Electronic Supporting Information

CO_2 Sequestration by Enzyme Immobilized onto Bioinspired Silica

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EXPERIMENTAL

Chemical reagents: Anhydrous disodium hydrogen orthophosphate (Na_2HPO_4), ethanol (99.5% v/v), hydrochloric acid (1M), bovine CA (CA), p-nitrophenol, p-nitrophenyl acetate, diethylenetriamine ($C_4H_{13}N_3$), sodium dihydrogen orthophosphate dihydrate (NaH_2PO_4•$2H_2O$), sodium metasilicate ($Na_2O_3Si \cdot 5H_2O$), tris base and calcium chloride were purchased from Sigma-Aldrich, and used without further purification. Experiments were performed at 20˚C, unless stated otherwise. Experiments were carried out in triplicate, and Minitab 16 was used to evaluate errors (reported as one standard deviation).

The immobilised enzyme's performance was investigated for the direct hydration and sequestration of CO_2. Furthermore, a spectrophotometric colorimetric enzyme assay was used to assess the activity of the immobilised enzyme compared to free enzyme. Reuse potential, leaching and thermal stability were also reviewed, and the materials were characterised through infra-red spectroscopy and power D-ray diffraction.

Experimental data was collected in triplicates and Minitab-16 was used to evaluate errors; these were reported as one standard deviation.

Enzyme Immobilisation: CA immobilised on silica was prepared by adding CA during silica condensation, producing immobilised enzyme, as described elsewhere.[S1] Sodium metasilicate was chosen as a silica precursor since it is most commonly used in industrial silica production and also due to its low toxicity compared to toxic alkoxysilanes which are often employed in laboratory silica synthesis. In brief, separate solutions of sodium metasilicate and amine were prepared in deionised water, and were mixed to obtain a 30mM concentration of silicic acid, and maintain a molar ratio of [Si]:[N] = 1: 1 in the final solution at pH 7± 0.10. A known mass of enzyme was added immediately, and the resulting solution was given a gentle mix and left unstirred for 5 minutes, before being centrifuged for 15 minutes at 8000 rpm, to allow the immobilised enzyme to be separated. The supernatant was removed and stored (for further testing), and deionised water was added to make up the volume again. This process of centrifugation and washing was repeated another two times. The immobilised enzyme was then dried at 40˚C in a vacuum oven for 5 hours. Control experiments were carried out where silica was prepared without enzyme being added.

Hydration and sequestration of CO_2: Water saturated with CO_2 was prepared using a literature procedure by bubbling carbon dioxide in water at 18^0C in an open system until equilibrium was reached.[S2] A mass of immobilised enzyme corresponding to a 0.02mg/ml enzyme concentration was added to 10ml 0.5M pH 6.4 tris buffer and 25ml CO_2 saturated water. An alternative approach was also tried where CO_2 was added to water in a closed system leading to pressure little above 1 atm. However, it was not possible to measure the exact amount of CO_2 dissolved in this closed system, hence the data generated from this method was not reported. After stirring for 5 minutes, 5ml 5% $CaCl_2$ in 0.5M pH 10 tris buffer was added. The solution was checked regularly for calcium carbonate precipitation and after 5 minutes, was filtered to separate any precipitate (plus immobilised enzyme). This was dried at 85^oC for 24 hours and weighed (accounting for the mass of immobilised enzyme), prior to undergoing Fourier Transform Infra-Red (FTIR) spectroscopy and X-ray diffraction (XRD) analyses. This was repeated with free enzyme and without enzyme.

Yield calculations: The mass of CO_2 removed was estimated based on the mass of calcium carbonate produced. Following the solubility for CO_2 in water and adopting literature method,[S2] the amount of dissolved CO_2 was known. $CaCl_2$ added was in excess and hence the amount of calcium carbonate precipitated when using CA (free or immobilised) was used to estimate the yield (100% theoretical yield of $CaCO_3$ would lead to consumption of all the dissolved CO_2). The masses of $CaCO_3$ precipitated in each experiment are reported in Table S2 below.

Enzyme loading: It was necessary to deduce how much CA had become entrapped within the silica. This allowed the immobilisation efficiency to be determined, and was also necessary to ensure that the same concentration of enzyme was used for comparing the free and immobilised enzyme systems. This was achieved by measuring the protein content in the supernatant produced during enzyme immobilisation, using UV-spectroscopy (Shimadzu UVmini-1240) at 283nm with a quartz cuvette. A CA calibration curve (Figure S1) was produced and used to deduce concentrations of un-immobilised enzyme. Knowing the mass of enzyme initially added during immobilisation, the immobilisation efficiency was recorded.

Enzymatic assay: In order to understand enzyme performance, a well-established assay, which was different to the carbon capture experiments, was used and is described below. Note that the experiments pertaining to leaching, thermal stability, reuse and storage were all performed using this assay. The enzyme assay used for quantitative analysis of enzyme activities involved the hydrolysis of *p*-nitrophenyl acetate to *p*-nitrophenol (and acetate acid). At pH ≥7, the *p*-nitrophenol mainly exists as a yellow anion. UV-vis spectroscopy was therefore used to measure the absorbance of the samples, and the corresponding *p*-nitrophenol product concentration was deduced using a calibration curve. For the calibration curve (Figure S2), *p*-nitrophenol solutions of various concentrations were prepared by dissolving the corresponding mass of *p*-nitrophenol in 0.1M, pH7 phosphate buffer. The absorbance was measured at 405nm. This procedure was repeated for the various temperatures involved in the entirety of the experiment, since it was found that calibration did not remain unchanged for different temperatures. Since the assay

reaction can occur even without the presence of CA (although to a lesser extent), it was necessary to determine non-enzymatic reaction rates in order to allow the true increase in reaction rate, due the presence of CA, to be determined. A 1.0M stock solution of *p*-nitrophenyl acetate was prepared by dissolving the necessary mass in ethanol (99.5% v/v). This was diluted into 10, 20, 30, 40, 50, 60 and 70mM solutions using ethanol. The assay mixture was then prepared and consisted of: 3ml 0.1M pH7 phosphate buffer; 15µl 0.1M pH7 phosphate buffer or 1mg/ml CA (free or immobilised) in 0.1M pH7 phosphate buffer and 30µl *p*-nitrophenyl acetate (in ethanol) substrate solution. This was prepared for all *p*-nitrophenyl acetate concentrations, as well as a 0mM solution as a control.

The assays were kept gently stirred, and the absorbance was measured at 405nm, every minute for 10 minutes. The absorbance was then measured at various intervals after, until there was no further change. This indicated that the reaction was complete. The *p*-nitrophenol calibration curve was then used to convert the absorbance into product concentrations. Measurements were recorded for systems with and without enzyme present. The initial rates V_0 were determined from initial linear regions and these were used for Michaelis-Menten analysis to produce V_{max} and K_M. When CA was present (free or immobilised), the corresponding non-enzymatic reaction rates were subtracted from the total reaction rates deduced, giving the enzymatic reaction rates. The enzymatic reaction rates were then plotted against substrate concentration, to produce a Michaelis-Menten model. Through non-linear regression techniques, the kinetic parameters were deduced; for this, the Hill function in Origin 8.5 was used to describe the following equation:

$$v = \frac{V_{max}[S]}{K_M + [S]} \qquad \text{(S1)}$$

where: v = reaction rate; V_{max}= maximum rate; $[S]$= substrate concentration; K_M= Michaelis-Menten constant. The kinetic parameter V_{max} was used to estimate the specific enzyme activity of the free enzyme. An enzyme activity unit (U) was defined as the amount of enzyme activity that caused the disappearance of 1 µmol of substrate, or formation of 1 µmol of product, per minute. The specific activity (U/mg) was defined as the activity of the enzyme per mg of enzyme.

***Leaching**:* A known mass of immobilised enzyme whose CA content had been determined was placed in 5ml of buffer. The absorbance of the buffer at 283nm (using a quartz cuvette) was measured at various time intervals. The CA calibration curve was then used to obtain an enzyme concentration from this, and the mass of enzyme that had leached out was deduced.

Reuse potential: Immobilised enzyme that had been used in a prior reaction (and was present in the reaction medium for 1 hour) was separated from the surrounding liquid by centrifugation, and washed. The material was then reused in the next cycle of the assay. The product formation after 10 minutes was measured, and compared to its initial use. This procedure was repeated for additional cycles.

***Thermal and storage stability**:* The performance of the immobilised enzyme was tested under various temperatures to study thermal stability. For all stability tests, it was necessary to perform the assay without enzyme, with free enzyme and with immobilised enzyme, at each temperature chosen, to achieve a true comparison. Thermal stability experiments were carried out between 40˚C and 60˚C in 10˚ increments; these temperatures were established through the use of a water bath. The product formation after 10 minutes was measured, which allowed a comparison of activity of the systems at various temperatures. Similarly, activities were measured upon thermal denaturation at 85^0C. After vacuum drying, the immobilised enzyme was stored at -18˚C, 4˚C or 20˚C, and its performance after 1 week at these conditions was evaluated, to establish loss of activity, if any, due to storage conditions.

***Characterisation of materials**:* Silica with and without CA was dried prior to use in carbon capture and mounted on SEM sample holders with double sided sticky carbon tape. Upon gold coating by sputtering, samples were analysed on a HITACHI SU-6600 Field Emission Scanning Electron Microscope (FE-SEM) at 10kV. Porosity measurements were performed on degassed samples by nitrogen adsorption using a Micromeritics ASAP 2420 porosimeter. The data was processed using BET model to yield surface areas while BJH model was adopted for obtaining pore sizes and pore volumes (see Table S1). FTIR was used to analyse the dried immobilised enzyme samples, in order to detect the formation of silica and the presence of CA as well as to characterise the precipitated calcium carbonate. Attenuated Total Reflectance-FTIR analyses (ABB Miracle MB 3000) of dried samples were performed, with 32 scans at a resolution of 4 cm^{-1}. The presence of $CaCO_3$ and its crystalline form were determined by X-ray powder diffraction using a PANalytical X'Pert Powder diffractometer in theta-theta geometry. These data were collected under ambient conditions using Cu K_α radiation over the range $10 \leq 2\theta \leq 100^0$ using a single step size of ~0.017^0 2θ. The observed data were analysed by the Rietveld Method,[S3] as implemented in the GSAS suite of programs,[S4] using pseudo-Voigt and shifted Chebyshev functions to describe the peak shapes and background, respectively.

Supporting References:

S1. C. Forsyth, S. Patwardhan, 2012, Under Review.
S2. M. Vinoba, M. Bhagiyalakshmi, S. K. Jeong, Y. I. Yoon and S. C. Nam, *Colloids Surf. Sci. B*, 2012, **90**, 91.
S3. H. M. Rietveld, *Journal of Applied Crystallography* 1969, *2*, 65.
S4. A. C. Larson, R. B. Von Dreele, General Structure Analysis System (GSAS), Los Alamos National Laboratory Report, LAUR 86-748, 2004.

SUPPORTING TABLES AND FIGURES

Table S1 Porosities of silica with and without enzyme.

Sample	*Surface area* [m^2/g]	*Pore size* [nm]	Pore volume [cm^3/g]
Silica without CA	17	22	0.06
Silica with CA	14	19	0.037

Table S2 Amount of $CaCO_3$ precipitated and yields.

Sample	Mass of precipitate [mg]	CO_2 removed [mg]	Yield %
No enzyme	33	15	32
Free enzyme	92	40	90
Immobilised enzyme	88	39	86
Free enzyme (denatured)	30	13	29
Immobilised enzyme (denatured)	36	16	35

Solubility CO_2 at 18^0C = 1.8 g/kg water, the experiment used 25 ml CO_2 saturated water at 18^0C, therefore CO_2 in water = 45 mg.

% Yield = 100 x [*mass of CO_2 removed in mg* /45]

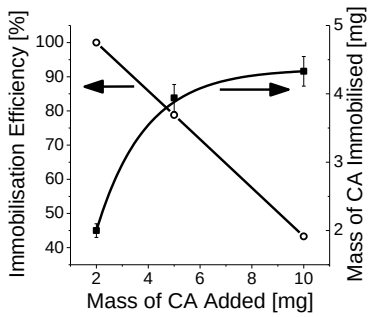

Fig. S1 Immobilisation efficiency and total amount of enzyme entrapped in to silica.

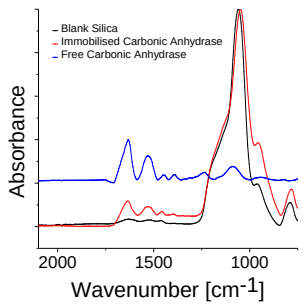

Fig. S2 FTIR spectra for silica with and without enzyme, and free enzyme.

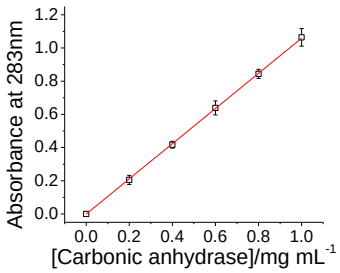

Fig. S3 Calibration curve for CA absorbance at 283 nm as a function of CA concentration.

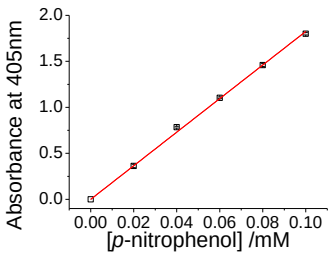

Fig. S4 Calibration curve for the product of CA assay showing absorbance at 405 nm as a function of product concentration.

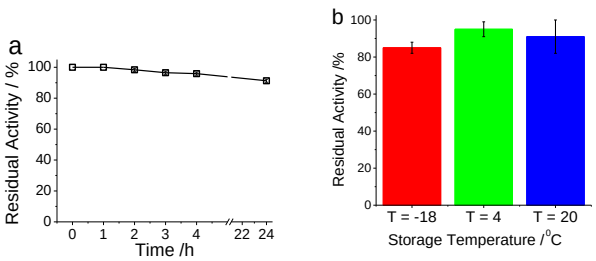

Fig. S5 A comparison of stability and storage potential for free and immobilised enzymes showing the extent of leaching (a) and loss of activity due to storage for 1 week (b).