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Process and biocatalyst development for CO_2 capture and conversion assisted by carbonic anhydrase

Maria Elena Russo*[a,b], Clemente Capasso[c], Antonio Marzocchella[a], Piero Salatino[a]

[a] Dep. of Chemical, Materials, and Industrial Engineering – *Università degli Studi di Napoli Federico II*, P.le V. Tecchio 80, Naples, 80125, Italy
[b] Inst. of Science and Technology for Sustainable Energy and Trasport – National Research Council, P.le V. Tecchio 80, Naples, 80125, Italy
[c] Inst. of Biosciences and Bioresources – National Research Council, Via P. Castellino 111, Naples 80131, Italy

**Corresponding author. Email address: merusso@unina.it*

Abstract

Carbonic anhydrase (CA) has been proposed as an industrial biocatalyst since the middle of the 20th century. In the last 20 years, consolidated technologies for recombinant enzyme production and immobilization have provided new opportunities to apply biocatalysis of CA in carbon capture utilization and storage (CCUS) processes. Indeed, the conventional CO_2 absorption process was reconsidered for post-combustion CO_2 capture by replacing amine-based solvents with CA-supplemented alkaline solutions. Moreover, hybrid processes for CCUS assisted by CA have been proposed, demonstrating the high potential of the combination of CO_2 enzymatic reactive absorption and biological/biocatalytic CO_2 fixation pathways. The successful development of these processes is related to the design of effective CA-based biocatalysts. Several immobilization techniques have been applied to thermostable CAs for the design of biocatalysts for CCUS. This contribution highlights the collaborative and multidisciplinary research approach undertaken by the Italian National Research Council and the University of Napoli *Federico II*. Three successful immobilization techniques applied to recombinant CA forms are discussed: covalent attachment to fine granular solids, carrier-free immobilization methods, and *in vivo*

immobilization techniques. Furthermore, we explore future perspectives on the integrated development of biocatalysts, bioreactors, and processes for CA-assisted CCUS.

Keywords: carbonic anhydrase, CO_2 capture, utilization, absorption

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1. Introduction

Biochemical engineering methodologies are rooted in the pursuit of optimal solutions that integrate the best operating conditions for biocatalysts with the operational limits of the target process, while leveraging the reliability of well-established technologies, such as those used in acid gas scrubbing units. This approach guided our research, beginning with our initial efforts to characterize carbonic anhydrase (CA) from *Sulfurihydrogenibium yellowstonense* YO3AOP1 as a promising candidate for industrial biocatalysis in carbon capture, utilization, and storage (CCUS) [Russo et al., 2013a]. Since then, it has been evident - and largely supported by early studies on acid gas reactive absorption [Alper et al., 1980] - that a sound lab-scale methodology is an essential tool to nurture the development of the process with reliable data about biocatalyst performance under conditions relevant for industrial application.

We developed a lab-scale stirred cell for CO_2 absorption tests, which allowed us to evaluate the contribution of recombinant CAs in enzymatic reactive absorption across a range of temperatures and solvent compositions [Russo et al., 2013a]. This method was further refined to assess the performance of CA immobilized on finely dispersed particles (see Section 3). The analysis of the data enabled us to evaluate the apparent kinetics of both free and immobilized CAs, which can inform the design of CO_2 absorption units using multiphase contact systems [Russo et al. 2016; Gladis et al., 2018; Shen et al., 2025].

The immobilization of CA is crucial, even though initial studies successfully demonstrated the feasibility of using free CA, as outlined in Section 2. Immobilizing CA on solid supports provides a strategy to confine the biocatalyst within CO_2 absorption units and protect it from the harsh conditions present in the desorption unit, where the solvent is regenerated, and CO_2 is recovered [Russo et al. 2013b; de Oliveira Maciel et al., 2022]. Recently, despite the maturation of CO_2 capture processes as reliable solutions for post-combustion applications in the energy sector, there has been a growing need for alternative methods of CO_2 utilization instead of relying on geological and marine storage. This need spurred the further development of CA-based processes. In this context, the immobilization of CA is especially significant, as it allows for the confinement of the biocatalyst, which is essential for converting CO_2 into valuable chemicals using continuous flow bioreactors.

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2. *Process design issues*

2.1. Solvent selection

Potassium carbonate solutions are amine-free solvents with good CO_2 absorption capacity, thus can overcome issues posed by amine corrosive effects and the high heat of regeneration. As a drawback, potassium-based solvents require promoters to enhance the absorption rate and achieve proper absorption column heights to replace amine-based technologies. Vacuum stripping is an intrinsically favored condition for CO_2 desorption from potassium carbonate solutions because of the low heat of reaction [Oexmann & Kather, 2010]. Under these conditions, the regeneration temperature can be lower than 100°C, which is consistent with the successful stability of several thermostable CAs [Reardon et al., 2014, Russo et al. 2013a,b]. This is a consistent strategy for integrating a biocatalytic process into a well-established CO_2 absorption process with thermal regeneration of the solvent. The need for versatile as well as reproducible methods for assessing CA activity under a variety of conditions is essential for the design of process layouts and proper gas-liquid contact systems for large-scale processes.

2.2. Methods for CA activity assay under CO_2 absorption conditions.

CA hydration activity assays, based on the direct conversion of CO_2 as a dissolved substrate, are limited to 0°C in alkaline buffers or 25°C with stopped-flow systems [Smith & Ferry, 1999]. The performance of CA in a variety of solvents used for CO_2 capture can be assessed through CO_2 reactive absorption tests. Systems with continuous gas feeding can be used for CO_2 mixtures, and the CO_2 concentration decay can be measured in the gas stream upon proper design. In this case, the gas-liquid contact is often based on gas dispersion by bubbling, thus the mass transfer rate is quite large and requires a high concentration of CA to substantially increase the overall CO_2 absorption rate. Closed systems can be easily handled with pure CO_2 as a gas phase (Figure 1). The mass transfer rate can be limited to 10^{-5} m/s [Russo et al., 2013; Kumar et al., 2003; Alper et al., 1980], therefore with a low enzyme concentration (1-10 mg/L, about 10^{-5}-10^{-4} mM in the case of pure CAs), a measurable contribution to the CA catalytic activity can be observed.

2.3. Gas-liquid contact systems

Absorption units for CO_2 capture processes are typically realized using towers with structured packings. In addition to considerations of material resistance to alkaline solvents, the main design issue is the compatibility of the packing geometry with biocatalyst use. In the case of free enzyme, apart from any foaming issues that may occur at high CA concentration (up to 3 g/L), the conventional structured packings offer a sound solution. Recently, rotating packed beds have been proposed to improve the mass transfer rate [Wojtasik et al 2019]. The design of the absorption unit is more complex in the case of immobilized CA. Indeed, in this case, the biocatalyst geometry should be designed to fit the conventional column packings (e.g. diluted slurries of fine particles) [Russo et al. 2016; Shen et al., 2025]. As an alternative, novel gas-liquid contact systems have been proposed to achieve both good mass transfer rates and efficient immobilized biocatalyst performance [Shen et al., 2022].

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3. Immobilization of CA

The immobilization of CA for CO_2 capture has been investigated using various techniques. This is a common practice in industrial biocatalysis, as it allows the stabilization of enzymes under operating conditions and enables the confinement of biocatalysts for continuous flow operations of a bioreactor. In the context of enzymatic reactive absorption for carbon capture, the use of free thermostable CA and solvent vacuum regeneration provided effective performance, reducing the immediate need for the development of immobilized CA. The motivation for immobilizing CA goes beyond merely stabilizing the enzyme. Whether combined with the use of carbonate-based solvents, immobilizing CA opens up possibilities for direct CO_2 utilization in the aqueous phase through biochemical, electrochemical, and biological conversion processes. Therefore, both innovative configurations and enzyme supports ready for use in conventional packed columns represent promising solutions for immobilizing thermostable CAs. Our studies focused on the use of dispersed granular solids as adaptable biocatalyst forms that are compatible with traditional absorption column configurations.

We compared three techniques to enhance CO_2 absorption rates using various forms of carbonic anhydrase (CA): an undisclosed thermostable CA from Novozymes, CA obtained from bovine erythrocytes, thermostable SspCA from *Sulfurihydrogenibium yellowstonense*, and thermostable DvCA from *Desulfovibrio vulgaris*. Table 1 shows the increase in CO_2 absorption rate when using free and immobilized CA, based on absorption tests conducted in the stirred closed cell depicted in Figure 1 [Russo et al., 2013a]. Both cross-linked enzyme aggregates (CLEA) and *in vivo* immobilization techniques proved effective in maximizing the immobilization yield and retained the activity. However, they did not enhance the CO_2 absorption rate as much as expected. Specifically, the particle size of CLEA ranged from 0.2 to 1 10^{-3} m, which resulted in poor biocatalyst effectiveness and limited access to the gas-liquid interface [Peirce et al., 2017]. The *in vivo* immobilization technique resulted in cell membrane debris that maintained good activity retention of the anchored CA relative to free CA, but suffered from low enzyme loading, which did not exceed 1-10 mg CA/g of biomass [Fabbricino et al., 2021]. The most effective technique involved attaching CA to magnetic nanoparticles via carbodiimide activation. This method maintained a consistent enhancement factor across various concentrations of immobilized CA, similar to that of the free enzyme. This result was observed for both undisclosed CA and DvCA [Peirce et al., 2018; Antonopoulou et al., 2025], with CA loading levels between 40 and 50 mg CA/g of nanoparticles.

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4. Conclusion

The selection of optimal technical solutions for enzymatic reactive absorption of CO_2 is primarily based on scientific and technical criteria focused on minimizing the desorption energy while ensuring compatibility between CA activity and stability, as well as the operational conditions of the CCUS process. Additionally, the need to upgrade existing CO_2 capture units or implement new technologies can influence the choice of different solvents, CA immobilization techniques, and gas-liquid contact systems that have emerged over the past 20 years. This variety of solutions highlights the effectiveness of the enzymatic reactive absorption of CO_2 as a versatile process with potential applications extending beyond post-combustion CO_2 capture and storage.

Moreover, a deep understanding of gas-liquid biocatalysis, transforming into gas-liquid-solid in the presence of immobilized enzymes, opens up new avenues for research on CO_2 utilization and other enzymatic conversions involving gas processing. Our contribution along this path provides a methodology to characterize CA-based biocatalysts in several solvents and temperatures relevant for post-combustion CO_2 capture. Moreover, thermostable CAs were successfully immobilized using covalent techniques, with magnetic nanoparticle attachment emerging as an effective method for deploying and recovering the dispersed biocatalyst in absorption columns designed to process dilute slurries of fine particles.

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Figures & Tables

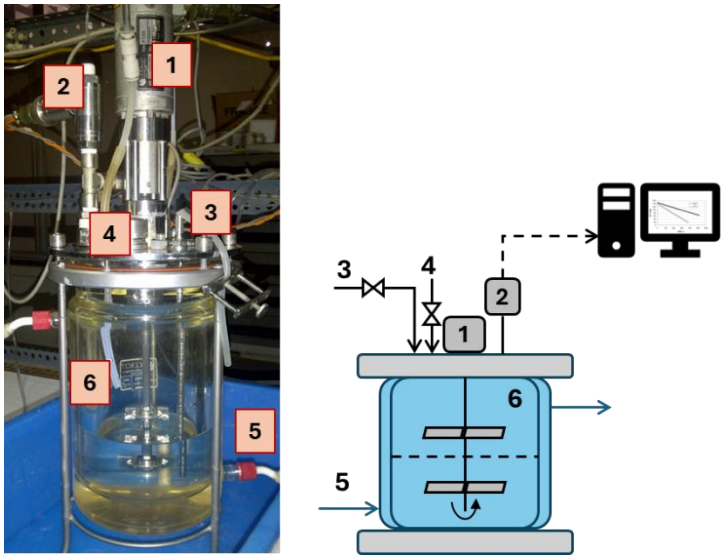

Figure 1: stirred cell system for CO_2 absorption tests with CA-promoted solvents. 1) stirrer motor with magnetic coupling; 2) differential pressure transducer (up to 350 mbarg); 3) liquid feeding port; 4) gas feeding and venting; 5) thermostatic bath water line; 6) Pirex® jacketed vessel 2L with flanged cap.

Type of biocatalyst	CA concentration, mg/L	Enhancement of CO_2 absorption rate* (-)	Ref.
Bovine CA	20	3.4	Peirce et al., 2017
Bovine CA as CLEA	100-1000	1.5-2	
SspCA	2.6-12.3	1.2-1.8	Russo et al., 2013
In vivo immobilized SspCA	6.7-22	1.3-1.8	Fabbricino et al., 2021
Thermostable CA (Novozymes) - ThCA	2.4-13	1.9-3	Peirce et al., 2018
ThCA attached to magnetic nano-particles	2-15	1.2-2	
DvCA	2-11.2	1.6-2.6	Antonopoulou et al., 2025
DvCA attached to magnetic nano-particles	2.6-11.8	1.7-2.7	

*Table 1: comparison between thermostable CAs as free and immobilized promoters of CO_2 absorption rate. *ratio between CO_2 absorption rate with biocatalyst and in pure solvent.*