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Enzymatically Assisted CO_2 Removal from Flue-Gas

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Abstract

The enzyme carbonic anhydrase is an enzyme known to enhance CO_2 absorption rates. However, for economic viability in enzyme based absorption technology long term stability under process relevant conditions is needed. Thus, here enzyme stability for extended times are investigated with respect to pH, temperature and solvent. Temperatures and pH stability were tested for up to 100 hours incubation and the enzyme was temperature stable up to 60 °C and in the pH range from 7 to 11, with some residual activity between pH 5 and 12. Furthermore, enzyme stability was tested for 7 different capture solvents for 150 days, at 1 M or 3 M solvent concentrations, 40 °C and pH between 8-9 and 10. Residual activity was found with all samples ranging from 12 to 91 % of the initial activity. This study show that this enzyme can indeed be used for extended periods in process relevant conditions, and thus shows promise for industrial implementation as a catalyst in carbon capture.

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Keywords: Carbonic anhydrase; post combustion carbon capture, biocatalysis

1. Introduction

The enzyme carbonic anhydrase (CA) (EC 4.2.1.1) catalyzes CO_2 fixation in nature, by hydrating CO_2 to bicarbonate (Figure 1). The reaction is catalyzed by a divalent zinc ion in the active site of the enzyme. CA is one of the fastest enzymatic reactions known, with reaction rates up to 10^6 s^{-1}[1]. The enzyme originates from a number of different sources and it has developed several times through convergent evolution. In fact five different enzyme

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classes which display little resemblance to each other, save the dependence on the zinc ion, are currently known[1]. Early research on the enzyme was focused on mammalian sources of the enzyme [2,3]. However, for industrial applications, such as carbon capture, today much focus has shifted towards microbial sources especially enzymes from thermophiles which often yield higher stability[1].

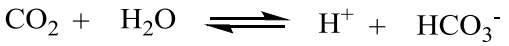

Figure 1. Carbonic anhydrase hydrolysis of carbon dioxide

To enable industrial implementation of CA in PCCC, it is vital that the enzyme is both stable and kinetically active under operating conditions. A recent review of thermostable enzymes[1] highlights the advances made in this field. However, in this study we take a holistic view of the process where a developmental enzyme supplied by Novozymes, is evaluated both in terms of temperature, pH and solvent loading stability for extended periods of time. All these factors will have a cumulative impact on enzyme deactivation during an actual process, making it important to understand the combined effects. It is important to note that the formulation of the enzyme may vary, to be used either as free enzymes, immobilized enzymes or particles, which all add benefits and drawbacks, not discussed here. Nonetheless, it is clear that the volume of CO_2 from an average power plant demands large capture equipment, thus any change in the capture set up, such as changing the solvent or enzyme packing will involve large capital and labor costs, and should therefore be avoided for as long as possible. Thus the enzymes used in such systems must be stable for long periods of time under operating conditions. In addition enzymes can only account for a small fraction of the overall cost of the capture process, since the cost of the technology must compete with current carbon taxes. The total cost of the process will also encompass solvents, stripping, compression and storage costs. To that extent a supplier which can deliver large quantities of enzymes at low costs, like we find in applications such as detergents and textile industry is needed for an economically viable process.

Nomenclature

AIB	2-Aminoisobutyric acid
AMP	2-Amino-2-methyl-1-propanol
CA	Carbonic anhydrase (Enzyme)
MAPA	3-(Methylamino)propylamine
MDEA	N-Methyldiethanolamine
MEA	Monoethanolamine
PCCC	post-combustion carbon capture
PNP	4-nitrophenyl acetate

2. Experimental

2.1. Materials

A developmental carbonic anhydrase of microbial origin was supplied by Novozymes A/S, Denmark in the form of a cell-free brown liquid. All other materials used were of reagent quality and purchased from chemical vendors.

2.2. Methods

2.2.1. Sample preparation

pH samples was prepared with a 0.2 M boric acid, 0.05 M citric acid and 0.1 M tertiary sodium phosphate complex buffer[4] suitable for the entire range of pH's tested.

Temperature tests were conducted with 0.1 M Tris-HCl buffer, pH 7.6, a Good buffer, which has a small pH change with temperature change.

Solvent solution was prepared by adding 1 M or 3 M in MilliQ water, and the pH adjusted by bubbling in CO_2 gas or by 6 M HCl solution.

Three separate vials of 3 mL solution were prepared for each sample described above, 2 % enzyme solution was added to two of the vials and the third was kept as a blank. The samples were then incubated as descried in the results section in a thermoshaker, at 450 rpm.

2.2.2 Activity assay

Activity was measured by a modified assay by Chirică and colleagues[5], Figure 2. Each sample was tested by adding 0.1 M Tris-HCl buffer, pH 7.6, in a plastic cuvette, 10 µL sample or blank was added, the sample was then left to equilibrate for at least 1 hour, and then 10 µL para-nitro-phenyl (PNP) solution (54.3 mg PNP in 3 mL acetonitrile) was added. The samples were mixed by inverting samples covered with parafilm twice. Each sample set ran in parallel contained two blank samples and three reaction samples. Absorption was recorded at 348 nM for 320 s, and the activity was determined from the slope of the absorption between 60 s and 300 s, subtracting the slope of the blank samples. The samples containing MEA and MAPA/MDEA had a high background absorption, thus the sample preparation was modified: 0.05 mL sample or blank was added to 0.45 mL 0.1 M Tris-HCl buffer, pH 7.6, in centricon vial 10 kDa cut off, spun down at 14,000 rpm for 10 min, 0.5 mL new buffer was added, the process was repeated 3 times. 0.1 mL of this sample was added to 0.9 mL buffer and the procedure was followed as indicated above.

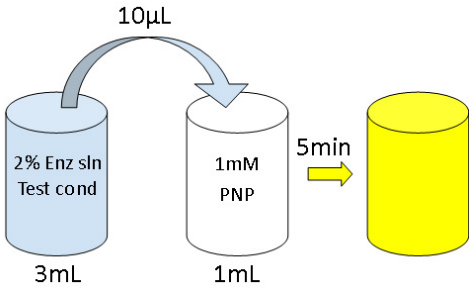

Figure 2. Experimental set up. 2 % CA solution in 3 mL test condition. 10 µL sample transferred to a 1 mM *p*-nitrophenyl (PNP) solution, color change (348 nm) detected over 5 min. All activities are % residual activity compared to initial activity under identical conditions

3. Results and Discussion

The study here evaluates the applicability of enzymes in PCCC. Enzyme stability was evaluated using an activated ester assay; PNP. Which measures the hydrolysis on the ester; this induces a color change. It should however be noted that the activity on this substrate may not accurately correspond to CO_2 hydrolysis rates. For this reason we do not report the activity values nor do we compare reaction rates between different samples in this study, with different pH's or solvents. Thus, here only the residual activity of the enzyme, under identical conditions, where background activity is thoroughly controlled for, is measured. In a PCCC application the conditions over the course of the process will vary with respect to temperature and pH (the pH drops as CO_2 is absorped in the solvent), in addition the solvent can both be varied in type and concentration. We therefore examine these three variables singularly and finally we compare the additive effects of the parameters, illustrated in Figure 3.

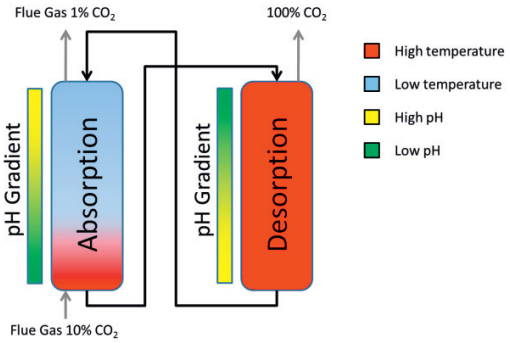

Figure 3. Example of varying pH and temperatures of the solvent in a typical post combustion solvent based capture process.

3.1 pH stability

pH stability was evaluated by measuring residual activity after 100 hours incubation at the respective pH at room temperature in the pH range from 4 to 12, with increments of 1 pH unit. Where only the pH values above pH 7 are initially interesting for PCCC applications, since below this pH the hydration rate of the reaction is strongly reduced[6]. Results are shown in Figure 4. No activity was detected after 100 hours at pH 4 and moderate activity (45-70 %) with pH's 5, 6 and 12. The enzyme proved stable in the pH range from 7 to 11 after 100 hours incubation. This defines the operating space for enzymes, and furthermore the maximal and minimal loading of any given solvent. Dependent on the solvent chosen this will also determine the maximum and minimum CO_2 loading used, since the pH of the solvent will depend on the loading.

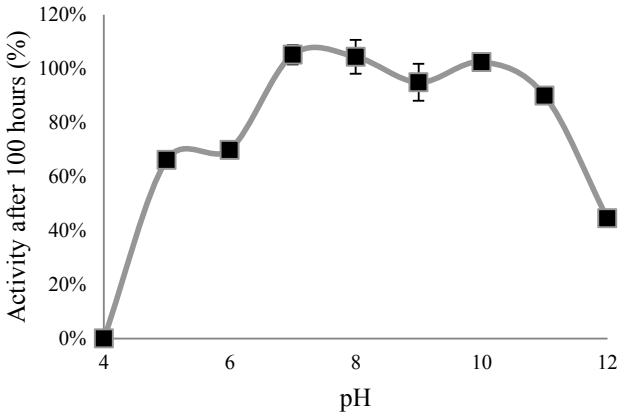

Figure 4. Residual activity after 100hours of incubation at pH 4 to 12

3.2 Temperature stability

Temperature stability was evaluated in a range from 50-80 °C, and the enzyme was found to have residual activity for at temperatures for up to 48 hours (Table 1), but the activity was drastically reduced at higher temperatures. In addition some experiments were made to recover activity from the high temperature experiments (70 °C and 80 °C) by incubating the samples at lower temperatures for some time before measuring the sample again (data not shown). From this up to 10 % activity was regained. After 48 and 72 hours no activity remained from the temperatures 80 °C and 70 °C, respectively. It should be noted that the accuracy of these values may be questionable as the activity is increasing over time with the 50 °C sample; this is likely due to the increased internal energy of the enzyme at the higher temperature, which in turn results in higher reaction kinetics. This may be an artifact stemming from that the activity assay was taken too soon after the enzyme was taken from the incubation temperature. This was corrected in later experiments below. Furthermore, this may suggest that some of the other residual activity values may be lower than the reported values. Nonetheless, the results give an indication that the enzyme is stable under operating conditions. The results correlate well with results found with other engineered CA's [1], which have been found to be stable at high temperatures. For example Codexis has previously engineered a thermostable enzyme, which retained 40 % activity after 40 hours incubation at 75 °C, comparable to what is found here[1]. In addition another engineered CA was found to retain up to 54 % residual activity after 2 hours incubation at 80 °C[7]. Thus, our results are comparable to the former results, indicating that the enzyme is stable at temperatures up to 60 °C for extended periods of time, although these results demonstrate that if the enzyme is to be exposed to temperatures above 70 °C, this must be limited to short periods of time. In practical terms this means that this enzyme is unlikely to survive a treatment in a reboiler for example.

Table 1. Residual activity after incubation at temperatures from 50 to 80 °C for up to 100 hours. All results are given in % residual activity

	Time (hours)				
T (°C)	1.5	25	48	72	100
50	105	121	132	196	280
60	84	87	64	66	74
70	47	41	49	36	0
80	7	9	2	0	0

3.3 Long term solvents stability

Here we also present a long term stability study, tested for 150 days, which is to the best of our knowledge the longest stability test under process relevant conditions for PCCC to date. The study was undertaken at two pH values and 7 different solvents, either using 1 M or 3 M concentrations. This was done in order to evaluate if the enzyme could be used long-term under operating conditions. The results show that the enzyme was highly stable for extended periods of time. The solvents tested were chosen as they were previously proven to be useful in CO_2 capture. Specifically the primary amine solvent MEA as it has been reported as a candidate for industrial applications, and is single most commonly used solvent[8], and thus serves as a good benchmarking solvent. It has excellent absorption rates, it is however haunted by problems such as corrosion, low stability, and high energy needed for desorption. AMP, MDEA and MAPA/MDEA have all shown great promise in CO_2 absorption, but with slightly lower absorption rates, thus they are good candidates for enzyme activation. AIB was previously shown to have higher desorption rates than MEA at 80 °C, but slower absorption rates[9], thus it serves as a good target for enzyme enhanced technologies, in addition AIB had higher solubility than other comparable amino acids like alanine. Potassium carbonate (K_2CO_3) has been used in enzyme based[10] and chemically enhanced[11] carbon capture on several occasions, and has the advantages of favorable thermodynamics and lowered desorption temperature, however a drawback with this solvent is lowered solubility. Finally the solvent AC was used as a cheap and available source of ammonium. Results for activity are showed in Table 2. All data points illustrate that activity is lost over time and a higher deactivation is found with a higher pH. Furthermore there is a negative correlation between pKa (calculated) values and stability. Finally the activity was compared after 100 hours and 150 days, to evaluate if a short term study could efficiently reveal which solvents were stable long the long term. The results correlated poorly and we conclude that within this data set a prediction of long term stability cannot be made from short term studies.

Table 2. Remaining activity after 5 and 150 days

Solvent (concentration)	pH	Residual Activity 5 days (%)	Residual Activity 150 days (%)
MEA (3M)	8.3	95 ± 0.4	73 ±1 0.8
	10	76 ± 1.8	33 ± 4.8
AMP (3M)	9	99 ± 0.3	42 ± 1.6
	10	104 ± 7.7	12 ± 0.6
MDEA (3M)	9	92 ± 2.8	62 ± 4.0
	10	91 ± 3.0	54 ± 2.5
AIB (3M)	8	106 ± 4.9	91 ± 3.0
	10	95 ± 0.1	35 ± 0.9
K_2CO_3 (1M)	8	116 ± 6.8	83 ± 3.6
	10	85 ± 1.2	29 ± 2.4
MAPA (1M)/ MDEA (2M)	8.6	86 ± 10.3	85 ± 0.5
	10	99 ± 4.8	69 ± 4.4
AC (3M)	8	99 ± 4.8	71 ± 5.1
	10	100 ± 4.7	22 ± 3.4

However as seen from Figure 5, a correlation was found between deactivation and pKa values. In comparison higher pKa values has previously been found to have a positive effect on solvent kinetics[12]. This suggests that a compromise might be made on implementation between enzyme activity and stability. However in the case of CA it it has been known since the 1930's that buffers and solvents can have more severe impacts on the catalytic activity of CA[2], albeit of mammalian source, thus the actual enchanced activity with the solvents should be investigated in

detail. Such work has been carried out on several occasions, in particular Haritos and colleagues has investigated enzyme enhanced CO_2 capture in a stirred cell reactor, where a rate enhancement was found with all eleven solvents tested[10]. This gives further evidence that the enzyme may indeed enhance reaction rates for CC applications. It should be noted however that at times higher enzyme stability can be associated with lower reaction rates[13].

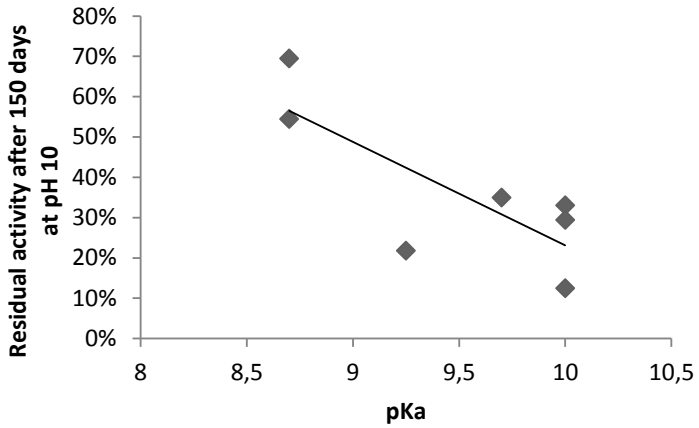

Figure 5. Correlation between pKa and long term (150 days) stability. Pka values were predicted with the software ChemDraw

3.4 Additive effects on stability

Finally, we have investigated the addititve effects of solvent strength and temperature with the solvents NACl, K_2CO_3, AMP and MDEA, Table 3. From these experiments we did not find a significant impact either by altering the concentation of the solvent or the temperature. When analyzing these results with the results we see that the two factors pH and solvent type seems to have a significant impact on enzyme stability but temperatures upto 50 °C and molar concentrations up to 3 M had no significant impact.

Table 3. Residual activity of CA after 100 hours with varying temperature and solvent concentration with the solvents, NaCl, K_2CO_3 , AMP and MDEA.

Solvent	1 M, 25 °C	1 M, 50 °C	3 M, 25 °C	3 M, 50 °C
NaCl	76%	91%	90%	78%
K_2CO_3	125%	100%	63%	80%
AMP	91%	87%	70%	79%
MDEA	88%	89%	83%	75%

4. Conclusion

We have evaluated enzyme stability in terms of pH, temperature and solvents, the latter at different concentrations and types of solvents, and the effect of these three factors added together. The carbonic anhydrase used here, showed long term stability for some, but not all process relevant conditions. In conclusion the findings in this paper hint potential future application of CA for use in PCCC applications.

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