may cause enzyme conformational changes and partial activity loss, and the fine particulate structure of conventional CLEAs hinders filtration recovery, restricting its industrial continuous application [105]. Early studies addressed the recovery issue by fabricating magnetic CLEAs via nanoparticle incorporation, achieving 95% activity retention after five cycles with facile magnetic separation [105]. Recent optimizations have further improved its practicality: modified magnetic CLEAs maintain 95% initial activity after 5 CO_2 hydration cycles with enhanced industrial stability [38], and CLEAs integrated into membrane contactors show stable long-term performance in continuous CO_2 capture [2]. Overall, despite residual activity loss challenges, CLEAs remain a highly promising strategy for CA-based CO_2 capture due to its operational simplicity, low cost and excellent stability [90].

Considering the physical and chemical properties of carrier materials and operational condition combination of immobilization techniques is often employed to establish robust linkages between CA and the support, thereby enhancing resistance to enzyme leaching and inactivation. For example, Woo et al. (2015) [106] first demonstrated BCA onto magnetic mesoporous foam silica, then used chitosan as an intermediate CA-support layer, followed by cross-linking with glutaraldehyde. The CA immobilized via this combined approach exhibited a half-life 353 times longer than that of the free enzyme and retained nearly all its initial activity after 30 cycles [58]. The main drawbacks of such combined methods are their complex and tedious preparation process.

5. CA-Driven Pathways for CO_2 Valorization

In the era of the circular economy, developing green and sustainable solutions for waste utilization is of increasing importance. Strategies that couple enzymatic catalysis with efficient industrial CO_2 capture and in situ conversion into high-value products are particularly pivotal, as they enable resource recovery while mitigating environmental pollution, forming the core of a CO_2-based circular bioeconomy [107]. Viewing CO_2 as a

resource rather than merely a greenhouse gas, and integrating it into biorefinery systems via biocatalytic routes, provides a transformative pathway for the development of value-added bio-based products and renewable energy, fundamentally changing its perception from an environmental threat into a sustainable industrial feedstock [108]. Building on the technical foundation of efficient CO_2 hydration by CA, and leveraging the enhanced stability and reusability achieved through the protein engineering and immobilization strategies discussed in the preceding sections, the established application pathways for CO_2 valorization primarily focus on two major directions: the synthesis of high-value-added products and the production of bioenergy, with the dual goals of carbon resource utilization and net carbon emission reduction.

5.1. Development of High-Value-Added Products

The enzymatic conversion of captured CO_2 into high-value compounds such as methanol, formate, and oxaloacetate is a key strategy for achieving carbon resource cycling. Compared with traditional photochemical or electrochemical conversion methods that require harsh conditions and high energy input, biocatalytic conversion offers significant advantages, including mild reaction conditions, high selectivity and efficiency. In recent years, researchers have made great progress in this field through the construction of multi-enzyme cascade systems and innovative immobilization techniques. Ji et al. (2016) [109] constructed a multi-enzyme-cofactor catalytic system by assembling CA on the surface of polyelectrolyte-doped hollow nanofibers, enabling efficient methanol synthesis from CO_2. This immobilized system remained stable over 10 consecutive cycles, achieving a methanol yield of 103.2%. Aleku et al. (2021) [110] further verified the synergistic effect of CA and downstream conversion enzymes, demonstrating that the combined catalysis of formate dehydrogenase and CA increased the reaction rate of CO_2-to-formate conversion by 4.2-fold. Building on these early works, recent studies have further optimized these multi-enzyme cascade systems through rational co-immobilization design. Chang et al. (2021) [111] successfully designed a synthetic enzyme complex comprising CA and phosphoenolpyruvate carboxylase, which can simultaneously capture CO_2 and convert it into the four-carbon platform compound oxaloacetate. These studies collectively demonstrate that constructing stable multi-enzyme systems with cascade reaction capabilities can provide an effective and sustainable platform technology that integrates point-source CO_2 capture with the synthesis of high-value-added chemicals.

5.2. Bioenergy Production

Microalgae-derived bioenergy is a core pillar of the CO_2-based circular bioeconomy, offering a suitable route to couple industrial CO_2 mitigation with renewable fuel production [15,112]. Compared to terrestrial energy crops, microalgae exhibit 10- to 50-fold higher photosynthetic efficiency and high lipid content suitable for biofuel feedstock [113], while the rate-limiting step of their photosynthetic carbon fixation is the slow hydration of gaseous CO_2 into bioavailable bicarbonate [15]. CA, with an ultrahigh CO_2 hydration turnover frequency of up to 10^6 s^{-1}. effectively breaks this gas–liquid mass transfer barrier, significantly boosting microalgal biomass and lipid accumulation for bioenergy production [114,115].

Two mainstream technical routes have been developed for CA-enhanced bioenergy production. The first route is exogenous CA addition, a non-transgenic approach avoiding the complexity and biosafety risks of genetic modification. Crude CA extract from *Bacillus halodurans* increased the CO_2 biofixation rate of *Tetraselmis* sp. from 0.64 g L^{-1} day^{-1} to 4.26 L^{-1} day^{-1} under simulated flue gas conditions (20% v/v CO_2) [112]. To address the poor stability of free CA, an optimized immobilized CA system via enzyme precipitate

coating on electrospun nanofibers achieved an ultra-long activity half-life of 797 days, and accelerated *Dunaliella tertiolecta* growth by 231% compared to carbon-free controls [114]. The second strategy is endogenous CA enhancement via genetic engineering, which strengthens the microalgal intrinsic carbon concentration mechanism. Heterologous expression of highly active CA in microalgae enhanced CO_2 fixation capacity and lipid accumulation, while cell surface display of CA on microalgae achieved a 1.6-fold increase in growth rate and 1.7-fold higher lipid production without exogenous carbon supplementation [114]. Lin et al. (2022) [116] enhanced CO_2 utilization and assimilation in *Chlamydomonas reinhardtii* via heterologous CA expression, with engineered strains showing ultra-high yields of lutein and lipids, increased carbon flux, and higher biomass production.

Despite the promising advances highlighted above, the practical implementation of CA-enhanced CO_2 valorization faces several critical challenges that temper near-term optimism. First, the stability of CA under industrial conditions remains a primary bottleneck: free CA rapidly loses activity at temperatures above 60 °C and in the presence of flue gas impurities such as SO_x and NO_x, which can cause permanent enzyme deactivation through pH changes and conjugate base inhibition [48]. While immobilization strategies significantly improve stability, achieving a balance between enzyme loading, mass transfer efficiency, and cost remains challenging [38]. Second, multi-enzyme cascade systems for high-value chemical synthesis, though highly efficient, currently rely on costly co-factors (e.g., NADH) and exhibit limited operational stability under continuous flow conditions, with most studies confined to batch reactors at laboratory scale [15]. Third, the scalability of microalgae-based bioenergy production is constrained by downstream processing costs (harvesting, lipid extraction) and the energy input required for CO_2 mass transfer, which together contribute to a levelized cost of biofuel that remains above that of fossil-derived alternatives [112]. Quantitative techno-economic analyses are still scarce for these emerging CA-based valorization routes, and future research should prioritize integrated assessments that couple catalytic performance metrics (e.g., turnover frequency, enzyme lifetime) with economic and environmental life-cycle evaluations to better benchmark their competitiveness against conventional technologies [2].

6. Conclusions and Future Perspectives

CA, as a highly efficient and specific biocatalyst, possesses unique advantages for CO_2 capture, fixation, and valorization. Whether employed in its free or immobilized form, CA acts as a non-toxic and highly efficient promoter in CO_2 capture processes. The synergistic innovation of strategies such as protein engineering, immobilization technology, and synthetic biology can significantly enhance CA's adaptability to industrial environments, operational stability, and economic feasibility, thereby advancing enzymatic carbon capture from proof-of-concept toward engineering demonstration. In the future, with deeper insights into the structure-function relationship of CA and the continued integration of interdisciplinary approaches, engineered microbial CA is poised to become a vital component within the key technology systems for carbon neutrality, offering green and sustainable solutions for climate change mitigation and resource circularity.

CA-based carbon capture processes represent a promising route for capturing CO_2 from industrial point sources and can be integrated with processes that convert bicarbonate into high-value-added products. Looking forward, to achieve breakthroughs and the industrialization of CO_2 capture and conversion technologies, focused efforts and cross-disciplinary innovation are required in several frontier areas: (1) development of novel CAs: employing diverse methods such as gene cloning, protein engineering, and synthesis biology to create new CA variants with superior activity and stability; (2) development of efficient, low-cost carrier materials: porous, biocompatible materials

with high specific surface areas are crucial for simultaneously enhancing enzyme stability and mass transfer efficiency. Such improvements will enable sustained high-performance operation and maximize CO_2 conversion rates; (3) integration of computational models and machine learning: combining these tools to identify effective mutation sites, predict the impact mutations on overall structure and activity, optimize decision-making processes, streamline development, and reduce reliance on trial-and-error methods. The convergence of advanced simulation techniques with rigorous laboratory testing holds promise for guiding the synthesis of novel materials with superior catalytic properties; (4) development of cost-effective immobilization strategies and optimization of process design: reducing enzyme replenishment needs, and improving catalyst recovery and reuse within reaction environments to lower application costs; (5) conducting Life Cycle Assessment (LCA) and Techno-Economic Analysis (TEA): to evaluate the economic and environmental viability of these systems. This provides a robust data foundation for optimizing and comparing technological pathways, understanding their broader impacts, supporting the scale-up of such technologies for industrial application, and informing policy and regulatory frameworks. Through coordinated, multidisciplinary, and multi-faceted efforts, engineered microbial CA technology holds significant promise for overcoming existing bottlenecks. It is anticipated to emerge as an efficient, green, and economically viable key technology within the future carbon neutrality landscape, making substantive contributions to the dual objectives of climate change response and sustainable resource utilization.

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