CHEMICAL ENGINEERING TRANSACTIONS
VOL. 27, 2012
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at: www.aidic.it/cet
Guest Editors: Enrico Bardone, Alberto Brucato, Tajalli Keshavarz

**ISBN** 978-88-95608-18-1; **ISSN** 1974-9791
DOI: 10.3303/CET1227031

# Kinetic Assessment of Thermostable Carbonic Anhydrase for $CO_2$ Capture Processes

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The most recent challenge to reduce greenhouse gas emissions is the development of Carbon Capture and Sequestration (CCS) processes for $CO_2$ removal from flue gases. The biomimetic strategy is based on the adoption of Carbonic Anhydrase (CA) as an industrial biocatalyst as an alternative to conventional additives (e.g. amines) to increase $CO_2$ absorption rate in aq. solutions.
The present contribution concerns the kinetic assessment of a recombinant CA (SspCA) identified and characterized in the thermophile bacterium *Sulfurihydrogenibium* sp YO3AOP1. The CA characterization - long term stability included - was carried out under operating conditions close to those typically adopted in CCS plants. The absorption rate of pure $CO_2$ into aq. solutions was assessed by working out time-resolved measurements of gas pressure decay in a batch stirred reactor. The first order enzyme kinetics for SspCA was assessed at 25 °C in buffer at pH 9.6. Long term stability of SspCA at 40 and 70 °C was promising compared with that of CA from bovine erythrocytes.

## 1. Introduction

Fossil fuelled power plants are the largest fixed source of carbon dioxide and they have been considered one of the main targets for greenhouse gas (GHG) control actions. Carbon Capture and Sequestration (CCS) processes have been proposed in the last decade to face such concern (Metz et al., 2005). In particular, CCS post-combustion treatments offer as a valid option to remove $CO_2$ from flue gases produced by power plants.
The most advanced CCS post-combustion process is based on $CO_2$ absorption into ammines aqueous solutions. The process consists in the continuous flow of the aqueous solution between the absorption unit and a desorption unit. The latter unit is devoted to: i) the thermal regeneration of the solvent, and ii) to the release of a concentrated $CO_2$ stream, to be compressed for long term underground storage (Metz et al., 2005). Main drawbacks of the process are the ammines oxidation and the release of volatile compounds (ammonia). Therefore, these processes require additional treatments for the removal of toxic products and gas-washing section in the absorption columns.
The biomimetic strategy has been proposed as a possible alternative to the absorption into ammines aqueous solutions. The enzyme Carbonic Anhydrase (CA) may be adopted as a catalyst alternative to amines for the enhancement of $CO_2$ absorption rate in aq. solutions. Lacroix and Larachi (2008) have

Please cite this article as: Russo M.E., Olivieri G., Rossi M., Capasso C., De Luca V., Marzocchella A. and Salatino P., 2012, Kinetic assessment of thermostable carbonic anhydrase for co2 capture processes, Chemical Engineering Transactions, 27, 181-186
DOI: 10.3303/CET1227031

recently reviewed the most relevant scientific papers and patents concerning the technical solutions for the adoption of CA in CCS processes.
CA (EC 4.2.1.1) is an ubiquitous enzyme devoted to the catalysis of the $CO_2$ hydration reaction (Tripp et al., 2001). Its activity can enhance the absorption rate of $CO_2$ into aq. solutions whenever it is close to the gas-liquid interface (Alper and Deckwer, 1980; Alper et al., 1980). $CO_2$ dissolved in the liquid phase takes part to two parallel reactions: hydration (1) and hydroxylation (2)

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \quad (1)$$

$$CO_2 + OH^- \leftrightarrow HCO_3^- \quad (2)$$

Reaction (1) can be catalysed by CA with a turnover number up to $10^6 s^{-1}$ (Steiner et al., 1975). The design and the optimization of absorption/desorption units ask for a detailed characterization of the $CO_2$ absorption assisted by CA under operating conditions typically adopted in industrial application. Accordingly, few recent studies addressed the assessment of CA performances as biocatalyst for $CO_2$ capture (Lu et al., 2011). It is worth to remember that flue gases contain fly ashes, $NO_x$, $SO_2$, mercury and chlorine. Post-combustion $CO_2$ capture processes would be implemented as the last step of the entire post-combustion treatment (fly ashes scrubbing, catalytic conversion of $NO_x$, and flue gas desulfurization). These features must be taken into account whenever the effect of flue gas pollutants on CA activity would be investigated.
The present contribution concerns the kinetic assessment of a recombinant CA (SspCA) identified and characterized in the thermophile bacterium *Sulfurihydrogenibium* sp. YO3AOP1 according to methodology typically adopted for the characterization of gas liquid absorption in the presence of a catalyst (Alper and Deckwer, 1980; Alper et al., 1980). The study regarded the set-up of an apparatus for the kinetics assessment and the study thermostability of SspCA. Since SspCA was confirmed to belong to the $\alpha$ class of carbonic anhydrases (Capasso et al., 2012), tests were also carried out with the $\alpha$ bovine CA (BovCA) as a reference catalyst.

## 2. Materials and Methods

### 2.1 Carbonic anhydrase

The gene coding for the new CA was identified in the thermophile bacterium *Sulfurihydrogenibium* sp. It was synthesized and inserted in the expression vector pET15-b (Novagen) to produce the recombinant enzyme SspCA. Transformed *E. coli* cells were grown overnight at 37°C in Luria-Bertani broth supplemented with 100 µg/mL ampicillin. The CA expression was induced by addition of 1 mM IPTG. After 5 h induction at 37 °C, cells were collected by centrifugation, broken by sonication, and centrifuged again. The cleared extract was heated at 90 °C for 30 min and centrifuged. The heterologously expressed SspCA was purified about 3 fold with the thermoprecipitation step (Capasso et al., 2012). BovCA was supplied by Sigma Aldrich® as well as all the other chemicals adopted.

### 2.2 Experimental apparatus

Figure 1 shows a schematic view of the apparatus for absorption tests. It consisted of: a stirred cell, a liquid vessel (5), a $CO_2$ cylinder, diagnostics and ancillary units. The stirred cell reactor was a 2.25 $10^{-3}$ $m^3$ Pyrex® jacketed vessel (Applikon Biotechnology ®). Steel head of the stirred cell was equipped with ports for: gas inlet (1); gas venting (2); pneumatic liquid transfer (3); electric motor shaft (6). Two impellers provided the liquid and the gas phase mixing respectively. Technical grade $CO_2$ was (RIVOIRA S.r.l.) fed through line 1 equipped with a pressure regulator. Line 3 allows the pneumatic transfer of the liquid from the vessel into the stirred cell.
The reactor temperature was kept constant by external circulation of tap water (4) with a thermo/cryostatic bath (Julabo F33). A differential pressure transducer (DPT) (Druck, PMP4165, full scale 35 kPa) measured the pressure in the reactor. The DPT signal was acquired at 0.2 Hz and processed with a LabView 7.1® home made code.

### 2.3 Experimental procedure

The reactor was operated batchwise with respect to both gas and liquid phases. The procedure consisted in: 1) fluxing $CO_2$ to displace air from the stirred cell; 2) verifying the absence of gas

leakages; 3) pneumatic transfer of the liquid (about $5 \cdot 10^{-4}$ $m^3$ of 0.5 M $Na_2CO_3$ 0.5 M $NaHCO_3$ buffer, pH 9.6); 4) acquisition of *p* decay during $CO_2$ absorption in aq. solution.
The adopted stirring rate during the absorption step assured a flat gas-liquid interface. Reactor leakage was checked at each run to avoid misleading decrease of pressure in the reactor. Three absorption steps were performed for each liquid composition. CA was supplemented through line 3.
Stability of SspCA and BovCA at 40 and 70°C was assessed in 12 mM TRIS buffer at pH 8.3 and 100 mg/L of CA. Activity was weekly measured by means of titrimetic assay in the same buffer at 0 °C according to Worthington (1988).

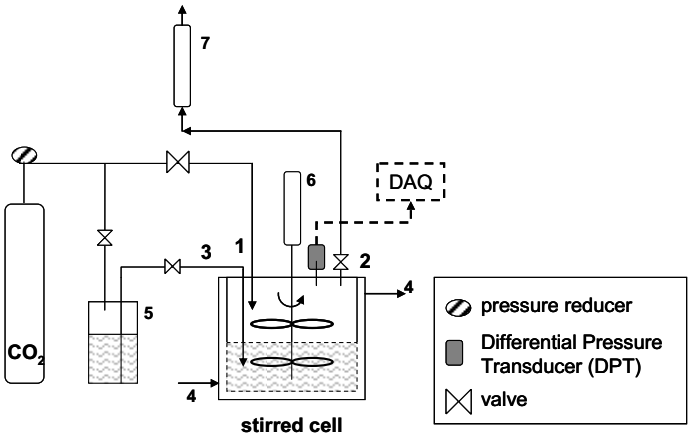


*Figure 1: schematic view of stirred cell apparatus*

## 3. Theoretical framework and data analysis

The $CO_2$ absorption rate was assessed assuming that: i) the system was isothermal; ii) the $CO_2$ behaved as an ideal gas; iii) the gas-side mass transfer resistance was negligible since pure $CO_2$ was adopted in each test; iv) the gas-liquid interface was flat and equal to the internal cross section of the vessel (*S*).
The rate of the pressure (*p*) decay in the reactor resulted form the linear regression of the *p* time-series measured during tests. Then, it was converted into the molar rate of absorption of $CO_2$ per unit surface $R_{CO_2}$ according to Eq. (3)

$$R_{CO_2} = \frac{dn_{CO_2}}{dt}\frac{1}{S} \tag{3}$$

The enhancement factor *E* is defined as the ratio between the absorption rate assessed with ($R_{CO_2}$) and without chemical reaction ($R_{CO_2,0}$). The straight physical absorption rate ($R_{CO_2,0}$) is (Danckwerts, 1970)

$$R_{CO_2,0} = k_L(c^*_{CO_2} - c_{CO_2}) \tag{4}$$

where $c_{CO_2}$* is the concentration of dissolved $CO_2$ at the gas-liquid interface assessed according to the Henry's law (H - Henry constant, $p_{CO_2}$ – $CO_2$ partial pressure):

$$c^*_{CO_2} = p^*_{CO_2} H \tag{5}$$

The rate equations of reactions 1 and 2 occurring in the liquid boundary layer are:

$$-r_{CO_2} = k_{H_2O}(c_{CO_2} - c^{eq}_{CO_2}) \tag{6}$$

$$-r_{CO_2} = k_{OH} c_{OH^-} (c_{CO_2} - c_{CO_2}^{eq}) \quad (7)$$

The concentrations of ionic species in the boundary layer may be assumed uniform during $CO_2$ absorption in carbonate/bicarbonate solutions if the criterion formulated by Danckwerts and Sharma (1966) is fulfilled:

$$c_{CO_2}^{*} \left( \frac{1}{c_{CO_3^{--}}} + \frac{2}{c_{HCO_3^-}} \right) \left( \sqrt{1 + \frac{D_{CO2} k_0}{k_L^2}} \right) << 1 \quad (8)$$

Once condition (8) is verified, that is $OH^-$ concentration is uniform in the liquid boundary layer, the linear combination of Eq.s (6) and (7) yields the overall first order kinetic constant $k_0$:

$$k_0 = k_{H_2O} + k_{OH} c_{OH^-} \quad (9)$$

Provided that the Hatta modulus $M = \sqrt{D_{CO_2} k_0 / k_L^2}$ is larger than 2, $CO_2$ absorption occurs in the presence of fast reactions. Taking into account the first order kinetics (9), the dependence of $E$ on $M$ is (Danckwerts, 1970):

$$E = \sqrt{1 + M^2} \quad (10)$$

Tests carried out adopting pure $CO_2$ as gas phase and aq. solutions at pH>8 allows to assume the $CO_2$ concentration in the liquid bulk negligible with respect to $c^*_{CO_2}$. Under these conditions, working out Eq.s (4) and (10), and taking into account the definition of $E$ it results

$$R_{CO_2} = \sqrt{k_L^2 + D_{CO2} k_0} c_{CO_2}^{*} \quad (11)$$

The mass transfer coefficient $k_L$ may be calculated from Eq. 11 by working out experimental values of $R_{CO_2}$, and values of $D_{CO_2}$, $k_0$ and $c_{CO_2}$* assessed as function of temperature and ionic strength in the adopted buffer according to Danckwerts and Sharma (1966).
The kinetics of the CA catalyzed hydration has been assessed as proposed by Alper and Deckwer (1980) assuming: i) Michaelis-Menten kinetics; ii) the typical values of dissolved $CO_2$ concentration in alkaline solutions; iii) the typical values of the $K_m$ constant about 10 mM (Steiner et al., 1975). Under these conditions it results:

$$-r_e = \frac{k_{cat}}{K_m} [CA] c_{CO_2} = k_e [CA] c_{CO_2} \quad (12)$$

where [CA] is the enzyme concentration, $k_e$ a second order kinetic constant, and $k_{cat}$ and $K_m$ are kinetic parameters. Under conditions that Eq. (8) applies, the overall first-order kinetic constant and the $CO_2$ absorption rate assisted by CA are:

$$k_1 = k_{H_2O} + k_{OH} c_{OH^-} + k_e [CA] \quad (13)$$

$$R_{CO_2} = \sqrt{k_L^2 + D_{CO2} k_1} c_{CO_2}^{*} \quad (14)$$

The ratio between Eq.s (14) and (11) gives:

$$\left( \frac{R_{CO_2}}{R_{CO_2,0}} \right)^2 = 1 + \frac{k_e [CA]}{\frac{k_L^2}{D_{CO_2}} + k_0} \quad (15)$$

Provided that operating conditions are consistent with hypothesis behind Eq. (15), the value of $k_e$ can be assessed working out the absorption rate measured at different CA concentrations. According to

this procedure, experimental values of $E^2$ were linearly regressed against [CA] and the value of $k_e$ was obtained as the constant of the regression.

## 4. Results

### 4.1 Assessment of recombinant enzyme kinetics

The experimental procedure reported in section 3 for the assessment of first order enzyme kinetics was validated by comparing the results with those reported by Alper et al. (1980) under the same operating conditions: 0.5 M $Na_2CO_3$, 0.5 M $NaHCO_3$ buffer, pH 9.6, 25 °C, 100 rpm. The mass transfer coefficient $k_L$ assessed according to Eq. (13) resulted 4.5 $10^{-5}$ m/s, close to the value reported in the literature (4.9 $10^{-5}$ m/s) for a similar system (Alper et al., 1980). Figure 2A shows the enhancement factor *E* vs. BovCA concentration. Data points refer to results by Alper et al. (1980) and to experimental assessments. According to Eq. (17), the linear regression of each data set provided the $k_e$ value: 0.836 L/(mg s) and 0.735 L/(mg s), respectively for literature data and present work data.

The successful comparison with results reported in the literature proves the soundness of the reported procedure for the assessment of first order $CO_2$ hydration kinetics of any kind of CA.

Figure 2B reports data assessed during absorption test in the presence of SspCA at 25 °C in carbonate/bicarbonate buffer at pH 9.6 expressed in terms of $E^2$ vs. CA concentration. The value of $k_e$ resulted 0.283 L/(mg s).

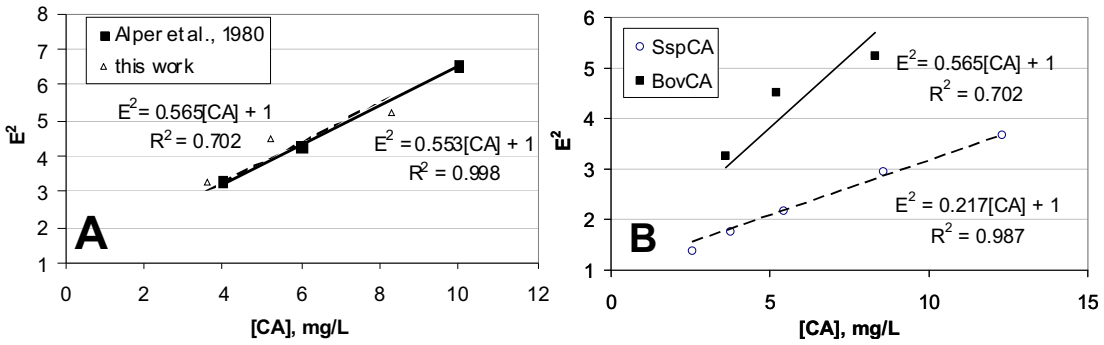


*Figure 2: $E^2$ vs [CA] for $CO_2$ absorption in 0.5 M $Na_2CO_3$ 0.5 M $NaHCO_3$ buffer (pH 9.6) at 25°C. A) BovCA data, comparison with data available in the literature. B) data related to BovCA and SspCA.*

### 4.2 Long term stability at high temperature

Figure 3 reports data related to stability tests carried with SspCA and BovCA in 12 mM TRIS buffer (pH 8.3) at 40 and 70 °C. Exponential regression of single set of data has been carried out and the half-life of each enzyme is reported in Table 1.

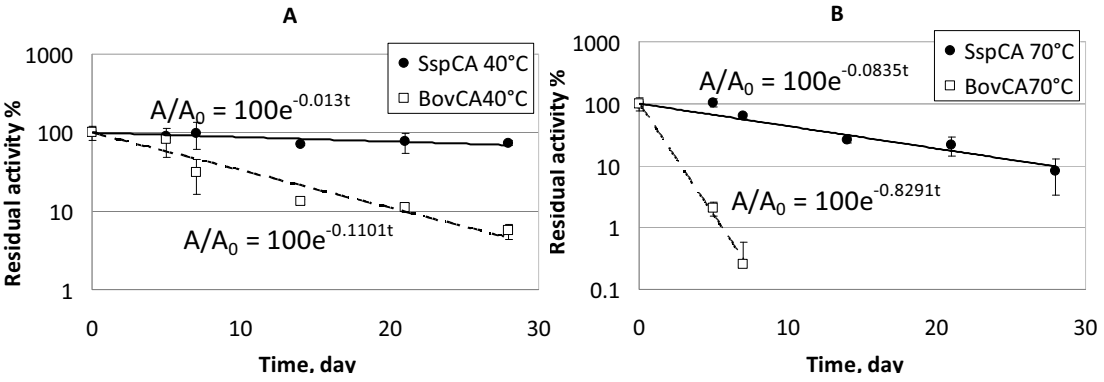


*Figure 3: Residual activity vs time assessed for SspCA and BovCA at: A) 40 °C, and B) 70 °C.*

*Table 1: Half life (days) of CA at different temperatures in 12 mM TRIS buffer (pH 8.3)*

| Temperature | BovCA | SspCA |
|---|---|---|
| 40 °C | 6 | 53 |
| 70 °C | 0.8 | 8 |

## 5. Final remarks

The reported results prove the soundness of the system adopted for the assessment of CA kinetics during $CO_2$ absorption. The first order kinetic constant assessed for bovine CA are confirmed by data reported in the literature.

The procedure has been applied to the recombinant SspCA. The enzyme was identified in the thermophile bacterium *Sulfurihydrogenibium* sp. and it has been considered as a possible industrial enzyme for CCS processes. On one hand, the kinetic characterization pointed out that the activity of SspCA at 25 °C was satisfactory even though about 2.5 times lower than that expressed by BovCA. On the other hand, the half-life of BovCA at 40 and 70 °C - operating temperatures of industrial plants - are definitely low with respect to that of SspCA. These results were expected since the temperature of 25 °C is likely closer to the optimal temperature of BovCA than to the optimal temperature of a thermophilic enzyme such as SspCA. Although the cost of SspCA has not been estimated yet, it can be expected that SspCA may be available at higher throughput and at lower price than BovCA.

Altogether, results point out that SspCA is a good candidate as homogeneous catalyst for the enhancement of absorption in $CO_2$ capture units. Further investigations will be accomplished to assess kinetics of SspCA into alkaline solutions at temperatures close to those adopted in absorption column.

**Acknowledgment**

Financial support from MiSE-CNR “Cattura della $CO_2$ e utilizzo pulito dei combustibili fossili” is acknowledged.

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