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Carbonic anhydrase to boost CO_2 sequestration: Improving carbon capture utilization and storage (CCUS)

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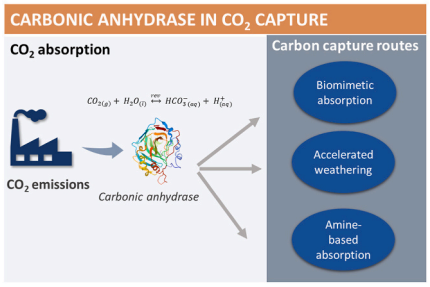
HIGHLIGHTS

- CO_2 capture by absorption can be enhanced by utilizing CA.
- Improvement techniques produce CAs more adapted to likewise industrial conditions.
- Biomimetic and CA-assisted amine absorption show superior CO_2 capture efficiency.
- CA-assisted accelerated weathering is an emerging approach for enhanced CO_2 capture.

GRAPHICAL ABSTRACT



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ABSTRACT

CO_2 Capture Utilization and Storage (CCUS) is a fundamental strategy to mitigate climate change, and carbon sequestration, through absorption, can be one of the solutions to achieving this goal. In nature, carbonic anhydrase (CA) catalyzes the CO_2 hydration to bicarbonates. Targeting the development of novel biotechnological routes which can compete with traditional CO_2 absorption methods, CA utilization has presented a potential to expand as a promising catalyst for CCUS applications. Driven by this feature, the search for novel CAs as biocatalysts and the utilization of enzyme improvement techniques, such as protein engineering and immobilization methods, has resulted in suitable variants able to catalyze CO_2 absorption at relevant industrial conditions. Limitations related to enzyme recovery and recyclability are still a concern in the field, affecting cost efficiency. Under different absorption approaches, CA enhances both kinetics and CO_2 absorption yields, besides reduced energy consumption. However, efforts directed to process optimization and demonstrative plants are still limited. A recent topic with great potential for development is the CA utilization in accelerated weathering, where industrial residues could be re-purposed towards becoming carbon sequestrating agents. Furthermore, research of new solvents has identified potential candidates for integration with CA in CO_2 capture, and through techno-economic assessments, CA can be a path to increase the competitiveness of alternative CO_2 absorption systems, offering lower environmental costs. This review provides a favorable scenario combining the enzyme and CO_2 capture, with possibilities in reaching an industrial-like stage in the future.

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1. Introduction

The climate change fight urges effective strategies to decrease global anthropogenic CO_2 emissions; according to IPCC, to limit global warming to 1.5 °C, emissions must decline by 45% from 2010 levels by 2030 (IPCC, 2018), requiring imperative actions. Carbon Capture Utilization and Storage (CCUS) could benefit the greatest CO_{2eq} global emitters (energy and industries) (Lamb et al., 2021) from the utilization of post-combustion methods (Rissman et al., 2020) such as chemical absorption (Asif et al., 2018) (Mumford et al., 2015).

The absorption benchmarking solvent, monoethanolamine (MEA), presents good CO_2 absorption (0.58 mol CO_2/mol amine) and fast kinetics (k_2) in the order of 4.8–5.5 m^3 mol s^{-1} (Bernhardsen and Knuutila, 2017) (El Hadri et al., 2017). However, MEA utilization brings some issues such high heat duty for regeneration, the formation of heat-stable salts, which makes difficult absorption and leads to corrosion problems, and its non-eco-friendly characteristics, have driven the development of alternatives to the traditional MEA-based scrubbing process. including the development of novel absorbents, less energy-intensive processes, and strategies to boost the kinetics of CO_2 absorption (Mumford et al., 2015). Methyl diethanolamine (MDEA) has been pointed as a possible substitute to MEA, exhibiting heat of absorption about 65% of the value observed for MEA, however lower CO_2 loading per kilo of solvent and slower kinetics (Svensson et al., 2013).

CA is an enzyme with spread occurrence in nature, found in several living organisms – from microbes and algae to vertebrates, as mammals (Sharma et al., 2020). CA plays a fundamental role in diverse biological-related mechanisms such as CO_2 transport, pH homeostasis, cell respiration and photosynthesis (Mondal et al., 2016). The demonstration that the enzyme successfully catalyzed reversible CO_2 hydration reaction to form bicarbonate, also under typical conditions of industrial flue gases, expanded the possibilities of capturing carbon from industrial

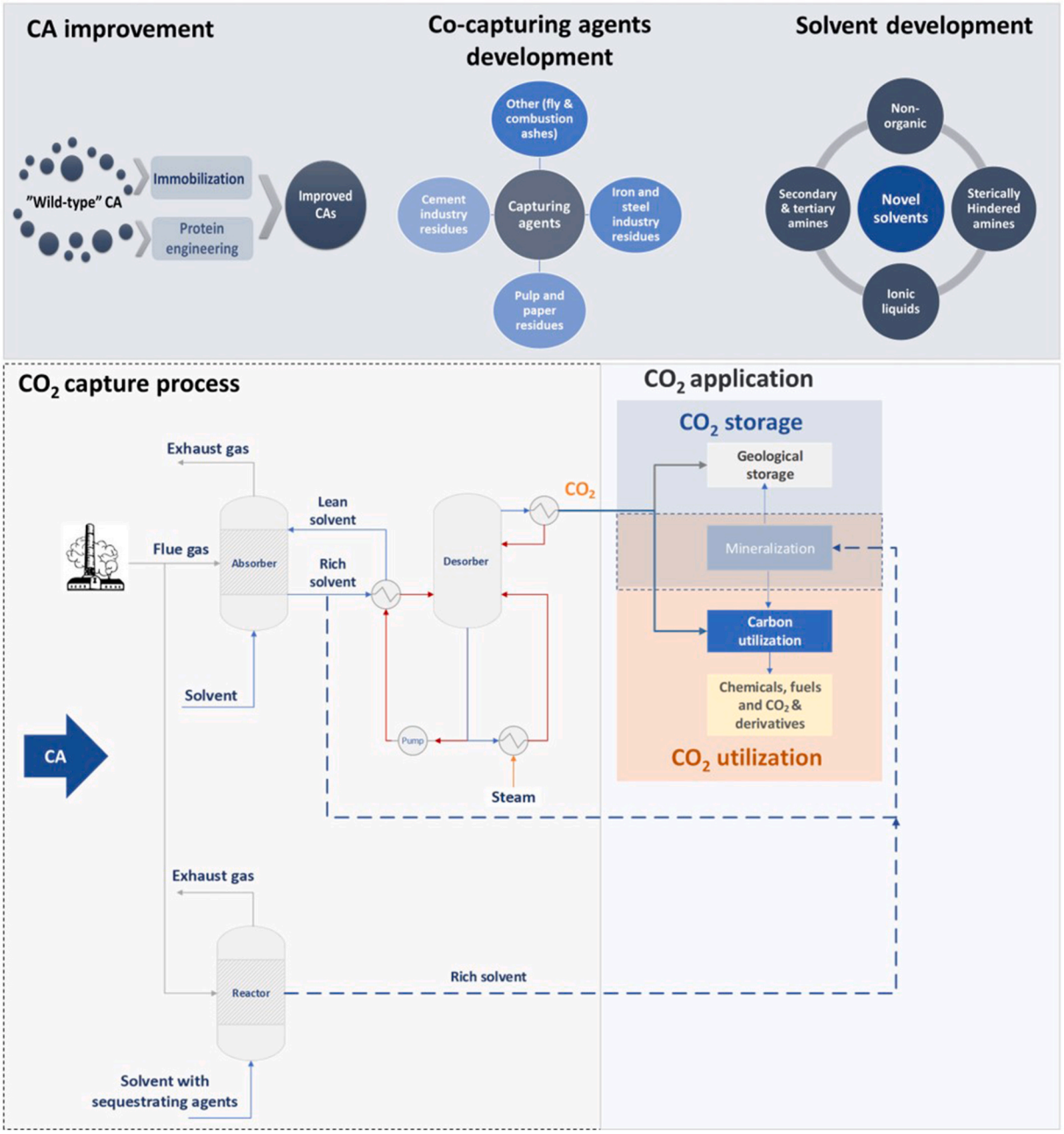

Fig. 1. Overview of the development of robust CAs for application in CCUS.

processes, from a biological route (Bond et al., 2000). This approach, also denominated as biomimetic CO_2 sequestration, has been targeted in studies of CO_2 capture, displaying so far good results – CA is a very fast enzyme whose turnover number can be as high as 10^4-10^7 s^{-1} (Sharma et al., 2020). In this way, CA utilization can be a smart strategy to achieve targets of carbon neutrality, as for example, by enabling net-zero emissions energy systems due to the increase in efficiency of carbon capture (IEA, 2020).

Furthermore, CA was also proven to bring interesting positive outcomes when utilized in absorption, such as amine-based scrubbing systems, accelerating the time spent for reaction, and addressing kinetics issues by improving the CO_2 reaction absorption rate. The enzyme integration to such well-established technologies enabled the utilization of solvents other than MEA, impacting CO_2 loading increase and characteristics such as lower reboiler energy duty and energy consumption in the regeneration cycle (Gladis et al., 2019). In Fig. 1, the improvements in absorption processes by utilizing CA, novel solvents and the potential application in CO_2 capture are summarized.

This review attempts to demonstrate the industrial importance of CA as promising catalyst for enhancing CO_2 absorption and the current stage of potential applications. Aspects about enzyme characteristics, as well as the most used techniques towards CA improvement, such as protein engineering and immobilization, are covered to understand their beneficial effects in CA applications. Information about the stability of distinct CAs is showed and compared. Besides, the enzyme kinetics under different conditions are displayed, and the impact of CA in CO_2 absorption for major applications, e.g. in biomimetic process, accelerated weathering and enzyme-assisted amine-based systems, are commented. Identification of enzymatic bottlenecks and overcoming strategies are also discussed. In the end, main challenges, prospects and CA mimics are briefly presented.

2. The carbonic anhydrase (CA)

2.1. Structure, families, and occurrence

CA (EC 4.2.1.1) constitutes a subclass of enzymes reported to exist in all life kingdoms (Supuran, 2016). CAs active structure most commonly contains a metal ion arranged in the center of tetrahedral geometry, bounded to three amino acids, and a hydrophilic part containing a water molecule or a hydroxide ion (Yadav et al., 2014) (Ozensoy Guler et al., 2016). Other geometries' configurations can exist under octahedral and bipyramidal forms (J. K. Kim et al., 2020). CAs are metalloenzymes, and the Zn is the most prevalent metal found in them. Nonetheless, other elements, such as Cd and Fe, were reported to occur in CAs of specific organisms (Tripp et al., 2004) (Akocak and Supuran, 2019) (Nocentini and Supuran, 2019).

Up to date, there are several types of CAs known which are classified into eight genetically distinct families: α-, β-, γ-, δ-, ζ- η-, θ- and ι- (Akocak and Supuran, 2019). α-CAs are the most numerous type in nature, being the first discovered exemplar in the early '30s, in the blood of humans (Meldrum and Roughton, 1933). Their oligomeric arrangement differs and can be found as monomer, dimer, and tetramer as quaternary structures (Cuesta-Seijo et al., 2011) (Supuran and Capasso, 2017). The α-CA occurrence in mammals is very well-documented, with 15 human isoforms so far identified, being intensively studied as drugs target research and diagnosis/therapeutic applications (Alterio et al., 2012). Other species, such as bacteria, fungi, plants, and algae have α-CAs as well, in which they regulate vital functions related to respiration, acid-basis homeostasis and the pH regulation, CO_2 transport, sexual development, and photosynthesis mechanism, respectively (Elleuche and Pöggeler, 2010).

The second group of most documented CA belongs to the β-CA type. They are present in microorganisms like bacteria and archaea, fungi, some types of high plants, and invertebrates. Typical quaternary structures so far discovered in β-CAs are dimer, tetramer, hexamer, and octamer (Kimber, 2000) (Murray and McKenna, 2019) (Urbanski et al., 2020). Plants and algae usually have CA in their chloroplasts and mitochondria, respectively, being believed they participate in the mechanisms of CO_2 fixation in plants (Mitra et al., 2004). Also, some fungal species possess this type of CA, with exemplars from *Candida* sp, *Saccharomyces cerevisiae, Sordara macrospara, and Aspergillus fumigatus* (Elleuche and Pöggeler, 2010) (Kim et al., 2020), where CA is a coadjuvant in mechanism related to pathogenicity. In bacteria, functions to pH regulation, by transporting bicarbonate (HCO_3^-) into the cell membrane, and virulence stimulation were also reported (Abuaita and Withey, 2009). The γ-CAs belong to a distinct family of CA presenting a left-handed parallel b-helix domain. Their presence occurs in bacteria, archaea, and some photosynthetic organisms, and so far, their quaternary structure revealed to be trimers (Ferraroni, 2019). Interestingly, evidence show that those enzymes might be one of the most ancient types among CAs (Smith et al., 1999). Ion transport and carbon fixation are some of the roles of CA in archaea and cyanobacteria. The other CA families, δ-, ζ- η-, θ- and ι-, occurs in nature in a scarcer way. For marine diatoms, both δ- and ζ-CAs are found to have be indispensable for enabling the process of carbon concentration (Capasso, 2019) (Langella et al., 2019); η-CA has only been identified in species of *Plasmodium* (Prete et al., 2014b). The θ- and ι-types can occur in marine diatom and bacteria, and θ-types in algae. θ-Cas, which contain Zn in their active site, are also reported for photosynthetic efficiency and growth in diatoms and (D'Ambrosio et al., 2019). For ι-types, besides the limited number of representatives and the poor report about their existence and properties, it is known that they can present their structure as dimers and can have their active site with Mn, instead of a Zn molecule (Prete et al., 2020).

2.2. Catalytic mechanism of CA

The catalytic mechanism of CA involving in CO_2 hydration/dehydration is known, however, CA also shows catalytic activity in other hydro catalytic reactions, such as the hydration of CS_2 and COS, hydrolysis of esters and cyanamide hydration to urea (Tanc et al., 2015). In nature, the reversible CO_2 hydration reaction occurs spontaneously, however, at limited rates. As a principle, CA acts in accelerating this reversible reaction towards the formation of a bicarbonate ion, as shown in eq. (.1). In this way, the CA could bring benefits in CCUS, by enhancing the efficiency and yield of CO_2 absorption. Compared to the non-catalyzed reaction, in which the catalytic turnover is in the order of magnitude of 0.15 s^{-1}, CA can potentially boost this reaction up to 1 billion faster – reported k_{cat}/K_m values ca be up to 10^8 s^{-1} (Effendi and Son Ng, 2019).

$$CO_{2(g)} + H_2O_{(l)} \overset{rev}{\leftrightarrow} HCO^-_{3(aq)} + H^+_{(aq)} \quad \text{eq .1}$$

For explaining the steps towards CO_2 hydration, a reaction mechanism was proposed (Supuran, 2007) (Kanth et al., 2013). The α-CAs, for example, have a metal-water molecule bond (EM-H_2O), which when ionized eq. (.2) (forming $EMOH^-$), presents a strong nucleophilic character. When a molecule of CO_2 approaches the enzyme structure, it is powerfully attracted by this part and linked to the hydroxyl group, conforming afterwards a bicarbonate ion to the metallic ion (eq .3). Further, the bicarbonate ion is released to the solution after being displaced from the active site by a water molecule (eq. (.4)); in the mass transfer limiting phase, thus, the rate-determining step of the reaction (Nocentini and Supuran, 2019), the metal-hydroxyl bond is regenerated: a proton transfer (H^+) from the water molecule will take place, driven by either the amino-acid chains present in the CA structure, or the buffers in the environment solution (eq .5), finally being bonded to the buffered media (eq. (.6)) completing the catalytic cycle CA (Lindskog, 1997). The Fig. 2 illustrates the mechanism of CO_2 hydration reaction catalyzed by CA.

$$EMH_2O + \ B \ \overset{rev}{\leftrightarrow} EMOH^- + BH^+ \quad \text{eq .2}$$

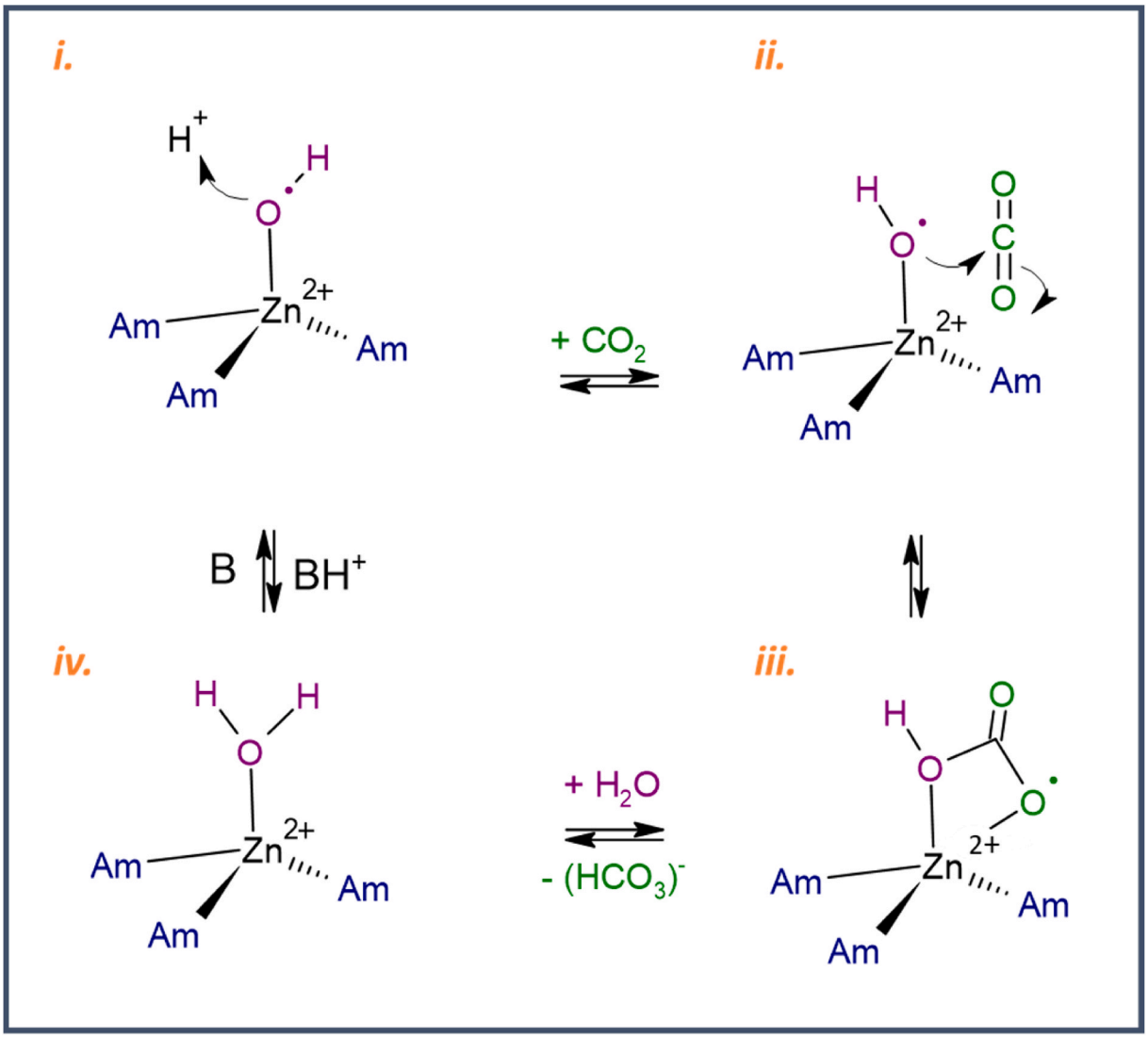

Fig. 2. The catalytic mechanism of CA. Reaction occurs following the order i) CA active site is deprotonated (H^+), ii) molecule of CO_2 approaches the active site, iii) CO_2 is coordinated into CA active site, iv) A molecule of bicarbonate is released by the substitution for a molecule of water followed by a deprotonation to restore the catalyst to the initial step. Am: amino acid, Zn^{+2}: zinc ion.

$$EMOH^- + CO_2 \overset{rev}{\leftrightarrow} EMOH - CO_2 \overset{rev}{\leftrightarrow} EMHCO_3^- \quad \text{eq .3}$$

$$EMHCO_3^- + H_2O \overset{rev}{\leftrightarrow} EMH_2O + HCO_3^- \quad \text{eq .4}$$

$$EMH_2O \overset{rev}{\leftrightarrow} + H^+ + EMOH^- \quad \text{eq .5}$$

$$H^+ + EMOH^- + B \overset{rev}{\leftrightarrow} BEMOH^- + BH^+ \quad \text{eq .6}$$

Besides, when determining the characteristics of enzymes properties, methods consisting of analytical assays are typically employed. Examples are the pNPA assay (Pocker and Storm, 1968) and the CO_2 hydration assay (Booth, 1946).

2.3. Improved CAs: protein engineering and immobilization methods

On the one hand, CA emerges to be a promising alternative to improve carbon fixation rate. due to its high catalytic efficiency; on the other hand, enzymes have a sensitive nature and might be susceptible to environment conditions such as high temperature, very acidic or very alkaline pH values leading to its deactivation. Besides, some metals and ions can inhibit the CA, affecting the enzyme activity in a temporary or permanent way.

When applied to industrial processes of CO_2 capture, thermostability and chemical resistance are crucial features to be achieved. Typically, the exhausted gases employed in the absorption column can reach up to 60 °C and the stripping column above 100 °C, with common solvents utilized to have strong alkaline character (Mumford et al., 2015). In this way, enhancement of enzyme properties targeting the obtention of more versatile and adaptable to adverse conditions of reaction are very desired. The utilization of protein engineering techniques such as directed evolution, rational design methods, and enzyme immobilization stands out for enabling more stable CAs, impacting the lifetime and resulting in lower operating costs.

2.3.1. Protein engineering: directed evolution and rational design

Directed evolution (DE) has emerged as one of the strategies employed to improve the stability of enzymes, from the simulation of natural evolution paths in lab environment (Yang et al., 2015). This technique is widely employed specially when particulars of the target structure are not well-known (Xu et al., 2019). For a succeeded DE experiment, high genetic diversity and appropriate selection or screening methods are necessary (Xiao et al., 2015). As a first step, for the creation of a diversified genetic library, strategies such as random

mutagenesis, EpCR and chemical mutagenesis, can be employed. Initially, expression of variants is performed, followed by screening to reveal which variants reached the desired properties intended by the mutation. In the last step, the genes identified are amplified (Sofia and Nogueira, 2013). Also, direct evolution is commonly utilized to enhance characteristics in CAs, such as thermostability and resistance to high-alkali medias.

Novel variants of a CA from *Thermovibrio ammonificans* (TaCA), with a potential to be applied in CO_2 capture, were produced also applying directed evolution. Modifications were performed in amino-acids located in the *N*-terminal region and resulted in variants with improved half-life compared to the original type. The variants were exposed to conditions suitable for CO_2 capture - at high temperature (>70 °C) and pH value 10. Among the constructed mutants, the variant S8R/G9P/E22P showed a 3-fold higher half-life in 1.45 M K_2CO_3 solution, at 85 °C and great stability when exposed to a solution of 2 M MDEA. Finally, in order to evaluate the TaCA stability under cyclic operation, an experiment simulating the operational conditions of an absorption/desorption cycle under the following approach was performed. A variant of TaCA, identified as SEQ ID N° 7, was dissolved in a K_2CO_3 solution loaded with CO_2, and repeatedly pumped in two different reservoirs, with two distinct temperatures (40 and 77 °C). After 20 days, the enzyme was capable of retaining up to 80% of its initial activity, showing that the DE strategy increased the enzyme stability in at high cyclic conditions and strong alkaline media, clearly evidencing its potential applicability under industrial conditions (Voyer et al., 2019).

Of course, the contribution of DE to enhance CA properties is meritorious and have still a great potential of application in the field given the possibility of optimizing enzyme characteristics and creating novel catalysts (Zeymer and Hilvert, 2018). However, some drawbacks of this technique should be noticed, particularly related to low efficiency and the need of large genetic library to increase the chance of successful results – which can lead to significant efforts during the screening phase, such as additional time and higher consumption of materials (Yang et al., 2015) (Zeymer and Hilvert, 2018).

As an alternative, utilization of rational protein design constitutes a powerful tool in the field of protein engineering. Its main features, however, relies on performing targeted adjustments, utilizing non-random molecule alterations. As a principle, the characteristics of the protein structure should be investigated and understood, and afterwards, modifications in the molecule can be proposed at specific sites (site-directed mutagenesis). Therefore, for a successful new enzyme design a deep knowledge about the structure and the enzyme mechanism of action is crucial (Steiner and Schwab, 2012), otherwise the alterations proposed might negatively impact on specificity and activity. So far, rational design happens via two ways: computer simulation or protein conformation (Dinmukhamed et al., 2021). The first is quite used in the research of improving CAs. With help of molecular dynamics and other computational tools, properties such as the increase of thermal stability could be verified, through the inclusion of intermolecular bonds in the CA molecule, such as disulfide and salt bonds and the inclusion of hydrophobic residues at the molecule surface (Jo et al., 2016) (Parra--Cruz et al., 2018).

In another work (Bharatiy et al., 2016), a highly thermostable CA variant from a mesophilic form of α-CA from *Neisseria gonorrhoeae* (ngCA) was designed. With the help of molecular dynamics, variables with an impact on stability were analyzed, such as salt bridges, hydrogen bonds, solvent-accessible area, and unfolding pathway. From this analysis, a mutant was designed from the insertion of three amino acid residues in the structure of the molecule. For evaluating the effect of the mutation on thermostability, the melting temperature was determined, and an enhancement of ΔT_m of 21 K was found. The modified ngCA contained more average number of hydrogen bonds and five additional salt bridges and presented more rigid structure. Furthermore, the activity of mutant CA showed evidence of being higher when compared to the wild type, from the calculations of pKa. As a conclusion, the new designed CA presented a more stable, a more active enzyme and with improved resistance to temperature, possibly due to new bonds created through the introduction of other amino acids into the enzyme, reflecting its potential to be utilized in high temperature conditions.

Also looking for a more thermostable form of CA, different variants of α-CAs were investigated to produce an ultra-thermostable form to be applied in carbon sequestration. Employing molecular dynamics simulations, TaCA was identified as the most stable enzyme among the studied CAs, indicated by the presence of the most rigid structure. For the design of the ultra-thermostable enzyme, alterations were targeted in amino acids which were determined with more flexibility and higher root mean square fluctuation (RMSF). Mutations were done by exploring two different approaches: the first, with an *in silico* mutation to form charged or small non-polar residues and a second, through the addition of a disulfide bonds to stabilize the most flexible zones. Also, five of eight designed mutants showed more rigid structures than the wild type at 400 K, reflecting their capability to retain activity at higher temperatures (Parra-Cruz et al., 2018). Further, a complementary experimental study was performed to verify the improved thermostability of the studied TaCA mutants via site-directed mutagenesis. The variants exhibited similar CO_2 hydration activities to the wild type, ranging from 0.6 to 1.04-fold. During the assessment of the enzyme thermostability at 90 °C, a CA variant, N140G, presented no loss of activity, meanwhile, the initial activity of wild type was decreased by 70%. Furthermore, an increase in 2.6-fold of half-life was observed for this same variant when incubated at 60 °C. To complement, all designed variants were subjected to an experimental procedure, where an absorption-desorption cycle was mimicked; the results showed that three variants (mutations C67G, N140G and T175P) were able to maintain their initial activity after 15 complete cycles. Finally, the enzymatic activity was assessed at 95 °C and an increase up to 53-fold for the mutant N140G and up to 23-fold for the other TaCA four mutants was constated when compared to 25 °C. During the same assay, the wild-type presented, though, an increase up to 14-fold testifying all the designed mutants could successfully have their thermostability enhanced (Parra-Cruz et al., 2020).

In the above studies, higher thermostability was reached by increasing the rigidity of CAs' structure, such as through the introduction of new salt bridges and hydrogen bonds, evidencing the correlation between high-temperature tolerance and less flexible residues. The utilization of rational design to produce ultra-thermostable CAs is valuable since it is possible to identify, by molecular simulations, the most susceptible regions and target them to be stabilized more assertively. Such technique can be of great interest for circulating CA through CO_2 absorption-desorption cycles as well, of which the latter requires high temperatures. Also, the improvement in thermostability of mesophilic variants, such as NgCA, is highly desirable to allow the enzyme to work in moderate higher ranges of operation – suitable to the conditions of CO_2 absorption which usually occurs at temperatures up to 40 °C. It is important, although, to highlight that more rigidity in CAs structure could bring some undesired effects, affecting enzyme activity, due to limited access to the active site or lower substrate affinity caused by the introduction of new residue(s) in the molecule.

With an approach other than thermostability enhancement, a halotolerant mutant from bovine carbonic anhydrase II (BCA-II) was created through a series of mutations by altering existing amino acid residues to an acidic form of them. From rational design, target residues were selected according to their localization in the CA structure and level of intramolecular engagement with neighboring residues – being given the preference for the ones located on the protein chain surface and with weaker interactions, to reduce the possibilities of causing molecule instability. In total, 18 residues were chosen for mutation, which took place in 3 stages – resulting in 4 different mutants (M1-M4), with amino-acid substitutions varying from 6 to 18. A mutant (M4) showed enhanced tolerance at high salt concentration, with increasing unfolding temperatures the more concentrated was the media (NaCl,

$NaSO_4$ – up to 3 and 2 M, respectively) while its wild-type faced an opposite effect in the unfolding temperature. In addition, positive correlation between salt concentration and activity were also observed; the ratio between apparent catalytic activity of a mutant and the wild type was about 5-fold, when assessed in a $NaSO_4$ (1.5 M) solution, and about 1.4-fold for NaCl (3 M). The results proved the engineered CA was capable to exhibit halotolerant characteristics while it was demonstrated that halotolerance can be generated in an enzyme solely by modifying surface residues (Warden et al., 2015). For application involving the utilization of non-conventional CO_2 capture sequestrant agents, such solid residues from combustion or mine tailings, the CA variant may prove a suitable candidate.

Other works, also aiming for thermostability, were able to show positive results towards the increase of half-life, where utilizing site-directed mutagenesis, a mutant from ngCA (N63C/P145C) reached 8-fold higher than the wild-type at 70 °C (Jo et al., 2016). Utilizing the same technique, a variant of hCA II (L204K) had its thermostability improved and was able to keep 100% of retention at 45 °C compared to its wild type, which at the same temperature was completely deactivated (Wu et al., 2020). Employing BCA II (Fisher et-al 2012), a mutant (TS4) designed through site-directed mutagenesis presented both thermostability and kinetics enhanced; melting temperature was improved by about 6 °C and the maximal proton transfer enhanced 6-fold compared to its wild-type (see Table 1).

The utilization of both DE and computational tools is possible, which can be referred as combinatorial approach or semi-rational design. With an objective of obtaining a more efficient CO_2 capture with MDEA in an absorption power plant, a variant of a β-CA from *Desulfovibrio vulgaris* (DvCA) was engineered to obtain a more suitable mutant to work under the harsh process conditions, such as the high temperature in the stripping unit and high reactive character of the solvent. The construction of libraries was performed utilizing saturation mutagenesis and statistical analysis to optimize the efficiency of screening; over 27,000 mutants were screened during 9 rounds of evolution, at high temperatures and MDEA at concentration 3.0–4.2 M. As result, the chosen mutant to operate in the experiment (DvCA 8.0), showed an improvement of 10.000-fold of half-life when compared to its wild form and presented 40% of remaining activity when stored during a period of 14 weeks in MDEA 4.2 M at 50 °C (Alvizo et al., 2014). Results proved a representative enhancement of wild CA properties, showing that the approach can be a strong tool to bring higher stability and to optimize enzyme use through recycling in consecutive absorption-desorption cycles. Table 2 displays studies of protein engineering techniques applied for the enhancement of CA properties and main results achieved.

2.3.2. Immobilization methods

More resistant and adapted CAs are indeed essential for enabling enzymes able to resist scrubbing and regeneration processes at industrial scale and conditions. However, as high costs are still involved in CA production, it is reasonable to direct efforts towards enzyme recovery and recycling to decrease operational costs in CA-assisted systems (Molina-Fernández and Luis, 2021). The small dimensions of the enzyme, as small as $5 \times 4 \times 4$ nm^3 in some α-types (Lindskog, 1997), makes its recovery hard and inefficient if executed through simple unit operations, such as filtration and/or centrifugation. Enzyme immobilization strategies have been widely employed to bring a longer operational lifetime for CA when compared to its free form. Also, the immobilization process is reported to create an additional protective effect in the enzyme (Ren et al., 2021). Consequently, besides reutilization, the enhancement of properties as thermostability, retention of activity and tolerance to hazardous chemicals, such as amines, sulfur, and nitrate compounds, can also be accomplished.

Five classic methods are used to immobilize enzymes, namely adsorption, covalent binding, entrapment, encapsulation, and cross-linking enzyme aggregates (Effendi and Son Ng, 2019). The choice of a specific immobilization method varies accordingly to the conditions in which the reaction takes place, such as type of solvent, reactor type, temperature, and how the chosen method might compromise secondary characteristics such as activity and kinetics.

In adsorption, the attachment of enzyme to the matrix happens via weak type of interactions, such as van der Waals force, hydrogen bonds and electrostatic forces. This method is very simple, and variables such as time of incubation, enzyme loading, pH, temperature, and agitation speed are generally studied to optimize its activity. Studies employing adsorption to immobilize BCA showed particular good results in term of specific residual activity (up to 98.8%) after immobilization and for the improvement of catalyst reusability, with remaining activity of 84% and 96% after 20 and 30 cycles of pNPA assays, respectively (Vinoba et al.,

Table 1
Families, occurrence, and structure of CA.

Family	Identified occurrence	Examples of species	Structure and metal ion bound at the active site	Reference
α-	i. Animal	*Homo sapiens* *Mus musculus*	Monomer, dimer or trimer – Zn	DiMario et al. (2018)
	ii. Bacteria	*Vibrio cholerae* *Sulfurhydrogenium. yellowstonense*		(Prete et al., 2014) (Di Fiore et al., 2013)
	iii. Algae	*Chlamydonomas reinhardtii* *Dunaliella salina*		(Karlsson et al., 1995) (Fisher et al., 1996)
	iv. Plants	*Arabidopsis thaliana* *Oryza sativa*		DiMario et al. (2018)
	v. Fungi	*Aspergillus oryzae*		Cuesta-Seijo et al. (2011)
β-	i. Bacteria	*Helicobacter pylori* *Vibrio cholerae*	Dimer, tetramer, hexamer, octamer – Zn	McGurn et al. (2016)
	ii. Archaea	*Methanobacterium thermoautotrophicum*		Smith et al. (2000)
	iii. Prokaryotes	*Trichonoma vaginalis*		Urbanski et al. (2020)
	iv. Fungi	*Cryptococcus neoformans* *Candida albicans* *Aspergillus fumigatus*		(Elleuche and Pöggeler, 2010) (S. Kim et al., 2020)
	v. Animal	*Drosophila megalongaster*		Syrjänen et al. (2010)
	vi. Algae	*Porphyridium purpureum,* *Coccomyxa* *Chlamydomonas reinhardtii*		(Mitsuhashi et al., 2000) (Huang et al., 2011) (Mitra et al., 2004)
	vii. Plants	*Pisum sativum*		Kimber (2000)
γ-	i. Bacteria	*Sulfurihydrogenibium yellowstonense* *V. cholerae*	Homotrimer – Zn	Angeli et al. (2018)
	ii. Archaea	*Myceliophtora thermophila,*		Kisker et al. (1996)
	iii. Plants	*Saccharina japonica,* *Arabidopsis thaliana*		Parisi et al. (2004)
δ-	i. Marine diatoms (algae)	*Thalassiosira weissflogii*	Monomer- Zn	Akocak and Supuran (2019)
ζ-	i. Marine diatoms (algae)	*T. weissflogii* *Thalassiosira pseudonana*	Monomer - Cd or Zn	Akocak and Supuran (2019)
η-	i. Eukaryote	*Plasmodium* ssp.	Not identified - Zn	Prete et al. (2014b)
Θ-	i. Marine diatoms	*T. weissflogii* *Phaedodactilum tricornutum,*	Not identified - Zn	(Akocak and Supuran, 2019) (DiMario et al., 2018)
	ii. Bacteria			
	iii. Algae	*C. reinhardtii*		
ι-	i. Marine diatoms	*T. weissflogii*	Dimer - Mn (II)	Jensen et al. (2019)
	ii. Bacteria	*Burkholderia territorii*		

Table 2
Improvement of CAs by protein engineering techniques.

Employed CA	Targeted optimization	Technique	Main Improvements	Best CA variant	Reference
DvCA	Thermo- and high alkali stability	Directed evolution (saturation mutagenesis) in combination with the protein sequence activity relationships (ProSAR) algorithm; 9 rounds (>27,000 variants)	Half-life: 10.000-fold improvement than wild-type T_{50}: 88.3 °C 40% activity when stored during a period of 14 weeks in MDEA 4.2 M at 50 °C	DvCA8.0, variant of 7th round of evolution 88.3% identity to the wild type	Alvizo et al. (2014)
TaCA	Thermo-, chemical, and high alkali stability	Directed evolution	Half-life: 3-fold Residual activity > 6-fold than higher than SEQ ID N^O7	Variant with 3 amino acids substitutions: S8R/G9P/E22P	Voyer et al. (2019)
ngCA	Thermostability	Rational redesign: molecular dynamics + site-specific mutagenesis, T = 300–500 K, time: 100 ns	ΔTm: 21 K - enhance in melting temperature pKa showed evidence of higher activity	Variant with 3 mutations: S44 R/S139 E/K168R. 5 new salt bridges were created	Bharatiy et al. (2016)
BCA II	Halotolerance	Rational redesign: molecular dynamics MD at T = 298 K, t = 600 ns – salt-protein interaction	Apparent activity: ~ 5-fold to wild-type	M4, variant with 18 substitutions in which 10 are Asp and eight are Glu residues	Warden et al. (2015)
TaCA	Thermostability	Rational redesign: molecular dynamics + site-directed mutagenesis MD: T = 343–400 K, t = 100 ns	Half-life: 2.6-fold Activity in high temperature (95 °C): 3.3- fold	Variant with mutation: N140G	(Parra-Cruz et al., 2018) (Parra-Cruz et al., 2020)
hCA II	Thermostability and kinetics enhancement	Rational redesign: site-directed mutagenesis	Enhance T_M in 6 °C more than its wild type. Kinetics: 6-fold maximal proton transfer	TS4, variant with 5 mutations: L100H/L224S, L240 P/Y7F/N67Q	Fisher et al. (2012)
ngCA	Thermostability	Rational redesign: Site-directed mutagenesis	Half-life: 8-fold higher than wild-type at 70 °C Thermo-stability: 11.4-fold vs 4.9 –fold higher stability for mutant and wild-type at 80 °C, baseline 25 °C	Variant with mutations: N63C/P145C	Jo et al. (2016)
hCA II	Thermostability	Rational redesign: site-directed mutagenesis	100% of retention at 45 °C – before enzyme deactivated at same temperature	Variant with mutation: L204K	Wu et al. (2020a)

DvCA: CA from *Desulfovibrio vulgaris,* TaCA: CA from *Thermovibrio ammonificans*, BCA II: bovine CA II, hCA II: human CA II, ngCA: CA from *Neisseria gonorrhoeae;* TM: melting temperature, T_{50}: half-life time.

2011) (Vinoba et al., 2012b). Besides, BCA was immobilized by adsorption in a metal organic framework (MOF) (Ren et al., 2018). The residual activity reached after immobilization was 75% at 60 °C, and 40% of initial activity was kept while the free form was completely deactivated. As an advantage, adsorption can offer a high loading capacity of CA per surface unit and does not necessarily require complex preparation. The weak/mild nature of chemical interactions between enzyme and carrier, however, can lead to the detachment of enzyme from the matrix, especially when subjected to high ionic media occasioning the leaching and loss of CA activity. Selection of materials which have more robust interactions with the CA, and pore size similar to the CA molecule size can improve catalyst reusability ref. For that, further steps to bring enhanced stability to the enzyme, such as activation of functional groups or post-modification are desired (Wu et al., 2020), once they can provide more stability by creating further interactions between CA-support, such as ionic and covalent bonds.

For entrapment, the enzyme is kept physically enclosed inside the matrix (Effendi and Son Ng, 2019). This method is relatively simple and can be performed with biobased polymeric materials origin such as sodium alginate and chitosan (Zhu et al., 2016a) (Oviya et al., 2012) (Yadav et al., 2012) or matrixes such as silica-based and other polymeric materials (Hsieh et al., 2021). Properties such as improved thermostability were reported, when after the process of immobilization in a bioinspired silica matrix, BCA was able to maintain its initial activity when incubated at 50 °C, while its free form was completely deactivated. Also here, 100% of the initial activity was retained after the immobilization process (Forsyth et al., 2013). In another evaluation, CA from *Bacillus subtilis* was entrapped in chitosan–alginate polyelectrolyte hydrogel and showed almost 94% of residual activity after immobilization. In addition, the immobilized could preserve 45% of initial activity when tested at pH 11, while the free form was totally inactivated (Oviya et al., 2012). Among the advantages of entrapment are the promptitude of the method and the utilization of inexpensive materials with low toxicity as support. The process of entrapment can offer extra protection once it (partially) coats the enzyme. When exposed to reactive solvents, CA lifetime can be prolonged due to the protection of the enzyme in the matrix and limited exposure to harsh reaction environment (temperature, alkali). Thus, in many cases higher thermostability is observed when compared to the free form. Main drawbacks can be the lower apparent activity as the support may restrict the substrate access to CAs' active sites and enzyme leaching due to the delicate nature of some biopolymeric matrices and their degradation over time. A further step after the entrapment, such as cross linking, could further improve the stability of the polymer network. Other popular technique of immobilizing enzymes, the covalent attachment (or bonding), consists of creating a strong link between enzyme and support through covalent bonds, normally via side chains of amino or epoxy groups (Effendi and Son Ng, 2019). For the CA, the attachment normally occurs via an amino acid group (lysine) as a nucleophile which can attack, i.e. an epoxide or aldehyde group present in the support (Sheldon et al., 2005). Covalent bonding is a very used method, as it promotes a stronger bonding to the enzyme to the matrix, when compared to other methods such as adsorption. Examples utilizing BCA, showed that the covalently attached CA presented high stability and improved capacity of recycling, keeping more than 87% and 75% of initial activity after 20 and 40 cycles, respectively (Vinoba et al., 2011) (Fei et al., 2016). The strong bonds which CA forms with the support in this method contributes in improving the catalyst reutilization capability, resistance to harsher environments, which is of great industrial interest. As drawbacks, the method can restrict the structure of the enzyme, decreasing the accessibility of active sites, culminating in loss of activity (Ren et al., 2021).

Like entrapment, encapsulation has as basis the confining of the

enzyme in a network structure. The method is reported to immobilize the enzyme with high efficiency, and results in high stability and specific activity (Wu et al., 2020b). Also, improved reusability with this method was proven to work, showing with residual activities up 90% after 10 cycles of successive pNPA assays when tested with a CA from *Hahella chejuensis* (Min et al., 2016). Besides, encapsulation is the easiest technique of immobilization when compared to other technologies and offers a micro-environment for the enzyme, helping it to maintain the CA structure.

With a different approach, CLEA (cross linked enzyme aggregates) is a method which does not need a support to immobilize the CA. This technique consists of a first step with enzyme precipitation by employing saturated salts (e.g. ammonium sulfate) or solvents, followed by cross-linking to avoid the enzyme to dissolve afterwards (Peirce et al., 2015). During cross-linking, lysine residues at the enzyme surface react with an aldehyde group from the cross-linker (typically glutaraldehyde) in a polymeric reaction, resulting in complex network where the CAs are covalently linked (Sheldon et al., 2005). Activity after immobilization with BCA were reported to as high as 84% (Peirce et al., 2015). Also, stability after repeated cycles was kept – with 95% of initial activity after performing 10 cycles. Once CLEA does not require a matrix for the immobilization process, costs related to immobilization process can be much lower. However, recover is still one of the main challenges when utilizing these techniques once the utilization of filters or other simple operations are not efficient to recycle the catalyst. Aiming to overcome this obstacle, utilization of magnetic nanoparticles with BCA in CLEA was tested, facilitating its recovery. After 5 cycles of CO_2 absorption in a glass reactor and utilizing an alkaline buffer, the magnetic CLEA was able to keep 95% of its initial activity (Peirce et al., 2017). The formation of covalent bonds during CLEA preparation, makes this method a good candidate to enhance CA recyclability similar to covalent bonding. The lack of a matrix during CLEA preparation leads to cost reduction when compared to other methods, while the use of glutaraldehyde as cross-linking agent is a highly economic choice.

Besides, utilization of combined immobilization methods is used to improve the susceptibility to leakage and deactivation of the enzyme, by creating a more robust CA attachment to the support. In other study, BCA was adsorbed in magnetic mesocellular siliceous foam (Woo et al., 2015). After, chitosan was adsorbed as CA-support, and then cross-linked via glutaraldehyde (GA) treatment. Utilizing pNPA assays, the immobilized CA had an improvement in half-life in 353-fold to the free form and kept almost all initial activity after 30 cycles of CO_2 absorption with further mineralization, which provides an indication that the cross-linking step presents positive effects on enzyme reusability. The material showed to be effective to prevent CA leaching, confirmed through a test where the immobilized enzyme was agitated for 85 days and still preserved the initial activity. In other example, experiments conducted with BCA and poly (acrylic acid-*co*-acrylamide)/hydrotalcite hydrogel, showed that the specific activity of the immobilized catalyst could reach near to 90%. The enzyme was embedded in the porous of the activated (with *N*-hydroxysuccinimide) hydrogel, forming covalent attachments with this matrix as well. The resistance to higher temperatures was verified as well, and after a period of 60 min, the immobilized form could keep 65% of residual activity and free form, instead, lost all the initial activity (Zhang et al., 2009), showing that the method could improve the thermostability of the enzyme. As drawback, the combined methods can demand more steps during preparation increasing complexity.

All methods of immobilization usually show some improvement in thermostability. The enzyme enclosure, such as entrapment and encapsulation methods, could preserve the CA structure from deformation once it restrains the enzyme mobility. However, it can limit the mass transfer diffusion of substrate. CA attached onto supports through weak nature interactions, such as physical adsorption, might not be recommendable under harsh environment conditions. Also, it is advisable to understand if the CA-matrix bonds can be affected in the presence of the chosen solvent. More stable catalysts, such as CLEAs and covalent bonded forms, are more likely to resist in solutions where there is no competition of the solvent with the amino acids that bond the CA to the matrix, like in buffered and salt solutions. On the other hand, they increase rigidity that may affect enzyme activity. Finally, the investigation of combined immobilized methods is highly recommendable since they help to overcome issues of catalyst stability and activity loss. It is worth to mention that most studies focus on immobilization are based on standard analytical assays, thus further evaluations of the immobilized forms in actual CO_2 absorption conditions is needed to comprehend their performance. An overview of different immobilization methods and their combinations applied to CA are presented in Table 3.

3. Enzyme kinetics and physicochemical properties

CAs may differ immensely in properties such as halo- and salts tolerance, thermostability, and pH resistance - all relevant for CO_2 absorption applications. Evaluating and identifying variants that combine such characteristics is a crucial step in enzyme selection. BCA, an α-type is a very well-known and commercially available enzyme, works with optimal temperature at 35 °C and pH operation range between 7.0 and 7.5 (Sharma and Bhattacharya, 2010). It can be quick inactivated at temperatures above 60 °C (Luca et al., 2013) which limits its application to absorption and desorption cycles, whose operation ranges range easily reach 40–60 °C and 70–80 °C, respectively (Ye and Lu, 2014). This scenario of challenges motivated numerous researchers committed to identify novel CAs with improved thermal and/or chemical resistance.

Among thermostable CAs, CA isolated from the liver of a camel kept 82% and 72% of its residual activity when exposed to temperatures of 50 and 60 °C, respectively (Chafik et al., 2020). A combination of 3 indigenous CAs isolated from *Pseudomonas fragi* (PCA), *Micrococcus luteus 2* (MTCA), and *Micrococcus lylae* (MLCA) showed an increased operating temperature of up to 10 °C as well as 3.42-fold higher CO_2 sequestration when compared to commercial BCA (Sharma and Bhattacharya, 2010). An α-CA from *Lactobacillus delbrueckii* (LdCA) presented a half-life of 370 and 177 h when exposed to temperatures of 40 and 50 °C, respectively (Li et al., 2015) and the α-type CA from Bacillus halodurans (BhCA) demonstrated to be extremely stable at 40 °C. However, a half-life of 65 min was observed after being incubated at 50 °C (Faridi and Satyanarayana, 2016). At 40 °C, both BhCA and LdCA presented high-temperature stability. However, at 50 °C LdCA showed a half-life almost 163-fold higher than BhCA, evidencing that BhCA may be an interesting choice for application at higher temperatures. In complement to that, CAs from extrethermophilic bacteria, such as *S. yellowstonense YO3AOP1* (SyCA) and *Sulfurihydrogenibium azorense* (SazCA) presented robust stability at very high temperatures, maintaining almost 100% of their activities at temperature ranges between 80 and 100 °C for about 180 min (Luca et al., 2013) (Capasso et al., 2012). The discovery of such CAs, which are not only stable at high temperatures but also present high activity in a broad range of temperature was remarkable. In this way, the existence of highly stable CAs working at high temperatures (above 80 °C) corroborates the potential of CA utilization not only in industrial stripping processes, but also in CO_2 regeneration cycles.

Temperature resistance is a critical factor to enable more competitive biomimetic absorption processes. Nonetheless, the enzyme performance in alkaline conditions is a topic of equal importance. BhCA, LdCA, and an α-CA from *Aeribacillus pallidus* (ApCA), respectively, showed noticeable stability at pH values up to 11, without significant loss of their initial activity (Faridi and Satyanarayana, 2016) (Li et al., 2015) (Bose and Satyanarayana, 2016). Furthermore, a recombinant CA obtained from *B. halodurans* TSLV1 (rBhCA) was able to keep 66% of initial activity after 24 h of incubation at pH 11.0. Also, when incubated for 2 h at pH 12.0, the enzyme kept 100% of its initial activity (Faridi et al., 2017). Compared to BCA, microbial CAs demonstrate a greater advantage in

Table 3
Techniques of CA immobilization and characteristics of immobilized forms.

Type of immobilization	CA type	Matrix	Load (mgCA/g)	Assay	% Residual Activity after immobilization	Thermal stability	Storage stability	pH stability	Reusability	Reference
Entrapment	SazCA	Biomimetic silica	N/A	pNPA, pH 7.6, room temperature	91	Activity 73%, 63%, and 60% at 50-, 60-, and 70 °C	Activity 62% immobilized, 30% free form, 35 days	At pH 12: 67% (free form - deactivated). Optimum both 10	10 cycles: 86%	Hsieh et al. (2021)
Entrapment	BCA	Sodium alginate	1160	CO_2 hydration, pH 8.3, 277 K	100 (in $CaCO_3$ pp)	Activity >80% immobilized and <50% free form at 40 °C	75.4 and ~35% - after 18 days at 277 K	Optimum at pH 8.5 both free and immobilized form	6 cycles: 67%	Yadav et al. (2012)
Entrapment	BCA	Chitosan	1[a)]	CO_2 hydration and pNPA, room temperature	9	Optimum at 25 °C	Half-life: 456 vs 406 h for immobilized and free	Optimum at pH 7.4 both free and immobilized	5 cycles: 45.7%	Arfin and Wanjari (2015)
Entrapment	CA from *Bacillus subtilis*	Chitosan–alginate polyelectrolyte hydrogel	N/A	CO_2 hydration assay and pNPA, pH 8.2, 37 °C	94	Activity 34% immobilized and 9.3% for free form at 70 °C	Activity 93% immobilized form, 50 days	At pH 11.0, 45% of activity immobilized and 0% for free form	N/A	Oviya et al. (2012)
Adsorption	BCA	Silver nanoparticles	307	pNPA, pH 6.4	99	N/A	Activity 89% immobilized, 77% free form, 20 days	N/A	30 cycles: 84%	Vinoba et al. (2012b)
Adsorption	BCA	Gold nanoparticles	289	pNPA, pH 6.4	97	N/A	Activity 98% immobilized, 69% free form, 20 days	N/A	20 cycles: 96%	Vinoba et al. (2011)
Adsorption	BCA	ZIF-8	N/A	pNPA, pH 7.0, 25 °C	75	Activity up 40% immobilized and ~0% free form at 60 °C	N/A	N/A	9 cycles: 85%	Ren et al. (2018)
Covalent bonding	BCA	SBA-15, modified with epoxy groups	222	pNPA, pH 8.5, room temperature	85	Activity up 85% immobilized and 70% free form at 55 °C	Activity 91% immobilized and 30% for free form, 30 days	At pH 10.0, 100% of activity for immobilized and ~45% for free form	20 cycles: 87%	Fei et al. (2016)
Covalent bonding	BCA	SBA-15, modified with amino groups	180	pNPA, pH 8.5, room temperature	81	Activity to up 82% immobilized and 70% free form at 55 °C	Activity 88% immobilized and 30% for free form, 30 days	At pH 10, 100% of activity for immobilized and ~45% free form	20 cycles: 87%	Fei et al. (2016)
Covalent bonding	BCA	Electropolymerized poly pyrrole film onto porous carbon support	1.25[b)]	pNPA, pH 7.6, 25 °C		Activity to up 40% immobilized and 9% free form at 90 °C	N/A	N/A	N/A	Merle et al. (2014)
mCLEA	BCA	CLEA with magnetic nanoparticles	500	CO_2 absorption experiment[c)]	84	N/A	N/A	N/A	5 cycles: 95%	Peirce et al. (2017)
CLEA	BCA	CLEA	193	CO_2 hydration, pH 7.0, 25 °C	95	N/A	Activity~95% immobilized and 5% for free form, 30 days	N/A	10 cycles: 97%	Peirce et al. (2015)
Encapsulation	HCA	Silica	N/A	CO_2 hydration and pNPA, pH 7.6 and 8.3, 25 °C and 0 °C	60	Activity 40% immobilized and 10% for free form at 80 °C	N/A	Negligible effect	10 cycles: 90%	Min et al. (2016)
Encapsulation	ngCA	Silica	N/A	CO_2 hydration and pNPA, pH 7.0 and 6.4–8.3; 25 °C	60	Activity of 50% for immobilized and 10% for free form at 80 °C	n/A	N/A	4 cycles: 89%	Jo et al. (2014)
Adsorption/ Covalent bonding	BCA	TiO_2	163	pNPA, pH 6.0	80	N/A	Activity >70% immobilized, 40% free form, 20 days	N/A	20 cycles: 40%	Hou et al. (2015)
Embedding + Covalent bonding	BCA	Polyacrylic acid-*co*-acrylamide)/	4.6	CO_2 hydration, pH 8.3, 2 °C	90	Activity of 65% immobilized and almost 0%	N/A	N/A	N/A	Zhang et al. (2009)

(*continued on next page*)

Table 3 (*continued*)

Type of immobilization	CA type	Matrix	Load (mgCA/g)	Assay	% Residual Activity after immobilization	Thermal stability	Storage stability	pH stability	Reusability	Reference
		hydrotalcite (PAA-AAm/HT) gel				for free form at 50 °C				
Adsorption + Crosslinking	BCA	Mesocellular siliceous foam coated with chitosan	N/A	pNPA, pH 7.6, room temperature	N/A	N/A	No detected loss in activity after 85 days under agitation at room temperature	N/A	30 cycles: almost no loss in activity	Woo et al. (2015)

[a] Unit: mg/ml, pNPA: Hydrolysis reaction of *para*-nitrophenylacetate, CO_2 hydration: CO_2 hydration assay.
[b] Unit: mg/ml; Unit: mg/cm^3.
[c] Experiment performed in a jacketed vessel, with 0.5 M $Na_2CO_3/NaHCO_3$ buffer at 25 °C.

operating at high pH environments with good residual activity, evidencing they can be a suitable choice with high salt concentrated solutions or organic solvents.

Besides occurring in many systems in nature, CO_2 hydration presents some limitations related to its low kinetics. CO_2 absorption benefits from higher pH values (>8.0) to form bicarbonate. Here, besides the CO_2 hydration reaction, the hydroxylation reaction also occurs. Khalifah (1971) valuated the influence of pH in two variants of human CA (hCA I and hCA II) and confirmed the positive correlation between pH and catalytic efficiency (k_{cat}/K_m). For the pH range of 5.8–8.8, an increase of 32 to 20-fold was detected for the higher pH value for hCA I and hCA II, respectively. The positive effect of pH for an increase of catalytic efficiency was shown to depend only on k_{cat}.

Gas scrubbing, utilizing carbonate solutions, has existed for decades, and this concept has been exploited and adapted for CO_2 capture as well. More recently, the utilization of carbonate-bicarbonate systems to CO_2 absorption has become a possibility in post-combustion absorption due to the lower heat of reaction and environmental impact. However, those systems have slow kinetics when uncatalyzed and can easily reach pH values greater than 10 when working at high salt concentrations (Zhang et al., 2013) (Ye and Lu, 2014). High alkali-stable CAs, therefore, can be understood to be a suitable option to be applied in such conditions – promoting the improvement of reaction rate, without losing their activity so promptly. Diverse studies utilizing alkaline media conditions assessing the CA effect in carbonate solutions were conducted to understand the kinetics mechanism behind this absorption as well as the conditions to promote higher CO_2 enhancement capture. In a system consisting of a wetted wall column with 30 wt% K_2CO_3 solution (and 0.04 of loading) and a thermostable CA (NZCA, from Novozymes) showed that 70% of its catalytic efficiency (initially at $5.3 \times 10^8\ M^{-1}\ s^1$) remained after 8 h at pH 10.6 to pH 10.8 and 323 K. In addition, when CA was added at a concentration of 5.2 μM, a 10-fold increase in CO_2 absorption to the uncatalyzed baseline was observed (Hu et al., 2017), demonstrating the high enzyme stability and no loss of CA specificity after the incubation In another evaluation, the catalytic constant rate of a commercial α-CA from Novozymes in 20 wt% carbonate-bicarbonate solution was estimated at different temperatures. The experiment took place in a stirred tank reactor working in batch mode at 298–323 K, pH 10.1–11.0, and 0.2 g/l CA. The k_{cat}/K_m at the studied conditions were estimated, and ranged from 1.2 to $1.3 \times 10^8\ M^{-1}\ s^{-1}$. This timid variation evidenced the temperature did not play a significant influence in improving enzyme kinetics at such conditions. Also, a decrease of the enhancement CO_2 absorption factor from 4.6 to 2.4 was observed (compared to the uncatalyzed reaction) when higher temperatures were utilized (313–333 K). The addition of the enzyme reduced substantially the reaction's activation energy, however, higher temperatures influence the kinetics. Also, the solubilization of CO_2 in water is affected negatively at the higher temperatures, limiting the available amount of this component to the reaction (Ye and Lu, 2014). Zhang et al. (2013) evaluated the k_{cat}/K_m of BCA in a carbonate-bicarbonate solution utilizing a stirred tank reactor, pressurized with CO_2, and temperature up to 298 K, and found a $5.97 \times 10^6\ M^{-1}\ s^{-1}$. Comparing to other evaluations at similar conditions, the k_{cat}/K_m obtained in those studies were still superior to the BCA, ranging between 1.2 and 20-fold (Russo et al., 2013) (Zhang and Lu, 2015) (Ye and Lu, 2014). The catalytic efficiency becomes even higher once some types of CA, such as the CA produced from Novozymes, which have proved to have their catalytic activity at higher temperatures than the mammalian CAs, reaching more than 97.2-fold when operating at 323 K and under high alkali conditions (Hu et al., 2017).

Luca et al. (2013) assessed the kinetic parameters of the CA from SazCA, SyCA, hCA II, and the CA from *Helicobacter pylori* (hpCA), in a stopped-flow experiment, at pH 7.5 and room temperature. The results revealed the SazCA to be one of the fastest CA so far known, showing a k_{cat}/K_m $3.5 \times 10^8\ M^{-1}\ s^{-1}$ and 3.2 to 23-fold higher catalytic efficiency than its counterparts. However, the experiments were conducted in a stopped-flow experiment, at pH 7.5 and room temperature, not employing highly alkaline solutions – which makes the comparison with other studies not straight forward. Utilizing K_2CO_3 solutions, the value of k_{cat}/K_m for NovoCA (Novozymes) varied $0.91–1.5 \times 10^4\ kg\ m^{-3}\ s^{-1}$, when assessed in a 2 M K_2CO_3 solution with carbonate to bicarbonate conversion (CTB) varying from 20 to 40% for temperatures between 298 and 313 K (Peirce et al., 2018a). Those results are similar to what was observed by Gladis et al. (2017a), where the kinetic parameters of NovoCA in potassium carbonate solutions (5–20 wt%) were assessed at 298–328 K, and showed values of k_{cat}/K_m ranging from 2.8×10^3-$2.1 \times 10^4\ kg\ m^{-3}\ s^{-1}$. Other studies assessing kinetic parameters under varied conditions of pH, temperature, and different types of CA, showed values of k_{cat}/K_m ranging 3.27×10^3-$1.2 \times 10^8\ M^{-1}\ s^{-1}$ (Peirce et al., 2018b) (Chafik et al., 2020) (Effendi et al., 2021). Table 4 summarizes kinetic parameters estimated for different CAs under varied conditions.

Favorable kinetics can imply more efficient and fast reactions; however, the enzyme reutilization is vital to make CA a competitive biocatalyst – possible through immobilization techniques. However, during the process of immobilization, the kinetics can be negatively affected. The CA's active sites might become less accessible due to the immobilization process, so it is crucial to assess the impact of the kinetic parameters as well. In Vinoba et al. (2012a), for example, BCA was immobilized via covalent bonding onto a support of (octa (aminophenyl)silses-quioxane)-functionalized Fe_3O_4/SiO_2 nanoparticles. From pNPA assays, the k_{cat}/K_m was found to be $734\ M^{-1}\ s^{-1}$, corresponding to 89.5% of the free form. From the results, the immobilization process did not substantially affect the catalytic efficiency. Nevertheless, the kinetics assessment in multiple absorption cycles to verify a loss in CA substrate affinity or eventual leaching could be valuable. Employing two different carbonate-bicarbonate solutions, one at 0.5 M Na_2CO_3–$NaHCO_3$, and the second at 10 wt% K_2CO_3 with CTB conversion 0–40%, were evaluated in terms of their kinetics. The tests were made at 298 and 313 K and a thermostable CA (NovoCA, Novozymes) with concentration varying from $0.2{\cdot}10^{-2}$ and $1.5{\bullet}10^{-2}\ kgCA\ m^{-3}$ of liquid volume. As result, the catalytic efficiency k_{cat}/K_m reached the highest values of $1.22 \times 10^3\ m^3\ kg^{-1}\ s^{-1}$, for the K_2CO_3 solution at 298 K and

CTB 0%, and 1.46 × 10^3 m^3 kg^{-1} s^{-1} for the sodium carbonates solution at 313 K. The experiments happened in a stirred cell, and the CA was immobilized by covalent bonding in a matrix of magnetic nanoparticles of Fe_3O_4 – facilitating its recovery after reaction. Furthermore, k_{cat}/K_m presented positive relation with temperature increase. The authors tested the stability of immobilized CA in carbonate solutions to verify the strength of the covalent attachment, and they reported enzyme leaching at high CA loads during catalyst preparation due to the high salt concentration environment in which causes detachment of the CA from the support. The overall results, though, showed the immobilized form could be utilized if applied under specified operation conditions (Peirce et al., 2018b). The immobilization process decreased the catalytic efficiency to 12–49% of the free form. Also, it was demonstrated that the bicarbonate ion had a negative effect on it. Despite that the enzyme tolerated high alkali salt conditions, kinetics was impaired. In a study also utilizing a high-alkali solution as solvent, a matrix composed of silica nanoparticles was chosen to minimize intra-particle effects of diffusion-phenomenon typically observed in porous materials. The kinetics parameters, using 3 different matrix particle sizes, a 0.1 M K_2CO_3–$KHCO_3$ (pH:10.5) solution, an enzyme loading varying from 45.1 to 54.9 mg/g of matrix - were estimated and revealed good results of catalytic efficiency – with values varying from 0.216–0.281 × 10^7 M^{-1} $s^{-1}$and immobilization factor between 0.36 and 0.47. Also, the immobilized form kept 80% of residual activity after incubation at 50 °C and pH 10.5 for 30 days, while its free form lost 40% of initial activity (Zhang et al., 2013). Comparing the kinetic parameters values to the previous work, where CA was immobilized in porous controlled pore glass, the immobilization factor (residual activity of immobilized enzyme over the free counterpart) has approximately doubled – confirming that the use of nanoparticles in fact improved catalytic efficiency (Zhang et al., 2011). Besides, the utilization of non-porous supports can decrease the high intra-particle diffusion resistance normally observed in porous matrixes, leading to higher activities to this form. Although kinetics play an important role, other relevant factors are important in enzyme selection for industrial applications, such as the suitability to the process, potential for reusability and robustness when exposed to harsh environments. Table 5 presents data of kinetics of immobilized CA types assessed at distinct conditions of operation.

4. CO_2 capture: boosting the process of carbon sequestration

4.1. Biomimetic CO_2 capture

4.1.1. Use of free enzymes

Over the years, the idea of employing the biomimetic route as an alternative over traditional CO_2 absorption paths has gained strength due to the simplicity of its mechanism, to evoke of the natural phenomenon of CO_2 fixation under the form of bicarbonate/carbonates. This concept integrates CA to accelerate the mechanism (Bond et al., 2000). Its eco-friendly appeal when compared to traditional absorption methods, by employing aqueous solutions instead of amine-based solvents and its lower energy requirements (Qi et al., 2016) makes the biomimetic approach to be an appealing route to be followed in the research field of CCUS.

Not only the selection of the most synergic CA types to improve the absorption reaction, but also the comprehension of how CA will perform in real or close to reality absorption systems is crucial. Different from assays, where the reaction conditions can be better controlled, the scale-up of a process introduces more variables in the system, typically affecting mass transfer in the gas-liquid interface, the overall reaction because mixing effects and of the presence of other compounds as well as operation conditions presenting instability over time.

Experiments to assess the biomimetic CO_2 absorption have been investigated by exploring both the CO_2 capture alone and CO_2 capture coupled with storage. In the first case, some possible set-ups are typically studied: the first consists of putting CO_2 in direct contact with the solution containing CA, such as by bubbling the gas into the solvent, and the catalysis happens at the same time CO_2 is provided to the solution. In the second approach, an aqueous solution is previously saturate with CO_2 and the enzyme is added to solution afterwards. The first concept, though, is quite exploited because it can mimic better the conditions of absorption columns found in the industry, where normally a stream of

Table 4
Kinetic parameters for CAs assessed under diverse conditions for free enzyme.

CA type	k_{cat}/K_m (M^{-1} s^{-1})	k_{cat} (s^{-1})	K_m (M)	Reaction conditions	References
NZCA (Novozymes)	0.27 and-5.8 × 10^8[a)]	3.40 × 10^5	12.5 × 10^{-3}	pH 7.7, 298 K, buffer solutions 10 mM[b)]	Hu et al. (2017)
SyCA	0.86–1.25 × 10^8	3.3–5.25 × 10^6	3.3–3.5 × 10^{-2}	pH 8.3, 273 K, buffer Tris HCl 20 mM + saturate CO_2 solution	Effendi et al. (2021)
NovoCA (Novozymes)	1.2–1.0 × 10^4[c)]	N/A	N/A	298–313 K, 0.5 M Na_2CO_3 $NaHCO_3$ and 10 wt% K_2CO_3–CO_2 pressurized reactor	Peirce et al., 2018b
Alpha-CA (Novozymes)	1.21–1.28 × 10^8	N/A	N/A	pH 10.1–11.0, 298–323 K, 20 wt% K_2CO_3–$KHCO_3$, CO_2 pressurized reactor	Ye and Lu (2014)
CA camel	3.27 × 10^3	47.37	1.45 × 10^{-3}	pH 7.4, room temperature, 4-NPA 0–4 mM – esterase activity	Chafik et al. (2020)
PCA	5.07 × 10^6	8920	1.76 × 10^{-3}	pH 8.0, room temperature, pNPA assay	Sahoo et al. (2017a)
SazCA, hCA II, hpαCA[d)]	3.5-,1.5-, 0.15 × 10^8	4.40-, 1.4-, 0.25 × 10^6	12.5-, 9.3-, 16.6 × 10^{-3}	pH 7.5, room temperature, 10–20 mM HEPES buffer- stopped-flow method – CO_2 saturate solution	Luca et al. (2013)
SyCA	1.1 × 10^8	0.96 × 10^6	8.4 × 10^{-3}	pH 7.5, room temperature, 10–20 mM HEPES buffer- stopped-flow method – CO_2 saturate solution	Luca et al. (2013)
NovoCA (Novozymes)	0.91–1.5 × 10^4[c)]	N/A	N/A	pH 10, 298–313 K 2 M K_2CO_3 CTB: 20–40%	Peirce et al. (2018a)
NovoCA (Novozymes)	2.8 × 10^3-2.1 × 10^4[c)]	N/A	N/A	298–328 K, 5–20 wt% K_2CO_3 – pressurized CO_2 reactor	Gladis et al. (2017a)
SspCA	9.2 × 10^6	N/A	N/A	pH 9.6, 298 K- 0.5 M Na_2CO_3–0.5 M $NaHCO_3$	Russo et al. (2013)
CA (Novozymes)	9.7 × 10^7	N/A	N/A	pH 11.0, 323 K 20 wt% PC solution – pressurized CO_2 reactor	Zhang and Lu (2015)
β and γ-CA (Bacillus sp. SS105)	N/A	N/A	1.36 (γ)-, 1.54 × 10^{-3} (β)	pH 8.3, buffer Tris-sulfate + saturate solution CO_2	Maheshwari et al. (2019)
BCA	5.97 × 10^6	N/A	N/A	pH 10.5, 279 K 0.1 M K_2CO_3/$KHCO_3$-pressurized CO_2 reactor	Zhang et al. (2013)

[a] Assessed in WWC reactor with 30% w/w potassium carbonates, at 328 K pH:11–12.
[b] Buffer using imidazole with 4-nitrophenol, dimethylimidazole with m-cresol and Ampso with thymol blue in purified water.
[c] Unit: kg m^{-3} s^{-1}.
[d] *Helicobacter pylori*, N/A: not assessed.

Table 5
Immobilized CA and kinetics characteristics under varied conditions.

CA type	Immobilization type	Matrix	k_{cat}/K_m ($M^{-1}s^{-1}$)	k_{cat} (s^{-1})	K_m (mM)	Conditions	Type of experiment	References
BCA	Cross linking + *in situ* encapsulation	Magnetic nanogel	1.89×10^4	1372.9	72.6	pH 7.0, room temperature	pNPA assay	Xu et al. (2018)
BCA	Covalent binding	Epoxy magnetic composite	34.1	0.94	27.6	pH 8.0, 313 K	pNPA assay	Ai et al. (2019)
PCA	Covalent bonding	Hetero-functional support (HFS): $CaCO_3$/H_3BO_3/SiO_2	234.7[a)]	N/A	N/A	pH 8:0, 298 K	pNPA assay	Sahoo et al. (2017b)
BCA	In-site encapsulation	ZIF-8	N/A	N/A	1.4	pH 10.5, 313 K	CO_2 experiment[c)]	Du et al. (2020)
BCA	Covalent bonding + cross-linking	Octa (aminopheny)silses-quioxane) Fe_3O_4/SiO_2 NP	783	N/A	N/A	pH 8.0 room temperature	pNPA assay	Vinoba et al. (2012a)
NovoCA (Novozymes)	Covalent bonding	Para magnetic Fe_3O_4 NP	1.82×10^2-1.46×10^3	N/A	N/A	298–313 K, 0.5 M Na_2CO_3 $NaHCO_3$ and 10 wt% K_2CO_3	CO_2 pressurized reactor	Peirce et al. (2018b)
CA[b)]	Covalent bonding (covalent coupling method)	Silica NP	0.217–0.287 $\times 10^7$	N/A	N/A	pH 10.5, 278 K 0.1 M K_2CO_3/$KHCO_3$	CO_2 hydration	Zhang et al. (2013)
BCA	Covalent bonding	Chitosan/SiO_2/γ-Fe_2O_3	303.2	4.21	13.9	pH 8.0, 298 K	pNPA assay	Sahoo et al. (2012)
BCA	*In situ* encapsulation	ZIF-8	471.8	3.0	6.4	pH 8.0, 298 K	pNPA assay	Asadi et al. (2019)
CA from *E. coli* MO1	Entrapment	Chitosan-alginate polyelectrolyte complex	N/A	N/A	19.1	pH 8.2, 310 K	CO_2 hydration	Oviya et al. (2013)

NP: nanoparticles, CO_2 hydration: CO_2 hydration assay, pNPA: Hydrolysis reaction of *para*-nitrophenyl acetate, CO_2 pressurized reactor: experiment of CO_2 capture where the drop of pressure is utilized for estimate absorption, ZIF-8: zeolitic imidazolate framework-8, N/A: not available, a) Reaction occurred through pNPA assay and the immobilized CA was incubated in MEA 30% per 1 week before experiment, b) New designed CA by a leading company in the market, c) CO_2 experiment in STR, 1 M MDEA at 313 K, c) Variant of manometric method - experiments in a STR using CA, MDEA 1 M – reactor previously pressurized with CO_2.

gas passes through a solution.

In a study conducted in a stirred tank reactor and utilizing 20 wt% K_2CO_3 solutions as solvent, BCA varying from 0 to 600 mg/l and temperatures from 298 to 303 K reached up to 12.8-fold CO_2 absorption than the uncatalyzed system. The experiments showed that higher concentrations of BCA led to high absorption rates. However, for BCA concentrations above 300 mg/l, the absorption rates did not present a proportional enhancement. Also, the temperature played an important role on the reaction, being the CO_2 sequestration inversely proportional to the temperature when employed the same concentration of the enzyme - the catalytic enhancement factor dropped from 8.8 to 3.4 when tested with 300 mg/l of the CA, 20% of carbonate to bicarbonate conversion and temperatures of 298 and 323 K, respectively (Lu et al., 2011). Differently, by using a wetted wall column, an enhancement of 10-fold in the first order rate coefficient was reached when compared to reaction without the catalyzer. This system operated for 8 h at 323 K, with NZCA concentration of 0.22 g/l and used as solvent a 30 wt% K_2CO_3–$KHCO_3$ solution (Hu et al., 2017). Both cases evidenced the potential of CA in boosting CO_2 capture more than 10-fold to the uncatalyzed forms. In the first study, higher loads of CA did not affect the absorption, meaning that substrate availability was a limiting factor. Also, higher temperatures limited the CO_2 absorption, as a result of the decreased gas solubility.

In another interesting assessment of an integrated regeneration system composed of a scrubber and stripping unit, an improvement up to 4.6-fold of CO_2 capture efficiency in the absorption column, and 4 times less energy in its desorption unit were attained. The system worked with the stripping unit at temperature of 348 K and pressure of 0.35 bar – and had the energy required for the regeneration process reduced. The evaluation tested a CA from Novozymes with concentrations varying from 1 to 4 g/l and a plateau was reached after concentrations above 2.5 g/l. The maximum CO_2 capture efficiency (for the absorption) reached during the experiments was 89% (Qi et al., 2016). The authors highlighted the possibility of increase CO_2 desorption driving force by increasing the temperature at strip unit, once the utilized CA in the round of experiments had limited tolerance to temperature. This case illustrates a good example of opportunity for the employment of an ultra-thermo stable CA variant, such as SyCA or SazCA, to promote regeneration efficiency without changing the settled process. The development of a less energy-intensive configuration adds competitivity to the technology when compared to traditional absorption processes.

CO_2 capture aiming for the formation of carbonates is possible, also referred as mineralization process. The difference between the first and this method consist in a further step, where the bicarbonate formed through absorption, is precipitated, usually by adding a solution of $CaCl_2$, forming then $CaCO_3$. Besides, this technique is generally applied to quantify the amount of CO_2 captured, and it demands no sophisticated procedure. From simple chemical reactions and unit operations easily reproduced in the lab, such as filtration and drying, total CO_2 absorbed in a reaction can be estimated.

In Faridi et al. (2017), BCA was tested for precipitation of bicarbonate onto $CaCO_3$, in a reaction with total volume of 75 ml and CA concentration 2 mg/l. CA dissolved in 15 ml of a 1 M Tris buffer with 1.5 wt% $CaCl_2.2H_2O$ was added to 60 ml of CO_2 saturate solution. The onset of $CaCO_3$ precipitation for the catalyzed reaction was 32 s, much faster than the control, which required 139 s. Also, the mass of $CaCO_3$ obtained during the precipitation revealed a 5-fold increase when compared to the uncatalyzed reaction. Employing a similar procedure, Bose and Satyanarayana (2016) performed mineralization experiments using ApCA at room temperature and Tris buffer. The improvement in the onset of $CaCO_3$ precipitation was 3.6-fold when compared to uncatalyzed reaction. The same experiment was performed utilizing BCA and results showed the ApCA was 1.6-fold faster than the mammalian type, evidencing the superiority of the CA from bacterial origin.

Sharma and Bhattacharya (2010), for example, performed CO_2 absorption in a bench scale reactor (20 ml) with a mix of PCA, MTCA and MLCA (at 4.1 mg/l) and utilized BCA as baseline for comparison. The precipitate was recovered and characterized, and from the solid weighted it was possible to determine improvement of 3.6-fold CO_2 sequestration efficiency compared to BCA. Also, Chafik et al. (2020) assessed the mineralization potential of CA from the liver of a camel, at pH 9.0 and room temperature and reached 966.7 mg $CaCO_3$ precipitated

per mg of enzyme. Comparing to Vinoba et al. (2012a), which performed mineralization experiments with BCA, this amount of $CaCO_3$ obtained for the first was 16.7-fold higher.

In experiments utilizing brucite dissolved in water (50 g/l), BCA was employed to investigate the impact on the acceleration of the carbonation reaction. The results showed the enzyme had a positive effect increase the carbonation rate in 240% over the control, when assessed at a flow 270 ml/min of CO_2 and enzyme concentration at 0.2 g/l (Power et al., 2016).

Other studies with results to be certainly highlighted presented meaningful increase of CO_2 capture, ranging between 2.4 and 10 fold (Ye and Lu, 2014) (Kunze et al., 2015) (Hu et al., 2017) (Qi et al., 2018) (Peirce et al., 2018a), enhanced kinetic rate of 1.5-fold (De Castro et al., 2020) and mass transfer increase up to 6-fold (Gladis et al., 2017a) are presented in Table 6. As demonstrated in this section, CA under free form was proved to be a feasible and promising biocatalyst in biomimetic processes. Enhancement in CO_2 absorption up to 10-fold and gas to liquid mass transfer coefficient was observed. Given the substrate availability limitations caused by high temperatures, operating such systems at moderate temperatures is recommended.

When CA was coupled with the mineralization step, a decrease in the onset of $CaCO_3$ precipitation happened. Furthermore, CA variants tested at similar conditions differed in CO_2 capture, and consequently, precipitate formation. Studies with a comprehensive assessment, including temperature effect, substrate concentration, and CA load, are scarce in the field, representing an opportunity for future research works.

Table 6
Biomimetic CO_2 absorption catalyzed with CA.

Type of reactor	CA	Reaction volume	Reaction media	CA concentration	Temperature	Observed improvement	Reference
Bench scale: jacketed stirred cell – CO_2 capture	NovoCA	⌀ 1.3 cm H = 17 cm	K_2CO_3 2–3 M, CTB: 0–40%	0–1.8 × 10^{-2} and 0.3–0.6 kg/m^3	298–313 K	E_{CA}: up to 8.2	Peirce et al. (2018a)
Bench-scale absorption regeneration system – CO_2 capture	NovoCA	⌀ 7.6 PVC scrubber H = 200 cm packing. ⌀ 7.6 cm stainless steel stripper H = 200 cm packing	23.5% K_2CO_3–$KHCO_3$ CTB:37–43%	1–4 g/l	313 K	3.9–4.6-fold CO_2 capture efficiency, 4-fold less energy in stripping column	Qi et al. (2016)
Wetted wall column - CO_2 capture	NZCA[a)]	51.7 cm^2 of surface contact between liquid and gas	30 wt% K_2CO_3–$KHCO_3$ solution	0.22 g/l	323 K	10-fold CO_2 absorption	Hu et al. (2017)
Gas liquid contactor wetted wall column - CO_2 capture	CA Novozymes	⌀ 1.2 cm H = 8.4 cm	15 wt% K_2CO_3	0.2 wt%	298–323 K	CA mass transfer enhancement: 5.9 to 6.6	Gladis et al. (2017a)
Stirred tank reactor - CO_2 capture	BCA	⌀ 10.2 cm H = 17.8 cm	20 wt% PC solutions	0–600 mg/l	298–323 K	12.8-fold more CO_2 absorption than uncatalyzed reaction	Lu et al. (2011)
Stirred tank continuous reactor (STCR) – CO_2 capture	Alpha-CA Novozymes	⌀ 10 cm H = 17 cm	20 wt% K_2CO_3–$KHCO_3$ CTB 10–40%	200–300 mg/l	298–323 K	E_{CA} 2.4–4.6	Ye and Lu (2014)
Laboratory scale spray reactor and packed absorption – CO_2 capture	CA Novozymes	Spray reactor: Basis = 10 × 10 cm ⌀ 25 mm Packed column = ⌀ 56 mm H = 230 cm packing height	10 wt% K_2CO_3	0.2 wt%	296 and 317 K	CE 4.8 at Spray reactor and 4.0 at packed column	Kunze et al. (2015)
Lab scale - Stainless-steel cell – CO_2 capture	BCA	Reaction volume: 4 ml	$NaCO_3$–$NaHCO_3$ 100 M pH 10	0.2–3 g/kg	303 K	143% more initial kinetic rate	De Castro et al. (2020)
Bench-scale absorption/ regeneration system - CO_2 capture	CA (Novozymes)	⌀ 7.6 PVC scrubber H = 200 cm packing; ⌀ 7.6 cm stainless steel stripper H = 200 cm packing	23.5% K_2CO_3 solution salts	2.5 g/l	313 K	4.7-fold of CO_2 sequestration efficiency for absorption	Qi et al. (2018)
Bench scale - CO_2 capture and mineralization	ApCA	Reaction volume: 75 ml	Buffer Tris HCl 1.5 M + $CaCl_2{\cdot}2H_2O$	0.67 mg/l	room temperature	3.6-fold faster precipitation than uncatalyzed	Bose and Satyanarayana (2016)
Bench scale - CO_2 capture and mineralization	rBhCA	Reaction volume: 75 ml	Buffer Tris HCl 1 M + $CaCl_2{\cdot}2H_2O$	2 mg/l	310 K	5-fold more precipitation of $CaCO_3$ over uncatalyzed	Faridi et al. (2017)
Bench scale reactor- CO_2 capture and mineralization	BCA	Reaction volume: 5 ml	Buffer Tris HCl 125 mM pH 8-9	30 ml/l	room temperature	966.67 mg $CaCO_3$/mg enzyme	Chafik et al. (2020)
Bench Scale reactor – CO_2 capture and mineralization	Mix microbial CAs[b)]	Reaction volume: 20 ml	Buffer Tris + saturate CO_2 solution	4.1 mg/l	303–313 K	CO_2 capture enhancement up to 3.6-fold compared to BCA	Sharma and Bhattacharya (2010)
Bench scale: Batch 125 ml sidearm flasks CO_2 capture & mineralization	Novo CA	Reaction volume: 100 ml	Solution with NaOH 0.125 M + $MgCl_2$ and 1 g/l of brucite + $MgCl_2$	0.05–0.2 g/l	297 K	Enhancement 240% carbonation rate	Power et al. (2016)

PC: potassium carbonate solution, ⌀: diameter, H: height, CTB: conversion carbonate to bicarbonate, a) CA from Novozymes, b) PCA, MTCA and MLCA, CE: catalytic effect – ratio of volume per time of CO_2 absorbed for reactions with and without CA. ECA: ratio between enhancement factors of catalyzed and uncatalyzed reactions – being the enhancement factor defined as the rate of absorption enhanced by the chemical reactions in the liquid phase to the rate of physical absorption.

Such good prospects, though, can face some limitations – especially in terms of enzyme production cost and the inability to be reused at multiples cycles. Because of that, efforts aiming to prolong the lifetime and catalytic activity are still one of the main keys to enable CA to be competitive.

4.1.2. Use of immobilized enzymes

Comparing BCA covalently immobilized on Fe_3O_4 microspheres, the free form presented higher absorption rate than the immobilized one – about 25% greater, however the reusability studies, performed through assays, showed that the immobilized enzyme was capable of keep almost 60% of its initial activity after 10 cycles, potentially increasing its overall CO_2 capture efficiency over the free form. Since the utilized matrix had magnetic properties, the biocatalyst recovery was facilitated in the end of each cycle (Lv et al., 2015). Still regarding BCA, features such as kinetics parameters, were evaluated at similar conditions of pH, temperature, and assay, but utilizing different immobilization techniques. In the first case, BCA was covalently immobilized onto a magnetic chitosan/SiO_2/Fe_2O_3 composite support, and the CO_2 absorption rate was quantified by measuring the pH drop over time. The experiments were conducted in a glass reactor with deionized water and bubbled with CO_2. A decrease in about 3 of pH value was observed in the immobilized form with the CA, but the support without the enzyme presented about 1.5 in pH value drop. Addition of CA evidenced the faster formation of bicarbonate and H^+ ions and showed a k_{cat}/K_m 303.1 M^{-1} s^{-1} (Sahoo et al., 2012). For the second approach, an *in-situ* encapsulation in microporous zeolite imidazolate framework (ZIF-8) to immobilize BCA resulted in higher activities of immobilized over the free form – about 18% more active. Also, the evaluation of the kinetic parameters revealed a k_{cat}/K_m of 471.8. M^{-1} s^{-1}. The increased activity was confirmed through a CO_2 absorption reaction of a solution pre-saturated with CO_2 in Tris HCl buffer at 25 °C, with posterior mineralization. In total, 34.3 mg and 29.5 mg of $CaCO_3$ were obtained for immobilized and free enzyme, respectively, in a reaction with volume of 30 ml and CA concentration of 0.3 mg/ml. Furthermore, after 10 repeated cycles of $CaCO_3$ precipitation, the immobilized enzyme could retain more than 60% of the initial catalytic activity of the free enzyme, revealing the notable potential for this type of catalyst in CO_2 capture (Asadi et al., 2019). Both immobilization approaches kept similar CO_2 sequestration capacity after ten cycles. Nevertheless, they differed in initial residual activity. From the *bottle-around-a-ship encapsulation* approach, where the *in situ* encapsulation occurs within the framework during it synthesis, the ZIF-8 network encages the enzyme, helps to keep the integrity, still allowing a good transfer of substrate to the CA active sites. That can explain the superior performance of this latter to the covalent bonding, which can rigidify the CA structure, decreasing the enzyme activity. Using a recombinant variant of BhCA covalently immobilized onto iron magnetic nanoparticles, the CO_2 capture to form $CaCO_3$ was evaluated. The reaction occurred using a CO_2 saturated solution, at 310 K, with enzyme concentration of 2 mg/l and 1 M Tris buffer The onset of $CaCO_3$ was 10 s – only 7.1% of the uncatalyzed reaction and the amount of $CaCO_3$ formed from the CO_2 sequestration was 140 mg and 138 mg for free and immobilized forms, respectively. The utilization of a nanoparticles support can reduce the CA unfolding and improve its performance, which can explain the similar apparent activity for free and immobilized form. Also, the amount of $CaCO_3$ precipitated was 5-fold higher than the reaction without the CA (Faridi et al., 2017).

Other examples, in which CO_2 absorption to mineralization was also performed, showed an enhancement ranging from 5 to 11-fold $CaCO_3$ precipitation (Oviya et al., 2013) (Faridi et al., 2017). For the first evaluation, CA from *E. coli* was entrapped using a chitosan-alginate polyelectrolyte complex and tested in a saturate CO_2 solution with CA at an approximate concentration of 4.3 mg/ml of immobilized enzyme per volume of solution. The reaction with the free enzyme showed 5.5% more carbonate precipitation than the immobilized form – demonstrating that the immobilization process did not affect too much the activity of the enzyme when compared to its free form. CA addition improved CO_2 capture 11-fold. When assessing the recyclability performance of the catalyst, the sequestration capacity was 53% of the initial after eight cycles of absorption (Oviya et al., 2013). The presence of bicarbonates from CO_2 absorption can destabilize the structure of some matrices, such as in chitosan and calcium alginate, probably by an occurring ion exchange between the matrix and the solution. In the case of alginate, the transfer of calcium ions into the solution, could lead to matrix collapse and enzyme release. To mitigate the problem, adding soluble alkali metal salts in the media reaction, such as calcium chloride, could provide a source of metallic ions for the bicarbonate to form $CaCO_3$, maintaining the alginate's structure. High concentrations of $CaCO_3$, though, could hinder the action of biocatalyst once it can accumulate on its bead surface, creating a barrier to the substrate reaching the CA.

The other study, where BhCA was immobilized through covalent bonding on a matrix of iron magnetic nanoparticles, indicated that the capability of precipitate $CaCO_3$ was almost the same for both free and immobilized form – approximately 920 mg of $CaCO_3$ per mg of enzyme. The reaction happened at CA concentration of 2 mg/l in a saturate CO_2 solution with buffer Tris-HCl added to calcium chloride. In this set of experiments, the catalyst could successfully keep, after 22 repeated cycles, about 50% of its initial activity. Remarkably, the time spent to initiate the precipitation of the carbonate dropped from 132 s to 10 s, for the uncatalyzed and CA immobilized reactions, respectively, and increased the $CaCO_3$ precipitation in 4.9-fold (Faridi et al., 2017). In both immobilization methods, the initial sequestration capacity was like the free form, near 100%. The covalently bonded form, though, presented superior recyclability – about 2.8-fold than the entrapped CA to reach about 50% of initial sequestration capacity, showing the covalent attachment was more robust to keep the enzyme properties and to prevent leaching, as sometimes observed in some entrapped forms. The precipitated $CaCO_3$ per mg of the enzyme was 3.8 higher for the BhCA, even with a lower enzyme concentration than the CA from *E. coli, inferring* BhCA a more suitable choice for CO_2 capture followed by mineralization. Also, it is valuable to highlight the presence of ions that could have an inhibitory effect on some enzymes, such as calcium and carbonates, which can affect enzyme activity.

Comparing to the results found by Wanjari et al. (2012), the amount of $CaCO_3$ precipitated per enzyme was much lower – around 16.14 mg $CaCO_3$/mg enzyme, and after ten cycles of absorption the catalyst showed 19% of initial capacity to form $CaCO_3$. The CA was adsorbed in mesoporous aluminosilicate and presented a catalytic efficiency of k_{cat}/K_m of 1.2×10^4 M^{-1} s^{-1}. In other study confirming the effect of CA in CO_2 capture to mineralization revealed a decrease in 5.5-fold of onset of $CaCO_3$ precipitation. The enzyme utilized (NgCA) was encapsulated in tetramethyl orthosilicate, and the immobilized form showed about 60% of CO_2 capture capacity than the free form (Jo et al., 2014).

Besides various examples of immobilized CA applied to biomimetic route, examples addressing this approach at pilot-scale are still few. However, some good examples exist where immobilized CA was tested in experimental set-ups with conditions more realistic to the ones occurred in commercial absorption processes. In a three-phase trickled bed reactor, the biomimetic CO_2 absorption was performed using SspCA (from the extreme thermophilic bacteria *Sulfurihydrogenibium yellowstonense* YO3AOP1) immobilized in a polyurethane foam and water at 298 K and liquid to gas ratio varying from 0.15 to 0.2. After rounds of experiments, it was possible to achieve up to 45% of CO_2 conversion efficiency of absorption, 19-fold more than the same absorption cycle without the enzyme. Additionally, the immobilized CA was very stable and showed no loss of activity after 30 days (Migliardini et al., 2014). It is interesting to note that despite the described thermophilicity of the enzyme, improvement of CO_2 capture at 298 K was observed with CA addition. This indicates, that SspCA may operate at a wide range of temperatures having a positive effect on CO_2 capture.

Additionally, a vertical reactor employing water as solvent and loaded with BCA cross-linked in alginate beads, and concentrations ranging up to 4 mg/l, demonstrated an increase of CO_2 absorption rate through experiments where pH values were assessed over time. Using enzyme load of 1 mg, pH values could reach almost 5.5 after 150 s of reaction, while the uncatalyzed reaction presented pH values above 6.1 for the same period. During the experiments, the reusability of the biocatalyst was tested - which maintained 61% of its initial activity after 6 rounds of CO_2 absorption. Furthermore, the biocatalyst proved to resist at lower pH values – caused by the formation of bicarbonate ions after the absorption (Zhu et al., 2016b). The authors also emphasized the viability of adapting such system to an on-site scrubber, given the positive results with the immobilized CA.

From the results displayed above, there are immobilized forms suitable to apply in biomimetic CO_2 capture since the catalytic performance seemed to be stable over repeated cycles. Further studies targeting the effect of operation conditions, such as gas liquid flow rate, the presence of high concentration of salts in the catalyst degradation (i.e. erosion effect, accumulation of solids in its surface) could be advantageous since most of current studies do not address the issue. In Table 7, CO_2 capture studies catalyzed by immobilized enzymes as presented.

4.1.3. CA-assisted accelerated weathering

More recently, the idea of employing materials and residues of industrial processes as sequestrating agents, such as silicates, carbonates, and oxide-based sub-products to promote CO_2 absorption has gained attention. For exploiting such materials in CO_2 absorption systems, though, an approach inspired in the process of mineral chemical weathering (rock and soil breaking/dissolution), where the material has its chemical composition modified (Jackson and Sherman, 1953), can be utilized. For rich-carbonate sources (i.e calcite and dolomite), for example, the weathering process occurs through the dissolution of carbonates into bicarbonates (CTB), eq. 10, similar to the Karst process. The process occurs in two steps, the first is related to the CO_2 hydration (eq. (.1)) and the second is the dissolution of the carbonate itself.

$$CO_{2(g)} + H_2O_{(l)} \leftrightarrow {}^{CA}HCO^-_{3(aq)} + H^+_{(aq)} \quad \text{eq .1a}$$

$$CaCO_3 + H^+ \leftrightarrow {}^{rev}\ HCO^-_3 + Ca^{2+} \quad \text{eq .10}$$

In nature, the limiting step of this process is associated to the natural slow kinetic of CO_2 hydration (eq. (.1)), making the CA utilization a good strategy to increase the CTB: the increase of CO_2 hydration enables higher concentration of H^+ in the solution, speeding the reaction of $CaCO_3$ dissolution. In Shen et al. (2017), CA isolated from *Bacillus cereus* was utilized to accelerate the Karst process in a CO_2–H_2O-carbonate system. The study, performed in a glass column, utilized limestone and dolomite as carbonate sources, CO_2 at concentrations ranging 350 to 10^6 ppm and CA a final concentration equivalent to 3 U/mL (unit of activity). In the catalyzed reaction, the production of bicarbonate was up to 1.34 and 1.64-fold over the positive control (with water, non-catalyzed) for the calcite and dolomite, respectively. Also, exploiting the effect of CA in carbonate systems, Liu et al. (2005) studied the effect of BCA (at 0.2 μM) in the dissolution rate (measured through increase on conductivity) of limestone and dolomite and at partial pressure of CO_2 varying from 30 to 10^5 pa. The results pointed the enzyme had a strong effect on the rock dissolution, with an enhancement up to 8.57 and 88.9-fold for limestone and dolomite, respectively.

Other materials, such as with lime-mortar and hydroxides, can react with CO_2 forming carbonates as final product (mineralization). The reaction also can be divided in two steps, where the CO_2 hydration provides the bicarbonates ions for the process of carbonation, as displayed in eq. (.11). In Cizer et al. (2018), BCA showed to bring enhancement in the overall kinetics of carbonation, by accelerating the hydration of CO_2, in saturated lime putty solutions under atmospheric conditions. The time spent for the reaction – measured from the pH value drop – was smaller in the samples where the enzyme was added to. Also, Power et al. (2016) utilized BCA to accelerate the carbonation of brucite ($Mg(OH)_2$). The experiments were conducted in a stirred reactor bubbled with CO_2, and the CA-added brucite solutions showed 240% of increase in the carbonation rate when compared to the uncatalyzed one.

$$CO_{2(g)} + H_2O_{(l)} \leftrightarrow {}^{CA}HCO^-_{3(aq)} + H^+_{(aq)} \quad \text{eq .1b}$$

$$Ca(OH)_2 + HCO^-_{3(aq)} \leftrightarrow {}^{rev}\ CaCO_3 + 2H_2O \quad \text{eq .11}$$

In Di Lorenzo et al. (2018), the effect of BCA in the carbonation of

Table 7
Biomimetic CO_2 capture experiments with immobilized CA.

Type of reactor	CA type	Volume reaction	Immobilization method	Matrix	Solvent, pH, CA concentration	Temperature	Improvements observed	Reference
Lab scale plant - trickled-bed reactor – CO_2 absorption	SspCA	⌀ 4 cm H = 60 cm	Covalent bonding	Polyurethane foam	Buffer Tris HCl 10 mM, pH 8.0, 10 mg	298 K	19.5-fold CO_2 conversion efficiency	Migliardini et al. (2014)
Sealed glass stirred – CO_2 capture	BCA	Reaction volume:100 ml	Covalent bonding	Microspheres of Fe_3O_4	Buffer Tris, pH 8.0, 2 mg/l	303 K	1.33-fold CO_2 absorption rate	Lv et al. (2015)
Water-jacketed organic glass vessel – CO_2 capture	BCA	⌀ 5 cm H = 80 cm	Entrapment + cross-linking	Sodium alginate	Deionized water, 4 mg/l	303 K	Maintenance of 61% of activity after 6 cycles of CO_2 absorption	Zhu et al. (2016b)
Lab scale – CO_2 capture and mineralization	BCA	Reaction volume: 10 ml	De novo method *in situ* encapsulation	ZIF-8 composite	Buffer Tris, pH 8.0, 3 g/l	298 K	1.16-fold higher than free enzyme and 60% of initial activity after 10 cycles of absorption	Asadi et al. (2019)
Bench scale - CO_2 capture and mineralization	rBhCA	Reaction volume: 75 ml	Covalent bonding	Iron magnetic nanoparticles (silane sized iron oxide particles)	Buffer Tris HCl, pH 8.0, 2 mg/l	310 K	4.9-fold more precipitation than uncatalyzed reaction	Faridi et al. (2017)
Bench scale - CO_2 capture and mineralization	CA[a)]	Reaction volume: 22 ml	Adsorption	Mesoporous aluminosilicates	Buffer Tris 1 M, pH 8.0, 45.5 mg/l	298 K	Reduction of time for precipitation in 3-fold	Wanjari et al. (2012)
Bench scale - - CO_2 capture and mineralization	ngCA	Reaction volume: 40 ml	Encapsulation	Tetramethyl orthosilicate	Buffer Tris 1 M + 20 mM $CaCl_2$, pH 11.0, 30 mg/l	303 K	Reduction of time for precipitation in 5.5-fold	Jo et al. (2014)

a) CA not specified, ⌀ diameter, H height.

wollastonite ($CaSiO_3$) (eqs. (.12) and (.13)) was studied. The results pointed the CA did not have effect on the silicate dissolution, however it accelerated the precipitation of the carbonates formed. Also, the authors verified that the addition of Zr-MOFs enhanced the wollastonite dissolution and highlighted that the blend of MOFS with other biocatalyst could be beneficial to the overall reaction (dissolution + carbonate precipitation). CA immobilized into MOFs, for example represents a good opportunity to be explored in this context. Also, the utilization of whole cells expressing intracellular CA have been used for enhancing the accelerated weathering of steel slag, a residue is rich in oxides and silicates. The carbonated material is an excellent candidate as filler for cement-based materials (Yi et al., 2020) (Jin et al., 2021a) (Jin et al., 2021b) (Wang et al., 2021).

$$CO_{2(g)} + H_2O_{(l)} \overset{CA}{\leftrightarrows} HCO^-_{3(aq)} + H^+_{(aq)} \quad \text{eq .1c}$$

$$CaSiO_3 + H^+_{(aq)} \overset{rev}{\leftrightarrows} HCO^-_{3(aq)} + SiO_2 + Ca^{+2} \quad \text{eq .12}$$

$$Ca^{2+} + HCO^-_{3(aq)} \overset{rev}{\leftrightarrows} CaCO_3 + 2H_2O \quad \text{eq .13}$$

Studies focusing on CA-assisted accelerated weathering for CO_2 capture applications are quite limited, having an enormous chance to expand. The research coupling CA and materials rich in carbonates, oxides, and silicates, such as combustion (and fly) ashes, cement, paper and pulp industrial residues and mining tailings, as CO_2 co-sequestrating agents, still lack. Since such industrial wastes may also contain other chemicals (sulfates, nitrates, metals) that could act inhibitory to CA, discovery, selection and characterization of novel CAs that could operate stably under such conditions is needed. Besides, re-purposing industrial waste to capture CO_2 is a valid path to promote both circular economy and climate change mitigation.

4.2. Integration of CA with amine-based CO_2 capture

Over the years, industrial CO_2 absorption capture have extensively used amine-based compounds, mostly MEA, as solvent - enabled by the fact it has a favorable kinetics and high capacity of CO_2 loading in ratio of g of CO_2 per kg of solution (Bernhardsen and Knuutila, 2017). MEA belongs to the category of the so-called simple alkanolamines which comprehends primary, secondary, and tertiary amines – including diethanolamine (DEA) and MDEA. The other group, named sterically hindered amines, has their basis on a primary or secondary amine containing alkyl radicals linked to their amino group. 2-Amino-2-methyl-1-propanol (AMP) a classic representative of this class. However, the most studied amines for applications in CO_2 sequestration are the ones belonging to the group of alkanolamines, and typically utilized in aqueous solutions of 20–30% w/w.

The mechanism in which CO_2 is bonded to amine-based solvents varies accordingly to its structure; when it happens with primary and second amines, there is the production of carbamate ion, which behaves as a weak base. The reaction occurs through a two-step reaction, with the formation of an intermediate zwitterion ion according to eq .7 and .8

$$R_1R_2NH_{(g)} + CO_{2(aq)} \overset{rev}{\leftrightarrows} R_1R_2NH^+COO^- \quad \text{eq .7}$$

$$R_1R_2NH^+COO^- + B \overset{rev}{\leftrightarrows} R_1R_2NCOO^- + BH^+ \quad \text{eq .8}$$

For tertiary amines, instead, the bicarbonate ion is formed as shown in eq. (.9)

$$CO_2 + H_2O_{(l)} + R_1R_2R_3N \overset{rev}{\leftrightarrows} HCO^-_{3(aq)} + R_1R_2R_3NH^+ \quad \text{eq .9}$$

On one hand, primary and secondary amines have a fast kinetics with CO_2, on the other hand, carbamates need a high demand of energy to be decomposed. Tertiary amines, instead, presents slower reaction rates, however their regeneration cycles are less energy-intensive and have theoretical absorption higher than primary and second amines - 1 mol CO_2/mole amine – what has been encouraging their use as potential solvents as well. The search of competitive substitutes for MEA is a topic of concern of diverse studies, and CA can play a fundamental role towards enhancing kinetics by promoting high mass transfer to the gas-liquid interface as well as decrease the time needed to reach equilibria.

Commonly, MEA is used as benchmarking for prospecting new solvents assisted by CA in CO_2 absorption – being the baseline for comparison of characteristics such as reaction rate, catalytic effect, and CO_2 absorption rate. On the other hand, MDEA, is frequently considered the baseline for desorption process, given its lower absorption heat, but much lower CO_2 absorption rate. In this way, the development of a solvent in which can reunite both characteristics – good CO_2 capture and less energy intensive desorption is targeted.

4.2.1. Use of free enzymes

Given the aggressive nature of amine compounds, the use of CA that can keep the catalytic properties when in contact with such chemicals is also crucial – under the free form, which is the most susceptible to be harmed. In a study employing a developmental CA from Novozymes, Gundersen et al. (2014) tested the solvent stability of 7 different solvents, including MEA, MDEA, and AMP, – to assess the suitability of the enzyme to be applied in post-combustion carbon capture. The CA was incubated in all selected solvents (whose concentrations ranged 1–3 M) for 150 days, at 313 K, and in pH values varying from 8 to 10. Through pNPA assays, results revealed that the enzyme kept 54% of initial activity after incubation in MDEA 3 M, versus 33% reached in MEA 3 M. Incubation in 1 M MAPA/2 M MDEA resulted in higher remaining activity than MDEA (69%) while all the others had an inferior performance. The effect of solvent concentration (1–3 M) on enzyme stability after 100 h of incubation, at 298 and 323 K, was also evaluated and revealed that the activity loss was not so accentuated. More specifically, the CA kept 75 and 78% of its initial residual activity in AMP and MDEA, respectively, at 3 M and 323 K. The results revealed the potential of this novel CA as very beneficial when looking for substitutes to MEA, given its long-term stability in amine and at varied temperatures.

A study evaluated the CO_2 capture with MDEA (15–50 wt%), AMP (15–30 wt%), and benchmarked to MEA (30 wt%) at a temperature range from 298 to 328 K, utilizing commercially supplied CA from Novozymes at 0.2 wt%. For the liquid side mass transfer enhancement, 2.8–8.8-fold increase for MDEA and a more discrete increase ranging from 1.3 to 1.4-fold for AMP were observed in the presence of the CA. When the enzyme was tested with MEA, no further increase in the mass transfer was detected – something expected given the already fast kinetics of MEA solutions and CO_2. In terms of kinetics, the solvent concentration influenced little the catalytic efficiency for MDEA, with a maximum deviation of 11.3% among all the tested solutions. However, temperature showed an inverse correlation with k_{cat}/K_m. In experiments with MDEA 30 wt%, this value was 2.3 higher at 298 K than at 328 K. Higher temperatures and concentration had an overall positive effect on the k_{cat}/K_m, for the AMP solution. Nonetheless, the more concentrated the solution, the less the temperature influenced the kinetics, which is an effect also observed when utilizing carbonate solutions. The catalytic efficiency showed 3.6 and 1.3-fold increase at 298 and 328 K, at concentrations of 15 wt% and 30 wt%, respectively (Gladis et al., 2017a).

When Leimbrink et al. (2017) assessed CO_2 absorption in a packed bed reactor utilizing MDEA at 30–50%, and CA at 0.2 wt%,a good improvement in CO_2 absorption rate compared to the uncatalyzed reaction was found. At 298 K and using different specific loads varying from 8 to 24 $m^3\ m^{-2}\ s^{-1}$, an enhancement of 7.2–9.1-fold higher CO_2 was measured. Higher temperatures (323 K), though, had a negative impact on CO_2 mass transfer to the liquid phase, and reduction the CO_2 absorption enhancement to 1.7–2.7. Also, MDEA 50 wt% was tested, and a decrease in 11–43% of the absorption capacity of the solvent was measured, being this effect proportional to higher specific liquid loads. Possibly, the high concentration of MDEA limits the formation of bicarbonate ions (and CO_2 absorption) once the amount of water available in this solution is lower than at 30 wt% MDEA solution, evidencing that

higher amine concentrations had a negative impact on CO_2 capture performance of CA. As consequence, in further enzymatic CO_2 capture studies with MDEA, the solvent concentration could be limited to values near 30 %w.

Utilizing a pilot scale unit, Kunze et al. (2015) evaluated the performance of amine solvents (MDEA and diethylethanolamine DEEA both at 30 wt%) to capture carbon, besides MEA. In an initial assessment in a spray reactor at 296 K, and CA concentration at 0.2 wt%, MDEA and DEEA showed an enhancement of CO_2 absorption rate (named catalytic effect) of 4.3 and 1.9-fold, respectively, compared to the uncatalyzed reaction. Agreeing with Gladis et al. (2017a), CA had no effect in the increase of liquid side mass transfer coefficient, showing the enzyme did not change the mass transfer kinetics in a perceptible way. Test in a packed column with 2.3 m height, MDEA solution at 317 K, and same CA concentration showed that the enhancement in CO_2 capture (named catalytic effect) was almost the same of the one observed in the spray reactor. Besides, the liquid load (u_L) in the column was varied, and a proportional positive influence in the catalytic effect was found – 3.3 to 4.2 when u_L ranged to 8–24.4 $m^3\ m^{-2}\ h^{-1}$. In comparative terms, the MDEA solution was able to capture 14 and 35% of the total of CO_2 absorbed by a MEA solution, when tested without and with the CA at 313 K. The overall mass transfer coefficient reached around 30% of the one observed with MEA. In relative terms, the enzyme enhanced the CO_2 absorption rate by 237% in the MDEA solution, however, when confronted to MEA, the overall absorption was still almost 3-fold smaller, evidencing the opportunity window for potential improvement in this type of system. As an example, for future evaluations, the system integration to a desorption unit can bring insights into CO_2 regeneration efficiency and energy demand, which could provide a more reliable comparison with MEA absorption systems. Remarkably, Liu et al. (2017) conducted an evaluation to explore if BCA would have effect in improving kinetics in aqueous solutions of MEA with high loads of CO_2. The experiments were done with MEA at 30 wt%, and BCA was added to the solutions at a concentration of 10 mg/l in a stirred reactor pressurized with CO_2. The lower the amount of CO_2 load in the solutions, the faster were the reactions of CO_2 absorption. For the high-loaded CO_2-MEA systems, CA had a positive effect both in increasing the amount of CO_2 absorbed and in decreasing the time of reaction – an effect showing the CA indeed influenced the kinetics. The results brought a different perspective in terms of CO_2 absorption with MEA assisted by CA, once diverse studies assessed it before, however not completely exploring the effect of CA in high CO_2 loading solutions. Consequently, CA could benefit MEA absorption column design once the acceleration on the CO_2 capture at higher stages of the absorber (near saturation), potentially would eliminate some absorption stages, and then reduce the column size.

Employing a pilot experimental arrangement, in a 10-m packed column with different sample collection points, parameters such as the column height, temperature, and solvent loading were evaluated to determine their influence in absorption performance. A solution of MDEA 30 wt% was tested with and without using CA. Results showed the temperature had a negative effect on the absorption, reducing from around 73 to 40 mol/h of absorbed CO_2 in the column, when evaluated at 313 and 291 K, respectively. The effect was probably caused by the lower amount of CO_2 dissolved at higher temperatures. Column height impacted also in the CO_2 absorption, being observed a sharp increase of CO_2 absorption up to 6 m-height – then, at heights of 8 and 10 m a more discrete increase was observed. Additionally, at higher liquid loads, higher the mass transfer was observed – increasing in almost 80% the moles transferred in the gas to liquid interface at 291 K when liquid load increased from 10 to 22 $m^3\ m^{-2}\ h^{-1}$. Another interesting aspect to highlight, was the positive effect of the enzyme in the desorption process: at the height of 6 m, around 77 mol/h and 32 mol/h were transferred to the amine solution in the catalyzed (at 323 K), and the uncatalyzed solution (328 K) respectively. The results evidenced CA can influence the regeneration cycle in the desorption step as well (Gladis et al., 2017b). In another study performed by the same research group, aqueous solutions of MEA and MDEA added to CA were experimented and compared. The liquid-gas ratio was varied for both solvents and showing a CO_2 efficiency capture up to 98% and 83% for MEA and MDEA with 3.5 g/l of CA, respectively. Higher L/G ratios impacted positively on efficiency capture and the enzyme has increased CO_2 absorption and mass transfer reached for MDEA experiments was almost 80% of the one observed with MEA (Gladis et al., 2019). Compared to other studies, the level of CO_2 capture efficiency was remarkable and reached values near to conventional technologies (MEA), thanks to the combination of enzyme use and technology scale-up. Performing the assessment of CO_2 efficiency capture after multiple cycles could bring a more comprehensive picture of CA activity loss after long exposure.

Alvizo et al. (2014) also performed a study in a pilot scale plant utilizing DvCA 8.0 (0.2 g/l) and with MDEA at 25%. The carbon capture plant consisted of two coupled absorption (packed ring column) and desorption units installed at NCCC facilities (National Carbon Capture Center in Wilsonville) and worked at 293–313 K and at 360–373 K, respectively. The experiments lasted 60 h, distributed in 5 days of 12 h of daily operation. As result, the enzyme successfully could maintain its activity over the period of the experiment and the carbon capture observed during the run had an average of the cycle efficiency of CO_2 capture of 63.7%. Over a period of a day and working under continuous operation, around 150 lb of CO_2 was captured. This work showed a great potential of an engineered ultra-stable CA to work in regenerative cycles and under conditions near-to-reality of industrial processes.

The utilization of new solvents rather than alkanolamines and/or MDEA have been also targeted. Ionic liquids, for example, stands out once they demand lower regeneration energy when compared to other traditional solvents, such as MEA. Also, sterically hindered amines, which have a high theoretical CO_2 loading per mol of amine, making them interesting options to be exploited in CO_2 capture processes.

In a more recent work, DvCA8.0 catalyzed CO_2 capture through the employment of novel solvent composed by amine and ionic liquid. In Sjöblom et al. (2020), an alternative solvent, consisting of a mix of MDEA (20%) and pentaethylenehexamine prolinate (PEHAp) (5%), was proposed aiming to reach both higher CO_2 absorption rates than a MDEA based absorbent and lower energy requirements in its desorption process when compared to conventional alkanolamines, such as MEA. The addition of an ionic liquid containing proline brings a protective effect for the enzyme, due to its hydrophobic character, and enhanced properties of regeneration, once it has a lower heat of absorption, given the formation of bicarbonates instead carbamate when compared to alkanolamines. During the absorption experiments, the developed solvent exhibited 5-fold higher CO_2 absorption rate than a solution utilizing MDEA 25%. Also, an increase in 31.7% of the CO_2 mass absorbed was reached for the new solvent when compared to the uncatalyzed reaction, as well as an increase in 1.4-fold in the initial reaction rate. For the desorption, the MDEA/PEHAp solvent showed 2-fold better regeneration than MEA. Here, the combination of a MDEA-stable CA variant and solvents with distinctive properties enabled not only an increased CO_2 absorption when compared to MDEA, but also aimed to enzyme preservation from inactivation during the reaction. Additionally, the benefit in the regeneration improvement is equally important to create more sustainable CO_2 absorption processes. Using sterically hindered amines, the kinetics of absorption for two different amines, 2-amino-2-ethyl-1, 3-propanediol (AEPD) and 2-amino-2-methyl-1,3-propanediol (AMPD), was evaluated in reactions with and without BCA. The experiments were performed in stopped flow experiments using both solvents over a spectrum of concentration ranging from 0.1 to 0.5 M, temperatures varying from 298 to 353 K and CA fixed at concentration of 0.1 g/l (Cihan and Orhan, 2020). Above 338 K, a sharp decrease was observed, possibly occasioned by the enzyme denaturation. Using a thermostable CA could be helpful to elucidate if the findings are related only to the loss in CA activity, or CA is still active but cannot compensate for the extent of CO_2 desorption that occurs under high temperatures.

Other studies also employing MDEA and BCA, showed improvement in of CO_2 absorption rate up to 18-fold, in a rotating packed (Wojtasik et al., 2019) and up to 1.9-fold in a vapor equilibrium device (Vinoba et al., 2013). When BCA was tested with AMP, the increase in CO_2 absorption rate varied from 1.12 to 4-fold (Li, 2011) (Novick and Alvizo, 2012). More information about the studies is displayed in Table 8.

Studies focused on assessing the CA performance over repeated absorption cycles as well as of pilot plants employing non-conventional solvents are still limited, representing a great opportunity for further research.

4.2.2. *Use of immobilized enzymes*

In Ai et al. (2019), the kinetics characteristics of CO_2 absorption of a system utilizing MDEA 10 wt% and immobilized BCA in microspheres of epoxy magnetic, were evaluated. The assessment of the kinetics parameters was performed and showed k_{cat} of 0.94 s^{-1} (1.15 s^{-1} of free form) and K_m of 27.61 mmol/l. The temperature indicated a positive effect in the k_2 of CO_2 absorption, with an increase of 2.2-fold when compared to the non-enzymatic reaction (at 313.15 K). Also, at the same temperature, the concentration of CA was varied and showed 3.4-fold higher k_2 when compared to the baseline – the higher loads of the BCA, the higher values of k_2. In terms of stability, the immobilized enzyme could keep about 92% of initial activity when incubated in MDEA at 277 K. Also, reusability tests were done, and the catalyst lost its activity at its 7th cycle showing a decreasing trend over the performed experiments. A potential limitation for immobilization techniques such as covalent bonding and CLEAs, which involve the formation of a covalent bond involving the amino group of lysine residues at the surface of the enzyme, is the potential competition for bond formation with a reactive amine instead with the amino group of lysine. Thus, a potential reason for loss of activity in covalently immobilized enzymes when used in amine-based systems could be in fact the enzyme leaching due to destabilization of covalent bonds formed between CA lysine amino groups and the support. For the CO_2 absorption experiments, the reaction took place in a stirred cell reactor, at 313.15 K and concentration of CA 5.33 mg/l. Results showed a 1.4 higher reaction rate in its beginning – increasing from 0.45 to 0.63 mmol min^{-1} for the uncatalyzed and enzymatic reaction, respectively. Additionally, the time needed to reach saturation was smaller than the baseline without catalyst, evidencing the higher constant rates for the catalyzed system and the positive effect of the CA with MDEA.

Utilizing a novel matrix composed of iron and zinc oxides and a thermal resistant CA (PCA),. Sahoo et al. (2017a) immobilized this CA through encapsulation to test its potential for utilization in both absorption and desorption processes in MEA 30 wt%. Stability studies revealed the catalyst could keep about 70% of its initial activity when exposed to a temperature of 363 K for 1 h. Furthermore, repeatability tests proved the enzyme was highly attached to the matrix – results showed no loss on activity after 15 repeated cycles, showing the method held the enzyme enclosed in the matrix. For the kinetic parameters, K_m and k_{cat} were found to be 0.26 mM and 0.97 $\times$ 10^2 s^{-1} when assessed from pNPA assays. As results of the CO_2 absorption experiment, the load of CO_2 per mol of MEA increased 2-fold compared to the uncatalyzed one. During the regeneration process, the immobilized CA enabled a desorption 2.6 higher than the neat solvent. Upon reaction with aqueous MEA solutions, the CO_2 captured forms carbamates and bicarbonates, being this second favored to desorb in the presence of CA. Because bicarbonates have lower energy of absorption, the desorption demands less energy, resulting in an overall benefit in terms of energy spent for mole CO_2 desorbed. Comparing the above immobilization methods, encapsulation brought a more superior CA stability than the covalent bonding since reactive amines can act competitively to the covalent

Table 8
CO_2 absorption with amine-based solvents and CA under free form.

Type of reactor	CA type	Volume reaction	Solvent and CA concentration	Temperature	Main improvement observed	Reference
Absorption packed column	Novozymes	∅ 0.1 m H = 10 m height of filling elements 8.2 m	MDEA 30 wt%, 0.85–3.5 g/l	323 K	Up to 3.6-fold of CO_2 sequestration efficiency	Gladis et al. (2019a)
Single-stage RPB unit	BCA	∅ 146 mm (of packing) Axial height 10 mm,	MDEA 30 wt%, 0.2 wt%	293 K	5-18-fold of CO_2 absorption rate	Wojtasik et al. (2019)
Absorption Packed column	Novozymes	∅ 0.1 m H = 10 m height of filling elements 8.2 m	MDEA 30 wt%, 0.2 wt%	293–313 K	4-fold increase of CO_2 moles absorbed per time	Gladis et al. (2017b)
Packed column with Katapak SP packing	hCA II	∅ 0.056 m packing height = 2.31 m	30-50 wt% MDEA, 0.2 wt%	293–313 K	CE: 3.3–4.2 at 313 K and 30 wt% MDEA, CE: 7.5–9.1 293 K, CE: 2.92–3.14, at 313 K and 50 wt% MDEA[a)]	Leimbrink et al. (2017)
Thermostated stirred cell type reactor	BCA	Reaction volume: 500 ml	MEA 30%, 10 mg/l	Room temperature	Time of reaction: 7000s/4500s uncatalyzed vs catalyzed	Liu et al. (2017)
Packed column with pall rings	DvCA	∅ 150 mm H = 3.15 mm	MDEA 25 wt%, 0.2 g/l	298–308 K	25-fold enhancement of overall mass transfer coefficient and carbon capture efficiency in average 63%	Alvizo et al. (2014)
Vapor−liquid equilibrium (VLE) device reactor	BCA	Reaction volume: 300 ml (with 150 ml of amine)	MEA, DEA, AMP and MDEA 5–10 wt%, 5 ppm	313 K	Enhanced K_{app}: 36-, 72-, 75-, 198% for MEA, DEA, AMP, MDEA higher than control. MDEA 1.9 -fold higher absorption rate than control	Vinoba et al. (2013)
Wetted wall column	BCA	N/A	AMP 4.1 M, 0.001 g/l	313–333 K	1.15–1.25-fold higher CO_2 absorption rate	Li (2011)
Batch scale experiments - three neck flask	DvCA	Reaction volume: 100 ml	MEA, MDEA, PEHA, PEHAp/ MDEA – 25–30 wt%, 1.5/100 v/v of CA lysate per volume of solvent	313 K	1.32-fold CO_2 absorbed (mass) for MDEA/ PEHAp compared to uncatalyzed reaction, 2-fold better regeneration MDEA/PEHAp to MEA	Sjöblom et al. (2020)
Stirred cell reactor	BCA	N/A	AMP 1–3 M, 0.1 g/l	Room temperature	1.2-4-fold higher CO_2 absorption rate	Novick and Alvizo (2012)

N/A: not available, ∅: diameter; H: height, CE: enhancement of CO_2 absorbed – molar basis – ratio catalyzed and uncatalyzed reaction, a) Experiments done with different specific liquid loads ranging 8–24 m^3 m^{-2} h^{-1}, Enhanced Kapp (%) = $(E_{app} + C_{app})/C_{app}$ where *Ekapp* is the overall absorption apparent rate constant of enzyme based system and *Capp* is the overall absorption apparent rate constant of control (pristine absorbent) system.

attachment of the enzyme with the support. Also, the system CA-MEA presented a lower value of regeneration heat, explaining the better performance for CO_2 regeneration than the neat solvent. Besides, the authors highlight that the possible interaction among support-carbamates could cause instability on the *N*–C bond, resulting in higher CO_2 desorption at lower temperatures. Other works utilized ZIF-8 to immobilize BCA, through *in situ* encapsulation. In Du et al. (2020), the enzyme kinetics parameters for free and immobilized enzyme were evaluated in home-made stirred tank utilizing 1 M MDEA at 313 K. As results, the V_m and K_m found – 1.85 mM s^{-1} and 1.39 mM were slightly different from the free form, suggesting the immobilization process decreased the CA activity. Also, the reusability was tested, and after 6 cycles 70% of the initial CO_2 absorption rate was kept. With encouraging results, Zhang et al. (2018) tested the performance of the immobilized enzyme (BCA in ZIF-8) for CO_2 absorption with MDEA at 1 M and 313 K. The biocatalyst showed improved activity 1.5-fold higher than its free form, had high stability to temperature, and great reusability once 134% of apparent catalytic activity after 6 cycles was kept. The CO_2 absorption experiments showed 5.27-fold more absorption than baseline without the catalyst and happened at 313 K. So far, very few studies utilizing amines and immobilized CA reached similar level of catalytic activity, stability to solvent, and enhancement of CO_2 capture, evidencing MOFs and CA could be a promising coupling to be investigated in this research area. Also, it is important to emphasize that, besides stability and overall activity, enzyme recovery is an essential factor to be explored when utilizing immobilized CA.

For that Xu et al. (2018) proposed a method to immobilize BCA in magnetic nanogel, through an initial step of cross-linking followed by an in-situ encapsulation. Kinetic parameters were assessed from pNPA assays and showed a value of k_{cat}/K_m 1.89×10^4 M^{-1} s^{-1} – about 39% of the free form – evidencing the immobilization process affected the enzyme activity. However, the immobilized form showed high stability at 333 K and kept almost its activity after 80 min. 10 absorption cycles for testing the reusability of the catalyst were performed, and they showed that the enzyme activity almost did not change over the cycles, evidencing the combined immobilization methods were effective for the enhancement of CA recyclability. To recover the catalyst, a magnet was used – avoiding other unit operations such as filtration or centrifugation. Absorption rate improved in 170% and 145% at 303 and 333 K, respectively, and increased overall mass and transfer coefficient in 4.1-fold at 333 K. The biocatalyst was quite stable at the high temperatures as well. The authors highlighted the importance of the biocatalyst of bearing higher temperatures given the temperature of flue gases from of industrial site be typically high as well. In conclusion, the catalyst proposed seemed to be a suitable alternative to application in industrial context, given its easy preparation, suitability to high temperatures, and with little loss of activity over multiple cycles.

Combining a matrix with magnetic properties and a MOF, Ying et al. (2021) elaborated a biocatalyst utilizing ZIF and Fe_3O_4 to immobilize BCA by physical adsorption for application in CO_2 absorption using MDEA. To test the CA stability, the catalyst was stored in MDEA 1 M at 313 K for nine days and maintained about 58% of its initial activity. Also, the reusability was evaluated, and only 54% of initial activity remained after the 4th cycle, evidencing that the adsorbed CA was unstable when exposed to the amine environment. The study also highlights that the catalyst recovery was very fast, in only 9 s, which brings a potential benefit to apply in large-scale processes, potentially decreasing the number of unit operations needed in the catalyst recovery process.

Interestingly, a novelty in terms of solvent development was proposed by Sahoo et al. (2017b) in a work in which a thermostable CA from *P. fragi* was covalently immobilized and tested in a blend composed of MEA 30 wt%, piperazine 5 wt%, and potassium carbonate 15 wt%. The enzyme and a Zn-Imidazole complex were bonded to hetero-functional support (HFS) and showed improved characteristics when tested for the developed solvent. To assess the enzyme stability in the designed solvent, the immobilized CA was stored in the blend and solution of MEA 30%. After, through a pNPA assay, the k_{cat}/K_m was calculated and revealed the newly designed blend 1.48-fold higher catalytic efficiency when compared to the only MDEA based solution. For both only CA and CA with Zn-Im complex, the HFS was able to guarantee high stability after storing the hybrid solvent and the k_{cat}/K_m decreased less than 5% after incubated for 1 h. When the enzyme was integrated with MEA in CO_2 capture, an improvement of 19% in CO_2 capture was detected. Also, the desorption process could benefit from the CA presence and showed 1.57 higher kinetics than the neat solvent. The proposal of a MEA-based solvent compatible with CA is inspirational, once it combines the high CO_2 capture typically observed with MEA but offering with lower regeneration heat due to formation of bicarbonate by CA.

Besides studies encompassing CO_2 absorption in pilot plants with amines and immobilized CA (Novozymes) are still limited in this field, however some examples exist, such as the work developed by Leimbrink et al. (2017). Employing a packed column with 2.3 m height and CA at free or immobilized on porous silica particles, CO_2 capture reaction was performed using MDEA at 30% and temperatures ranging 293–313 K. The solvent and CO_2 mixture streams run in counter-current mode. The immobilized CA enhanced CO_2 absorption in 1.47-fold, when the specific liquid load of the column was 8 m^3 m^{-2} h^{-1}. The experiments with free enzyme were carried out with a CA load 50 times higher than the immobilized form, showing only 2.3-fold higher CO_2 capture yield, which could be related to the mass transfer phenomena limiting substrate availability. Also, higher specific enzyme loads led to limiting the enhancement of CO_2 capture, however, an overall improvement occurred during all experiments. The immobilized biocatalyst increased the mass transfer resistance in the column, impairing the diffusion of the substrate towards the enzyme, which also explains the lower CO_2 capture performance compared to the free counterpart. Tests increasing the current CA concentration and the use of CAs would be helpful to verify the equipment performance limitations. Other information CO_2 absorption studies employing immobilized CA and amines are shown in Table 9. CAs have been proved to be compatible with amine-based solvents, improving CO_2 absorption. However, other variables would be valuable to explore in future research works. Experiments employing industrial exhausted gases, as done in Alvizo et al. (2014), can elucidate the inhibitory effect of NO_x and SO_x in the absorption reaction. Also, studies of enzyme kinetics amine-derivate solvents are still few.

Also, it is worth addressing efforts in process design optimization since limitations in mass transfer occasioned by the CO_2 capture devices can meaningfully affect the reaction. Improving the total mass transfer coefficient by altering the conditions of liquid and gas flow rates could be recommended. Issues related to liquid stagnation and the creation of solvent preferential paths can be especially relevant with immobilized CAs. It should be noted, though, that increasing the convection and turbulence can be detrimental to the biocatalyst structure, causing undesired effects as enzyme leaching and support wearing.

4.3. CA mimic compounds

The validation of CA for catalysts that have similar properties in CO_2 hydration has been also a topic in the context of CO_2 absorption, where the synthesized compound contains a structure that mimics the CA active site. The utilization of metallic ions (such Zn^{+2}) connected to other ligands, such as amino acid residues and chelates generally occurs in such approaches (Sahoo et al., 2018). Indicatively, mimics based on a zinc-cyclen, MOF, zinc-based eutectic solvents, zinc complexes of tripodal peptides, and other compounds have been reported the past decade as possible alternatives to CA.

The mimics have been demonstrating positive results in improving temperature and pH stability and reusability (Floyd et al., 2013) (Jin et al., 2018) (Widger et al., 2019). However, significant drawbacks related to the low activity compared to CA, and performance decrease under the presence of bicarbonates have been reported. Other major

related challenges concern the lower selectivity compared to CAs, as the mimic may have a tendency to dimerize, which introduces undesirable side reactions in the system. Moreover, the difficulties in orientating the CO_2 molecule towards the active site due to the lack of hydrophobic pocket as occurred in CAs, reduces the catalytic efficiency of the compound (Kelsey et al., 2015). Thus, mimics have not yet surpassed one of the most important advantages of enzymes as biocatalysts, which is the high specificity and exceptional selectivity, eliminating byproduct formation.

Another factor regarding the impact and cost effectiveness of (bio) catalysts is their upstream and downstream production process. Enzyme production involves the use of microorganism and their growth entails the utilization of simple and low cost chemicals, such salts and culture media components. Downstream processing is defined with simple unit operations (i.e. filtration) which do not require high consumption of hazardous chemicals or high demanding energy equipment. On the other hand, in chemical synthesis of CA mimics high temperatures can be demanded, as well as, additional purification processes, which can be a disadvantage. Additionally, the possible need of high pressure and/or temperature of some mimics to initiate the reaction can limit their application in existent CO_2 absorption systems.

5. The challenges for CA-mediated absorption scale-up

Utilization of CA as a promoter for enhancing CO_2 capture seems to be a very tangible opportunity. On the other hand, obstacles related to costs, scaling up and lack of integration to the industrial environment can impede the participation of the enzyme in CO_2 absorption at greater scales in a near future. On the other hand, CA can bring benefits if applied in industrial scales. Among them, there is the possibility of reducing investment costs, by reducing size of absorbers. Besides, decrease on operational costs are also possible, given the CA can result in lower energy duty for the regeneration step. Also, due to the enhancement of CO_2 capture per mass of solvent, lower amounts of solvent are required.

Penders-van Elk et al. (2013) assessed how CA addition in the process of CO_2 absorption would affect the absorber size. Through process simulations and employing two difference solvents (MDEA and Na_2CO_3) with 0.5–1.0% kg m^{-3} of the enzyme, the height of absorption columns was estimated. The CA had a dramatic effect in the kinetics and led to a decrease on absorber height more than 90% in both cases. As consequence, a direct impact in reducing the capital costs of the absorber unit can be expected. Economic assessments also including a stripper unit would be valuable to compare the economic feasibility of the system with traditional MEA-based systems.

Nonetheless, to reach efficient, environmentally friendly, and economically viable CO_2 capture by absorption, it is necessary to encompass all the bottlenecks that limit this process. Employment of sorbents with improved performance solvents are a must and have been continually assessed over years. As demonstrated in several studies, the gold standard MEA faces a series of limitations, such as low load capacity (0.5–0.61 mol/mol amine), corrosion and high regeneration energy. For absorption processes, solvents with good capacity of absorbing CO_2 at low pressures are imperative as well, since these set-ups operate typically up to 3 bar.

In El Hadri et al. (2017) 30 different amine-based solvents and their blends were evaluated to characterize their potential for implementation in CO_2 capture processes. As results, diamines showed a potential of CO_2 loading (per mol of solvent) higher than MEA, ranging from 0.81 to 1.35, followed by monoamines with results of 0.8–0.87 mol CO_2/mol amine (AMP and 2 TBAE). Among the compounds screened, 6 of them (2-(dimethylamino) ethanol (2DMAE), 3-dimethylamino-1-propanol (3DMA1P), 1-dimethylamino-2-propanol (1DMA2P) *N,N*-diethylethanolamine, MDEA, N,N,N'N'-Tetramethyl-1,3-propanediamine (TMPAD) and 2-ethylaminoethanol (2 EAE)) showed good CO_2 loading

Table 9
CO_2 absorption experiments with CA immobilized and amines.

Type of reactor	CA type	Immobilization method	Matrix	Volume reaction	Solvent and CA concentration	Temperature	Improvements observed	Reference
Bench scale – stirred reactor	BCA	Cross linking + encapsulation	Magnetic Fe_3O_4 nanoparticles (MNP)	Reaction volume: 0.4 l (100 ml of solvent)	MDEA 1 M, 1.12 g/l	303–333 K	1.70-fold CO_2 absorption, 45-fold of absorption rate at 60 °C, K_G 4.61-fold at 60 °C	Xu et al. (2018)
Double-stirred cell	BCA	Covalent bonding	Epoxy-based composite microsphere	∅ 6.1 cm L = 19.0 cm reaction volume:150 ml	MDEA 10.0 wt%, 5.33–26.7 mg/l	313 K	1.4-fold in initial absorption rate, 3.55-fold in mass transfer coefficient	Ai et al. (2019)
Stirred batch reactor	PCA	Covalent bonding	Hetero-functional support (HFS): $CaCO_3/H_3BO_3/SiO_2$	Reaction volume: 100 ml	CA/Zn-Im: HFS +30% MEA+7.5 wt% PZ+15 wt% K_2CO_3, 537.5 mg/l	Room temperature	1.35–1.57-fold CO_2 uptake, 1.6-fold improved desorption kinetics	Sahoo et al. (2017b)
Stirred batch reactor	PCA	Encapsulation	ZnO–Fe_2O_3 nanoparticles supported in silica	Reaction volume: up to 20 ml	MEA 30%, 0.5 wt %	Room temperature, 363 K for desorption	2.3-fold higher load of mol CO_2/mol MEA, 2.6-fold higher solvent desorption	Sahoo et al. (2017a)
STR pressurized tank	CA commercial type	*de novo method, in situ encapsulation*	MOF	∅ 4 cm H = 6 cm	MDEA 1 M, 0.05–1 g/l	313 K	2.5–5.5-fold in absorption rate, residual activity:134% after 6 operation cycles	Zhang et al. (2018)
Lab scale column-packed bed	CA microbial origin extracellular (Novozymes)	N/A[a)]	Porous silica particles	∅ 0.056 m packing height = 2.3 m	MDEA 30 wt%, 0.0038 wt%	313 K	1.47-fold absorbed CO_2 mole flow	Leimbrink et al. (2017)
Stirred tank reactor - STR	BCA	*De novo* approach *in situ* encapsulation	ZIF	∅ 4 cm H = 3 cm	MDEA 1 M, CA: N/A	313 K	kept 70% of initial activity after 6 cycles of CO_2 absorption	Du et al. (2020)

a) CA was spray coated in the porous silica particles and immobilized in pockets of a Sulzer Katapak SP, ∅: diameter, H: height, L: length, KG: total mass transfer coefficient in the gas phase.

per mol of amine and low heat of CO_2 absorption >-70 kJ/mol of CO_2. Finally, in a last assessment of the CO_2 absorption kinetics, the 2-ethylaminoethanol (2 EAE) showed a high value of k_2 to MEA – being then an interesting amine to be applied in CO_2 processes (El Hadri et al., 2017). Here, it is interesting to highlight that the study did not assess the use of CA, however there is the potential of them to be studied and combined with CA to boost its kinetics, opening a new range of opportunities for developing novel CA-solvent systems suitable to CO_2 absorption.

Under the spectra of processes, improvements can also be done. In a technical assessment performed by Beiron et al. (2019) the length of an absorber column utilizing water and NaOH as solvent was estimated as well as the mass flow needed in four industrial plants –with different levels of CO_2 emissions each one, ranging from 0.33 to 80 kg/s. The study was performed through simulations in ASPEN Plus, assuming CA is utilized under its immobilized form in a packed column filled with Sulzer-Katapak-SP. The results showed the amount of water flow rate needed varied from 3 to 6200 kg/s and the NaOH from 0.35 to 64.4 kg/s. All the assessments were found to be technically feasible. However, for plants with high levels of CO_2 emissions per year, the high demands for both water and sodium hydroxide make this alternative likely to not be economically sustainable. The study brings a perspective of substitution of alkali solvents (carbonate or amine-base) for water, which in terms of environmental impact and further disposal, is easier to be handled. However, the implementation of such system implies that a reliable water access might exist, limiting its construction to specific geographic regions. Once the model was based in existent experimental kinetic parameters for the immobilized CA, it exists the opportunity of exploring other variables such as transport phenomena effects, and different types of material for immobilization to reach a more favorable CA kinetics - which could result in more advantageous scenarios in term of CO_2 capture efficiency and costs. For that, an economic assessment is necessary to determine the real feasibility of implementing such solution.

Aiming to evaluate the techno-economic feasibility of capturing CO_2 from exhaust gases with sequestration efficiency of 90% in a power plant with capacity of 600 MW_e, two different configurations of CO_2 capture systems were assessed: one with membrane separation technology and the second composed of a CA enzymatic-assisted absorption/desorption system, utilizing potassium carbonate solution as solvent. Simulations followed by an optimization model were performed in both scenarios, reaching to minimize the costs, CAPEX and OPEX for each solution, and created solutions were compared. For the membrane technology case, the driving force of the separation process was maximized; for the enzymatic absorption technology, the relationship between the lean conversion of carbonate to bicarbonate and energy for CO_2 regeneration were verified. The scenario utilizing the aqueous CA absorption system provided more advantageous economic results and slightly lower internal electricity consumption (89.13 vs 95.14 MW, without CO_2 compression) than the one using membrane-based separation process. As a matter of comparison, the first case presented total CO_2 capture annual costs of 27.85 and 47.34 \$M/ton CO_2, the second. Thanks to the use of vacuum desorption at 0.4 bar, the employment of lower steam quality from the turbine is possible, which permits a better more efficient electricity production, especially when compared to MEA whose stripping processes requires higher temperatures (Gilassi et al., 2020). In a further work, the same authors evaluated a hybrid plant using both technologies to sequestrate CO_2. Compared to the standalone CA-assisted CO_2 absorption/desorption unit studied in their previous work, both the total costs of CO_2 captured, and total annual costs increased, being them between 36.1 and 36.5 \$M/ton_{CO2} and 137.9 to 139.3 \$M per year. However, there were some reduction in the total amount of energy required and decrease of the amount of circulating solvent from 33.4 to 33.6 to 13–19 m^3/h, what also impact as well in the consume of enzyme, potassium carbonate and electricity destined to pumps and vacuum devices. Overall, the studies show the potential of the technologies to capture CO_2 instead of MEA conventional systems; specially the enzymatic-assisted absorption, which is a most mature technology than the membrane. Drawbacks are basically related to the fact of high enzyme costs (here assumed to be \$ 480.00/kg) and overall CAPEX/OPEX, high electricity and water consumption, and still, a lower absorption capacity compared to other conventional amine processes (Gilassi et al., 2021).

Not only to determine which technology guarantees better cost-benefit in CO_2 capture, it is important also to understand the overall picture in terms of embodied CO_2 emissions and the environmental impacts are brought during the construction and life operation of a new facility. Comparing 3 different technologies, Saunier et al. (2019) performed a cradle-to-gate life cycle assessment for CO_2 capture in a coal-fired power plant with capacity of 550 MW. The systems chosen consisted of a MEA-based absorption technology (MEA process), an enzyme-assisted system employing potassium carbonate solution as solvent (CIS process) and, a separation technology which is based on the precipitation of potassium carbonate (UNO MK3). The plants were assumed to have CO_2 capture efficiency of 90%. The results showed that CIS had, for the four endpoint indicators assessed (climate change, human health, ecosystem quality and resources), 50–54% of the environmental impact the MEA-based presented. The UNO MK3 system was comparatively better than the MEA, however still had high impact than the CIS (approx. 70–78% of MEA system). The lower impact scores for the CIS technology happened basically because of no need of steam in its regeneration process and favorable kinetics. The environmental performance of the CIS process is more attractive, especially in a scenario where mitigation of climate change is a priority. For the proper selection of enzyme-assisted CO_2 capture systems, a compromise between cost-benefit, CO_2 capture efficiency and environmental costs should be targeted. Other facts such technological spillovers, market changes impacting the enzyme costs could also strengthen the technology competitiveness.

6. CA commercialization

Commercialization of CA is still a market in development, being employed by few players around world. Among them, Prospec-Tany Technogene, MP Biomedicals, Yuanye Biology, Cida, Hangzhou Fanda Chemical, Jinpin Chemical Technology and Novozymes can be mentioned (Reports absolute, 2021). By 2021, CA sales were mostly focused on lab and medical industries. The increasing interest of CA utilization in CO_2 capture systems represents a real chance of this market to soon expand. From 2016 to 2022, more than 100 new patents relevant in CA assisted carbon sequestration have been registered. Currently, CA presents high costs, which makes its application to specific niches with high aggregated value or research purposes.

In 2021, Saipem and Novozymes announced a collaboration to build a CO_2 capture facility utilizing CA. The process plant optimization will happen through enzyme innovation, and it represents an opportunity to validate the technology. This has a potential to deeply impact the CA market, once its utilization in CO_2 capture would open a completely new segment, which has great attractivity for new entrants, given the global interest in CCUS. As consequence, prices could be strongly affected with a trend to decline over the years.

7. Conclusions

Currently, the research moves towards enabling CA utilization in CO_2 absorption. The advances of techniques for enzyme improvement, the identification of more resistant novel CAs are crucial to select more suitable biocatalysts needed for operating in industrial CO_2 absorption conditions. In particular, immobilization has a fundamental role in providing enzyme stability and reusability, where the combination of immobilization methods not only leads to more stable enzyme-support attachment, but also handles with issues related to loss of specificity and mass transfer limitations characteristic from the methods.

The application of CA to enhance CO_2 capture is demonstrated to bring meaningful effect for biomimetic and enzyme-assisted amine-

based CO_2 capture, with more accentuated effect in the first group. Also, CA boosts the carbonate to bicarbonate conversion in accelerated weathering applications, highlighting that this route can gain space in the close future in CO_2 sequestration. The global industrial sector (e.g. mining, steel, paper and pulp, cement industries) produces huge volumes of fine wastes that could be utilized as feedstock for enhanced weathering in combination with CA. The construction of competitive CA assisted absorption facilities remains a challenge, since the operational costs are an important bottleneck to be targeted. CA market expansion for CO_2 capture applications can contribute to decrease of the enzyme price, as well as the development of new solvents with higher CO_2 loading and novel CAs, enabling more efficient capture.

The development of processes utilizing CA showed potential to reduce capital costs by decreasing absorption column size. Also, the environmental impact demonstrated potential to be twice less when compared to a conventional MEA absorption system. Finally, we believe that the combination of a good solvent performance allied to more efficient and environmental solutions has the potential to drive the scale-up of CA-assisted carbon capture technologies and revolutionize fast towards a carbon neutral economy.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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