Development of Integrated System for Biomimetic CO_2 Sequestration Using the Enzyme Carbonic Anhydrase

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The enzyme, carbonic anhydrase, is the biological catalyst responsible for the interconversion of CO_2 and bicarbonate in living organisms. The present research is aimed at the development of a CO_2 scrubber that can be used to reduce CO_2 emissions from, for example, fossil-fuel-burning power plants. In this system, the enzyme works as a catalyst to accelerate the rate of CO_2 hydration for subsequent fixation into stable mineral carbonates, the counterions for which may be supplied from such sources as brines from saline aquifers, waste brines from desalination operations, or seawater. Proof of principle has already been demonstrated. One of the requirements for the enzyme will be that it must be able to function in the presence of other chemical species likely to be present in the industrial application. The present results show excellent enzyme activity in the presence of low levels of SO_x and NO_x (that might be expected from flue gases) and also in solution representative of seawater. The effects of SO_x and NO_x are of interest because, although emissions of these species are strictly controlled, some very low level will still be present. The reason for examining enzyme performance in seawater-like solutions is to give a better approximation of the compositions likely in actual process streams based on either seawater or other brines.

Introduction

The possible contributions of gases generated by human activities to the atmosphere, and more specifically their role in determining global surface temperatures through what is called "the greenhouse effect", have been discussed for several years. Recently, the part played by anthropogenic CO_2 has received much attention. While CO_2 is not in itself a particularly strong greenhouse gas (compared to, for example, methane (CH_4)), the contribution to the atmosphere since the beginning of the industrial revolution through the burning of fossil fuels has been very considerable. Data exist showing the increase in global CO_2 levels over the last hundred years or more, and the change has been quite substantial. Furthermore, there is some indication that there has been an increase in global temperatures, which may correlate with the increase in atmospheric CO_2. Whether there is a causal connection is still a matter of controversy, but a number of international meetings, most particularly that in Kyoto in December 1997, have recommended that efforts need to be initiated with the aim of reducing, and eventually even eliminating, further human contributions.

In the United States, there are three more or less equal contributors to the problem. These are: (1) domestic, industrial, and commercial use of fossil fuels; (2) transportation; and (3) electricity generation. Of these, the contribution of large coal-fired utilities is in many ways the easiest to address, not least because they are large fixed sources. Currently, close to half of the electricity generated comes from these units. In 1996, coal accounted for 1797 billion kilowatt hours, or 52% of the total electricity generation,[1] and this figure is predicted to increase to 2304 billion kilowatt hours by 2020. In each of the last three years, the coal consumed by the electric utilities has been close to 900 000 000 tonnes[2].

It may be helpful to understand the magnitude of the problem by considering a single coal-burning station. In 1991, for example, EPRI analyzed the situation for a hypothetical new 300 MW(e) plant in a Kenosha, WI, location, burning an Appalachian coal. The coal contains 71.3% carbon, 6.0% moisture, 9.1% ash, 4.8% hydrogen, 4.8% oxygen, 2.6% sulfur, and 1.4% nitrogen (by weight; Ultimate Analysis). The heating value is 13 100 Btu/lb (30 470 kJ/kg). The net efficiency of the unit (from the coal in, to the electricity delivered to the system busbar) is 35%. The corresponding coal burn rate is approximately 125 tonnes per hour. Typically, boilers operate with approximately 5% excess oxygen, and the flue gas

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(1) Energy Information Administration. Monthly Energy Review, DOE, Washington, DC, January 1999.
(2) Energy Information Administration. Monthly Energy Review, DOE, Washington, DC, August 2000.
(3) Electric Power Research Institute. *Electricity Technology Road Map, Powering Progress, 1999 Summary and Synthesis*; EPRI, Palo Alto, CA 1999.

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characteristics are (in units of tonnes per hour) (principal components only) the following: O_2, 93; CO_2, 290; SO_2, 6; H_2O, 73; and N_2/NO_x, 1134.

From this, it can be seen that the quantity of CO_2 is very large; if it were to be 100% sequestered as calcium carbonate, $CaCO_3$, this would correspond to 666 tonnes of product per hour, or close to 16 000 tonnes per day. The cation source would amount to the equivalent of 9000 tonnes per day. This compares with a coal supply of approximately 3000 tonnes per day, and an ash production of 270 tonnes per day. The masses involved are a factor of over fifty times that involved in flue-gas desulfurization, which is a standard procedure for most U.S. coal-burning plants, and has some analogies with the carbon issue; (in this process, the sulfur is captured as calcium or magnesium sulfate). This "typical" plant produces 2.32 tonnes of CO_2 per tonne of coal, and hence the utility industry is currently producing 2.1×10^9 tonnes of CO_2 per year from burning coal. (This does not take account of the CO_2 produced from units burning natural gas).

There have been a number of meetings discussing the overall topic of "carbon management" over the past few years. In broad terms, the approaches to the problem include (a) improving the efficiency of fossil-fuel-fired generation; (b) continuing the historical increase in the hydrogen-to-carbon ratio in the fuels used; (c) replacing all fossil fuels with hydrogen; (d) replacing fossil fuels as the heat source in thermal generation by nuclear fission or perhaps by geothermal heat; or (e) replacing thermal generation by electrochemical methods such as hydrogen fuel cells, or renewables such as wind or photovoltaics. While all these have their virtues, it is unlikely that large changes can be achieved in the next several years, and in the meantime the problems of dealing with the products of carbon combustion must be dealt with.

The discussion above has concentrated on the situation in the United States. It is even clearer that coal is likely to remain a major fuel for some time on the global scale, as electrification becomes more widespread in the developing countries. The achievement of universal global electrification by 2050 would require some 10 million MW of new electricity generating capacity worldwide in that time frame.[3] Some of the most densely populated developing countries have large coal reserves, and it is unrealistic to think that these will not be exploited.

The issues relating to the different options have been summarized in *Carbon Sequestration: State of the Science*, a U.S. Department of Energy Report from April 1999.[4] It is usual to treat the process as composed of two (perhaps three) distinct steps. The first is the capture of the CO_2 from the combustion system, and the second is the sequestration, where "sequestration" means a long-term capture or immobilization of the CO_2. The third step involves the transportation of the CO_2 from the capture site (presumably the power station) to the sequestration site.

Many possible approaches to carbon sequestration are being investigated by researchers worldwide (see, for example, refs 5−12). This is good, because it is clear that the scale of anthropogenic CO_2 emissions is so huge that sequestration of anything more than a small fraction of it is likely to require a combination of different approaches. Most sequestration studies have been based on the assumption that CO_2 would first have to be separated from the remainder of the exhaust gases from fossil-fuel combustion. It could then be disposed of, for example, in DOG (depleted oil and gas) wells, in deep saline aquifers, in the deep ocean, or through deposition into minerals such as peridotites or serpentinites. It is likely that the best choice of strategy for CO_2 capture and sequestration will be site specific, probably determined by access to an appropriate sequestration site, to minimize the problems associated with long-distance transportation of a potentially hazardous material. In this context, it should be noted that a leak in a natural gas pipeline may result in a flame, which destroys the leaking gas, or a dissipation of the gas upwards because of its low density; however, CO_2 does not burn, and has a high density, so that it will tend to concentrate in low-lying regions near a leak.

A common theme for all of these approaches is that the capture step involves the need to concentrate CO_2 from a very dilute combustion gas stream, a large fraction of which is nitrogen. However, while it is technically feasible to remove CO_2 from flue gases with existing technology,[8,12] the removal systems require large amounts of capital and energy, and could raise the cost of busbar electricity, for example, by 50%.[7] Transportation of CO_2 by pipeline as a supercritical fluid is a well-established technology in EOR (enhanced oil recovery), but still is not cheap.

Our present research is aimed at the development of a novel biomimetic approach to CO_2 sequestration, and is primarily addressed at the issue of CO_2 emitted by fossil-fuel-fired, principally coal-fired, thermal systems for the generation of electricity, based on the use of an enzyme or biological catalyst. The resulting sequestration system would offer several potential advantages, including a plant-by-plant solution to emission reduction; no costly CO_2 concentration and transportation steps; a safe, stable, environmentally benign product; and an environmentally benign process. Proof of principle has already been demonstrated.[5,6] The present emphasis will be on the performance of the enzyme in the presence of other chemical species likely to be present in the industrial situation. It is necessary first,

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(8) Hendriks, C. *Carbon Dioxide Removal from Coal-Fired Power Plants*; Kluwer Academic Publishers: Dordrecht, 1994.

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however, to summarize the biomimetic approach, to put the present results in context.

Biomimetic Approach

Atmospheric levels of CO_2 are much lower today than they were early in the earth's history. Carbonate minerals, such as calcite, aragonite, dolomite, and dolomitic limestone, comprise a massive CO_2 reservoir, estimated[13] to contain an amount of carbon equivalent to $150\,000 \times 10^{12}$ metric tons of CO_2. Thus carbonate minerals offer a geologically proven, safe, long-term repository for CO_2. If anthropogenic CO_2 can be fixed into solid carbonate form, such as calcium carbonate, then we have a stable and environmentally friendly product. The problem, of course, is one of rate.

To address the problem of rate, we adopted a biomimetic approach.[5,6] It is useful to keep in mind here that we are defining a biomimetic approach as one in which a particular aspect of a biological process or structure is identified and applied to solve a specific nonbiological problem.[5] In other words, it is an approach in which (1) we have a specific engineering problem to solve; (2) we identify a biological system in which an analogous engineering problem has been solved; and (3) we use the enabling part of that system, whether it be a structural design, a processing route, or a biochemical component to solve our engineering problem.

In the present instance, we examined the rate-limiting step in the chemistry of CO_2 fixation into calcium carbonate in aqueous solution, and then considered what lessons could be learned from biological systems in order to accelerate that step. Calcium carbonate can be precipitated from aqueous solution, given a suitable saturation of calcium and carbonate ions, and so the issue becomes one of how to produce carbonate ions rapidly from CO_2 and H_2O,[14] a process which first requires the formation of bicarbonate ions. One important parameter to be considered is pH, because of its strong effect on the proportions of the carbonic species present,[15] and because, at low pH, carbonates will tend to dissolve rather than precipitate. Although carbonate could be formed rapidly at high pH, this would pose both economic and environmental concerns, and hence a process that operates at very mildly basic pH values would be desirable.

Gaseous CO_2 dissolves rapidly in water to produce a loosely hydrated aqueous form.[16,17]

$$CO_{2(g)} = CO_{2(aq)}$$

This reaction is rapid. The aqueous CO_2 may then react either with water or, at high pH, with hydroxyl ions:

$$CO_{2(aq)} + H_2O = H_2CO_3 \quad (1a)$$

$$H_2CO_3 = H^+ + HCO_3^- \quad (1b)$$

$$CO_{2(aq)} + OH^- = HCO_3^- \quad (2)$$

At pH < 8, reaction 2 is negligible due to the absence of OH^- ions. At 8 < pH < 10, both reaction sequence 1 and reaction 2 occur, while, at pH > 10, reaction 2 predominates. Except in high-pH situations (such as the absorption of CO_2 into lime), calcium carbonate precipitation involves reaction sequence 1. Once bicarbonate ions are present in solution, they will, to an extent dependent on pH, dissociate to form carbonate ions.

The rate-controlling step in the fixation of gaseous CO_2 into carbonate ions is the hydration (reaction 1a) of CO_2 (except at high pH). The rate constant of the forward reaction 1a is 6.2×10^{-2} s^{-1} at 25 °C and zero ionic strength. Reaction 1b is very rapid, the rate being virtually diffusion controlled.[17] Dissociation of bicarbonate ions to yield carbonate ions is slower than 1b, but is still much faster than reaction 1a. Thus, if a viable means to accelerate reaction 1a (the hydration of CO_2) could be found, it should be feasible to fix large quantities of CO_2 into calcium carbonate at moderate pH.

A solution to the problem of accelerated CO_2 hydration, in fact, already exists in biological systems. The carbonic anhydrases (CAs) are a broad group of zinc metalloenzymes that are ubiquitous in nature.[18−20] They are among the fastest enzymes known, and they catalyze the reversible hydration of CO_2. The fastest CA isozyme known is the human isozyme HCA II, each molecule of which can hydrate at least 1.4×10^6 molecules of CO_2 per second;[21] the catalyzed hydration occurs at or near the diffusion-controlled limit for the encounter rate of enzyme and CO_2. Thus, if we use CA to catalyze the hydration of CO_2, it should be possible to fix large quantities of CO_2 into carbonate form, without recourse to caustic conditions. [Another group has, in fact, been looking at CA as a catalyst for short-term aqueous sequestration of CO_2 for use in completely closed systems, such as a space station (M. Trachtenberg, personal communication).]

Feasibility and Issues

Feasibility of our biomimetic approach was demonstrated,[5,6] based on two types of experiment. One was designed to show acceleration of the overall process of forming a solid product (calcium carbonate) in the presence of CA. This involves a series of steps beyond the hydration of CO_2, but is vital to show potential industrial applicability. The other was designed to

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(17) Keene, F. R. In *Electrochemical and Electrocatalytic Reactions of Carbon Dioxide*; Sullivan, B. P., Krist, K., Guard, H. E., Eds.; Elsevier: Amsterdam, 1993; pp 1−18.

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(21) Khalifah, R. G.; Silverman, D. N. In *The Carbonic Anhydrases: Cellular Physiology and Molecular Genetics*; Dodgson, S. J., Tashian, R. E., Gros, G., Carter, N. D., Eds.; Plenum Press: New York, 1991; pp 49−70.

demonstrate the accelerated hydration of CO_2 in the presence of CA. This shows catalysis of a single reaction, and hence is applicable also to comparisons of enzyme performance for different isozymes, and under different conditions. Initial experiments were performed with bovine carbonic anhydrase (BCA), which is available in purified form from Sigma Chemical Corporation. Since an economical source of the enzyme would be essential for commercial application, tests were also performed on crude (dilute) extracts from plant sources (spinach and parsley) and yeast (*Saccaromyces cerevisiae*).[5] Very large accelerations of both CO_2 hydration and precipitation of calcium carbonate were seen with the purified BCA, but significant accelerations were also obtained even with very crude, dilute plant extracts. The simplest and cheapest means of obtaining large amounts of CA will be by means of overexpression by a genetically modified bacterial system. Hence we then turned our attention to HCA II obtained by bacterial overexpression, following the generous donation of the phage vector by Drs David Silverman and Peter Laipis at the University of Florida. Excellent results have been obtained,[6] again with rather crude (though in this case less dilute) extracts; this is important because enzyme purification is expensive.

Following the successful proof of principle, several topics were identified as needing further study, and some of these are discussed in refs 5 and 6. Among these topics are optimization of the catalyst, and source of the cations. We are currently considering three possible sources of cations (particularly calcium ions), each of which could serve a dual function as both aqueous process stream and cation source. These are seawater, waste brines from desalination operations, and brines from saline aquifers. Selection of the source is anticipated to be site specific, depending on the location of the particular power plant.

It is interesting to note that shell-forming marine organisms produce calcium carbonate and not magnesium carbonate, even though the concentration of magnesium ions in seawater is around five times that of calcium ions. Not only does magnesium carbonate have a somewhat higher solubility than calcium carbonate, but also magnesium ions are more strongly hydrated than calcium ions, which inhibits crystal growth.[22,23] We are, however, going to be investigating the possibility of forming magnesium as well as calcium carbonate, though not necessarily with seawater as the cation source.

In any approach to CO_2 sequestration, the huge quantities involved cannot be ignored, and the proposed biomimetic approach is no exception. For example, if the CO_2 were to be sequestered by the pumping of seawater through a separation vessel at the utility site, with a calcium ion concentration in the seawater of 400 g per tonne, 100% removal from a unit of the size of the model Kenosha plant would require a flow of 18 million tonnes of seawater per day. This is a very large number indeed. It is, however, less than an order of magnitude higher than the cooling water flow through such a unit, which would be of the order of 2.4 million tonnes per day

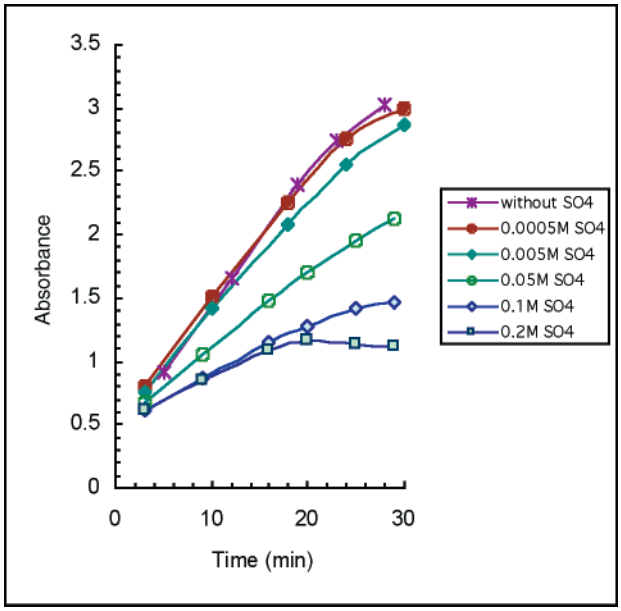

Figure 1. *p*-NPA activity of BCA in various concentrations of SO_x, in tris buffer.

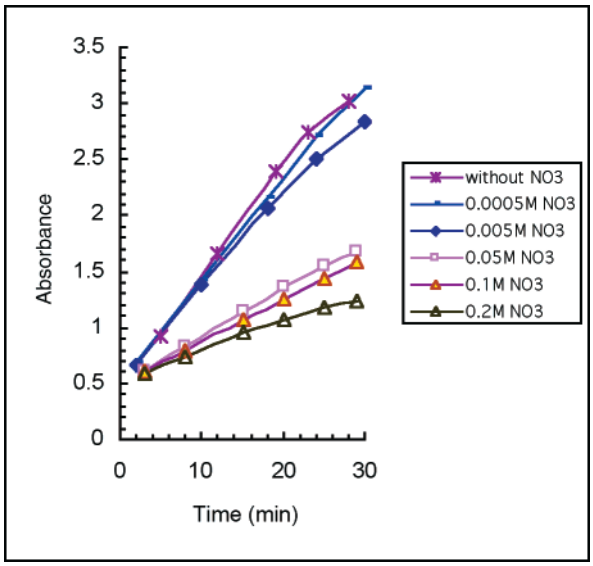

Figure 2. *p*-NPA activity of BCA in various concentrations of NO_x, in tris buffer.

(based on a typical requirement of 800 tonnes of cooling water per tonne of coal burned). It should be noted also that seawater is the most dilute of the brines being considered as possible cation sources, and so it is by no means certain that such a large flow through the plant would be necessary.

It was pointed out above that the amount of solid product would also be very large. For example, if 100% of the CO_2 produced by the model Kenosha plant were to be sequestered as calcium carbonate, $CaCO_3$, this would correspond to 666 tonnes of product per hour, or close to 16 000 tonnes per day. The masses involved are a factor of over fifty times that involved in flue-gas desulfurization (FGD). A more useful comparison in terms of solid handling, however, is with the amount of coal consumed, which is approximately 3000 tonnes per day, from which the scale-up would be by a factor of a little over five (for 100% sequestration of the CO_2 produced).

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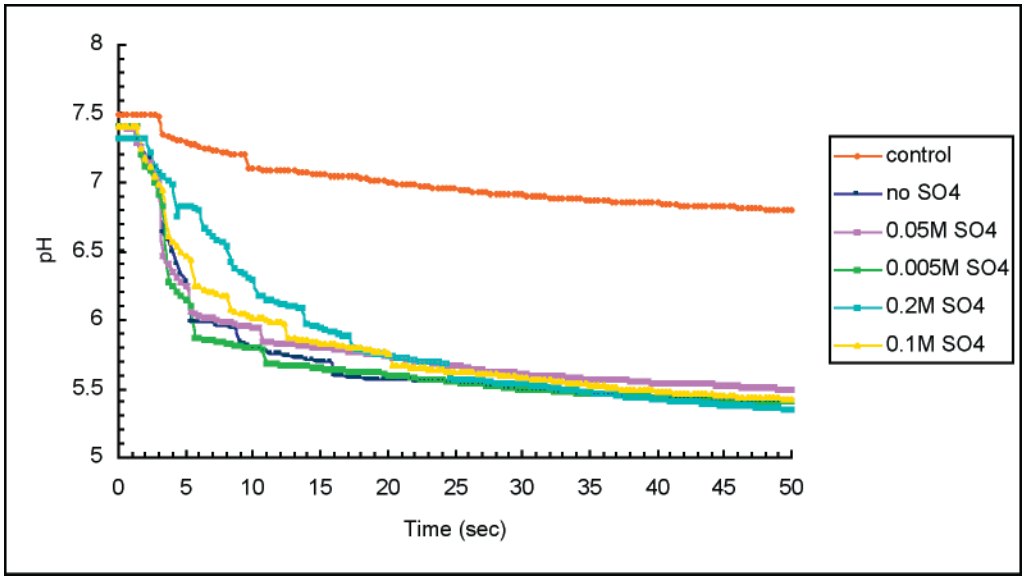

Figure 3. Delta-pH activity of BCA in various concentrations of SO_x, in tris buffer.

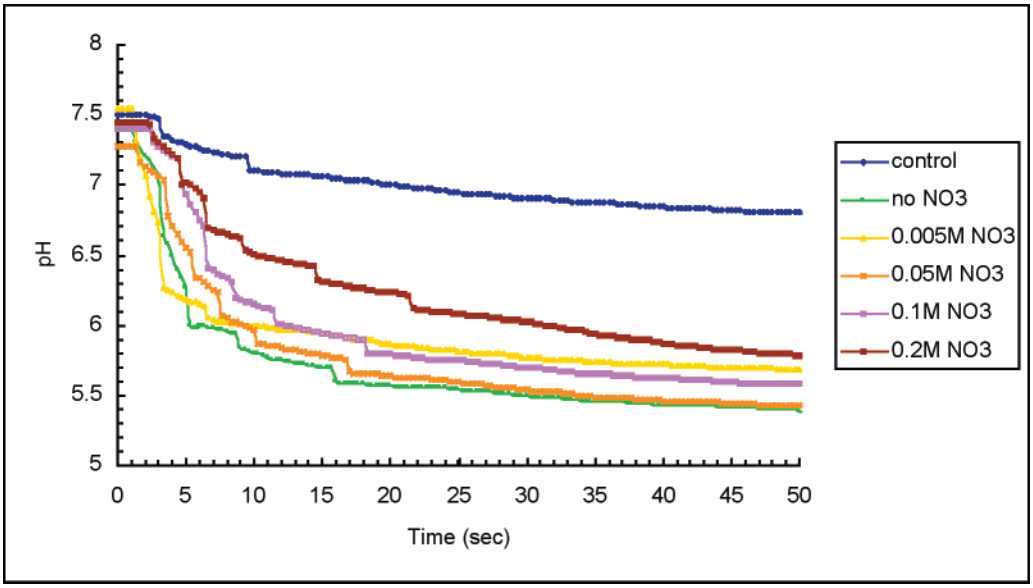

Figure 4. Delta-pH activity of BCA in various concentrations of NO_x, in tris buffer.

One of the issues that arise in relation to sequestration products is their potential marketability, although it should always be remembered that it is dangerous to try to justify remediation processes on the basis of product marketability. For example, the product of FGD is gypsum, and this can be sold, but the amount produced by FGD has quickly exceeded the market. This is a well-known fact. Nevertheless, potential markets are definitely of interest. At first thought, there may appear to be little potential market for a solid carbonate, because carbonates are such common minerals. In most commercial applications, however, the requirement of a fine particle size would make the (powder) product of our proposed process likely to compete very well with mined and crushed carbonate.

We are actively looking at possible geometries of a commercial system based on our proposed biomimetic sequestration method. At this time, there is still too much research remaining to be done, for us to describe a potential scrubber in detail. The different types that have been used or proposed for FGD will probably not be directly scalable to the requisite size, but the gas/liquid contacting systems are likely to be the same in general principle.

Some inhibition of CA activity by various anions has been reported previously (see, for example, refs 24 [delta pH] and 25 [*p*-NPA assay]). By far the most potent of the inorganic anionic inhibitors of CA, however, is CN^-, which is not an issue for the proposed application. Very small amounts (unlikely to exceed 100 ppm) of SO_x[26] and NO_x may be present in the flue gases. Higher concentrations of anions are to be expected in the process water used, particularly, of course, if it is brine or seawater. There are other contaminants besides SO_x and NO_x, the effects of which will need to be determined. It is our belief that a carbon-scrubbing device based on the proposed method would follow an FGD system at

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Table 1. Content of Ions in Seawater[28]

Cl^-	19.304 g/kg	0.546 mol/L
Na^+	10.77 g/kg	0.468 mol/L
Ca^{2+}	0.412 g/kg	0.0102 mol/L
K^+	0.399 g/kg	0.0102 mol/L
Mg^{2+}	1.29 g/kg	0.0053 mol/L
HCO_3^-	0.142 g/kg	0.00238 mol/L
This Includes Total Co_2 Measurement		
SO_4^{--}	2.712 g/kg	0.0028 mol/L
Br^-	0.0673 g/kg	8.4×10^{-4} mol/L
B	0.0045 g/kg	4.1×10^{-4} mol/L
F^-	0.0013 g/kg	6.8×10^{-5} mol/L
Other Ions:		
I	60 μg/kg	
As	2 μg/kg	
Ni	490 ng/kg	
Zn	390 ng/kg	
Se	170 ng/kg	
Cu	120 ng/kg	
Cd	70 ng/kg	
Fe	40 ng/kg	
Ga	10−20 ng/kg	
Mn	10−20 ng/kg	
Hg	6 ng/kg	
Ag	3 ng/kg	
Co	2 ng/kg	
Pb	1 ng/kg	

an actual power plant, and hence relatively few other contaminants in the flue gas would reach it. Nevertheless their effects should be investigated, and work on this is in progress. Flue gas that had passed through an FGD system would have been in contact with a relatively basic (calcium hydroxide) environment, and so other acid gases would have been removed. The only issue in relation to pH would then be the acidity of the CO_2 itself. We know that a buffering system may well be necessary, and we are looking at possible buffering systems.

Experimental Section

A suitable catalyst (or isozyme) for an industrial-scale CO_2 scrubber will have to be fast, robust, and capable of being produced in large amounts cost-effectively. For this we anticipate the use of enzyme produced by bacterial overexpression, and we are currently optimizing production of HCA II, the fastest known CA isozyme, as indicated above. In the short term, however, initial experiments on anionic inhibition have been performed on BCA (bovine erythrocyte CA), purchased in purified form from Sigma Chemical Corporation. BCA is quite similar to HCA II, and should give a good initial indication of robustness.

The method that has been used to show the accelerated hydration of CO_2 is a delta pH method.[27] CA catalyzes the reversible hydration reaction between CO_2 and H_2O, producing HCO_3^- and H^+. This production of protons leads to a change in pH as the reaction proceeds toward equilibrium. Measurement of this pH change as a function of time forms the basis of the delta pH method. Measurements are usually made at temperatures in the range 0−5 °C, to slow the enzyme-catalyzed reaction, which is otherwise so rapid that initial rates are hard to measure.[27] A World Precision Instruments Bee-Trode pH electrode and Dri-Ref system, with an ATC (automatic temperature compensation) probe, connected to an Orion Sensorlink pH data acquisition system, was used for temperature-compensated pH monitoring. Activities were

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Table 2. Content of Ions in ASW (based on data on Sigma Chemical Corp. web page)

chloride	19.251 g/L
sodium	10.75 g/L
sulfate	2.659 g/L
magnesium	1.317 g/L
potassium	0.402 g/L
calcium	0.398 g/L
carbonate/bicarbonate	0.192 g/L
Trace Elements Approximate Values:	
strontium	0.007 g/L
boron	0.004 g/L
bromide	0.060 g/L
iodide	0.00006 g/L
lithium	n/a
nitrate	n/a

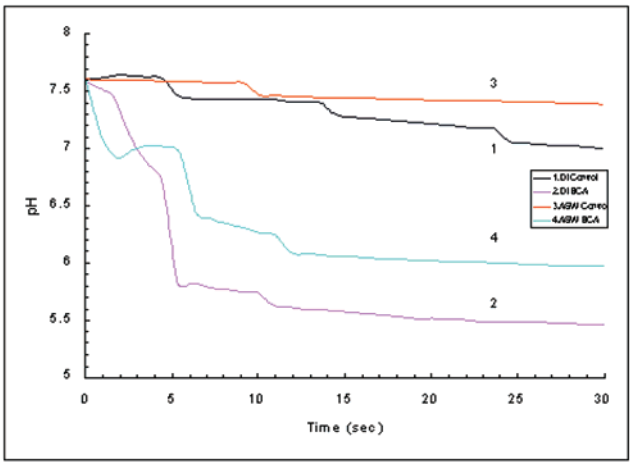

Figure 5. Delta-pH activity of BCA in artificial seawater (ASW) and de-ionized water (DI) tested in barbital buffer.

measured and compared for 30μg/mL BCA in 2.5 mM aqueous CO_2 solution, in the presence of different concentrations of Na_2SO_4 and $NaNO_3$, in 25 mM pH 7.4 tris buffer, at 1−3 °C. CA activity in ASW-based solution (artificial seawater) was also compared to that in deionized-water-based solution. ASW was produced by dissolution of Sigma artificial-seawater salts, 38 g of which were added to 1 L of deionized water prior to bubbling with CO_2. Activities were also measured and compared in the ASW-based solutions, in the presence of different concentrations of Na_2SO_4 and $NaNO_3$, at 1−3 °C. In the case of ASW-based solutions, the weak buffering was achieved with either barbital or tris buffer.

A *p*-NPA assay has also been used to monitor the activity of CA, based on the enzyme-mediated hydrolysis of *p*-nitrophenyl acetate.[25] Activities were measured and compared for 20 μg/mL BCA in the presence of different concentrations of Na_2SO_4 and $NaNO_3$, in 50 mM pH 7.4 tris buffer, at 25 °C. Stock solutions of 0.0025 g/mL *p*-NPA were prepared in acetonitrile, which was used to prevent spontaneous decomposition of *p*-NPA in air or in water. 10% (v/v) was added into each sample solution. The absorbance intensity at 405 nm for the yellow product, *p*-nitrophenol, was followed versus time with a Hitachi-330 spectrophotometer. The slope of absorbance versus time was defined as the activity gradient.

Results

As indicated above, the *p*-NPA assay has been used to monitor the activity of CA, in addition to the delta pH assay; the *p*-NPA assay is based on the enzyme-mediated hydrolysis of *p*-nitrophenyl acetate.[25] The reason that this assay is often used in studies of the carbonic anhydrases is that the same enzyme active site

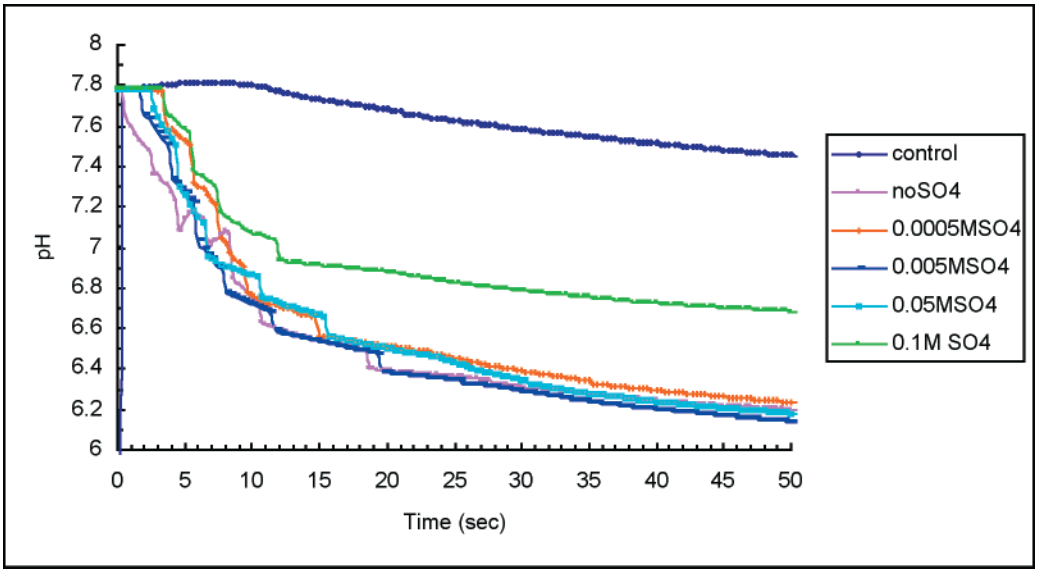

Figure 6. Delta-pH activity of BCA in various concentrations of SOx, in ASW.

that is responsible for acceleration of CO_2 hydration also accelerates this hydrolysis reaction, which yields a bright-yellow product that absorbs at 405 nm and can be determined spectrophotometrically. Due to stearic factors, however, the reaction rate is much slower for the hydrolysis reaction than for the hydration of CO_2. Thus the enzyme-accelerated hydrolysis rates can be conveniently monitored without cooling (in contrast to the delta pH measurements of accelerated CO_2 hydration). Therefore we are using the *p*-NPA assay for a first look at the influence of different parameters on CA activity, since it is simpler to perform, and does not require cooling of the solutions to slow the kinetics, but still involves the same active site on the enzyme. We are then using the delta pH assay to obtain results more directly relevant to acceleration of the hydration reaction.

Absorbance-versus-time plots from the *p*-NPA assay are shown for SO_4^{2-} concentrations ranging from 0.5 up to 200 mM in Figure 1. A higher gradient demonstrates a more rapid reaction, and so any marked reduction of the gradient is indicative of a potentially problematic inhibition. There is little inhibition at concentrations up to 5 mM (480 ppm), but there is significant inhibition by 50 mM. Similar plots are shown for NO_3^-, again over a concentration range of 0.5 up to 200 mM, in Figure 2. Again, large-scale inhibition starts somewhere between 5 mM (310 ppm) and 50 mM.

Delta pH data for different SO_4^{2-} and NO_3^- concentrations, ranging from 5 to 200 mM, are shown in Figures 3 and 4, respectively, for solutions based on CO_2-saturated deionized water containing BCA. Here, a more rapid drop of pH to the equilibrium (plateau) value demonstrates more rapid hydration of CO_2, and slowing of that drop indicates inhibition. Contrary to the results obtained with the *p*-NPA assay, there is little indication of inhibition at concentrations below 100−200 mM.

Experiments have also been performed in ASW-based solutions, since the actual process stream may well be based on seawater, or some other brine, as discussed above. A typical composition for seawater is given in Table 1, and the composition of the ASW is given, for comparison, in Table 2. (Experiments with solutions based on more concentrated brines will be performed, following the identification of the most potentially relevant compositions.) Figure 5 shows a comparison of enzyme activity in ASW-based solution versus DI-water-based solution. The enzyme is seen to perform well in both solutions. The delta pH assay was preferred for assessment of the effects of SO_4^{2-} and NO_3^- ions in the ASW-based solutions, in light of the discrepancy between the results of the two assays indicated above for the experiments in DI-water-based solutions. Delta pH data for different SO_4^{2-} and NO_3^- concentrations, ranging from 0.5 to 100 mM, are shown in Figures 6 and 7, respectively, for solutions based on CO_2-saturated ASW containing BCA. Noticeable inhibition does not occur until 100 mM.

Discussion

It is interesting to note that the enzyme appears to be less susceptible to inhibition as determined by the delta pH technique, than by the *p*-NPA assay. As indicated above, the *p*-NPA assay is being used for a first look at the influence of different parameters on CA activity, since it is simpler to perform, and does not require cooling of the solutions to slow the kinetics, but still involves the same active site on the enzyme. It should be remembered, however, that the enzyme is a much less efficient catalyst for the hydrolysis of *p*-NPA than it is for the hydration of CO_2, (which is why this assay is simpler and does not require cooling), and hence it is perhaps not surprising that that already somewhat difficult catalytic action should be more easily inhibited. It is clear that, for the present purposes of catalyst optimization, the *p*-NPA assay has a useful role to play for convenient initial screening, but the final assay should be delta pH.

Given the low levels of SO_x and NO_x that would be present in flue gases reaching a CO_2 scrubber located behind a sulfur scrubber, it appears very unlikely that either of these species would present an inhibition problem to the enzyme in such a system. It also appears unlikely that the type of water used will pose an

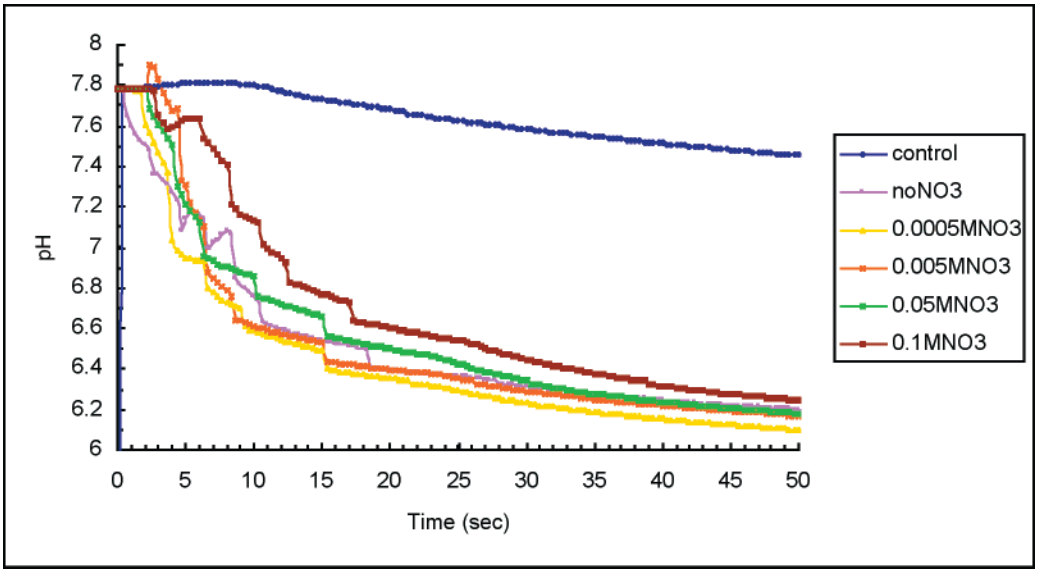

Figure 7. Delta-pH activity of BCA in various concentrations of NO_x, in ASW.

inhibition problem, even for seawater, as represented here by the ASW-based solution. Similar experiments will be performed on the other isozyme, produced by bacterial overexpression, that we are currently investigating, and also on solution chemistries representative of other brines.

The results are extremely encouraging, because they indicate that the robustness of mammalian CA II may well be adequate for its application in a CO_2 scrubber, as envisaged. Given sufficient robustness of these isozymes, our first preference would be to make HCA II the isozyme of choice. It is the fastest isozyme known, and it is already being successfully produced by bacterial overexpression. Furthermore, we have already immobilized it successfully in an environmentally friendly matrix, based on porous alginate beads with a relatively hard chitosan coating.[29,30]

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