

Review Article

Enzymatic Carbon Dioxide Capture

Alain C. Pierre

*Institut de Recherches sur la Catalyse et L'environnement de Lyon, Université Claude Bernard Lyon 1 CNRS, UMR 5256,
2 Avenue Albert Einstein, 69626 Villeurbanne, France*

Correspondence should be addressed to Alain C. Pierre, valerie.pierre.sed@numericable.com

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In the past decade, the capture of anthropic carbonic dioxide and its storage or transformation have emerged as major tasks to achieve, in order to control the increasing atmospheric temperature of our planet. One possibility rests on the use of carbonic anhydrase enzymes, which have been long known to accelerate the hydration of neutral aqueous CO₂ molecules to ionic bicarbonate HCO₃⁻ species. In this paper, the principle underlying the use of these enzymes is summarized. Their main characteristics, including their structure and catalysis kinetics, are presented. A special section is next devoted to the main types of CO₂ capture reactors under development, to possibly use these enzymes industrially. Finally, the possible application of carbonic anhydrases to directly store the captured CO₂ as inert solid carbonates deserves a review presented in a final section.

1. Introduction

One of the main problems our world is presently facing, concerns the capture of anthropic carbon dioxide rejected in the atmosphere by human activities. This gas is considered as one of the main atmospheric components responsible for a greenhouse effect and an increase of the earth atmosphere temperature [1, 2], with many unwanted consequences, including the development of infectious diseases [3]. According to a report by the International Panel on Climate Change (IPCC) on the earth climate evolution, dating from 2007, the release of this gas in the atmosphere has increased by 80% from 1970 to 2004 and it accounted for 76.7% of the “Greenhouse Effects Gases” in 2004 [4]. An international agreement termed the “Kyoto Protocol,” established by the United Nations Framework Convention on Climate Change, was initially signed in 1997 by 37 countries in order to reduce greenhouse gas (GHG) emissions [5]. This treaty was enforced in 2005 and the number of countries who ratified the convention increased to 191 in 2011. The target was to reduce the CO₂ emission by an amount depending on the country by comparison with a defined basis (8% in Europe, 7% in USA), over the five-year period 2008–2012.

Several methods are being developed or studied for this purpose [6, 7] and progress is being followed by the International Energy Agency (IEA) of the Organization for

Economic Co-operation and Development (OECD) [8]. A general review was also published in a book chapter by Muradov [9]. Amongst them, one group of technology is proposing to use enzymes of the carbonic anhydrase type. The specificity of these enzymes is to catalyze the reversible transformation of neutral aqueous CO₂ molecules, termed CO₂(aq) in this paper, to the ionic species H⁺ and HCO₃⁻. Very few reviews have specifically addressed these enzyme projects. To our knowledge, these comprise a recent publication by Shekh et al. [10] and a bibliography in a recent Ph.D. thesis by Favre [11]. However, the number of new research articles published has also significantly increased during the same time span, and the aim of the present paper is to present an up-to-date synthesis of this field.

2. Place of Enzyme Technologies amongst the Main CO₂ Capture and Storage (CCS) Techniques

Three major steps are being considered to tackle the anthropic CO₂ problem: the capture of this gas from the atmosphere, its transport to storage places, and its storage under various forms. These 3 steps are often gathered under the abbreviation “CCS,” for “CO₂ Capture and Storage.”

Enzymes are concerned by the first step, that is, CO₂ capture, and also to some extent by the third one, to transform the captured CO₂ to carbonates for a safe storage, or possibly to more valuable products.

The main techniques developed to capture the CO₂ from industrial fumes can be classified as “postcombustion,” “oxycombustion” or “precombustion” methods [12]. The enzymatic capture techniques can be classified within the first group of methods, where CO₂ is withdrawn from the industrial fumes produced by the combustion of hydrocarbons. Within this group, different CO₂ capture and storage techniques are actually in competition and they were reviewed in the 2005 report from the Intergovernmental Panel on Climate Change (IPCC) [13]. They comprise amine scrubbing, membrane separation, wet and dry mineral carbonation, pressure storage, and adsorption on solids or in liquids.

Typically, industrial fumes contain from 10% to 20% CO₂, mixed with nitrogen as the major component plus some lower percent of O₂ and H₂O vapor and a variety of other pollutants, in particular sulfur compounds. The processes most extensively studied rest on reversible carbonation reaction with amines. For instance, when an aqueous monoethylamine (MEA) solution is used, some ammonium carbamate partly hydrolyzed to a carbonate is produced [14]. To recover the CO₂ from the carbamate, it is then necessary to increase the temperature in order to displace the carbonation equilibrium towards CO₂ release. In the enzyme techniques, the amines are replaced by an aqueous solution of enzyme of the carbonic anhydrase family. As previously mentioned, the latter proteins can catalyze the reversible transformation of CO₂(aq) neutral species to ionic HCO₃[−] species, provided adequate conditions can be satisfied as further detailed in the present paper.

By comparison, in the “oxycombustion” methods, combustion of the hydrocarbon is achieved in pure O₂ or in a mixture of O₂, H₂O vapor and CO₂. Consequently, the fumes are mostly constituted of H₂O vapor and CO₂, from which CO₂ can simply be separated if H₂O is condensed to the liquid state, by cooling [13]. At last in the “pre-combustion” methods, the fuel used is first converted to a mixture of CO₂ and H₂, often termed “syngas” [15].

In most cases, the recovered CO₂ can then be compressed to liquid CO₂ under a moderate pressure (e.g., 2 MPa at −20°C), to be transported by ships or trains. It can also be transported by pipeline, usually when brought to the supercritical fluid state, (temperature >31°C, pressure >7.4 MPa) [15].

The main storage methods which are being tested consist in injecting the captured CO₂ at great geological depth, at least 800 m, where it can hopefully remain for a time as long as possible [13, 15, 16]. The main geological sites considered for such storage comprise exhausted oil fields, unexploited coal seams where CO₂ could possibly react with the coal to produce some methane, and deep underground salinas which are actually evenly dispersed and abundant on Earth, so that they could offer a storage volume of the order of 10 times that from the other geological sites [13, 15–17]. Injection of the CO₂ in the ocean, at a depth beyond 1000 m

where dense solid CO₂ hydrates could form, is also being considered [13]. A number of fundamental research studies also addressed the adsorption of CO₂ on solids, mainly basic solids. Carbonic anhydrase enzymes are concerned by a storage technique of CO₂ as solid carbonates. Such storage is often considered to be of lesser importance, because it would require abundant and cheap basic cation sources (Ca²⁺, Mg²⁺, Na⁺, etc.) to be economically applicable. However, a number of basic scientific publications have addressed the use of carbonic anhydrase for this purpose, and they are reviewed in the last section of this paper.

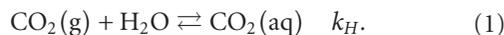
CO₂ could also be used as a substrate to synthesize valuable chemicals, as reviewed by Sakakura et al. [18]. In particular, combined with a dehydrogenase, CA enzymes could be used to transform the captured CO₂ to methanol by a fully enzymatic process [19]. Besides, other biological techniques are also in progress such as the use of marine algae to perform a photocatalytic transformation of CO₂ to biofuels [20–24]. However, these subjects are outside the scope of the present paper.

3. The Physical Chemistry of CO₂ Capture in Aqueous Media

The general mechanism of CO₂ capture in aqueous media and its separation from other gases, can be decomposed in the 5 following steps [25].

- (1) Dissolution of the CO₂ gas molecules in water on the CO₂ capture side, at the gas/aqueous medium interface, according to the Henry’s equilibrium [26–28]. As a result, neutral aqueous CO₂(aq) molecules are introduced in the aqueous film in direct contact with the gas.
- (2) Reversible conversion by deprotonation of the neutral CO₂(aq) species, usually termed hydration, to form anionic bicarbonate species HCO₃[−], according to a chemical equilibrium which is pH dependent.
- (3) Transport of both the neutral and anionic aqueous CO₂ species, from the CO₂ capture side towards the CO₂ release side, by molecular diffusion inside the aqueous medium and/or by forced fluid circulation.
- (4) Reverse conversion of the anionic HCO₃[−] species to the neutral CO₂(aq) ones, according to the same chemical equilibrium as in step 2.
- (5) Evaporation of the CO₂(aq) in the gas to liberate CO₂ gas species, on the CO₂ release side, according to the same Henry’s equilibrium as in step 1.

Regarding steps 1 and 5, the Henry’s chemical equilibrium can be written as



The equilibrium constant k_H of (1) is known as the Henry’s constant, and it is usually written as in (2) known as the Henry’s law:

$$N(\text{CO}_2(\text{aq})) = \frac{P(\text{CO}_2(\text{g}))}{k_H}. \quad (2)$$

According to this law, the molar fraction $N(\text{CO}_2(\text{aq}))$ of the $\text{CO}_2(\text{aq})$ species in the aqueous film, in equilibrium with a gas phase with which it is in direct contact, is proportional to the partial pressure $P(\text{CO}_2(\text{g}))$ in this gas. This equilibrium equation concerns both the capture side and the release side. After conversion of the molar fraction $N(\text{CO}_2(\text{aq}))$ to the molar concentration $[\text{CO}_2(\text{aq})]$ in water, (2) can be transformed to

$$[\text{CO}_2(\text{aq})] = \frac{P(\text{CO}_2(\text{g}))}{(0.018k_H)}. \quad (3)$$

The exact nature of these neutral $\text{CO}_2(\text{aq})$ species is controversial. It is generally admitted that they essentially comprise CO_2 molecules more or less loosely solvated by H_2O molecules to which they can be linked by fluctuating hydrogen bonds [26, 29, 30]. One of these neutral molecular species is the carbonic acid molecule H_2CO_3 , which could actually be synthesized in a virtually pure state in special conditions, from an exact stoichiometric molecular ratio $N(\text{CO}_2)/N(\text{H}_2\text{O}) \approx 1$ [31]. However, these H_2CO_3 molecules are metastable and they become very unstable in the presence of a slight excess of water. Hence they remain present in very low molar ratio solutions ($<3/1000$) in CO_2 saturated water at 25°C , by comparison with the simply solvated $\text{CO}_2(\text{aq})$ species [28, 31–34].

The Henry's equilibrium is a direct consequence of simple molecular collisions at the interface between the gas phase and the liquid phase, which do not involve chemical reactions. Hence, regarding the first layers of liquid water molecules in direct contact with the gas, it is implicitly considered that this equilibrium is very rapidly established and maintained, independently of further diffusion or transformations of the neutral $\text{CO}_2(\text{aq})$ species [30]. Consequently, for a given $P(\text{CO}_2(\text{aq}))$ partial pressure, the concentration $[\text{CO}_2(\text{aq})]$ in the aqueous strata in direct contact with this gas can be reasonably considered as being constant.

On the other hand, equilibrium with a thicker water layer, such as needed for instance to experimentally determine the Henry's constant, is much slower. The reason is this requires a diffusion of both the neutral and anionic CO_2 species, from the aqueous strata in direct contact with the gas towards the whole liquid volume. Fortunately also, when the CO_2 Henry's constant is determined in pure water (no electrolyte added), the neutral $\text{CO}_2(\text{aq})$ species are by very large dominating over the anionic ones, as summarized further on. Consequently, (3) practically concerns the neutral species only. At last, to favor the dissolution of $\text{CO}_2(\text{aq})$ species on the capture side, as well as the release of CO_2 gas on the release side, the exchange surface between the gas phases and the aqueous medium must also be designed to be as high as possible. This point is very important to design efficient CO_2 "scrubbers."

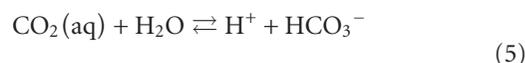
The solubility of CO_2 in pure water under a partial pressure $P(\text{CO}_2(\text{aq}))$ ranging from 0.1 MPa (1 atm) to 100 MPa was reviewed in 2003 by Diamond and Akinfiev [26]. For lower $P(\text{CO}_2(\text{g}))$ partial pressures more in line with CO_2 capture from industrial fumes, it was reviewed in 1991

by Carroll et al. [27] and by Crovetto [28]. For instance, according to Crovetto:

$$\ln(k_H) = \frac{4.800 + 3934.40}{T} - \frac{941290}{T}, \quad (4)$$

where the Henry constant k_H is expressed in bar (1 bar = 10^5 Pa) and the temperature T in Kelvin. As an example, for distilled water saturated in CO_2 under a partial pressure $P(\text{CO}_2(\text{g})) = 0.1$ MPa (= 1 bar \approx 1 atm), this equation indicates concentrations $[\text{CO}_2(\text{aq})] \approx 33.7$ mmol L^{-1} at 25°C and 76.5 mmol L^{-1} at 0°C . The temperature is therefore an important parameter, since the $\text{CO}_2(\text{aq})$ concentration in water increases significantly when the temperature decreases.

Regarding steps 2 and 4, the first deprotonation equilibrium or so-called hydration of $\text{CO}_2(\text{aq})$ species to form bicarbonate anions HCO_3^- , can be written [35]:



$$K_{a1} = 10^{-6.35} = 4.47 \times 10^{-7} \text{ at } 25^\circ\text{C}.$$

According to (5), the pH rapidly falls below 7 as soon as CO_2 is dissolved in distilled water at an initial pH 7, as this is indeed the case to determine the Henry's constant in pure water. On the other hand, if the pH can be maintained at a value $> pK_{a1} = 6.35$ with the help of a buffer, the formation of ionic HCO_3^- species is favored, although the concentration of neutral $\text{CO}_2(\text{aq})$ species remain fixed at the gas liquid interface by the Henry's law. Overall, because the HCO_3^- anions are much more soluble in water than the neutral $\text{CO}_2(\text{aq})$ species, a much larger total CO_2 concentration can be dissolved in aqueous solution. This result is at the base of the idea to use a catalyst to capture CO_2 in aqueous media, where the catalyst role is simply to accelerate the formation of HCO_3^- anions.

The kinetic mechanism underlying (5) largely depends on the nature of the catalyst used and carbonic anhydrase enzymes only constitute one type of catalyst. Without any catalyst, hence at an acidic equilibrium pH, the forward reaction to produce HCO_3^- anions from $\text{CO}_2(\text{aq})$ species is first order with a rate constant $\approx 0.15 \text{ s}^{-1}$. The reverse reaction is faster, with a rate constant $\approx 50 \text{ s}^{-1}$ [33, 34]. Possibly, it could be considered that the H_2CO_3 molecule constitutes the transition state. In basic conditions where OH^- anions are abundant, the main mechanism involves a direct attack of these anions on the $\text{CO}_2(\text{aq})$ species. Hence any base is a catalyst of the CO_2 capture and competes with the carbonic anhydrase enzyme. The enzyme catalytic mechanism involves its active site, as briefly summarized further on. Overall, as any catalyst, the enzyme only modifies the kinetics rate of both the forward and reverse reactions, not the thermodynamic equilibrium.

It is important to note that the Henry exchange mechanism operates for all gas components present on the capture side, including O_2 and N_2 . However, with the latter species, no formation of highly soluble anions such as HCO_3^- occurs. Hence the overall concentration of these components in water, and their further transport rate towards the release side, remains much lower than that of CO_2 , provided the pH

is such that HCO_3^- species are abundant. Unfortunately, this is not the case of other pollutant species such as SO_2 , which can also produce very soluble anions such as SO_4^{2-} .

Step 3 of a CO_2 capture system, which is the transport of the CO_2 aqueous species from the capture side to the release side, applies both to the neutral $\text{CO}_2(\text{aq})$ species and HCO_3^- anions. The liquid medium transport itself can be forced, with the help of circulating pumps as in one type of process under development presented in the next section, or it can be spontaneous by simple molecular diffusion. In both cases, the CO_2 transport must be fast enough to not be the rate limiting step. This implies a fast circulating pump system in the former case, or a very short diffusion distance such as for instance across thin water films in the latter case. In the CO_2 capture systems based on such thin aqueous films, transfer of the $\text{CO}_2(\text{aq})$ and HCO_3^- species from the capture face towards the release side by diffusion of these species is illustrated Figure 1, which gathers the 5 previous steps. Besides, the diffusion coefficients of $\text{CO}_2(\text{aq})$, HCO_3^- and other gas species such as N_2 and O_2 , are of the same order of magnitude, because their molecular weights are relatively close to each other. Hence, as previously mentioned, it is indeed necessary to maximize the HCO_3^- concentration on the capture side, with the help of a catalyst and in the appropriate pH range, so as to increase the overall separation selectivity in CO_2 relative to other species.

In the case of a thin aqueous membrane, the overall CO_2 transfer rate across the membrane is described by (6) where $\Phi(\text{CO}_2)$, expressed in $\text{mol s}^{-1} \text{m}^{-2}$ is the CO_2 flux density carried per second across 1m^2 of liquid membrane; $P(\text{CO}_2(\text{capture}))$ and $P(\text{CO}_2(\text{release}))$ are the $\text{CO}_2(\text{gas})$ partial pressures, in Pascal, on both sides of the liquid membrane and \mathcal{P} defines the membrane permeance, measured in $\text{mol s}^{-1} \text{m}_{\text{membrane}}^{-2} \text{Pa}^{-1}$:

$$\Phi(\text{CO}_2) = \mathcal{P}[P(\text{CO}_2(\text{capture})) - P(\text{CO}_2(\text{release}))]. \quad (6)$$

Another direct consequence of the CO_2 capture in aqueous media concerns the influence of the partial pressure $P(\text{CO}_2(\text{capture}))$ on the capture side. According to Henry's law (3), the $[\text{CO}_2(\text{aq})]$ concentration dissolved in water increases with the CO_2 partial pressure $P(\text{CO}_2(\text{g}))$ in the gas in contact with the aqueous medium. In turn, the $[\text{H}^+]$ and $[\text{HCO}_3^-]$ equilibrium concentrations increase with $P(\text{CO}_2(\text{g}))$, as a consequence of the hydration equilibrium reactions in (5), unless an increasingly stronger and faster reacting buffer can be added to maintain a $\text{pH} > \text{p}K_{a1}$. But this becomes increasingly difficult to achieve as $P(\text{CO}_2(\text{g}))$ increases. Accordingly, experimental results on thin water films gathered by Bao et Trachtenberg [36] and reproduced in Figure 2, confirm that the enzyme efficiency to accelerate the CO_2 capture decreases as the partial pressure $P(\text{CO}_2(\text{g}))$ increases on the capture side.

4. Enzymatic CO_2 Capture

Carbonic anhydrase enzymes are labelled as belonging to the group E.C.4.2.1.1 by the International Enzyme Commission, in agreement with the International Union of Pure and

Applied Chemistry (IUPAC). These numbers indicate that they belong to class 4, the class of lyases which gathers the enzymes able to catalyze a reaction of addition on a substrate carrying a double bond (such as $\text{O}=\text{C}=\text{O}$), to sub-class 2 corresponding to the creation of a single C–O bond by addition of an oxygen atom (Carbon-oxygen lyase), with an oxygen atom brought by an aqua group (Hydro-lyase, first number 1), HCO_3^- being the product, or substrate (inverse reaction) amongst of a list of possible substrates (carbonate dehydratase-second number 1) [37]. Enzymes of this group are actually present in the 3 classes of the living world: prokaryotes, archaea, and eukaryotes [38, 39].

Historically, the first carbonic anhydrase (CA) was discovered in 1933 by Meldrum and Roughton when studying the factors responsible for a rapid transition of the bicarbonate anions HCO_3^- from erythrocytes towards the lung capillary [40]. In 1939, CA of plant origin were shown to be different from the previously known CA [41]. In 1940, Keilin and Mann purified AC extracts from bovine erythrocytes and they showed that the CA contained a Zn atom in their active site [42–44]. In 1963, Veitch and Blankenship discovered AC enzymes in prokaryotes [45] and the first purified AC extracted from such a source was achieved in 1972 from *Neisseria Sicca* [46]. The first genetic sequence of a purified AC of prokaryote origin (bacteria *Escherichia coli*) was established in the 1990's [47] and this metalloenzyme of molecular weight of 24 KDa was the first β class CA [48], while the previous ones were classified in the α class.

In α CA, the active catalytic center is built about a Zn atom in tetrahedral coordination with 3 histidine residues, plus 1 water molecule [49, 50]. For instance, as illustrated in Figure 3 for a human α CAII, this active center is located in the cavity of a protein comprising a polypeptidic chain of 260 amino acids of molar mass 29 kg mol^{-1} (29 kDalton) [34].

β CA predominate in plants and algae. Their main difference with α AC is that they have an oligomeric quaternary structure composed of 2 to 6 monomers, which are each roughly similar to a full α CA. In each monomer, the Zn atom is moreover coordinated to 2 cysteines, 1 histidine, and to 1 aspartate by the intermediate of its carboxylate termination. The first CA from an Archaeon was isolated and purified in 1994 by Albert and Ferry from *Methanosarcina thermophila* [51]. It showed a different amino sequence by comparison with the previous α and β CA, hence it was placed in a new γ -CA class. Contrary to the previous CA, their Zn atom is coordinated in a penta mode to 3 histidines and 2 water molecules.

During the following years, much progress was achieved to discover new AC varieties and to understand the catalytic mechanism of these enzymes, particularly regarding the AC of human origin [52–54]. While α and β CA were found to predominate in Eukaryotes, γ CA were mostly present in Archaea [39, 46]. A new CA of molar mass 27 KDa was extracted from *Thalassiosira weissflogii* [55] and it showed a different amino acid sequence, compared to α , β and γ CA. Hence it opened a new δ CA class [39]. To this one must add another type of CA purified and sequenced from the shell of *Halothiobacillus neapolitanus*. This enzyme can also be found in the shells of Marine cyanobacteria *Prochlorococcus*

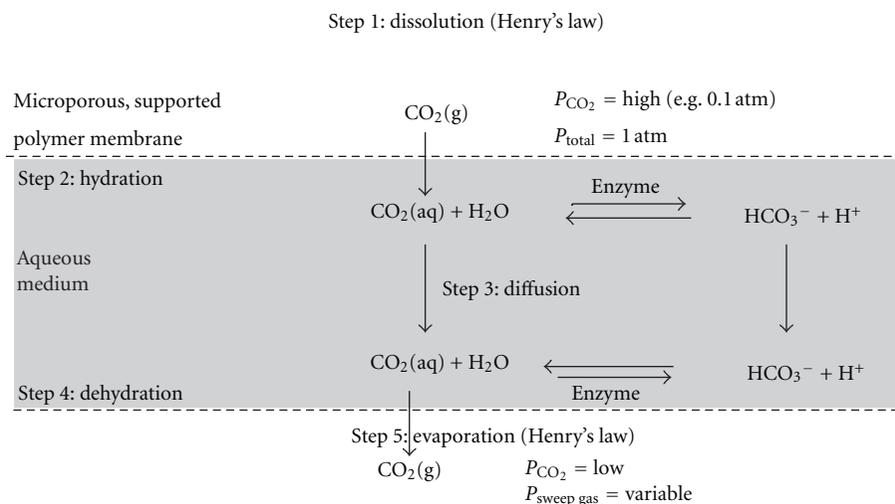
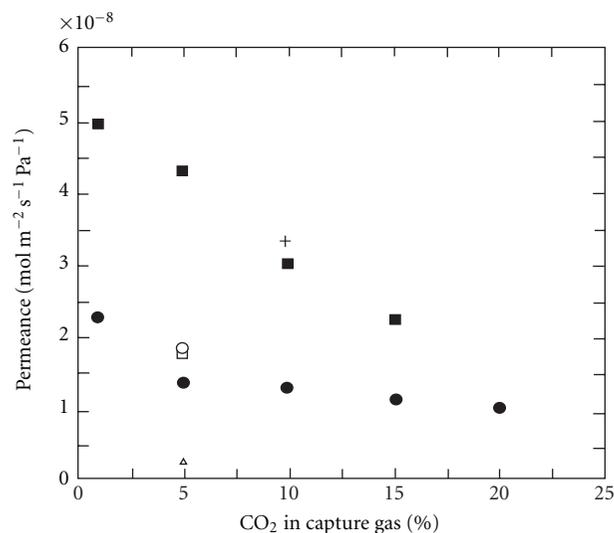


FIGURE 1: Illustration of the CO_2 transfer mechanism inside a thin liquid membrane [140].



- DEA (20% by weight): Bao and Trachtenberg
- CA (3 mg/mL) + NaHCO_3 (1 M): Bao and Trachtenberg
- △ CsHCO_3 (6.4 M): Ward and Robb
- CA (2 mg/mL) + KHCO_3 (2 M): Ward and Robb
- CA (0.5 mg/mL) + NaHCO_3 (1 M): Suchdeo
- + CA (0.2 mg/mL) + NaHCO_3 (1 M) + SiO_2 : Favre and Pierre

FIGURE 2: CO_2 permeance as a function of the CO_2 percent in the capture gas after the results of Bao et Trachtenberg [36], completed by those of Ward and Robb [107], Suchdeo and Schultz [108], and Favre and Pierre [140]. Adapted from Bao and Trachtenberg [36].

and *Synechococcus*. Its molecular weight of 57.3 KDa and its tertiary structure showed two domains, similar to the β -CA, except that only one of these domains had a Zn binding site. This CA converts HCO_3^- to CO_2 inside shells where the CO_2 is incorporated within the biomass by the enzyme Ribulose Bis phosphate Carboxylase (Rubisco) [56]. It was placed it in a new ϵ -CA class. δ and ϵ CA are present in

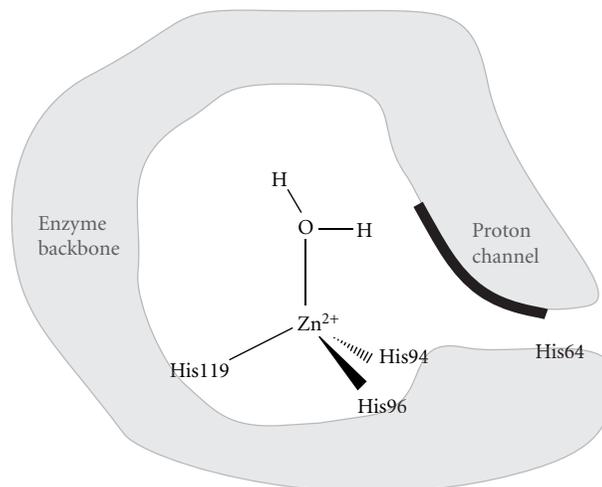


FIGURE 3: Schematic structure and active center of human α ACII [34].

eukaryotic algae and phytoplankton. Their Zn is coordinated 2 cysteines, 1 histidine, and 1 water molecule. At last, a ζ -CA class, comprising CA of molar weight 69 KDa containing a cadmium atom in their active site in place of a zinc, was isolated from the marine diatom *Thalassiosira weissflogii*. [57].

Globally, the AC enzymes based on Zn atoms are classified in 5 groups labelled α , β , γ , δ , and ϵ which all have in common to catalyze for the interconversion of HCO_3^- and CO_2 [34, 52, 58, 59]. However, in more details regarding the mammals α AC, 4 sub-classes can distinguished [53, 58, 60].

- (i) Cytosolic α CA, present in the cytoplasm of cells, themselves comprising several sub-sub-groups labelled CA-I, -II, -III, -VII, and -XIII.
- (ii) Mitochondrial α CA present in cells mitochondria (groups CA-VA and -VB).

- (iii) Secreted α CA present in saliva and milk (group CA-VI).
- (iv) Membrane binding α CA (groups CA-IV, -IX, -XII, -XIV, and -XV).

To these one must add 3 “acatalytic” CA isoforms with unclear functions (CA-VIII, -X, and -XI). Overall, 16 different forms of isozymes (or isoenzymes), which are different forms of an enzyme type coexisting in a same living organism, could be identified in the mammals, of which 10 in humans.

The panel of CA enzymes available to capture CO_2 is indeed large and it keeps increasing. For instance recently, Ramanan et al. reported the isolation, purification, and sequencing of CA from the Enterobacter bacteria *Citrobacter freundii* and *Bacillus subtilis*. [61]. Progress regarding CA enzymes also concerns their extraction and purification techniques. Da Costa et al. compared 2 different purification techniques of bovine CA (BCA): one by extraction with the organic solvents chloroform and ethanol, and the other by ammonium sulfate precipitation [62]. In a CO_2 hydration assay, the first technique provided the highest enzyme activity, for a recovery of 98% and a purification factor of 104-fold. Im Kim et al. compared the hydration activity of a cheaper recombinant α -type CA from *Neisseria gonorrhoeae* (NCA) which they highly expressed in *Escherichia coli*, with a more expensive commercial BCA. The activity of both CA was found to be equivalent. Even the nonpurified NCA showed a significant activity, which opens the route to less expensive enzymatic CO_2 capture processes [63]. On the other hand, Trachtenberg patented a new γ -carbonic anhydrase enzyme which could operate in the temperature range of 40–85°C [64]. Regarding this last point, an interesting geological discovery must also be mentioned. Along the mid-ocean ridge system where tectonic plates are moving away from each other, sea water penetrates the fissures of the volcanic bed and is heated by the magma. This heated sea water rises to the surface and, although this environment seems very hostile, many microorganisms happen to prosper. Amongst them, some micro-organisms have developed efficient CO_2 assimilation processes [65]. In a quite different domain, an artificial, bifunctional enzyme containing both a CA moiety from *Neisseria gonorrhoeae* and a cellulose binding domain (CBD) from *Clostridium thermocellum* was synthesized. This new biocatalyst opens the route to the development of new immobilized enzyme CO_2 capture systems [66]. Besides, the synthesis of biomimetic analogs of CA enzymes is also being investigated. After immobilization on a support, these catalytic complexes could be used to design “biomimetic” CO_2 capture systems more robust than the true enzymatic ones [67, 68]. Overall, new more efficient and cheaper enzymatic systems for CO_2 capture may reasonably be expected to progressively appear in the future.

Carbonic anhydrase enzymes are known to catalyze 2 different types of equilibrium reactions [35]. First, as a “hydrase,” they catalyze the equilibrium hydration and dehydration reactions of $\text{CO}_2(\text{aq})$, previously presented (5). Secondly, as an “esterase,” they hydrolyze substrates such

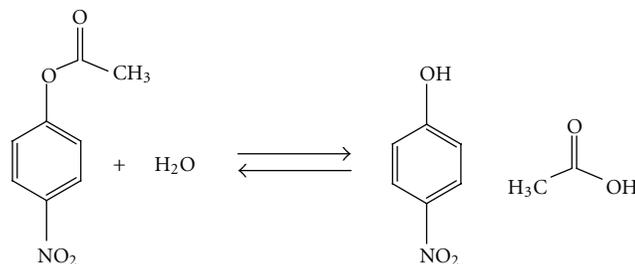


FIGURE 4: Hydrolysis reaction of para-nitrophenylacetate (p-NPA) to para-nitrophenol (p-NP) [35].

as the para-nitrophenylacetate (p-NPA) to para-nitrophenol (p-NP) according to Figure 4.

The capture of CO_2 is concerned by the hydrase activity. The catalytic properties and mechanism of CA enzymes in CO_2 hydration were the subject of many papers which are only partially reviewed here. Overall, the experimental techniques used to measure this activity were generally derived from an electrochemical method first designed by Wilbur and Anderson [69]. These scientists studied 3 types of techniques, based on manometry, colorimetry, and electrochemistry respectively. The manometric techniques rest on a measure of the gas pressure, in a CO_2 containing atmosphere in equilibrium with an enzyme solution in a buffer. The colorimetric technique rests on a measure of the time for a change in color, when a color indicator is mixed in the enzyme solution. Actually, the latter technique was first investigated by Brinkman in 1933 [70] and later successively modified by Meldrum and Roughton [40], Philpot and Philpot [71], and finally Wilbur and Anderson [69]. The electrochemical technique rests on a measure of the pH decreasing rate with a pH electrode, during CO_2 capture. This is often done at low temperature (e.g., $\approx 4^\circ\text{C}$) [69] in CO_2 saturated water to which a buffer at a pH slightly above 8 is added, altogether with a variable mass of enzyme m_{enz} (a few mg). The pH decreasing rate with time, $(d[\text{H}^+])/dt$ is usually determined in a pH range about pH 7.

Because $\text{CO}_2(\text{aq})$ hydration also occurs without any enzyme, it is necessary to subtract the non-enzymatic contribution from the data obtained with the enzyme. This operation really gives the “added” contribution to CO_2 hydration, due to the enzyme. For instance, let t_0 and t_{enz} designate the times measured when the pH decreases from 7.5 to 6.5, respectively, without enzyme and with a mass m_{enz} of CA. The activity per mg of enzyme, really a “relative added activity” ν_r , can be expressed in Units per mg (U mg^{-1}) according to Wilbur equation (7), where ν_{enz} is the pH decreasing rate with the enzyme and ν_0 the pH decreasing rate without any enzyme:

$$\nu_r (\text{U mg}^{-1}) = \frac{\nu_{\text{enz}} - \nu_0}{\nu_0 m_{\text{enz}}} = \frac{t_0 - t_{\text{enz}}}{t_{\text{enz}} \cdot m_{\text{enz}}} \quad (7)$$

In most papers, ν_r is often simply termed ν .

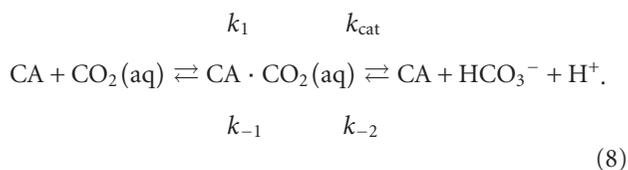
Biochemists traditionally measure the enzyme concentration in preparations in “units” (“U”), where 1 U is defined as the quantity of enzyme which catalyzes the transformation

of 1 μmol of substrate in 1 minute in conditions which must be specified (substrate nature and concentration, temperature, liquid medium, pH). However, regarding $\text{CO}_2(\text{aq})$ hydration, the “units” defined through (7) are not of a same nature. They rather designate an “added relative activity” which largely depends on the buffer used, because the non-enzymatic contribution significantly depends on this buffer. The latter brings OH^- anions which are catalyst competitors to the enzyme, as previously mentioned. Hence (7) cannot be used to indicate the “enzyme concentration” of a CA preparation. For this purpose, the hydrolysis of paranitrophenyleacetate (p-NPA) into para-nitrophenol (p-NP) according to Figure 4 has to be used instead. This reaction is typically followed by measuring the UV-visible absorption due to p-NP, at a wavelength of 400 nm and 25°C, after mixing an aqueous CA solution at pH 7.5 with a p-NPA solution in acetonitrile. Because p-NPA also undergoes self dissociation, the rate of the self dissociation measured in the same conditions without enzyme must be subtracted from the data obtained with the enzyme [72].

On the other hand, it is necessary to keep using Wilbur’s equation (7) when the aim is to study the factors affecting CO_2 hydration. Different scientific groups have applied this equation with variations regarding the enzyme concentration, the CO_2 saturated water volume and the buffer nature and molarity. Overall, the hydration kinetics of $\text{CO}_2(\text{aq})$ by CA enzymes was extensively studied [44, 49, 50, 73–76]. However, the oldest data were often affected by significant error magnitudes, as reported by Bond et al. [30], Mirjafari et al. [77] or Ozdemir [72]. In the most recent developments, CO_2 saturated water and an enzyme solution in a buffer are rapidly mixed in a stop-flow cell. The pH time evolution is then followed by recording the visible light absorption at a characteristic wavelength of a pH dependant color indicator [44, 74].

It must also be mentioned that other CO_2 -capture assaying methods have also been developed for many decarboxylating enzymes. In particular, some methods involve a radiometric measurement of the trapped $^{14}\text{CO}_2$ by scintillation counting, which can be performed in capped tubes or in the μL wells of titration plates [78].

The simplest model of enzyme kinetics applied to CA enzymes is the very classical Henri-Michaelis-Menten model [79]. The chemical reactions underlying this model can be summarized by (8) in the case of CO_2 hydration, where $\text{CA}\cdot\text{CO}_2(\text{aq})$ stands for a so-called enzyme-substrate Michaelis complex:



In 1913, next to a work by Henri [80, 81], Michaelis and Menten [82] considered that at the beginning of product formation (presently HCO_3^-), the second inverse reaction corresponding to the kinetic constant k_{-2} was very slow and

could be neglected, so that the initial formation rate ν_0 of this product could be written as:

$$\nu_0 = \frac{d[\text{HCO}_3^-]}{dt} = k_{\text{cat}} [\text{CA} \cdot \text{CO}_2(\text{aq})]. \quad (9)$$

This mechanism also implies that the species CA, $\text{CO}_2(\text{aq})$, and $\text{CA}\cdot\text{CO}_2(\text{aq})$ were in quasi-thermodynamic equilibrium, described by a thermodynamic equilibrium constant K_m termed the Michaelis constant defined by

$$K_m = \frac{k_{-1}}{k_1} = \frac{[\text{CA}] [\text{CO}_2(\text{aq})]}{[\text{CA} \cdot \text{CO}_2(\text{aq})]}. \quad (10)$$

Overall the following Michaelis-Menten rate equation (11) previously established by Henri [80, 81] is applied to $\text{CO}_2(\text{aq})$ hydration:

$$\nu_0 = \frac{\nu_{\text{max}} [\text{CO}_2(\text{aq})]}{K_m + [\text{CO}_2(\text{aq})]}. \quad (11)$$

In this equation, ν_{max} is the maximum initial formation rate of the product HCO_3^- , obtained when the total enzyme concentration $[\text{CA}]_t$ is engaged in a Michaelis complex $\text{CA}\cdot\text{CO}_2(\text{aq})$.

Consider

$$\nu_{\text{max}} = k_{\text{cat}} [\text{CA}]_t. \quad (12)$$

Amongst the many publications related to this simple model, a number of them report some values for k_{cat} , K_m , k_{cat}/K_m , and possibly ν_{max} . An example of such kinetic constants are for instance gathered in Table 1 for the most efficient human CA (isoenzyme CA II), regarding both the forward and reverse reactions, for which $\text{CO}_2(\text{aq})$ and HCO_3^- are, respectively, the substrates. Table 1 shows that the enzymatic turnover number of the forward hydration reaction is high, $k_{\text{cat}} \approx 10^6 \text{ s}^{-1}$, as well as the ratio of this turnover to the Michaelis constant $k_{\text{cat}}/K_m^{\text{CO}_2} \approx 8.33 \times 10^7 \text{ s}^{-1} \text{ M}^{-1}$. These high numbers are at the origin of the idea to investigate the enzymatic capture of CO_2 .

However, according to (5), a proton H^+ is also exchanged during $\text{CO}_2(\text{aq})$ hydration. This is illustrated in Figure 5 regarding the active site of a αCA enzyme, for the most simple mechanism model. The latter model involves 4 successive steps [49, 50, 75] as follows.

- (1) The enzyme ligands close to the enzyme active site induce a polarization of the O–H bond in the H_2O molecule coordinated to the Zn atom. This facilitates the deprotonation of this aqua ligand which is transformed to an OH^- ligand, while the lost proton is captured by another histidine residue close to the active site.
- (2) The oxygen atom of this OH^- ligand performs a nucleophilic attack onto the C atom of an incoming CO_2 molecule.
- (3) As a result of this nucleophilic attack, an HCO_3^- anion is bound to the Zn coordination center.

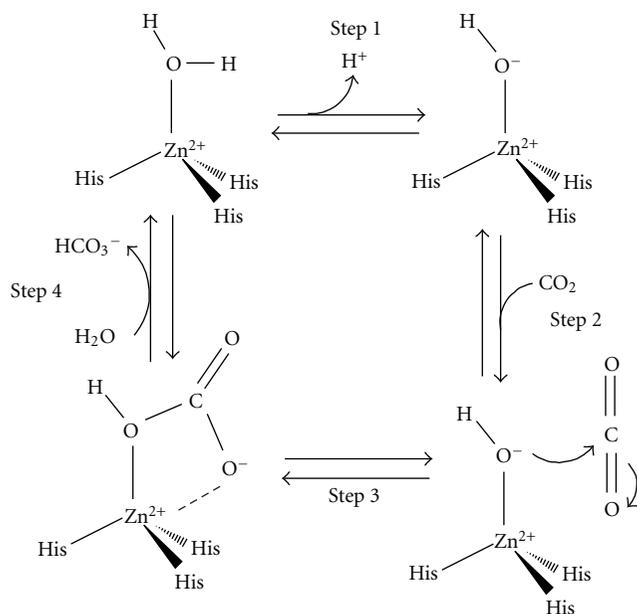


FIGURE 5: Simplified catalytic mechanism of an α CA enzyme [49].

TABLE 1: Michaelis-Menten kinetic constants for the forward and reverse equilibrium reactions involved in $\text{CO}_2(\text{aq})$ hydration, according to references [73, 76].

Substrate	K_{cat} (s^{-1})	K_m (M)	K_{cat}/K_m ($\text{s}^{-1}\text{M}^{-1}$)
$\text{CO}_2(\text{aq})$	10^6	0.012	8.33×10^7
HCO_3^-	4×10^5	0.026	1.54×10^7

- (4) This HCO_3^- ligand is labile and in turn it can be rapidly exchanged for an H_2O ligand, which regenerates the enzyme active site in its initial state.

However, the Michaelis-Menten rate equation model implies that this proton exchange is not rate limiting, while many studies later showed that the nature of a buffer mixed in the enzymatic solution could significantly modify the CO_2 hydration kinetics. Indeed, as previously mentioned, the base form B of a buffer couple (B/BH^+) is in competition with the enzyme to exchange a proton, hence to catalyze the hydration reactions in (5). Hence, other models were developed to specifically address this point. The various kinetic models of CO_2 hydration catalyzed by a CA of human source (HCAII) were reviewed and analyzed in detail by Larachi [74]. His result was that the kinetic model most consistent with the reliable experimental data published by different investigators, was a model termed pseudo random Quad Quad Iso Ping Pong. This model involves a proton transfer competition between, on one hand the enzyme active site and the $\text{CO}_2/\text{HCO}_3^-$ couple, on the other hand between the later couple and the B/BH^+ buffer couple. The term Ping-Pong indicates that the HCO_3^- anions must first leave the enzyme active site, before they can interact with the buffer. The term Pseudo indicates that the enzyme mechanism requires at least 2 different enzyme-substrate complexes, since a proton must be transported from inside

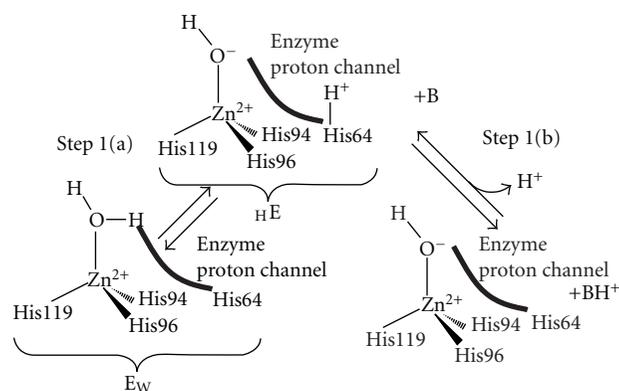


FIGURE 6: Steps 1(a) and 1(b) of the “Random Quad Quad Pseudo Iso Ping Pong” CO_2 hydration model, catalyzed by human α CA (H α CA), adapted from Larachi [74] and Rowlett and Silverman [44].

the enzyme active site, towards the active site entrance, before it can leave the enzyme and react with the buffer. The 2 different complexes of the protonated enzyme, designated by E_W and ${}_{\text{H}}E$ in Figure 6 indeed constitute 2 different isomers of this enzyme. In the first one (E_W) the proton belongs to the H_2O ligand inside the enzyme active site. In the second one (${}_{\text{H}}E$) this proton is located near the entrance of the active site cavity, although it still belongs to the enzyme, while the H_2O active site ligand is transformed to OH^- . Hence, the most important modification brought by the pseudo random Quad Quad Iso Ping Pong model to the simplified model presented in Figure 5, concerns previous step 1. As illustrated in Figure 6, it involves a transformation of the enzyme from the E_W isomer to the ${}_{\text{H}}E$ one (Step 1(a)). In this process, the proton H^+ withdrawn from the H_2O active site ligand in the E_W isomer, is transported along a “proton tunnel” to the external entrance of the channel leading to the active site. This tunnel is constituted by a series of H_2O molecules covering lateral sites of the channel. At the end of this transfer, the proton remains linked to the enzyme via the imidazole ring of a histidine residue (His64) located on the external surface of the enzyme, a conformation which corresponds to the ${}_{\text{H}}E$ isomer. In a further step 1(b), this ${}_{\text{H}}E$ enzyme proton is finally exchanged with the buffer B, to produce BH^+ .

Step 3 of the simplified model presented in Figure 5 is itself subdivided in 2 sub-steps. First, a CO_2 molecule links via a single bond the O atom of the OH^- enzyme ligand (step 3(a) in Figure 7 leading to a $\text{HO}-\text{CO}_2$ ligand). Secondly, this single bond transforms to a chelate bond via 2 oxygen atoms, with transfer of the H atom to the CO_3 group terminal position (Step 3(b) in Figure 7 leading to a HCO_3^- ligand). Step 4 is itself unchanged as it involves the replacement of an HCO_3^- ligand by H_2O , while the HCO_3^- product is liberated. The active site with a full H_2O ligand corresponds to the previous E_W enzyme isomer.

A reverse protonation of the liberated HCO_3^- to form back some $\text{CO}_2(\text{aq})$ is also possible outside the enzyme, depending on the pH and the buffer couple B/BH^+ used,

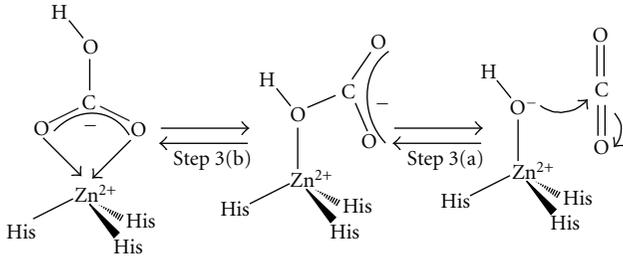


FIGURE 7: Step 3 of the “Random Quad Quad Pseudo Iso Ping Pong” CO_2 hydration model, catalyzed by human CA (HCA), adapted from Larachi [74] and Rowlett and Silverman [44].

in particular when the concentration of HCO_3^- is high. Moreover, an inhibition of the enzyme active site by the products HCO_3^- or CO_2 , respectively, for the forward and reverse CO_2 hydration reactions, must be taken into account when any of these compounds are present in excess in the solution.

The full set of kinetic equations describing this model is very complex and would require a large development to present them. The readers interested in this point are recommended to refer to the publication by Larachi [74].

A more simple and more approximate equation (13) applicable to CO_2 capture in conditions when the substrate concentration is low enough to ignore inhibition reactions, was proposed by Rowlett and Silverman [44]. It only takes into account the Michaelis constant $K_m^{\text{CO}_2}$ and the corresponding turnover number k_{cat} , as well as a constant k_4 which describes the proton transfer kinetics between the enzyme isomer ${}_H\text{E}$ and a buffer B,

$$\frac{[\text{E}]_t}{\vartheta} = \frac{1}{k_4 [\text{B}]} + \frac{1}{k_{\text{cat}}} \left(1 + \frac{K_m^{\text{CO}_2}}{[\text{CO}_2]} \right). \quad (13)$$

When inhibition by HCO_3^- and/or CO_2 must be taken into account, this equation can be transformed to (14)

$$\frac{[\text{E}]_t}{\vartheta} = \frac{k_{\text{cat}} [\text{B}]}{K_m^{\text{B}} \left[1 + \left(\frac{[\text{HCO}_3^-]}{K_i^{\text{HCO}_3^-}} \right) \left(1 + K_i^{\text{CO}_2} / [\text{CO}_2] \right) \right] + [\text{B}] \left(1 + K_m^{\text{CO}_2} / [\text{CO}_2] \right)} \quad (14)$$

with K_m^{B} = Buffer Michaelis constant, $K_i^{\text{CO}_2}$ = inhibition constant by CO_2 , $K_i^{\text{HCO}_3^-}$ = inhibition constant by HCO_3^- . The previous effective buffer constant k_4 is given by:

$$k_4 = \frac{k_{\text{cat}}}{K_m^{\text{B}}}. \quad (15)$$

An apparent HCO_3^- inhibition constant can also be defined by

$$K_{i,\text{app}}^{\text{HCO}_3^-} = \frac{K_i^{\text{HCO}_3^-}}{1 + K_i^{\text{CO}_2} / [\text{CO}_2]}. \quad (16)$$

Some kinetic constants for a few buffers are gathered in Table 2. Overall, the influence of the pH on the CA activity in $\text{CO}_2(\text{aq})$ hydration is therefore complex. However, according to data on k_{cat} provided by Berg et al. [34] and reported in Figure 8, the human CA reaches its maximum activity at $\text{pH} > 8$, hence when a buffer is added and the base buffer B can compete with the enzyme. These results are consistent with those of Ramanan et al., who reported that the enzyme from *Bacillus subtilis* was stable in the pH range 7.0 to 11.0, with a maximum activity in the pH range 8 to pH 8.3 [61, 85]. The stability of CA actually depends on the enzyme source, as shown in a study on CA from *Pseudomonas fragi*, *Micrococcus lylae*, *Micrococcus luteus* 2 and commercial bovine CA (BCA) in a pH range 8.0–9.0 and temperature range 35–45° [86]. In the latter study, the stability was the highest for the *Micrococcus luteus* 2 CA.

The influence of temperature on the human and bovine CA activity in $\text{CO}_2(\text{aq})$ hydration was studied by Ghannam et al. in a pH = 9 buffer [87]. The data on k_{cat} and $K_m^{\text{CO}_2}$

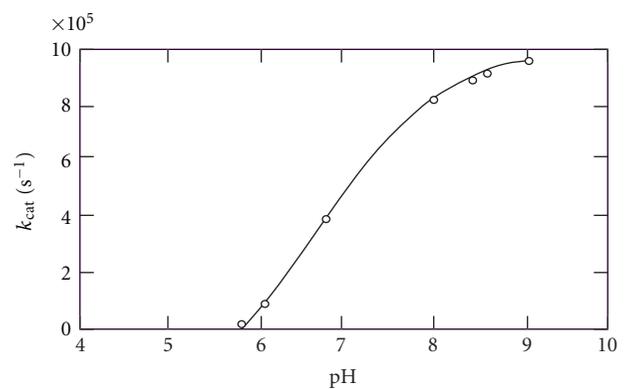


FIGURE 8: Effect of the pH on a human CA, in $\text{CO}_2(\text{aq})$ hydration. Adapted from [34].

are respectively reported in Figures 9 and 10 for these 2 enzymes. For both enzymes, k_{cat} and $K_m^{\text{CO}_2}$ increased as the temperature increased in the range from $\approx 5^\circ\text{C}$ to $\approx 30^\circ\text{C}$. HCAII was somewhat more active than BCA (higher k_{cat} for HCA), and it showed a better affinity for CO_2 (lower K_m).

However, an increased denaturation of CA enzymes occurs when the temperature increases because the enzyme conformation is progressively altered. Hence the lifetime when they remain active is shortened. This particular aspect was studied in the hydrolysis reaction of par-nitrophenylacetate [88, 89] by BCA enzyme, in which the thermal denaturation kinetic could be described by an Arrhenius type equation (17):

$$-\frac{d[\text{E}]}{dt} = k_d[\text{E}]. \quad (17)$$

TABLE 2: Magnitude of the kinetic constants in (13) to (16) for a few buffers, according to reference [44].

Buffer	pH	pK_a	K_m^B (mM)	k_4 ($M^{-1} s^{-1}$)	$K_{i,app}^{HCO_3^-}$ (mM)
Triethanolamine	8.4	7.8	1.2	5.4×10^8	10 ± 1
HEPES*	8.2	7.5	3.5	2.1×10^8	10 ± 1
4-methylimidazole	8.4	7.8	1.3	1.2×10^9	5.4 ± 0.3
1-methylimidazole	8.0	7.2	2.4	2.9×10^8	6.5 ± 0.4

*HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

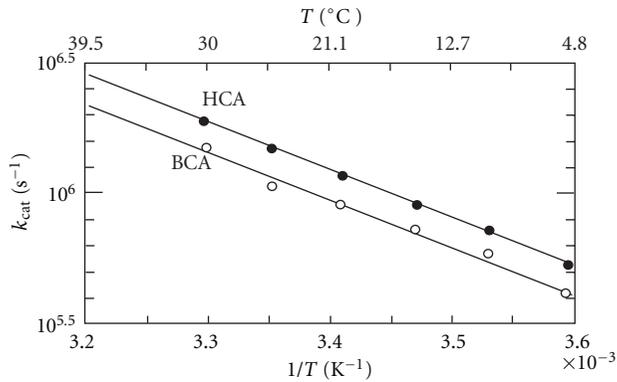


FIGURE 9: Influence of the temperature on k_{cat} for human CA (HCA) and bovine CA (BCA). Adapted from [87].

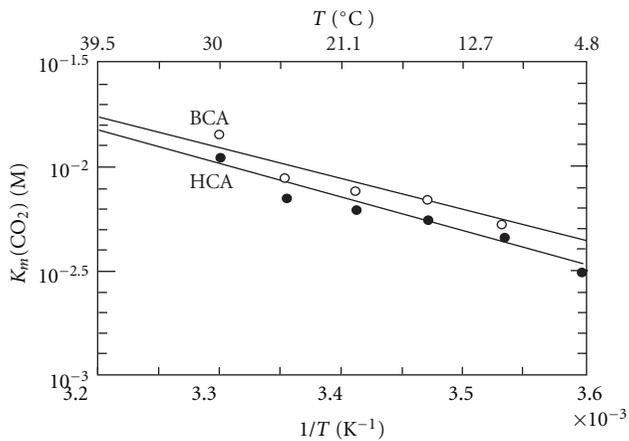


FIGURE 10: Influence of the temperature on $K_m^{CO_2}$ for human CA (HCA) and bovine CA (BCA). Adapted from [87].

In this equation, $[E]$ designates the active enzyme concentration and k_d a kinetic denaturation constant. The latter constant itself followed an Arrhenius type law as a function of the temperature T , according to (18) where E_d is a denaturation energy

$$k_d = A_d e^{(-E_d/RT)}. \quad (18)$$

Some numerical values of k_d were determined by Kanbar and Ozdemir and they are reported Figure 11 [88]. Practically, after 120 min at 40°C, 50°C or 60°C, the residual enzyme activity was respectively 90%, 70% and 20% of its initial activity.

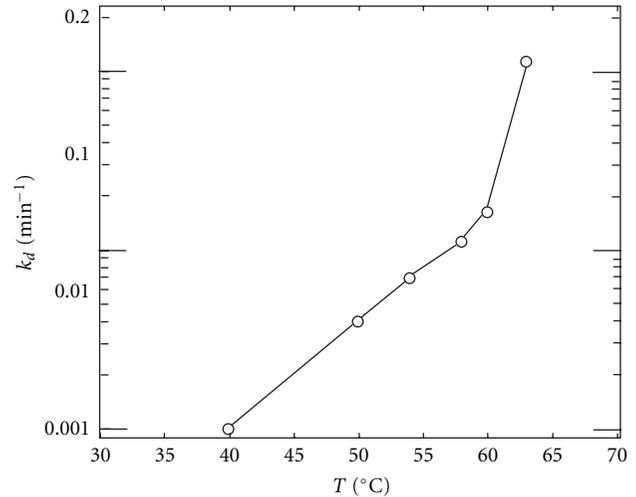


FIGURE 11: Denaturation kinetic constants of bovine erythrocyte CA (BCA) as a function of the temperature in the hydrolysis reaction of para-nitrophenylacetate, according to [88].

At last, various chemical species inhibit the CA activity, in particular some present in industrial flue gases from which CO_2 must be captured. A study was carried out by Ramanan et al. on CA from *Bacillus subtilis* [61, 85]. The anions Cl^- , HCO_3^- , and CO_3^{2-} and the metal cations Pb^{2+} and Hg^{2+} were found to significantly inhibit the CA activity, while Ca^{2+} and Mn^{2+} were weak inhibitors and Co^{2+} , Cu^{2+} and Fe^{3+} were found to enhance this activity. Regarding SO_4^{2-} , a major pollutant in industrial flue gases, they found this anion activated CA, contrary to the results of Bond et al. [30]. The inhibition by Cl^- , SO_4^{2-} , NO_3^- , HCO_3^- and the cations As^{3+} , Ca^{2+} , Mg^{2+} , Hg^{2+} , Mn^{2+} , Cd^{2+} , Cu^{2+} , Zn^{2+} , Co^{2+} , Pb^{2+} , Fe^{2+} , Ni^{2+} , Se^{2+} , Na^+ , and K^+ was also studied on CA from *Pseudomonas fragi*, *Micrococcus lylae*, *Micrococcus luteus* 2, and BCA by Sharma and Bhattacharya [86]. The level of inhibition was found to depend on the ion and the enzyme. It was significantly higher for BCA and *M. luteus* 2 CA, in particular by the anions.

5. Enzymatic CO_2 Capture Scrubbers

In this paper, CO_2 scrubbers designate systems to separate $CO_2(g)$ from other gaseous components. In 2008, Lacroix and Larachi reviewed the different types of CA enzymatic scrubbers in development [90]. These comprised membrane contactors using free CA solutions, to release gaseous CO_2

as well as to precipitate calcium carbonate, contactors using immobilized CA, namely counter-current and cross-current packed columns, and contactors using either free or particle-immobilized CA. These authors also examined a list of possible CA enzymes to capture CO₂ and to potentially produce useful organic compounds.

Overall, three enzymatic CO₂ capture techniques are being industrially developed, to which other scientific research publications must be added. In a first process developed by the company "CO₂ Solution Inc." and schematically illustrated in Figure 12, the enzyme is immobilized on a solid support, itself packed in a bed reactor [30, 91–95]. An aqueous solution is sprayed through a nozzle at the top of the reactor. It washes a counter flow of the gas containing the CO₂ to be captured, itself injected at the lower end of the reactor. Capture of the CO₂ occurs when the opposite flows of aqueous solution and gas from which CO₂ must be scrubbed out, percolate through a supported enzyme bed. A second reactor in which the former aqueous CO₂ solution is sprayed, for instance in a carrier gas or in a partial vacuum, makes it possible to recover the CO₂ gas. In reactors of this type, the exchange mechanisms between the liquid and the gas in the presence of the CA enzyme, are critical. In order to favor these exchanges, Fradette et al. deposited a patent according to which the liquid layer flows as droplets on the enzyme immobilized on elongated supports, in such a way that the CO₂ hydration reaction occurs within the flowing liquid [96]. According to the authors, a prototype was tested in 2004 in an aluminum foundry of the Alcoa Inc. company, during a non-stop one month period. It made possible to capture 80% of CO₂ from the industrial fumes [30]. The enzyme permitted to reduce the reactor size by comparison with the same process without any enzyme. The process was also found to be more economical than a process based on CO₂ capture by an amine solution, which required to heat the amine solution in order to recover the CO₂ [14, 93]. In a variant of such a process, Bhattacharya et al. immobilized the enzyme by covalent grafting on silica coated porous steel and water was sprayed down through the flue gas. The best results were obtained with an enzyme support pore size of $\approx 2 \mu\text{m}$ and an enzyme load of 2 mg mL^{-1} [97]. Besides, it was also shown that CA enzymes could promote the absorption kinetics of CO₂ in potassium carbonate or aqueous amine solvents [91, 98–101].

The gas-liquid and liquid-solid mass transfer exchange mechanisms were examined in details by Iliuta and Larachi, for Robinson-Mahoney and packed beads reactors [102]. These authors showed that, for immobilized enzyme, these mechanisms could significantly alter the CO₂ hydration kinetics. The most remarkable increase in CO₂ removal was obtained by integrating immobilized-enzyme absorption with ion-exchange resin microparticles to remove excess enzyme inhibiting HCO₃⁻ anions [103]. As a consequence, they developed a 3-phase reactor, comprising HCAII enzyme, immobilized in the longitudinal channels washcoat of a post combustion monolith, in which an aqueous slurry containing resin exchange beads was flowed [102]. Other researchers proposed to enhance the exchange mechanisms

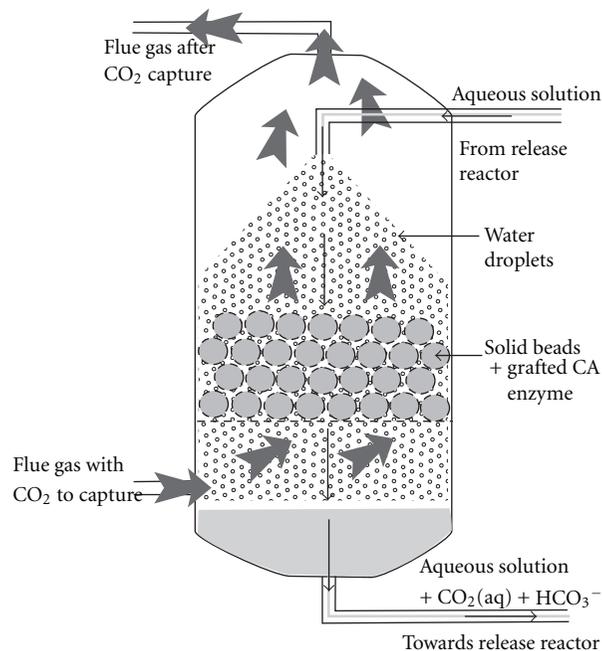


FIGURE 12: Schematic illustration of a CO₂ capture unit by gas washing, of the CO₂ Solution Inc. Company. Adapted from [93].

limiting the CO₂ hydration rate, by ultrasonic techniques [104].

In a second type of process initially developed by the National Aeronautics and Space Administration (NASA) to purify the ambient atmosphere of confined inhabited cabins, the CO₂ is captured through thin aqueous films in which some CA is dissolved [25, 105]. The CO₂ concentration of such atmospheres is low, typically of the order of 0.1% or less. A schematic illustration of the membrane sandwich involved is presented in Figure 13. The core of the liquid membrane comprises a thin (e.g., $330 \mu\text{m}$ thick) layer of enzymatic solution in an aqueous phosphate buffer, squeezed in between 2 microporous hydrophobic polypropylene membranes, themselves retained by thin metal grids to insure the liquid membrane thickness and rigidity. The CO₂ from the atmosphere to purify, spontaneously dissolves inside the liquid membrane on one face of the membrane. It diffuses across the liquid membrane and evaporates out the other liquid membrane on the opposite face, either in vacuum or in a carrier gas. Analysis of the capture and release gases with a mass spectrometer showed that the enzyme permitted a selective diffusion of CO₂, in ratio of 1400 to 1 by comparison with N₂ and 866 to 1 by comparison with O₂. As previously discussed, this result is due to the fact the N₂ and O₂ can only dissolve as neutral molecules; hence their solubility in water is limited by comparison with CO₂. These selectivities were superior to those achieved with a 20% (by weight) diethaloamine (DEA) solution, respectively, 442 to 1 and 270 to 1. By comparison with the same liquid membrane without enzyme, the CA permitted to decrease the overall resistance to CO₂ transport through the membrane, by 71%. Besides, the diffusion of HCO₃⁻ and H⁺ ions

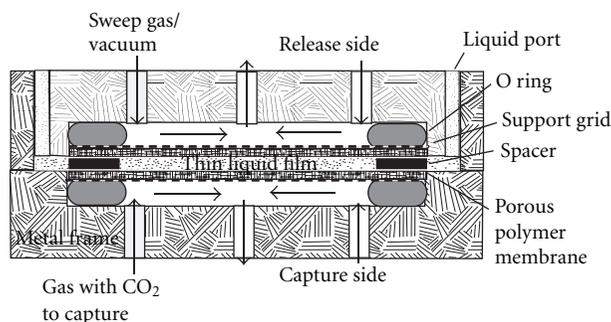


FIGURE 13: Schematic illustration of a thin liquid membrane system such as developed by NASA to capture CO_2 . Adapted from [25].

across a liquid membrane could possibly be accelerated by an electrochemical process [106]. For a gas containing 0.1% CO_2 , Cowan et al. showed that a liquid membrane with CA enzyme was very stable, even in the presence of dry feed, and had a CO_2 permeance of $4.71 \times 10^{-8} \text{ mol m}^{-2} \text{ Pa}^{-1} \text{ sec}^{-1}$ at ambient temperature and pressure [105]. Ward and Robb were the first to apply simple diffusion liquid enzyme membranes to gases containing 5% CO_2 , in which the enzyme was dissolved in cesium or potassium bicarbonate solutions [107]. Suchdeo and Schultz used enzyme solutions in sodium bicarbonate [108]. Matsuyama and colleagues extended such enzymatic liquid films to gases containing up to 15% de CO_2 , more representative of industrial fumes [109, 110].

Technical difficulties may appear due to the drying of aqueous film during long time operation. Humidifiers such as based on polysulfone were proposed to humidify the capture and release gases [105]. However, to better solve this problem, Trachtenberg et al. adapted the technique to networks of hollow microporous fibers in which the flue gas and the release gases could flow [36, 111]. Next to this progress, the Carbozyme company developed a technology which is schematically illustrated in Figure 14, based on such hollow microporous propylene microfibers, separated by control separators made with thin oxide powders, the whole system bathing in an excess aqueous enzyme solution. The enzyme was directly immobilized on the external faces of the microfibers. Water vapor under moderate vacuum (15 kPa) was used as the sweeping gas at a low flow rate in the release microfibers. The CO_2 content in the sweeping gas reached $\approx 95\%$, for a flue gas containing $\approx 15\%$ de CO_2 . No significant loss of enzyme activity was observed during a 5 days continuous run [112], and a conservative run time of 2500 hours was selected before needing to change the enzyme [111]. Several refinements were later designed, regarding the microfiber network geometry, the nature of hollow microfibers and the variety of CA used. This system was found to be efficient for a flue gas containing from 0.05 to 40% CO_2 , at a temperature ranging from 15 to 85°C with a particular γCA isozyme [39, 112]. Trachtenberg et al. also confirmed that the system permeance, as well as the selectivity of CO_2 transfer with respect to O_2 , N_2 , and Ar, decreased when the CO_2 content in the flue gas increased [36, 112]. Hollow fiber membrane reactors were

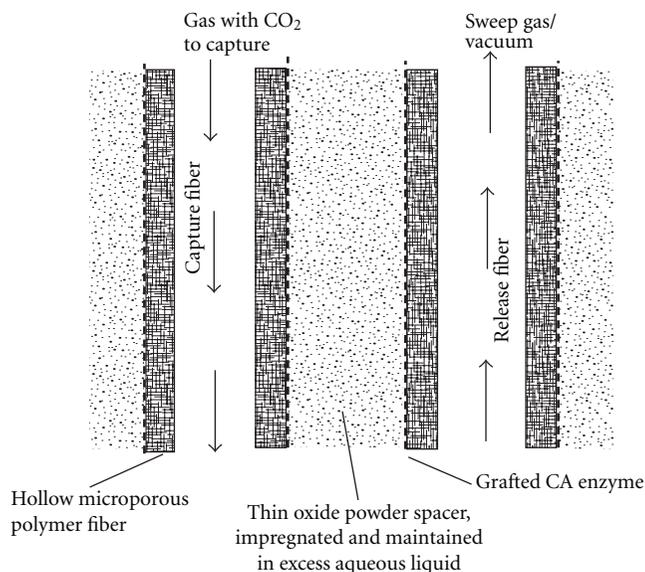


FIGURE 14: Schematic illustration of a membrane CO_2 capture system based on microporous microfibers, such as developed by the Carbozyme Inc. company. Adapted from [111].

also designed and modeled with success by Zhang et al. Their set-up comprised some CA immobilized in nanocomposite hydrogel/hydrocalcite thin films, used as thin layers to separate the fibers [113–115]. With 0.1% (v/v) of CO_2 in the flue gas, a permeance of $1.65 \times 10^{-8} \text{ mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ was achieved for a selectivity of CO_2 of 820 over N_2 and 330 over O_2 , and a stable performance during a 30 h run [114].

Some patents were also deposited by the Novozymes company. The latter proposed to combine various CO_2 capture and release units, such as those developed by the CO_2 Solution or Carbozyme Companies, interconnected by fluid circulation pipes [83]. Some results obtained with hollow microfiber units containing $0.03 \text{ mg mL}^{-1} \alpha\text{CA}$ extracted from the bacteria "*Bacillus Clausii* KSM-K16," dissolved in a 1 M pH 8 sodium bicarbonate solution and applied to a flue gas containing 15% CO_2 , are gathered in Table 3.

The CO_2 scrubbers presented above mostly use immobilized CA. One reason is that commercial CA are costly and their immobilization on a support permits to use them for a longer time before losing them by leaching, hence to decrease the operational cost. This explains that a number of researchers addressed the problem of CA immobilization.

Actually, the immobilization of CA on solid supports is not recent. In 1988, Crumbliss et al. published a paper on the immobilization of BCA on silica beads and graphite rods [116]. The enzyme was covalently linked after activation of the graphite with amide bonds, while glutaraldehyde was used as an intermediate between the enzyme and the beads. The BCA surface coverage on the silica beads was reported to be superior to previously reported data on silica beads and polyacrylamide gels and comparable to that on other organic matrix supports. In 2001, Bond et al. immobilized BCA by adsorption on chitosane and alginate beads, which they applied in a sequestration process of CO_2 as stable solid carbonates

TABLE 3: Percent CO₂ in the release gas after a 75 min. capture test, with or without αCA enzyme, according to [83]. The flue gas composition was 15% CO₂ and 85% N₂.

Liquid	% de CO ₂ in the release gas after 75 min test	Initial liquid pH	Final liquid pH
Water, no enzyme	0	—	—
NaHCO ₃ , 1 M, no enzyme	3.3	8	8.3
NaHCO ₃ , 1 M + 0.03 mg mL ⁻¹ enzyme	10	8.1	8.8

[30, 117, 118]. In 2003, Hosseinkhani and Nemat-Gorgani adsorbed partially unfolded CA on hydrophobic alkyl substituted sepharose 4B supports. The octyl substituted support provided the best thermal stability and highest k_{cat}/K_m (app), which was attributed to an irreversible thermal inactivation of the enzyme by interaction with the alkyl support groups [119]. Dilmore et al. studied the adsorption of CA on wet polyacrylamide gel beads functionalized with amino groups facilitating the adsorption of CO₂ [120]. Adsorption on chitosan and alginate supports was often used, such as for CA from *Pseudomonas fragi*, *Micrococcus lylae*, *Micrococcus luteus* 2, and *Bacillus pumilus* [121–123]. The immobilized enzymes showed improved storage stability and retained up to 50% of the initial activity after 30 days [123]. The immobilization of CA from *Bacillus pumilus* on chitosan beads was also studied by Wanjari et al. [124]. In p-NPA hydrolysis, they determined that K_m and v_{max} were 2.36 mM and 0.54 $\mu\text{mole min}^{-1} \text{mL}^{-1}$, respectively, for the immobilized CA, versus 0.87 mM and 0.93 $\mu\text{mole min}^{-1} \text{mL}^{-1}$, respectively, for the free CA [124]. For CA immobilized on ordered mesoporous aluminosilicate, K_m , v_{max} , and k_{cat} were 0.158 mM, 2.307 $\mu\text{mole min}^{-1} \text{mL}^{-1}$, and 1.9 s⁻¹, respectively [125]. Besides, whole cells of *Bacillus pumilus* were immobilized on different chitosan and sodium alginate based materials, which also improved their esterase activity by comparison with the free cells [121]. For the same *Bacillus pumilus* CA immobilized on chitosan activated alumina-carbon composite beads, the K_m and v_{max} values were 10.35 mM and 0.99 $\mu\text{mole min}^{-1} \text{mL}^{-1}$ [126]. Overall, in p-NPA hydrolysis, it was found that K_m often decreased after immobilization, which denoted a greater affinity of the CA for the substrate, while v_{max} increased.

The main drawback of adsorption techniques is the enzyme progressively desorbs and is leached out during repeated tests. To solve this problem, the enzyme can be covalently grafted on a support, and a few techniques have been experimented in this direction. Bhattacharya et al. immobilized CA on iron particles coated with γ aminopropyltriethoxysilane, by grafting via dicarbocarbodi-imide (DCC) bonds or via dicarboxy bonds after conversion of the support surface groups with succinic anhydride. Cyanogen bromide coupling on an intermediate thin glass coating was also applied. Immobilization was also carried out by CA copolymerization with gluteraldehyde in methacrylic acid polymer beads. All these methods were reported to provide excellent results regarding the activity (98% activity retention) and leaching, in particular the DCC and dicarboxy coupling methods [97]. Belzil and Parent grafted human CA on nylon 6.6 Raschig rings [127]. The best activity in CO₂ hydration from a gas containing 20% CO₂ at 1°C was

obtained by grafting CA from an enzyme solution contained 0.5 mg mL⁻¹ CA. 73% of the enzyme was actually grafted on the support and 45% of this enzyme was active. However the relative hydration yield for the immobilized enzyme, defined by (19) itself derived from (7), was only 20% compared to 85% for the free enzyme:

$$\text{Yield} = v_r (\text{U mg}^{-1}) m_{enz} = \frac{v_{enz} - v_0}{v_0}. \quad (19)$$

Zhang et al. investigated the covalent grafting of CA on a hybrid Poly(acrylic acid-co-acrylamide)/hydrotalcite nanocomposite termed “PAA-AAm/HT” [113, 114]. Hydrotalcite is a basic inorganic material of composition Mg₆Al₂(CO₃)(OH)₁₆·4(H₂O) [128, 129] and coupling was achieved by the intermediate of N-hydroxysuccinimide (NHS) and DCC. Up to 4.6 mg of enzyme per gram of support could be grafted and 76.8% of the initial enzyme activity could be retained after immobilization. A covalent coupling method was also developed to graft CA enzymes onto silica nanoparticles made by spray pyrolysis [130]. These immobilized enzymes exhibited a significantly improved thermal stability compared to the free counterpart. Lee et al. studied the immobilization of CA by single or multiple attachments to polymers, themselves deposited onto Fe₃O₄ magnetic aggregates [131]. Yadav et al. immobilized CA on silylated chitosan beads, to precipitate CaCO₃ [132]. They observed that the immobilized CA had a longer storage stability than the free enzyme and retained 50% of its initial activity up to 30 days. They also developed core-shell single enzyme nanoparticles (SEN-CA), by covering the CA surface with a thin layer of chitosan, which showed an improved stability by comparison with the free enzyme [133].

Vinoba et al. compared BCA immobilized on SBA-15 by various techniques comprising covalent attachment (BCA-CA), adsorption (BCA-ADS), and cross-linked enzyme aggregation (BCA-CLEA). They found all were promising reusable catalysts [134]. In the hydrolysis reaction of p-nitrophenyl acetate (p-NPA), the k_{cat}/K_m values were 740.05, 660.62, and 680.11 M⁻¹ s⁻¹, respectively, by comparison with 873.76 M⁻¹ s⁻¹ for free BCA. In the hydration of CO₂, the k_{cat} values were 0.58, 0.36, 0.78 s⁻¹ by comparison with 0.79 s⁻¹ for free BCA, respectively, indicating that BCA-CLEA showed a comparatively higher hydration rate than the other immobilized CA, although it remained lower than the free CA [135]. Vinoba et al. also immobilized human carbonic anhydrase (HCA) via electrostatic interactions on silver nanoparticles confined in amine-functionalized mesoporous SBA-15 [136]. The latter retained ≈87% of its initial activity after 30 days. Similarly, they immobilized HCA on Au nanoparticles assembled over amine/thiol-functionalized,

mesoporous SBA-15 [137]. Depending on the grafting agent K_m ranged from 22.35 to 27.75 mM and k_{cat}/K_m from 1514.09 to 1612.25 $M^{-1} s^{-1}$ in p-NPA hydrolysis. With HCA simply covalently immobilized on SBA-15 via various amines, the k_{cat} values ranged from 7182 to 7569 $M^{-1} s^{-1}$ [138].

Besides adsorption and covalent grafting, enzymes can also be efficiently entrapped within porous supports. Such entrapment can be done within polyurethane foams, next to an initial protocol developed by Wood et al. in 1982 [139]. This technique was extended with success to CA enzymes for CO₂ capture, by Kanbar and Ozdemir [88] hydrolysis reaction of para-nitrophenylacetate (p-NPA) to para-nitrophenol (p-NP) and by Ozdemir [72]. Polyurethane immobilized CA could be used without any activity loss in aqueous media for 7 successive CO₂ capture tests and the optimum operational temperature was in a range from 35°C to 45°C. Simple entrapment in the open pores of a porous polymeric membrane is possible by immersion of the membrane in an enzyme solution, such as done by Favre and Pierre [140] with BCA, for a thin membrane system. In a 1 M NaHCO₃ solution at initial pH \approx 8, the existence of an optimum enzyme concentration of 0.2 mg mL⁻¹ was observed. The permeance was quite comparable with the data gathered by BaO and Trachtenberg and reported in Figure 2. The NaHCO₃ solution helped to maintain a high [HCO₃⁻] concentration by displacing (5) towards a higher pH, while the electrical neutrality was insured by the Na⁺ cations. As a further development hybrid sol-gel membranes were moreover made by impregnation of the previous polymeric membranes with a SiO₂ sol made from tetramethylorthosilicate (TMOS) [140]. After SiO₂ gelation and drying, the hybrid polymer-SiO₂ membranes were then impregnated with an enzyme aqueous solution in a buffer. It was observed that SiO₂ moderately increased the membrane permeance. Based on these results, a moderate catalytic action of silica in the capture of CO₂ could be proposed. It relied on the fact that the isoelectric point (i.e.p) of SiO₂ is low: i.e; p. \approx 2.5 à 3 [141]. At pH > i.e.p, as this is the case in CO₂ capture, SiO₂ carries an excess of \equiv SiO⁻ negative surface charges. Hence the equilibrium between \equiv SiOH and \equiv SiO⁻ surface sites in SiO₂, is similar to that between -Zn-OH₂ and -ZnOH⁻ in the active enzyme site, so that a catalytic mechanism similar to that of the enzyme could be proposed. However this possible catalytic effect remained moderate and could be impeded by a necessary diffusion inside of CO₂(aq) inside the gel fine pore texture, depending on the membrane architecture.

In a variation of the process, nylon membranes was impregnated with a silica sol in which the enzyme was dissolved. SiO₂ gelation then directly occurred during the CO₂ transfer [11]. Leaks were rapidly induced by shrinkage of the gel about the nylon the fibers, followed by rapid drying, but these leaks occurred more rapidly with the CA enzyme than without it. This result led to investigation the action of CA enzyme and of CO₂(aq) on the gelation of silica sol made from TMOS [142]. It was found that both additives accelerated the gelation of SiO₂, although in a different way. While CO₂(aq) acted as an acidic gelation catalyst, leading to a so-called "polymeric" SiO₂ gel characterized

by a very high specific surface area and small mesopores mixed with micropores [143], the CA enzyme acted as a basic catalyst resulting in a more "colloidal" gel, characterized by a lower specific surface area, mesopores of bigger size, and a reduced contraction during drying. These results were consistent with several other reports on the capability of other enzymes or proteins to catalyze the formation of silica from a liquid precursor. Such effects were indeed observed with polypeptides [144, 145], silicatein [146], lysozyme [147, 148], papaïn and trypsin [149], and a lipase from *Burkholderia cepacia* [150]. Monolithic SiO₂ gels were synthesized by Frampton et al. by hydrolysis of tetraethoxysilane (TEOS), catalyzed by α -chymotrypsin or trypsin and by hydrolysis of phenyltrimethoxysilane (PhTMOS) catalyzed by pepsin, in a time scale where gels were not obtained without any enzyme [149].

Because SiO₂ is slightly acidic, the deposition of a basic solid was also attempted in nylon and hybrid nylon-SiO₂ membranes. This was done by impregnating these membranes with a CaCl₂·2H₂O solution containing 0.2 mg mL⁻¹ enzyme at pH \approx 10.5, so as to deposit some CaCO₃ in situ during CO₂ capture. In the nylon membranes, the best permeance was obtained with a CaCl₂·2H₂O concentration of 0.146 M and was equivalent to that obtained with a 1 M NaHCO₃ solution at pH \approx 8. SEM micrographs showed that small calcite crystals had deposited on the nylon fibers. On the other hand, in hybrid nylon-SiO₂ membranes, the permeance was slightly lower than that of similar hybrid nylon-SiO₂ membranes impregnated with in a 1 M NaHCO₃ solution. SEM micrographs and EDX microanalysis showed that besides calcite, some calcium silicate was also formed. Because SiO₂ is slightly soluble at pH 10.5, it is therefore possible that coprecipitation of the solubilized SiO₂ with the calcium from CaCl₂ may have produced this calcium silicate.

6. Enzymatic CO₂ Storage as Solid Carbonates

The storage of CO₂ as solid carbonates requires the carbonation of basic cations dissolved or in contact with an aqueous medium. The most noteworthy candidates are alkaline or alkaline earth cations available in hydroxides such Ca(OH)₂ or oxides such as MgO and CaO [13, 30, 61, 77, 85, 117, 151–153]. Natural minerals such as wollastonite (CaSiO₃) [154], serpentine (Mg₃Si₂O₅(OH)₄) [155], and olivine (Mg₂SiO₄) [156] provide such cations. More interestingly, metallurgical slags, lignite ashes, or chemical brine rejects such as from the oil industry [157] contain a significant concentration of such cations and could be used for carbonation. Besides, materials such as concrete do contain a high concentration of such cations and are abundantly used in civil engineering. Actually, the incorporation of CA enzyme in cement compositions was attempted with success, with the aim of designing civil engineering materials able to capture the CO₂ from the air and directly sequester it as solid carbonates within porous coatings of building walls [158].

The products of these carbonations are stable solid carbonates, for exemple, MgCO₃ and CaCO₃ which are themselves the major components of natural limestone.

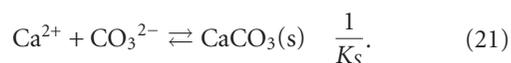
Hence they present no environmental inconvenience for long term safe disposal [13, 61, 85], and they could possibly be reused in civil engineer constructions.

A first important drawback is that, considering the molar mass of the silicates mentioned above, from 1.6 to 3.7 tons of silicate source and from 2.6 to 4.7 tons of products would have to be handled, per ton of CO₂ to be stored. Hence, huge geological deposits would have to be mined when the cation source has to be extracted as a solid from such sites.

A second drawback is that the pH decreases when the carbonation reaction proceeds, due to the fact that the first deprotonation (5) as well as the second deprotonation equilibrium of CO₂(aq) to form the carbonate anions CO₃²⁻ according to (20) [159], both decrease the pH:



Given the magnitude of K_{a2} , CO₃²⁻ anions predominate at pH > 10.5. Hence, it is necessary to maintain a high pH to induce the precipitation of a solid carbonate. Indeed, precipitation of a solid carbonate, such as CaCO₃, is itself the result of a dissolution/precipitation equilibrium as described in (21), where the solubility product $K_S = [\text{Ca}^{2+}] [\text{CO}_3^{2-}]$ relates the ion concentrations in the liquid solution in equilibrium with the solid being precipitated:



According to (21), CO₃²⁻ anions are continuously withdrawn from the liquid solution during precipitation of the solid phase. Hence, new CO₃²⁻ anions must be continuously supplied in the solution in order for precipitation to keep proceeding. This supply is achieved in accordance with (20), which simultaneously brings supplementary H⁺ cations, so that the pH keeps decreasing unless a buffer is continuously supplied. If this is not done, the HCO₃⁻ anions again predominate when the pH reaches a value < pK_{a2} (20), so that precipitation stops, and the previously precipitated solid carbonate may even redissolve. This explains that in laboratory batch studies with a given initial buffer concentration, the final mass of CaCO₃ precipitated was relatively the same with or without enzyme. Only the initial rate to reach this final mass changed. Depending on the CO₂ capture system used, it was actually shown that the mass of CaCO₃ precipitated could only be used as an indicator of the CO₂ capture rate at the beginning of precipitation [140]. This problem can be solved by carefully monitoring the continuous rate of addition of basic ashes or brines relative to the flow rate of CO₂ to sequester [157].

Moreover, the deposit of CaCO₃ from natural brine solutions supersaturated in both bicarbonate HCO₃⁻ anions and Ca²⁺ cations, was extensively studied by geochemists, for instance Dreybrodt et al. [160]. In this case the deposit of CaCO₃ occurs when the solution is placed in contact with an atmosphere where the P(CO₂(g)) partial pressure is lower than that corresponding to equilibrium with the brine solution. When this occurs, some CO₂ is not captured but released from the brine by reverse dehydration of HCO₃⁻, according to the equilibrium equation (5). The situation is

equivalent to the release of CO₂ on the release side of a thin liquid membrane. However, simultaneously, a proton H⁺ is captured, which increases the pH and in turn displaces the equilibrium reaction in (20), in favour of the formation of CO₃²⁻ anions. The latter in turn induce the precipitation of CaCO₃. Overall, one mole of CO₂(g) is released per mole of deposited CaCO₃. If this was applied to the captured CO₂, half of this CO₂ would be released in the air. Obviously, such a situation must be avoided when the aim is to capture and fully sequester CO₂(g). For this purpose, the P(CO₂(g)) partial pressure in the gas in contact with the brine, must not be lower than the equilibrium partial pressure corresponding to the brine [HCO₃⁻] concentration.

From the kinetic point of view, the second deprotonation of CO₂(aq) (20) is much faster than the first one (5) and it does not a priori necessitate to use a catalyst. However, because a CA enzyme catalyzes the formation of HCO₃⁻ from CO₂(aq) (5), which in turn displaces successively the equilibrium reactions in (20) to form more CO₃²⁻ anions, and the precipitation of CaCO₃ (21), this CaCO₃ precipitation is indirectly catalyzed by the enzyme. This is particularly true when carbonation is directly made in the medium where CO₂ is captured, where the first and second deprotonation steps of CO₂(aq) occur in the same medium.

At a given temperature, the solubility limit K_S in (21) is a thermodynamic constant. However, its value depends on the solid phase which first nucleates, as indicated in Table 4 [84]. In this table, the final solid phase most often obtained is the stable thermodynamic phase calcite. The other crystalline forms are metastable, although they may easily nucleate and grow first, before calcite, depending on the conditions. When this occurs, they eventually redissolve in a second stage to reprecipitate as calcite, as illustrated further on. Overall, these phases are not very soluble at high temperature, but their solubility increases as the temperature decreases.

The precipitation kinetic of CaCO₃ in a CaCl₂ solution, or of other alkaline earth cation carbonates, was studied by several authors, in particular Pocker and Bjorkquist [29], Bond et al., [30, 117] and Druckenmiller and Maroto-Valer [161]. Various techniques were used to follow such precipitation, in particular the precipitation onset time according to turbidity data, the [Ca²⁺] concentration before and after precipitation, the total "inorganic carbon" concentration in solution, and the pH decreasing rate in industrial brines containing Ca²⁺ cations. The enzyme itself could directly be present in the precipitation medium, or used to first catalyze the formation of HCO₃⁻ anions in a pH range from 8.55 to 8.7, while the Ca²⁺ containing brine was added in a second step. Ramanan et al. [61, 85] compared CaCO₃ precipitation with the enzymes from *Citrobacter freundii* and *Bacillus subtilis*. They showed that the crude enzymes were much less active than the purified ones. Li et al. showed that CA of microbial origin and bovine CA both accelerated the precipitation of CaCO₃ and favoured the formation of the calcite phase [162]. An acceleration of CaCO₃ precipitation was also observed by Da Costa et al. on bovine CA (BCA) extracted by 2 different techniques [62] and by Kim et al. with a cheaper recombinant α CA from *Neisseria gonorrhoeae* (NCA) [63]. With purified CA from

TABLE 4: Solubility product K_S as a function of the temperature T , for various crystallographic forms of CaCO_3 , according to Gal et al. [84].

Mineral name	Crystallographic structure	$-\log K_S$ (at 25 °C)	$-\log K_S$ as a function of T (K) or θ (°C)
Amorphous	Amorphous	6.40	$= 6.1987 + 0.005336\theta + 0.0001096\theta^2$
Ikaite	Monoclinic	6.62	$= 1696/T + 0.9336$
Vaterite	Hexagonal	7.91	$= 172.1295 + 0.077993T - 3074.688/T - 71.595 \log T$
Aragonite	Orthorhombic	8.34	$= 171.9773 + 0.077993T - 2903.293/T - 71.595 \log T$
Calcite	Rhombohedral	8.48	$= 171.9065 + 0.077993T - 2839.319/T - 71,595 \log T$

Pseudomonas fragi, immobilized by adsorption on chitosan, Anjaba et al. observed a more than 2 fold increase in calcite CaCO_3 sequestration by comparison with the free enzyme, in 5 minutes precipitation tests [122, 123]. With *Bacillus pumilus* CA adsorbed on chitosan beads, Wanjari et al. [124] showed that the precipitation of CaCO_3 was also accelerated by comparison with the free CA. Mirjafari et al. determined the mass of CaCO_3 precipitated in an aqueous $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution containing a buffer, when some CO_2 saturated water was added to the solution [77]. They also followed the evolution of the liquid turbidity as a function of time. Sharma and Bhattacharya compared CA extracted from *Pseudomonas fragi*, *Micrococcus lylae*, and *Micrococcus luteus* 2 with commercial bovine CA (BCA) [86]. The 3 bacterial CA exhibited enhanced CO_2 sequestration compared to the commercial BCA.

With CA immobilized on silylated chitosan beads, Yadav et al. determined apparent Michaelis constants K_m and v_{\max} for the precipitation of CaCO_3 [132]. For this purpose, they quantized the CaCO_3 by gas chromatography after decomposing it with HCl to release the captured CO_2 (g). They found that K_m was higher for the immobilized enzyme than for the free enzyme (respectively 4.547 mM and 1.211 mM), while v_{\max} was relatively unchanged (1.018 and 1.211 $\text{mmol min}^{-1} \text{mg}^{-1}$). The CO_2 sequestration capacity was found to be best enhanced with CA immobilized on core-shell CA-chitosan nanoparticles [133]. Kim et al. showed that the precipitation rate of CaCO_3 was about 3-fold faster with BCA and a CA enzyme extracted from oyster shell, than without enzyme [163]. In CO_2 capture and sequestration as CaCO_3 in 2 successive steps, BCA immobilized by various techniques on SBA-15 supports [134] displayed a similar CaCO_3 precipitation capability [135]. With human carbonic anhydrase (HCA) immobilized via electrostatic interactions on silver nanoparticles, themselves confined to amine-functionalized mesoporous SBA-15, they reported a CO_2 capture rate ~ 25 fold higher than that of free HCA after 30 cycles [136]. HCA was also immobilized on Au nanoparticles assembled over amine/thiol-functionalized mesoporous SBA-15 and the CaCO_3 final mass precipitated per test was similar to that of free HCA. However, the immobilized BCA retained its activity after 20 days storage at 25° and 20 recycling [137]. With HCA simply covalently immobilized on SBA-15 via various amines, the immobilized HCA efficiency in CO_2 hydration was 36 times greater than free HCA, and 75% of initial enzymatic activity was retained after 40 cycles [138]. Favre et al. investigated the deposition kinetics of CaCO_3 in a mixture of CO_2 saturated water, a buffer at different pH and an aqueous $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

solution, for different enzyme concentration [164]. Overall, it was shown that the enzyme could drastically increase the apparent precipitation rate of CaCO_3 (s), during the first minute. However, a maximum in this precipitation rate was observed, for an enzyme mass $\leq 0.3 \text{ mg mL}^{-1}$. This result could be explained by a faster increase of the formation rate of HCO_3^- and H^+ ions during the first deprotonation step, when the enzyme concentration increased. Hence, the pH decreased more rapidly to a low value, unfavorable to the formation of CO_3^{2-} ions. In turn, this stopped the precipitation of CaCO_3 (s) at an earlier time. Globally, if the pH was not maintained at a high enough value (e.g., 10) the total mass of CaCO_3 (s) precipitated did not depend on the presence of enzyme. The enzyme only modified the time to reach equilibrium where CaCO_3 (s) precipitation stopped. The final mass of CaCO_3 (s) precipitated only depended on the initial buffer nature, pH and concentration. The precipitates were also analyzed by X-ray diffraction [164]. At 20°C and initial pH 8.4 or 9.4, the only phase observed was calcite when no enzyme was added. At initial pH 10.5, vaterite was predominant. On the other hand, still at pH = 10.5, the enzyme favored the conversion of vaterite to calcite. The same observation was made at 5°C, although this conversion to calcite was uncomplete at this temperature. Scanning electron micrographs of these CaCO_3 deposits are shown in Figure 15. Both CaCO_3 phases have a hexagonal structure [165, 166], but vaterite is more complex than calcite. Solid vaterite particles displayed a porous spherical shape made by aggregation of nano crystallites, while calcite particles are characterized by a well defined rhombohedral shape with marked facets.

It was known that, in some cases, enzymes may possibly catalyze the formation of a given solid phase [142]. However, more common mechanisms are likely. Indeed, a spontaneous change in the crystallographic form and/or particle shape is often observed when solid particles nucleate and grow in liquid media [167]. Shape transitions without any crystallographic phase change can be due to a change in the concentration magnitude of the oligomers responsible for precipitation. These oligomers are formed from the chemical precursor of the solid, in the present case $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. Moreover, the first particles which nucleate do not necessarily correspond to the most stable thermodynamic phase. They really depend on the nature of those precursor oligomers which first reached a critical supersaturation for nucleation, and which may correspond to a metastable phase. On the other hand, the most stable thermodynamic phase (presently calcite) is likely to form in a second slower step, by dissolution of the first metastable solid phase, followed

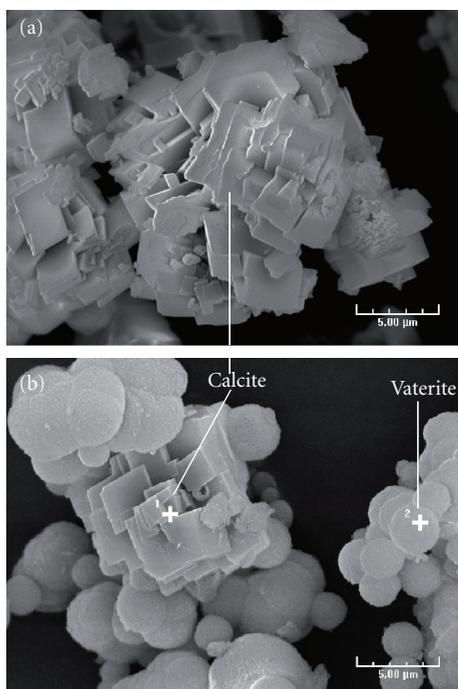


FIGURE 15: SEM micrographs of CaCO_3 solid particles precipitated from CaCl_2 at initial pH 10.5 and 20°C : (a) with CA enzyme; (b) without enzyme. Adapted from [164].

by re-precipitation to calcite. In the study of Favre et al., the first CaCO_3 phase nucleating at pH 10.5 was vaterite [164]. But this phase is also more soluble than calcite [84] so that it could redissolve and reprecipitate to calcite, at a lower rate. Consequently, metastable vaterite could more likely be observed during the first stage of precipitation, in particular when the overall precipitation kinetics was slow, hence at 5°C rather than at 20°C and/or when no enzyme was present.

7. Conclusion

Carbonic anhydrase are amongst the most well known enzymes, since they operate in most living organisms, including human beings where they play an important role. Their catalytic mechanism in the hydration of $\text{CO}_2(\text{aq})$ molecules has been extensively studied, and the summary presented in this review has stressed out the fact that this was a complex mechanism, requiring the use of pH buffers with which the enzyme was in direct competition. Nonetheless the well understood chemical physics laws underlying the capture of CO_2 in aqueous medium have permitted to develop several types of efficient CO_2 capture reactors. In particular, hollow microfibers reactors seem very promising for applications to industrial fumes. To improve their applicability, significant progress on several points may also be expected. These concern the cost of these enzymes, their catalytic activity, their stability in time and their resistance to pollutants such as sulfur compounds. Indeed, the large variety of carbonic anhydrase enzymes available in the living organisms, with a very different resistance capability to the

operational parameters involved, may permit to anticipate that their application to capture CO_2 will increasingly emerge as an efficient, environmental friendly technique, applicable with very moderate energy consumption, without requiring any heating.

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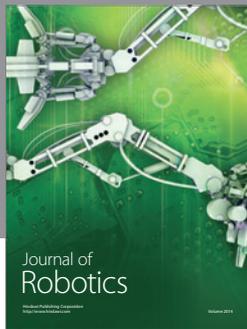
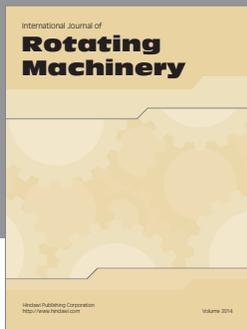
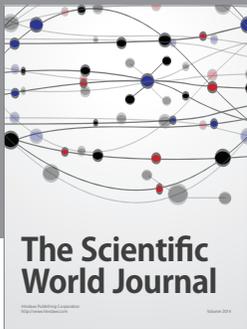
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