The Resilience of Carbonic Anhydrase Enzyme for Membrane-based Carbon Capture Applications

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Abstract

A microbial carbonic anhydrase was immobilized onto the surface of hollow fiber membranes to enhance the absorption rate of carbon dioxide into an aqueous 30 wt% potassium carbonate solvent at pH 10-12. The performance of the immobilized enzyme was investigated over long time frames to determine the suitability of this approach for post-combustion carbon capture. The immobilized enzyme was able to maintain activity for up to 80 days at 25°C even when initially exposed to pH 12, however lost activity rapidly when exposed to such high pH at 50°C and subsequently was completely deactivated within 4 days. The effect of sulfur dioxide and nitric oxide that are present in post-combustion flue gas streams was investigated by contacting enzyme immobilized onto flat planar membrane coupons directly with the dry gases and with solutions containing the anions associated with the gases, namely sulfate, sulfite, nitrate and nitrite. The immobilized enzyme maintained at least 80% of its activity even in the presence of 200 mM of these anions or 500 ppm of the dry gases. Our findings indicate that the enzyme is not significantly affected by these toxic gases or their associated anions. Rather, the combination of high pH and ionic strength of the solvent at a high absorption temperature causes a reduction in the enzyme activity and affects the performance of the absorption process.

1) Introduction

Carbonic anhydrase (CA) has been researched extensively for its rapid hydration of carbon dioxide (CO_2) into bicarbonate (HCO_3^-) ions. The enzyme acts through a coordinated metal cation, usually Zn^{2+} , which binds a water molecule to form a hydroxide ion in the first step of the catalytic cycle. This hydroxide ion then reacts with the CO_2 molecule to form a HCO_3^- ion, with hydration rates ranging from 10^4 - 10^6 molecules of CO_2 per molecule of CA per second [1-7]. This hydration can be beneficial for increasing the absorption efficiency of CO_2 into a solvent for carbon capture and storage (CCS) operations. The enhanced absorption rate is especially useful for solvents that exhibit absorption kinetics that are much slower than conventional monoethanolamine (MEA), including tertiary amines [8-11] or potassium carbonate (K_2CO_3) [12].

Within a carbon capture absorption unit, the enzyme must contend with not only elevated temperatures of 30-50°C [13, 14], but also with pH levels of up to 12 (for fresh 30 wt% K_2CO_3) [15] and a high ionic strength environment. Enzyme stability or activity data obtained at ambient temperature, lower pH and low ionic strength [16-18] do not necessarily translate into similar performance under these harsher industrial conditions. Recent work has focused on the development of pH and thermally stable CA variants that can withstand these harsher conditions [16, 17]. Zhang *et al.* [18] have shown that a microbial CA variant obtained from Novozymes was able to maintain 60% of its activity when kept in a 0.1 M K_2CO_3 - KHCO_3 solvent (pH 10.5) at 50°C for 30 days. Gundersen *et al.* [19] showed that a similar Novozymes CA was completely deactivated after exposure to pH 4 for 100 h at ambient temperature, while 45 to 70% of the residual activity was retained after a similar period at pHs between 5, 6 and 12. Enzyme stability in a range of capture solvents was also tested. For example, in 1 M K_2CO_3 , 30% of the initial enzyme activity remained after 150 days at pH 10. Salmon and House [20] demonstrated that a similar Novozymes CA variant

had a low activity loss rate at 40°C in 20 wt% K₂CO₃ at 37.5% carbonate-to-bicarbonate conversion, but that the rate of activity loss increased exponentially as the temperature increased, especially at temperatures above 60 °C.

In addition to the high ionic strength and pH of such carbon capture solvents, the CA must also be tolerant of the sulfur oxides (SO_x, which is mainly SO₂ with some SO₃) and nitric oxides (NO_x, typically 99% NO, with balance NO₂ and N₂O) found in post-combustion flue gas streams at concentrations of 100-300 ppm [2, 8, 21-26]. While NO is not readily soluble, it readily reacts with any oxygen present to form NO₂, which in turn is readily hydrated to nitrite and nitrate ions through the following reaction [27]:

Sulfur dioxide is readily hydrated to form sulfite ions through Equation 2 [28]:

However, in practice, these anions are readily oxidized to sulfate (SO₄²⁻) and nitrate (NO₃⁻) ions. The concentrations of SO₄²⁻ and NO₃⁻ found in a typical MEA solvent post-absorption are typically at 15-25 mM and 10-25 mM, respectively [29]. Conversely, the concentrations of sulfite and nitrite are much lower as well [29].

Most monovalent ions (including HSO₃⁻ [30] and NO₃⁻ [1, 2, 31]) are known to inhibit CA activity at neutral pH. In mammalian CAs, these anions bind to a site that is near the active metal cation and displace the ‘deep water’ molecule that is required for the hydration reaction to proceed [32], which results in the formation of an inactive enzyme complex [31] (see Figure 1). However, the effect of these anion inhibitors is said to be reversed by an increase of pH [33]. As an example, Kernohan shows that 0.1 M NO₃⁻ reduces the ability of bovine CA to catalyse CO₂ hydration to around 7% of the uninhibited value at pH 5.4 but only to around 70% of the uninhibited value at pH 8.3 [31]. Conversely, Pocker and Diets [34] indicate that by increasing the pH to around 9, anionic inhibition of CA-catalyzed HCO₃⁻

dehydration and p-nitrophenyl acetate (PNA) hydrolysis is abolished, while inhibition of CO₂ hydration persists.

Other reports indicate that monovalent ions are more likely to cause inhibition of CA than divalent ions [30-32], (including specifically SO₄²⁻ [35]). Indeed one report indicates that high SO₄²⁻ concentrations of up to 1 M could in fact enhance the esterase activity of human CA [32]. Conversely, Sharma and Bhattacharya observe activity losses to between 53 and 84% of the fresh enzyme upon exposure of four different strains of CA to 200 mM SO₄²⁻ at pH 8 [1]. Indeed, conflicting reports for different CA variants [1, 2, 36-38], make it difficult to make broad conclusions.

Figure 1. Schematic drawing of an uninhibited human CA showing the ‘deep water’ needed for CO₂ hydration (A) and its displacement by a bisulfite (B) anion. Reproduced from [30].

Furthermore, there has only been limited work to date considering the inhibition of CA activity in the presence of these monovalent and divalent anions at the pH and ionic strengths typical of carbon capture. Lu *et al.* [39] showed that the presence of 0.9 M sulfate, 0.7 M chloride or 0.2 M nitrate within 20 wt% potassium carbonate solutions with 20% CO₂ loading and at 50°C resulted in less than 10.2% loss of enzyme activity over short time frames. Zaks and Reardon [40] evaluated the impact of similar anions at 8°C in a similar 20 wt% potassium carbonate solution at 25% loading (pH 10.1) but again only over very short

time frames. They showed that the Novozymes enzyme was tolerant to any concentration of sulfate and retained 80% of its activity at 0.25 M nitrate concentration.

In our previous work [41], we immobilized thin layers of a modified thermostable CA onto the external side surface of a hollow fiber membrane via the layer-by-layer (LbL) adsorption technique to ensure that the CA was as close to the gas-liquid interface as possible for maximizing the efficiency of the adsorbed CA [42]. A schematic of this process is shown in Figure 2:

Figure 2. Polyelectrolyte layers (shown as random coils) and CA molecules (shown as ovals) are sequentially adsorbed into a film onto the surface of a membrane. CO₂ is pumped through the lumen side of the hollow fibers and diffuses through the membrane and the LbL film into the liquid solvent, which is pumped through the shell side. The CA is immobilized onto the shell side for facilitating the CO₂ absorption reaction.

These layers were able to reduce the wetting of the microporous membranes and increase the overall mass transfer coefficient for CO₂ transport significantly. The LbL coated enzyme retained 70 to 80% of activity after exposure to pH 12 at ambient temperature for 72 h [43]. However, other workers have shown that the LbL coating may be compromised at pH levels of more than 11 [44], due to a deprotonation of the polyallylamine hydrochloride (PAH) polyelectrolyte [45-47].

Our work to date has used pure solutions and gases that were free of NO_x and SO_x, as well as their corresponding ions. This paper aims to examine the effects of these gases and anions on the activity of immobilized CA at pH and ionic strengths comparable to the most aggressive intended industrial application and over extended time frames. We specifically analyze the impact of 30 wt% K₂CO₃ (2.8 M) with pH 10-12 corresponding to loadings of 0-0.15. The catalytic efficiency of the immobilized CA is also monitored over long-term operations with 30 wt% K₂CO₃ at different temperatures to determine the thermal tolerance of both the CA and the coating used to immobilize the enzyme.

2) Materials and Methods

2.1 Materials

Polyethyleneimine (PEI, 25 kDa), polystyrene sulfonate (PSS, 70 kDa) and polyallylamine hydrochloride (PAH, 10 kDa) were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). Hydrochloric acid (HCl, 37%) and sulfuric acid (H₂SO₄, 98%) were purchased from Scharlau (Gillman, SA, Australia). Sodium nitrite (NaNO₂), sodium nitrate (NaNO₃), sodium hydroxide (NaOH), sodium sulfite (Na₂SO₃), potassium sulfate (K₂SO₄), and trishydroxymethylaminomethane (Tris) were obtained from ChemSupply (Gillman, SA, Australia). Potassium carbonate (K₂CO₃, ≥ 99%) was obtained from Thasco Chemical Co. Ltd (Bangkok, Thailand) and dissolved in tap water to prepare a 30 wt% solution at pH 12. Purified water was obtained from an Elix purification system (Merck Millipore, Kilsyth, VIC, Australia) with a resistivity > 5.0 MΩ cm, while ultrapure water was obtained from a Milli-Q purification system (Merck Millipore, Kilsyth, VIC, Australia) with a resistivity of more than 18.2 MΩ cm. The gases, including carbon dioxide (CO₂, 99%), nitric oxide (NO, 1000 ppm in N₂), sulfur dioxide (SO₂, 1000 ppm in N₂) and H₂S (1000 ppm in N₂) were supplied by Coregas (Thomastown, VIC, Australia).

Tris buffer (50 mM, pH 7.2 or 6.4) was prepared in ultrapure water and the pH was adjusted by dropwise addition of hydrochloric acid (1 M), in line with the activity tests that were conducted in our previous work [43]. The polyelectrolyte (PEI, PSS, and PAH) solutions were all prepared in Tris buffer (pH 7.2) at a concentration of 1 mg mL⁻¹.

The CA variant used in this work was a developmental microbial CA produced by Novozymes A/S (Bagsværd, Denmark) via a fermentation and recovery process. The recovered liquid was stored at -4°C and only thawed to room temperature when required for use. This solution was diluted to 2% (v/v) with Tris buffer (pH 7.2) prior to LbL adsorption.

Flat sheet polypropylene (PP) membrane coupons were obtained from Sterlitech Corporation (Kent, WA, USA) with a pore size of 0.10 µm, a thickness of 75-110 µm and a diameter of 47 mm (17cm² membrane area) (information provided by the supplier). Nonporous polysulfone hollow fiber membranes that were coated with a 0.5 µm layer of polydimethoxysilane (PDMS) were supplied by Airrane Co. Ltd. (Daejeon, Korea). Hollow fiber membrane modules were prepared with 20 of these hollow fibers sealed within each membrane module (Table 1).

Table 1. Membrane Module Design Parameters

Fiber Internal Diameter d_{in} (mm)	0.30 ^a
Fiber External Diameter d_{out} (mm)	0.45 ^a
Module Inner Diameter (cm)	0.37
Effective Module Length (cm)	20.5
External Membrane Area (cm ²)	58
Module Hydraulic Diameter d_H (mm)	0.81
Packing Density (%)	30

^aThese values were obtained from cross-sectional scanning electron microscopy (SEM) images.

2.2 Layer-by-Layer Adsorption of Enzyme

The flat sheet PP membrane coupons were coated with polyelectrolyte solution to form a PEI/PSS/PAH/[PSS/PAH/CA]₂ film in the same manner as in our previous work [43]. Approximately 5 mL of polyelectrolyte solution was pipetted onto the membrane surface and spread evenly with a paintbrush at $23 \pm 2^\circ\text{C}$ to deposit a polyelectrolyte layer. These solutions were kept in contact with the membrane for 3 min before washing with excess ultrapure water for 5 min. Subsequent layers were then deposited in the same manner for fabricating a polyelectrolyte film via LbL adsorption.

Similarly, adsorbed polyelectrolytes in the form of a PEI/PSS/PAH/[PSS/PAH/CA]₂ film were coated onto the outside surface of the hollow fibers as described in our previous work [41]. In this case, polyelectrolyte solutions were pumped through the shell side of the hollow fiber membrane module using a peristaltic pump at $23 \pm 2^\circ\text{C}$. Each solution was kept in contact with the hollow fibers for 5 min to allow the polyelectrolytes to adsorb onto the hollow fibers, followed by a wash step with ultrapure water for 10 min.

2.3 Flat Sheet Membrane Experiments

In initial experiments, moist PP membrane coupons with adsorbed enzyme were placed in a stainless steel vessel. The vessel was evacuated and then pressurized with 1000 ppm of SO₂ or NO in N₂ to 2 bar abs, giving a final impurity partial pressure of 200 Pa. Other membrane coupons were dried under a stream of N₂ gas for 3 min before being placed in the vessel. In this case, the vessel was purged with N₂ at atmospheric pressure (1 bar abs) and pressurized with 1000 ppm of SO₂ or NO in N₂ to 2 bar abs for a final SO₂ or NO concentration of 500 ppm in N₂, resulting in a final impurity partial pressure of 100 Pa abs. The membrane coupons were removed from the vessel periodically to determine the enzyme

activity. After each removal, the vessel was purged with N₂ and re-pressurized again with 500 ppm of SO₂ or NO in N₂.

Other PP membrane coupons were immersed in Tris buffer (pH 6.4) containing NaNO₂, NaNO₃, Na₂SO₃ or K₂SO₄ at varying concentrations (50-200 mM). These solutions were kept at ambient temperature (23 ± 2°C) and the membranes were removed from the solutions periodically for testing as well after being dried under a stream of N₂ gas. In some experiments, the pH of the Tris solutions was adjusted by dropwise addition of NaOH (1 M) or HCl (1 M) to pH 2 and 12 for determining the CA activity upon exposure to extreme pH conditions. The pH of the solutions was monitored and found to remain approximately constant throughout the incubation period. The membranes were washed for 3 min with ultrapure water prior to conducting any activity tests to remove any residual HCl or NaOH on the membrane.

The enzyme activity on the flat sheet membranes was assayed according to the method used in our previous work [43] as well as by Vinoba and co-workers [48, 49]. A saturated CO₂ solution was prepared by passing a CO₂ gas stream into a bottle of ultrapure water at 23 ± 2°C for 1 hour. Approximately 40 mL of Tris (pH 6.4) was added to 100 mL of the saturated CO₂ solution before the membrane coupon was immersed into the mixture. The mixture was stirred for 1 minute followed by the removal of membrane coupon before the addition of 20 mL of CaCl₂·2H₂O solution (5.3% w/v) and 10 mL of NaOH solution (1 M) to quench the reaction and precipitate the Ca(HCO₃)₂ as CaCO₃. The precipitated CaCO₃ was then filtered, dried in an oven at 80°C overnight and weighed to determine the quantity of CaCO₃ produced. The results presented are the average of two replicate membranes, with the error bars based on one standard deviation.

2.4 Operation of the Hollow Fiber Membrane Contactors

A pilot plant gas absorption unit that was described previously [12] was utilized for these experiments. Pure CO₂ was fed to the lumen side of the enzyme-coated hollow fibers at 1.0-1.5 bar and 30 wt% K₂CO₃ solvent was pumped through the shell side of the fibers at a pressure differential of 0.1 bar above the gas pressure. The flowrate was controlled by the pump speed to achieve a solvent Reynolds number between 20 and 60. In long term experiments that lasted for up to 600 hours, spent solvent was pumped through a second PDMS hollow fiber module at elevated temperatures (70-100°C) at flow rates of 0.01-0.03 L min⁻¹ to release the excess dissolved CO₂ for solvent regeneration. After the first 10 h of absorber operation with fresh solvent at pH 12, regenerated solvent (pH 10-11) was pumped back into the absorber for the remainder of the experiment and then continuously recirculated between the absorber and the regenerator hollow fiber modules. The temperature of the regeneration operation and the solvent flow rate were controlled to maintain a consistent K₂CO₃ solvent concentration for recirculation into the absorption unit at a CO₂ loading of 0.1-0.15 and a pH of 10-11, while the feed solvent flow rate for the absorber was kept constant at 0.05 L min⁻¹. The CO₂ loading for this solvent is defined as the ratio of bicarbonate to potassium concentration (Equation 1):

$$Loading = \frac{[HCO_3^-]}{[K^+]} \quad (3)$$

Separate hollow fiber absorption experiments were conducted with 200 mM NaNO₃ and 200 mM K₂SO₄ added to fresh 30 wt% K₂CO₃ (pH 12) to determine the effect of the dissolved ions on the activity of the CA enzyme.

Samples of spent solvent exiting the contactor were analyzed for their K₂CO₃ and KHCO₃ concentrations via titration with a Metrohm–Titrando 809 (Switzerland) auto-titrator coupled with a pH probe. Solvent samples of approximately 2 mL each were diluted with 60 mL of RO water using a set volume liquid dispenser (Eppendorf, Varispec, Germany).

242 This mixture was then titrated with 0.4 M sulfuric acid to determine the HCO_3^- and the
 243 H_2CO_3 equivalence points. These bicarbonate concentrations were then used to determine the
 244 CO_2 flux (N_{CO_2} , mol/m².s) through the hollow fiber membranes based on the CO_2 - K_2CO_3
 245 reaction stoichiometry (Equation 3):

246 As reported in our prior work, for a pure CO_2 feed, the overall mass transfer coefficient (K ,
 247 m/s) can be related to this CO_2 flux by Equation 4 [41].

$$K = \frac{N_{\text{CO}_2} RT}{P} \quad (5)$$

248 where R is the gas constant (8.314 J/mol.K), P is the feed gas pressure (Pa) and T is the
 249 absolute temperature(K). This overall mass transfer coefficient can then be described as a
 250 function of the liquid phase mass transfer coefficient k_L^o (m/s) in the absence of any chemical
 251 reaction and the membrane mass transfer coefficient k_M (m/s):

$$\frac{1}{K} = \frac{1}{mEk_L^o} + \frac{1}{k_M} \quad (6)$$

252 where m and E are the Henry's law constant and the enhancement factor contributed by the
 253 chemical reaction, respectively (both dimensionless). As described in our previous work, the
 254 value of m can be calculated as 0.97 and k_M as $7.5 \times 10^{-3} \text{ m s}^{-1}$ [41]. The value of k_L^o can be
 255 obtained from a shell-side liquid phase mass transfer correlation provided by Li *et al.* [50] for
 256 hollow fibers with a packing density of 30% and for Reynolds numbers between 0 and 100,
 257 which is shown as Equation 6:

$$Sh = (0.52 - 0.64\phi) Re^{0.36 + 0.3\phi} Sc^{0.33} \quad (7)$$

258 Where ϕ refers to the packing density, Sh is the Sherwood number, Re is the Reynolds
 259 number and Sc is the Schmidt number (all dimensionless). This then allows for a

determination of the enhancement factor E provided by both the carbonate and the enzyme reactions.

3) Results and Discussion

3.1 Effect of Anions and Toxic Gases on the Activity of the Immobilized Enzyme

Initial experiments with moist flat sheet membranes indicated a dramatic loss of activity of the immobilized CA within the first 1-3 h of exposure to 1000 ppm of SO₂ or NO (Figure 3).

Figure 3. The activity of the immobilized CA on the moist membranes when exposed to 1000 ppm (200 Pa partial pressure) of NO or SO₂. The data were normalized to the activity of the fresh enzyme that was not exposed to any contaminant. The lines have been drawn to guide the eye.

This loss of activity may have resulted from reaction of the gas with the enzyme. However, it might also be attributed to a fall in the pH of the moisture that is present within the membrane. Both SO_2 and NO are known to form strong acids when dissolved in water in the presence of oxygen, with H_2SO_4 having a pK_a value of around -3; while HNO_3 has a pK_a of -1.45 [51]. Given the small quantities of water present within the samples, it is likely that the pH fell to these levels during exposure, particularly given the oxygen contamination when opening and closing the vessels.

To separate these two effects, the membrane coupons were exposed separately as dried coupons to the acid gases; and to their respective anions (NO_2^- , NO_3^- , SO_3^{2-} and SO_4^{2-}) within aqueous solutions of acidic, neutral and basic pH conditions, to determine the effect of the pH on the CA activity.

Figure 4. The effect of pH on the enzyme activity when the PP membranes were contacted at $23 \pm 2^\circ\text{C}$ with dry NO , 200 mM NaNO_3 for up to 200 h (a), or 200 mM NaNO_2 for up to 200 h (b). The data were normalized to the activity of the fresh enzyme that was not exposed to any contaminant.

Figure 4a indicates exposure to 500 ppm dry NO gas for up to 200 h or to 200 mM NO_3^- at near neutral pH results in only a small activity loss of less than 10%, which is possibly within the range of experimental error. Exposure to 200 mM of NO_2^- has no impact at all (Figure 4b) at this pH. This suggests that the enzyme variant used in this work may be more stable than that used by either Kernohan or Sharma and Bhattacharya, who observe much greater activity losses upon exposure to NO_3^- at moderate pH [1, 31]. However, other researchers have shown that NO_3^- does not significantly affect the activity of a common bovine CA, when the hydration of CO_2 is specifically considered [2]. De Luca *et al.* [36] show that the inhibition constants for both NO_2^- and NO_3^- can vary significantly with the CA variant used.

Importantly, the pH plays a significant role in enzyme activity inhibition. An alkaline pH of 12 causes an activity decrease of approximately 20%, which is similar to that observed in our previous work in the absence of any nitrate [43]. The result is also consistent with Li *et al.* [52], who observe a 50% loss in activity for a bacterial CA once the pH exceeds 6 irrespective of the ionic environment, with this activity stable from pH 8 to 11.0. The relatively small fall in activity also suggests that the LbL coating is still stable under these conditions, as the enzyme is not washed away during the three minute wash of the membrane with water prior to analysis. An acidic pH of 2 causes the activity to decrease by 30-40%. The activity decrease at this pH is independent of the NO_2^- or NO_3^- concentrations across the range from 50 to 200 mM (data not shown). It is thus likely that the results presented for the moist membranes (Figure 3) represented conditions of $\text{pH} < 2$ developing when the limited amount of water present in these structures was exposed to the acid gases.

Similar trends were observed for the immobilized enzyme activity upon exposure to SO_2 , SO_3^{2-} and SO_4^{2-} , as shown in Figure 5. The dry SO_2 gas again has a small effect, but it represents less than 10% activity loss and may be within experimental error. The SO_4^{2-} anions

do not negatively affect the CA activity at pH 6.4 within experimental error, consistent with the literature. The effect of SO_3^{2-} is also small at pH 6.4, although there is again evidence of a slight decline. However, the activity loss is heavily dependent upon the environment pH. Again, this is in agreement with the work from other researchers [2, 36].

Figure 5. The effect of pH on the enzyme activity when the PP membranes were contacted at $23 \pm 2^\circ\text{C}$ with dry SO_2 , 200 mM Na_2SO_4 for up to 200 h(a), or 200 mM Na_2SO_3 for up to 200 h(b). The data were normalized to the activity of the fresh enzyme that was not exposed to any added contaminant.

The immobilization of the enzyme onto hollow fiber PDMS membranes in a PEI/PSS/PAH/[PSS/PAH/CA]₂ film and its subsequent operation as a membrane contactor yielded CO_2 mass transfer performances as illustrated in Figure 6.

333

334 **Figure 6.** The overall CO₂ mass transfer coefficients in the presence of 200 mM of sulfate or
 335 nitrate anions. The data for the 30 wt% K₂CO₃ solvent were obtained from our previous work
 336 [41].

337 Figure 6 illustrates that the overall mass transfer coefficient in an enzyme coated
 338 hollow fiber module decreases by approximately 10% when 200 mM SO₄²⁻ or 200 mM NO₃⁻
 339 is added to a fresh 30 wt% K₂CO₃ solvent (pH 12), consistent with the flat sheet results. The
 340 reaction enhancement factor decreases by approximately 8% from 6.2 to 5.8 when these
 341 anions are added. In practice, these anions will not be present in the solvent at such high
 342 concentrations, so it is likely that the immobilized enzyme will retain most of its activity
 343 during typical flue gas carbon capture operations. A similar conclusion was reached by Zhang
 344 and co-workers [13, 14, 18] with a commercially available bovine CA enzyme.

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3.2 Effect of Temperature on Long-Term Mass Transfer Operations

It can be observed from Figure 7 that the overall mass transfer coefficient decreases exponentially as a function of time in 30 wt% potassium carbonate solutions (pH 12 for ten hours, then pH 10-11) due to enzyme deactivation, consistent with reports for bovine carbonic anhydrase [53]. At ambient temperatures (25°C), the CA is found to maintain a relatively stable mass transfer coefficient over a 100 h period (Figure 8). However, significant decreases in the mass transfer coefficient are observed at 35°C and 50°C, which are consistent with the kinetic data that has been presented by our colleagues [54].

Figure 7. The overall mass transfer coefficient for the CA-promoted absorption of CO₂ into 30 wt% K₂CO₃ as a function of time (a) at different temperatures over 100 h of operation, (b) at 30°C over 600 h of operation. The absorption was conducted with fresh K₂CO₃ solvent (pH 12) for 10 h before regenerated solvent with a 0.1-0.15 KHCO₃ loading (pH 10-11) was circulated into the absorber. K₀ refers to the overall initial mass transfer coefficient at the relevant temperatures.

Table 2 illustrates the initial overall (K₀) and liquid phase (k_L) mass transfer coefficients obtained at each different temperature, as well as the calculated initial enhancement factor (Equation 3). These enhancement factors are then monitored over time, which leads to Figure 8.

Table 2. The initial overall and liquid phase mass transfer coefficients at different temperatures and the corresponding enhancement factors.

Temperature (°C)	$K_0 (\times 10^4 \text{ m s}^{-1})$	$k_L (\times 10^5 \text{ m s}^{-1})$	E
25	1.34	2.26	6.2
30	1.58	2.76	6.6
35	1.76	3.12	6.7
50	2.76	3.87	7.6

Figure 8. The change in the enhancement factor over a period of 100 h at different temperatures.

This analysis shows that the immobilized enzyme can maintain an enhancement factor of a relatively constant 6.0-6.2 over 100 h of operation at 25°C. A similar enhancement factor was achieved under short term operations in our earlier work [41]. As the temperature increases from 25-35°C, the value of the liquid phase mass transfer coefficient (k_L^0) increases due to reductions in solvent viscosity and density as well as increases in the solute diffusivity. A further temperature increase to 50°C results in a larger enhancement factor, though it can

be observed that the enhancement factor at 50°C decreases rapidly because of a higher likelihood of enzyme deactivation.

After 70 h of operation, the enhancement factor decreases to 1.6, which was found to be the value of the base enhancement factor in the absence of any enzyme at 65°C [12]. Therefore, it appears that the CA appears to be completely deactivated after 3 days of operation in the 50°C absorption process.

The activity of an enzyme (a) is known to follow an exponential decay mechanism as a function of time (t), according to Equation 5 [53, 55, 56]:

$$a = a_0 e^{-\lambda t} \quad (8)$$

Where a_0 is the initial enzyme activity at $t = 0$ and λ is the decay constant. Fitting such an exponential decay equation to the data in Figure 10 allows a prediction of the time required for the CA to be completely deactivated (i.e. when the enhancement factor is unity).

Figure 9. The time required for a complete deactivation of the adsorbed CA.

As shown in Figure 9, the immobilized enzyme is predicted to survive in the 30 wt% K₂CO₃ solvent at 25°C for approximately 80 days. Conversely, at 50°C, it will only be effective for approximately 3 days. Although other Novozymes CA variants have been shown to withstand temperatures as high as 50-70°C with activity retention of 50-70% [17, 18], the activity tests carried out in these works involved the incubation of the enzyme with a 1 M sodium bicarbonate solution at pH 8 [17] or a 0.1 M K₂CO₃-KHCO₃ solvent at pH 10.5 [18]. These environments are not as harsh as the present analysis, where the CA is exposed to an elevated ionic strength environment (2.8 M K₂CO₃) at pH 10-12, which leads to an accelerated enzyme deactivation rate. In contrast, Alvizo *et al.* [57] showed that a *Desulfovibrio vulgaris* CA strain engineered for enhanced stability retained up to 40% of its activity over an absorption period of 14 weeks when operated as a free enzyme at 50°C in a 4.2 M methyl-diethanolamine solvent.

The decay constant (λ) usually follows an Arrhenius relationship with temperature (T) as shown in Equation 6 [58]:

$$\lambda = \lambda_0 e^{-\frac{E_D}{RT}} \quad (9)$$

Where λ_0 is the decay constant at T = 0 K, R is the gas constant and E_D is the deactivation energy. For the decay constants determined from Equation 5 this results in a predicted deactivation energy of the enzyme as 113 kJ mol⁻¹ (27.2 kcal mol⁻¹) (R² = 0.97).

The value of this deactivation energy is lower than the 160 – 630 kJ mol⁻¹ (40-150 kcal mol⁻¹) generally observed for other enzymes [58, 59]. Firstly, this suggests that the LbL coating is retaining the enzyme within the absorber. If the enzyme is desorbed from the LBL film into solution, it would flow with this solution into the regenerator, where temperatures are much higher (70-100°C). This would lead to much greater deactivation and the Arrhenius

relationship shown in Equation 9 would not hold. Secondly, it indicates that the enzyme is significantly more thermostable. In prior work, carbonic anhydrase variants isolated from *Lactobacillus delbrueckii* [52] and the *Gossypium hirsutum* cotton plant [60] exhibited deactivation energy values of 185 kJ mol⁻¹ and 146 kJ mol⁻¹, respectively, based on the Wilbur-Anderson activity assay method [61] for CO₂ hydration. Kanbar and Ozdemir [53] obtained values of 120 kJ mol⁻¹ for free BCA and 360 kJ mol⁻¹ for BCA immobilized within a polyurethane foam. However, their activity tests were conducted based on the hydrolysis of *p*-nitrophenyl acetate, which may not correlate well with the activity of CA in CO₂ hydration [62]. The greater thermal stability of the present enzyme reflects the inherent thermal stability of the enzyme variant employed, but may also be assisted by its immobilization on the membrane surface, which can increase the resistance to thermal denaturation [63].

These results indicate that if the present enzyme is to be used in a carbon capture operation with 30 wt% K₂CO₃ at high pH, lower absorption temperatures are preferred. The use of a K₂CO₃ solvent of lower ionic strength (such as 20 wt%) as well as a lower operational pH might also increase the viability of the enzyme.

4) Conclusions

The membrane-immobilized Novozymes carbonic anhydrase studied in this work appears to be more thermostable relative to other CA variants. However, given the harsh operating conditions used in the carbon capture operation of this study, including both high pH and ionic strength, it is still expected to become completely deactivated after only 3 days at an operating temperature of 50°C. Operation at a lower temperature of 25°C would allow for 80 days of immobilized enzyme use. It may therefore be more useful to use such an enzyme-coated membrane contactor for gas separation processes and other purposes operating at lower temperatures, such as the separation of CO₂ from methane in biogas purification processes [17]. It will also be beneficial to minimize the exposure to high pH

levels. In this work, we considered the most aggressive operating conditions, where the enzyme is exposed to fresh 30 wt% potassium carbonate. Pre-loading of the solvent with some carbon dioxide, or use of a lower potassium carbonate concentration, to reduce the pH below 12 would extend both the life of the enzyme and of the LbL coating.

Contrary to other published work with carbonic anhydrase enzymes, the immobilized enzyme shows a good tolerance towards the presence of SO₂ and NO or their associated anions. In contactor experiments, the performance loss upon exposure to 200 ppm of sulfate or nitrate was only of the order of 10%. Flat sheet experiments showed slightly greater performance losses at pH 12, of the order of 20%, when exposed to sulfate, sulfite, nitrate or nitrite anions. There was also a small loss in performance upon exposure to dry SO₂ or NO. However, the concentration levels of these toxic gases (500 ppm) or their anions (200 mM) was much higher than what would normally be obtained from post-combustion gas streams. The further development of an enzyme that retains this resistance towards gas impurities, but increases its thermostability under aggressive operating conditions would be desirable for the future of carbon capture operations that make use of K₂CO₃ solvents.

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Carbonic anhydrase enzyme immobilised on a membrane contactor

Exposure to pH 12 causes a performance loss of 10-20%

At least 80% activity maintained even with exposure to 200 ppm nitrate, nitrite, sulfate or sulphite

Exposure to dry SO₂ or NO has no impact

The enzyme coating is stable for 80 days at 25°C, but only 3 days at 50°C