Solubilization and Concentration of Carbon Dioxide: Novel Spray Reactors With Immobilized Carbonic Anhydrase

Sumana Bhattacharya,[1] Amiya Nayak,[1] Marc Schiavone,[1] Sanjoy K. Bhattacharya[2]

[1]ABRD Co., LLC, Cleveland, Ohio
[2]Department of Ophthalmic Research, Area I31, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44195; telephone: 216-297-9680; fax: 216-297-9892; e-mail: sbc-adrd@usa.net

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Abstract: Novel spray reactors are described that employ immobilized biocatalyst (carbonic anhydrase), enabling concentration and solubilization of emitted CO_2 by allowing catalytic contact with water spray. The reactors were fed with simulated emission gas. The performance of the reactors was investigated with respect to operation variable: emission flow rate; gas composition in the emission stream; water flow rate; area-to-volume ratio of immobilized reactor core; and the enzyme load within the core. The reactors were also investigated for pressure drop and extractability of CO_2 from the emission with single vs. multiple reactors (of combined equal volume). The biotechnological process of solubilization and concentration of CO_2 from emission exhausts or streams occurring in the spray reactors could be coupled for further biochemical/chemical conversion of the concentrated CO_2. © 2004 Wiley Periodicals, Inc.

Keywords: solubilization; concentration of CO_2; immobilized carbonic anhydrase; novel reactors; trickling spray reactors

INTRODUCTION

Anthropogenic CO_2 emission has a severe impact on climate. It is regarded as a global pollution problem and has been implicated in global warming (Joos et al., 1999; Schnur, 2002). Controlling CO_2 pollution is best achieved if abatement is implemented at the source. The fixation of carbon of CO_2 in the concatenated form in compounds is the best way of holding the carbon in the fixed state for a long period as compared with one-step terminal fixation, such as in the form of carbonate by combining with metal oxides (e.g., calcium oxide) (Bhattacharya, 2001; Bhattacharya et al., 2002). Biochemical fixation of CO_2 readily renders fixation in concatenated carbon compounds, which however, also requires a CO_2 concentration step. A biotechnological solution to fixation requires an aqueous solubilization step in addition to concentration before incorporating the CO_2 for biocatalytic fixation into concatenated carbon compounds.

Correspondence to: S. Bhattacharya

A recyclable bioprocess enabling continuous fixation was developed for the abatement of CO_2 pollution at the source (Bhattacharya, 2001; Bhattacharya et al., 2002). The device enabling the use of this recyclable process was based on a modular approach: in one module the CO_2 is fixed on 5-carbon-acceptor D-ribulose-1,5-bisphosphate (RuBP) using Rubisco (Bhattacharya, 2001; Chakrabarti et al., 2003a, 2003b), and in another module using a cohort of enzymes the RuBP is regenerated from 3-phosphoglycerate (3-PGA), the product of fixation. Energy for driving the recycling is derived from solar radiation (Bhattacharya, 2001); however, any other form of energy can also be used to propel RuBP regeneration.

The capture of CO_2 from the emission stream and main tenance of the concentration of CO_2 near the active site of Rubisco in the bioreactor (harboring immobilized Rubisco) are two great challenges. Direct capture of CO_2 from the emission stream using only immobilized Rubisco limits the capture rate and also leads to an increase in pressure of the outlet because the gas phase is required to pass through a solution phase holding immobilized Rubisco. A process for direct capture of CO_2 from emission of the exhaust/stream for concentration and solubilization (and feeding to immobilized Rubisco) is lacking. The notion that CO_2 pollution can be abated by fixation at the source has been discounted continuously in the scientific literature (Beckman, 1999). This belief has limited research and development in this area, such as the study of processes and devices for the concentration and solubilization of CO_2. Thus, any device employing a biotechnological process for concentration and solubilization of CO_2 directly from the emission steam for further biochemical (or chemical) conversion of the latter do not exist. This has resulted in the development of the present novel trickling spray reactor employing immobilized carbonic anhydrase, which enables concentration of CO_2 from the emission stream. Carbonic anhydrase is one of the fastest enzymes that make fast mass transfer from gas phase to aqueous phase, which may then be fed to coupled Rubisco reactors, enabling effective conversion of captured CO_2.

Carbonic anhydrase (CA; EC 4.2.1.1), a zinc metalloenzyme, catalyzes the reversible hydration of CO_2 and the dehydration of HCO_3^- and plays a significant role in a number of physiological processes (Badger and Price, 1994; Coleman, 1991; Tashian, 1989). CA activity is found in tissues of plants and animals (Badger and Price, 1994; Maren, 1967; Maren and Sanyal, 1983; Sultemeyer et al., 1993), archaea (Karrasch et al., 1989), cyanobacteria (Ingle and Coleman, 1975; Kaplan et al., 1990), and in a variety of eubacteria (Maren and Sanyal, 1983; Suzuki et al., 1994). CA plays an important role in photosynthesis and in the operation of the CO_2-concentrating mechanism (CCM) in archaebacteria, eubacteria, and cyanobacteria (Kaplan et al., 1990). As a process, CCM increases CO_2 concentration around the primary carboxylating enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), to levels several orders of magnitude above that present in the surrounding medium, which enables an enhanced rate of photosynthesis (Badger and Price, 1994; Miller et al., 1990; Price et al., 1992). Carbonic anhydrase has been immobilized by a number of different methods (Azari and Nemat-Gorgani, 1999; Bhattacharya et al., 2003; Manecke and Schlunsen, 1976; Manecke and Vogt, 1980; Salley et al., 1992; Simsek-Ege et al., 2002; Turkova, 1976). However, neither immobilized carbonic anhydrase nor any other biocatalyst or chemical agent has been applied for concentration and solubilization of CO_2 from emission streams. Also, it is widely anticipated that attempts to capture CO_2 from the emission stream would result in a decrease in pressure in the outlet (pressure drop), leading to an increase in pressure (backpressure) in the inlet part of the emission stream.

MATERIALS AND METHODS

Carbonic Anhydrase

The carbonic anhydrase from thermophilic *Methanobacterium thermoautotrophicum* was cloned in pET19b vector using standard molecular biology protocols, as described elsewhere (Smith and Ferry, 1999). The cloned enzyme was expressed in *E. coli* BL21DE3 plysS transformed with a plasmid vector (pET19b) carrying the DNA sequence and purified using an Ni–NTA resin column and was used in the reactor core after immobilization.

Assay of Carbonic Anhydrase

Carbonic anhydrase activity was assayed using an electrometric method (Wilbur and Anderson, 1948). In typical assays, 50 μL of protein solution was diluted to 4 mL of prechilled 50 m*M* HEPES (*N*-2-hydroxethylpiperazine-*N′*-ethanesulfonic acid) buffer, pH 8.0. For assay at different pH, 50 m*M* HEPES was used at above pH 7.0 and 50 m*M* MES (2*N*-morpholinoethanesulfonic acid) was used at below pH 7.0. The mixture was stirred and maintained on ice for several minutes. The assay was initiated by the addition of 10 mL of ice-cold, CO_2-saturated water into the reaction vessel. The change in pH from 8.0 to 7.0 at 25°C was monitored using a bench-top pH meter and semi-micro combination electrode and the signal was directed to a chart recorder. CA activity was expressed in Wilbur–Anderson (WA) units per milligram of protein and calculated using the formula: $[(t_0/t - 1) \times 10]$/mg protein, where t_0 and t represent the time required for the pH to change from 8.0 to 7.0 in a buffer control and CA sample, respectively. A micro method was also used to determine CA activity for select samples (Maren, 1960).

Immobilization

Carbonic anhydrase was immobilized using coupling methods on steel matrix coated with silica (Bhattacharya et al., 2003).

Preparation of Silanized Carrier

Iron filings from a lathe machine were collected and 30- to 45-mesh particles were used for silanization. For immobilization, about 10 mg of CA in Tris or HEPES buffer (pH 8.0) per gram matrix was used for immobilization. The inorganic support material was first treated with organofunctional silane, as described elsewhere (Bunting and Laidler, 1972). The silane reacts with available oxide groups on the carrier surface leaving an organic functional group available for coupling to the enzyme. Reaction of the carrier with γ-aminopropyltriethoxysilane was used for coupling. Silane polymerizes across the surface of the carrier anchored at various intervals (Bunting and Laidler, 1972; Kobayashi and Moo-Young, 1973). The amino derivative was covalently coupled using carbodiimides as described previously for other enzymes and matrices (Chakrabarti et al., 2003a), or converted into a carboxyl derivative using an alkylamine carrier with succinic anhydride according to a published protocol for other enzymatic entities (Harhen and Barry, 1990).

Determination of Mechanical Stability of Immobilization Matrix

The particle size distribution of controlled pore inert matrices was measured as a function of applied load to a standard volume of materials in a punch-and-die set within a pressure range 50 to 200 and 200 to 2500 psi (Eaton, 1974, 1976). Mercury intrusion porosimetry utilizing an Aminco–Winslow porosimeter (Messing, 1970, 1974) was used to determine the pore density of immobilized porous materials. For this purpose, both bovine serum albumin (BSA) and CA II were immobilized and operated under pressures of 50 to 200 and 200 to 2500 psi, and the average pore diameter was estimated to determine breakage of matrix.

Measurement of Pressure and CO_2 in Emission Gas

The pressure of the emission stream in the inlet and outlet was measured using an HD8804 K pressure and temperature kit equipped with the appropriate pressure probes. Some measurements were also made using a Testo 525 instrument (Hotek Technologies, Tacoma, WA). For CO_2 measurement in the emission gas, a Testo 400 IAQ kit, equipped with 0632 1240 and 0635 1240 CO_2 probes, was used (Hotek Technologies, Tacoma, WA). The percent of CO_2 in the emission gas with and without reactor attachment was measured. For most optimization studies, unless stated otherwise, an enzymatic core volume of 100 mL with an enzyme load of 1.5 mg/mL and average gas flow rate of 4 to 5 L/min, having 40% CO_2 in the emission stream, was used.

Description of Reactors

For the biotechnological process enabling concentration and solubilization of CO_2, novel immobilized carbonic anhydrase reactors were designed. The reactors have a highly porous core (harboring immobilized CA) that allows flow of emission gases and water in the form of spray. Components of the reactor are described in Figure 1. The gas in the reactor enters through a tube connected directly to the emission stream (part 1 in Fig. 1). The top of the reactor has a lid (part 2) connected to the water entry port (part 3), and the bottom of this top portion has a porous lid that allows water spray (part 4). The novel trickling spray reactor has a solid body (part 5), which houses the central immobilized matrix shell (part 7). The shell is encased in a wire mesh

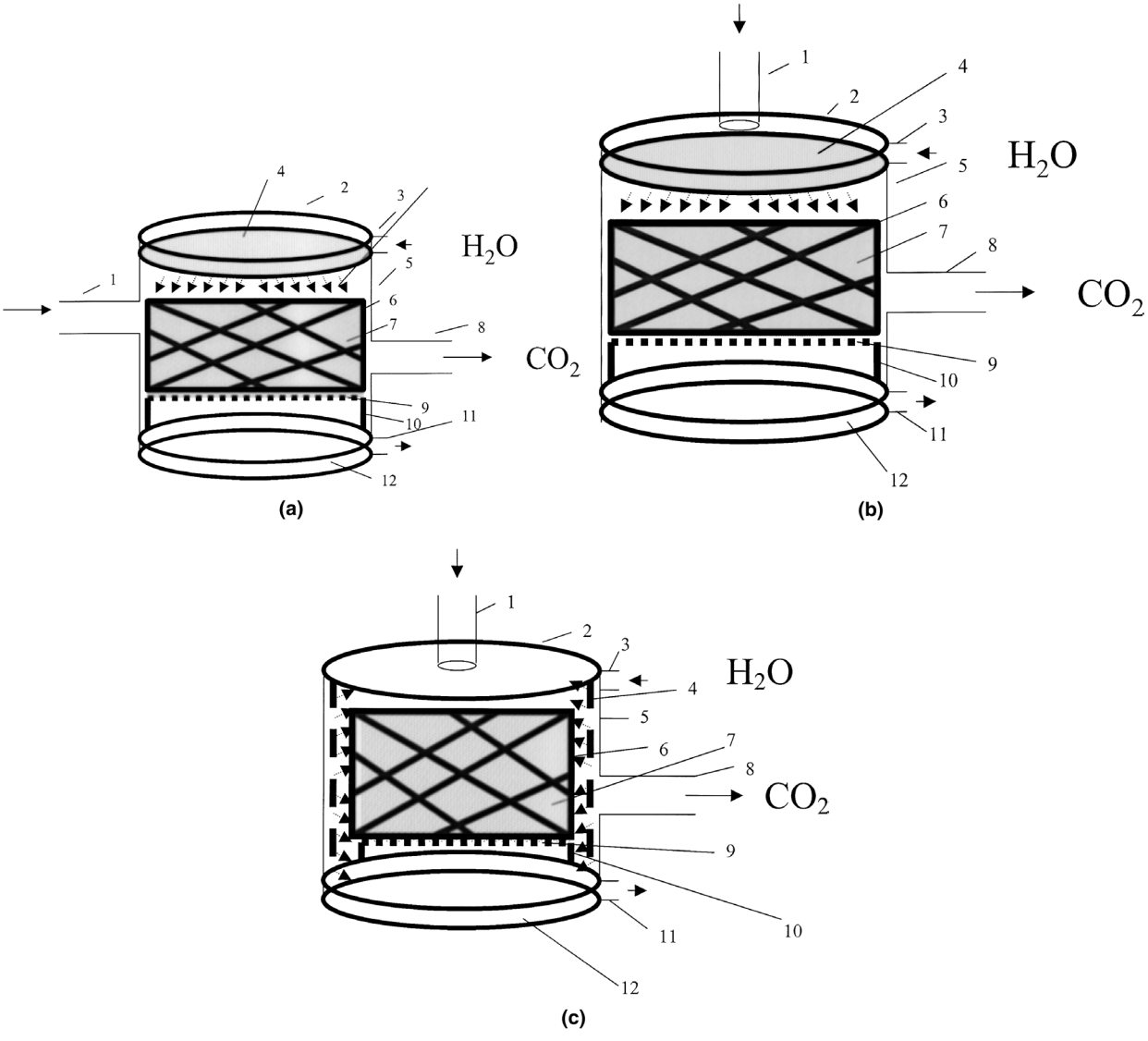

Figure 1. Design of the concentrator reactors. There were three basic designs: (a) with horizontal inflow and outflow of gas and vertical water spray; (b) with vertical inflow, horizontal outflow of gas (or vice versa), and vertical water spray; and (c) with vertical inflow, horizontal outflow of gas (or vice versa), and horizontal water spray. The parts are: 1—inlet nozzle for gas/emission; 2—outer lid; 3—water inlet; 4—sprayer mesh; 5—main vessel/reactor; 6—large wire container for holding immobilized enzyme core; 7—immobilized carbonic anhydrase core; 8—outlet nozzle for gas/emission; 9—bottom wire mesh for percolation of solution; 10—holder stand; 11—water outlet; 12—bottom solution holding chamber.

(part 6) and sits on a perforated metal plate coated with glass (part 9). The metal plate (part 9) has pores, 2 to 5 mm in diameter, and rests on a strand (part 10). The reactor has one entry port (part 1) and one exit port (part 8) for the flow of emission gas/stream. The reactor also has water inlet (part 3), spray mechanism (part 4), and water/solution outlet (part 11). The bottom of the reactor (part 12) collects the aqueous flow through a single tubing exit (part 11). This solution exit (part 11) could easily be connected to a coupled reactor harboring immobilized Rubisco. The dimensions of the cylindrical central part of the reactor are 50 cm × 30 cm (diameter × length). The diameter of the gas inflow and outflow tube is 10 cm. However, these dimensions can vary according to the emission stream and other parameters. The 5-cm portion from the top of this cylindrical central reactor houses water for spray. The water inlet (part 3) and solution outlet (part 11) have a diameter of 2 cm. The spray is governed by a lid having pores of 0.5 mm in diameter (part 4). The wire-mesh encasing (part 6) for immobilized enzyme is made up of steel material with pores 5 to 8 cm in diameter. The steel is coated with glass to withstand corrosion.

RESULTS

A biotechnological process has been developed where the CO_2 present in the emission stream (free of soot) could be contacted with water in the presence of immobilized carbonic anhydrase (Bhattacharya et al., 2003), resulting in catalytic solubilization of CO_2 in water. The contacting process of gas with water also results in the concentration of CO_2 from the emission stream in the aqueous phase.

Bioreactor Operation and Stability of Immobilized Biocatalyst

The reactor described in Materials and Methods houses the immobilized enzyme core harboring purified recombinant carbonic anhydrase from thermophilic *Methanobacterium thermoautotrophicum* (Smith and Ferry, 1999). The enzymes were immobilized (covalently coupled using carbodiimides) on a silica-coated steel matrix of different average mesh size using methods reported earlier (Bhattacharya et al., 2003). A thin film of water around the immobilized enzyme keeps the enzyme hydrated and active for a long period of time. Buffering of the enzyme is apparently not necessary for long periods. Buffer flushing of the reactors once every 24 h of continuous operation greatly enhances the shelf-life of the immobilized enzyme. The average temperature of the emission gas entering into the reactor in all experiments was maintained at 60°C. Three basic designs for the reactors are shown in Figure 1a–c. All three reactor designs allow control of two different flows, emission gas, and water spray, and with respect to flow of gases it was either horizontal inflow and horizontal outflow or vertical inflow and horizontal outflow (or vice versa). For water spray it was either vertical or horizontal. Therefore, the basic design of the reactor was reduced to one of three types: (i) that with horizontal inflow and outflow of gas and vertical water spray; (ii) that with vertical inflow, horizontal outflow of gas (or vice versa), and vertical water spray; or (iii) that with vertical inflow, horizontal outflow of gas (or vice versa), and horizontal water spray (Fig. 1a–c, respectively). The designs that employed vertical inflow of gas allowed inflow only from the top, never from the bottom. This is due to the stability of matrix in the presence of vertical inflow from the bottom; also, bottom gas inflow would lead to water spray going into the emission stream in some design settings. The reactor shown in Figure 1a (horizontal inflow and outflow of gas and vertical water spray) showed the best performance with regard to CO_2 reduction during the preliminary investigation, and all studies reported herein were done with this type of reactor. In all reactors investigated, simulated emission (generated using a mixture of gases/air and CO_2 derived from dry ice) rather than real stack emission was used. Nevertheless, on the basis of these operational studies, we believe that the device/reactors will work with different emissions, including stack emissions.

Effect of Emission Flow Rates and CO_2 Content in Emission Gas on CO_2 Reduction

The CO_2 percent in the simulated emission stream (CO_2 accounting for about 33% to 40% of the stream) was subjected to flow at varying rates. The water flow rate was held constant at 2.5 mL/min. The mean matrix pore size was 1 μm. The reduction in CO_2 as a function of varying gas flow rate showed an initial increase, reaching a plateau at between 5 and 7 L/min, and thereafter a progressive decrease (Fig. 2). At each point, the CO_2 in the stream without any treatment (without attachment of the reactor core) was taken as 100%. The decreases in the CO_2 calculations are based on this untreated CO_2 amount.

The reduction in CO_2 was also measured at a constant flow rate of 4.5 L/min. This measurement was achieved using an artificially enriched stream of CO_2. There was a progressive increase in reduction of CO_2 in the emission stream as a function of varying increased CO_2 composition in the emission gas. The reduction in CO_2 reached a plateau when the CO_2 concentration in the emission stream reached approximately 70% (Fig. 2).

Effect of Spray Area Versus Immobilized Core Volume on CO_2 Reduction

The area of spray with respect to core-immobilized CA volume affected percent CO_2 reduction when this biotechnological process was used. The spray area is defined as the immediate surface area of the enzyme core, which is first directly contacted by the spray generated by a definite pore size at a definite distance between spray source (pore) and immobilized reactor core. For consistency with respect to spray area measurement, and in all reactors and investigations herein, a constant spray pore size (0.5-mm

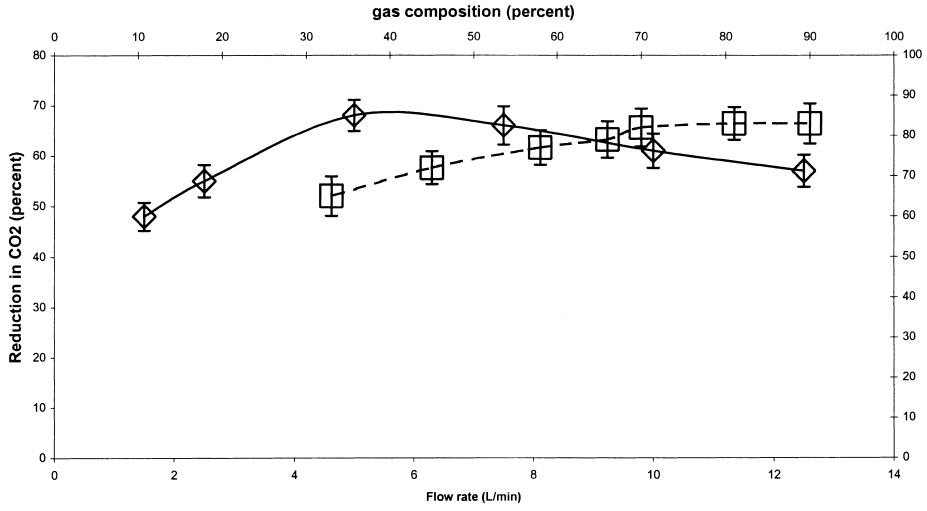

Figure 2. Percent CO_2 reduction with varying flow rate and varying gas composition in the emission. These measurements were done using a simulated stack-type emission, where parameters can be varied unlike actual emission. The measurements for both flow rate and gas composition were made (analyzed) per liter of solution used for extraction. The symbols (◇ and □) indicate flow rate and gas composition, respectively. The presented results are mean ± standard deviation of three independent experiments.

diameter) and constant distance between immobilized core and spray point (5 cm) was incorporated into the definition of spray area. To understand the effect of spray area to the volume of immobilized enzyme core, the diameter of the core was varied while keeping the volume constant (100 mL). Enzyme load was 1.5 mg/mL. The resultant L/D ratio was calculated and percent CO_2 reduced was determined, where L refers to length and D refers to diameter of the core (Fig. 1). As shown in Figure 3, the L/D ratio had an effect on CO_2 reduction. The longer length reduced the mass transfer, whereas shorter length led to decreased solubilization and a rapid escape of CO_2 from the immobilized CA core. The intermediate L/D ratio was optimal for proper mass transfer to the active site of CA and for hold-up of the gas within the immobilized core.

Effect of Flow Rate of Water (Spray) on CO_2 Reduction

The rate of water flow due to the spray also affected the catalytic solubilization of CO_2. Fast flow of water enabled a constant hydration and provided sufficient water near the active site for catalytic conversion. The flow rate of water was varied from 1 to 12.5 mL/min. The increased water flow rate showed an initial increase in the rate of CO_2 reduction and reached a plateau at around 8 mL/min

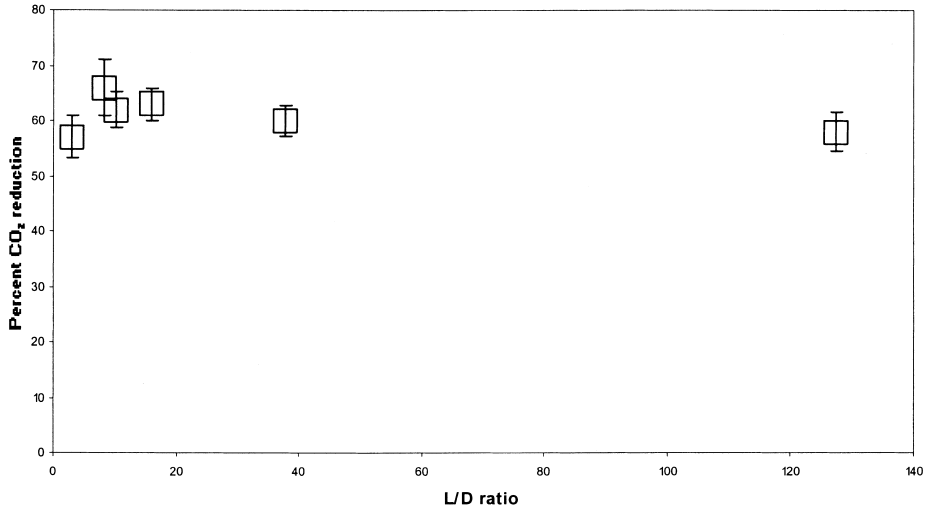

Figure 3. Percent CO_2 reduction with varying the ratio of water spray area to core immobilized CA volume [length (L)/diameter (D) ratio]. Results presented as mean ± standard deviation of five independent experiments.

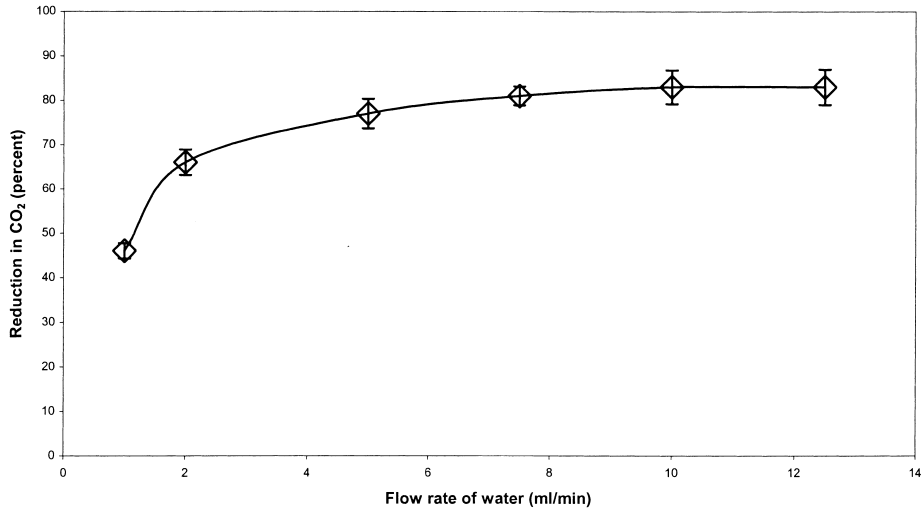

Figure 4. Percent CO_2 reduction with varying water flow rate. The water flow rate was varied between 1 and 12.5 mL/min. For correlation, the increase in pH was measured in every 1 L of extracted solution. The mean ± standard deviation of five independent experiments is shown.

(Fig. 4). The availability of water around the immobilized CA affected the CO_2 reduction, as measured by an increased reduction with increased flow rate. However, after the flow rate exceeds the limiting rate, any further increase in water will not allow further availability of reacting aqueous phase near the active site of the enzyme, and thereby the rate would remain unaffected.

Effect of Enzyme Load on CO_2 Reduction

Immobilized enzyme load had a profound effect on reduction of CO_2. The enzyme load was varied from 0.25 to 10 mg/mL. There was a progressive increase in CO_2 reduction at up to 5 mg/mL of enzyme load; beyond this point there was a decrease in CO_2 reduction from the emission stream. The decrease was possibly due to denaturation of the enzyme or to mass transfer limitation in the enzyme microenvironment with high protein load (Fig. 5).

Effect of Immobilized Matrix Pore Size on CO_2 Reduction

The matrix pore size influences CO_2 reduction. The average matrix pore size, which varied between 0.5 and 5 μm, was determined by mercury intrusion porosimetry with an Aminco–Winslow porosimeter (Messing, 1970, 1974), as described in experimental protocols. The increase in pore size increases the reduction, but beyond a definite size (2 μm) a further increase in pore size actually reduces CO_2 reduction (Fig. 6). The increase in CO_2 reduction with

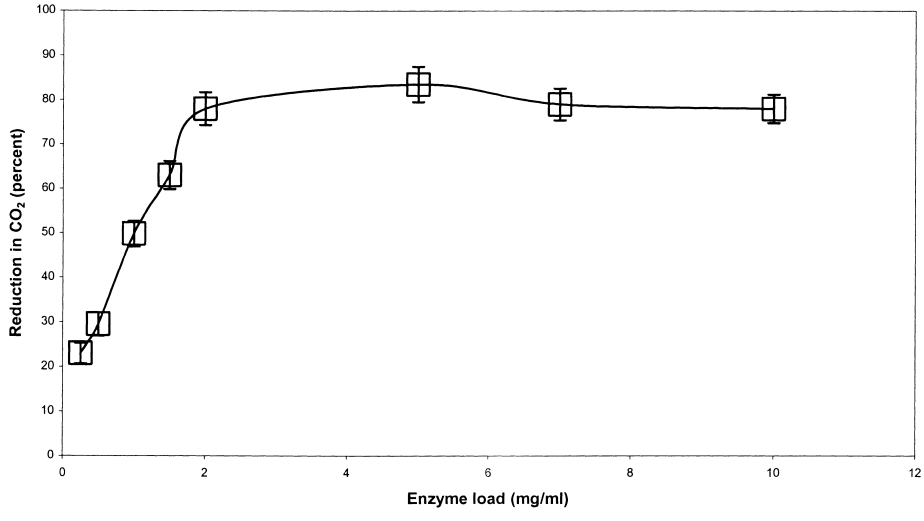

Figure 5. Percent CO_2 reduction with variation in enzyme load in the matrix of immobilization. The enzyme load was varied between 0.25 and 10 mg/mL. The enzyme (protein) load (in milligrams) was measured from initial applied load and drainage. The enzyme protein retained postimmobilization per unit core volume is expressed here as enzyme load (milligrams per milliliter). For correlation, the increase in pH was measured for every 1 L of extracted solution. The mean ± standard deviation of three independent experimental results is presented.

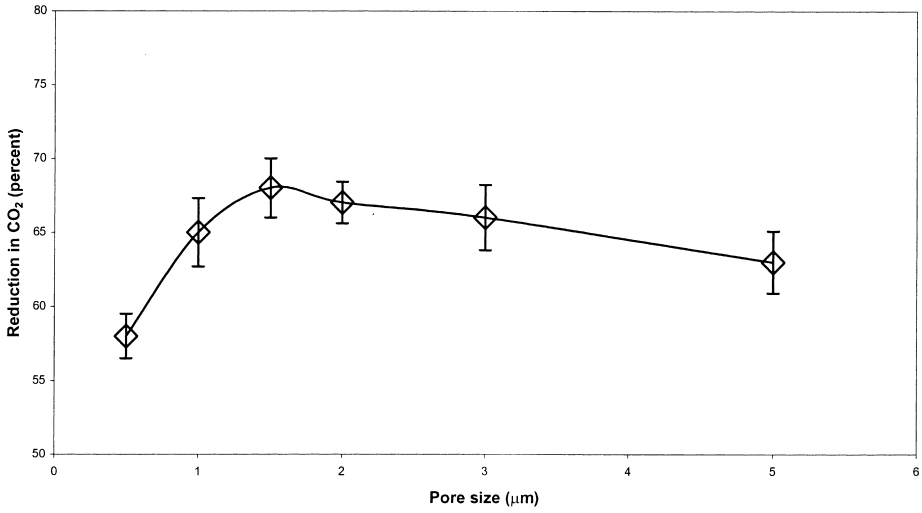

Figure 6. Percent CO_2 reduction with variation in immobilization matrix pore size. For these measurements, the average matrix pore sizes were between 0.5 and 5 μm. For correlation, the increase in pH was measured in every 1 L of extracted solution. The results represent mean ± standard deviation of five independent experiments.

increased pore size was due to an increased mass transfer of CO_2 near the active site of immobilized carbonic anhydrase. The observed decrease in CO_2 reduction with large pore size was perhaps due to an escape of CO_2 from reaching to actual active site of the enzyme immobilized in the matrix. Also, the availability of water and diffused CO_2 at the same rate in the large-pore matrix may affect the rate of CO_2 reduction.

Attachment of Reactor Module in Emission Stream and Pressure Drop Across the Stream

The attachment of the reactor is expected to cause a change in pressure of the outlet (after the reactor) and inlet (before the reactor) within the gas emission. To test this, the emission gas pressures before entry into the reactor and at the exit port of the reactor were determined. These pressure measurements were done with respect to thickness of the reactor core and varying matrix pores. The inlet stream was maintained at a pressure of about 104 Pa (without any reactor core). These experiments were also repeated with a very high-pressure simulated system (data not shown), where insignificant pressure changes were observed due to attachment of the reactor core. Pressure was measured in the reactor inlet and outlet using reactor cores of varying diameter (100 to 1000 cm; Fig. 7) and with varying matrix pores ranging between 0.5 and 5 μm (Fig. 8). The maximum pressure drop was only 17% for an immobilized reactor core with a

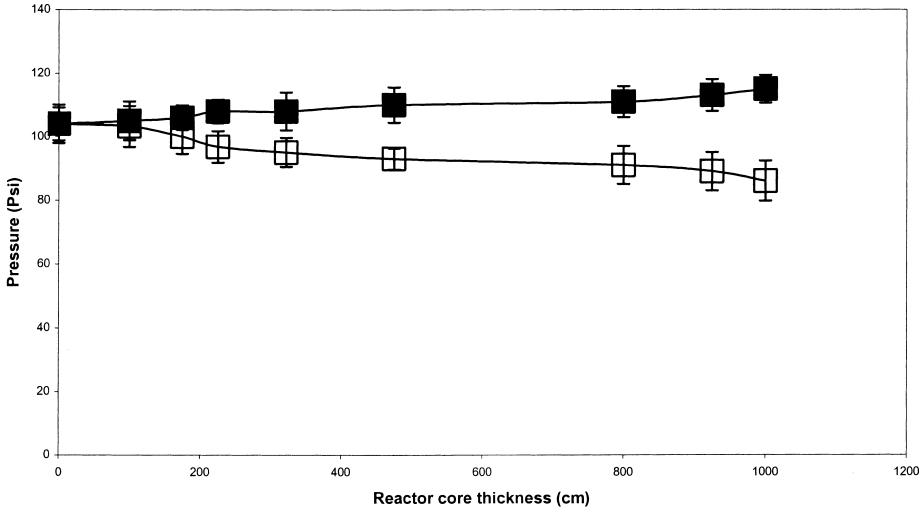

Figure 7. The determination of pressure drop as a result of attachment of a module in the emission stream. The effect of varying reactor diameter on pressure drop in the outlet and the backpressure in the inlet is shown. The reactor diameter is the barrier that the emission stream has to traverse (varied between 100 and 1000 cm). The symbols (□ and ■) represent outlet and inlet pressures, respectively. For correlation, the increase in pH was measured for every 1 L of extracted solution. The mean ± standard deviation of three independent experiments is presented.

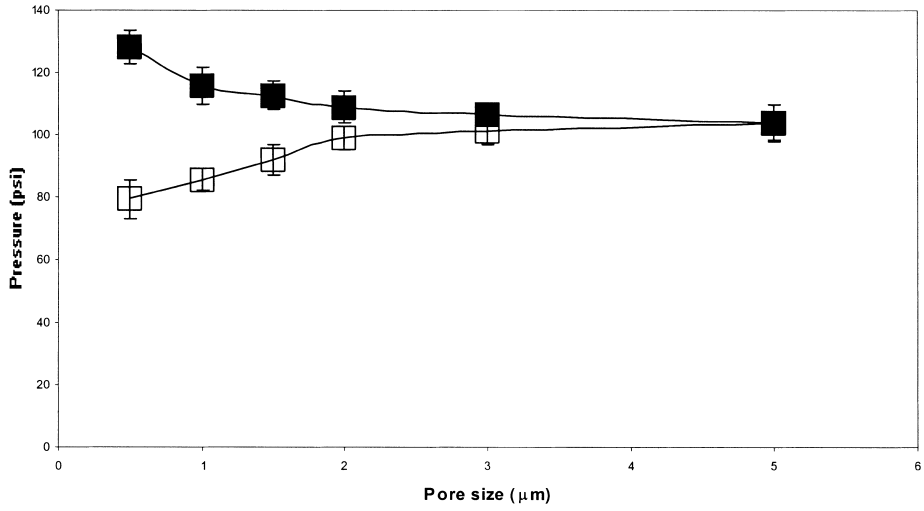

Figure 8. Determination of pressure drop in the outlet and the backpressure in the inlet with varying matrix pore diameter. The matrix pore diameter was varied between 0.5 and 5 μm, all other parameters were same as described in Figure 7 as (□) outlet pressure and (■) inlet pressure, respectively. The experimental results are mean ± standard deviation of three independent experiments.

diameter of 1000 cm and the pressure drop in the outlet was <11% up to a core diameter of 500 cm. A commensurate but insignificant increase in inlet pressure (backpressure) was also observed when the immobilized reactor core was attached than when without a core attachment (Fig. 7). Using a reactor vessel without an immobilized core, water flow alone did not show a significant effect on inlet or outlet pressures (data not shown). The matrix pore size also had an effect on pressure. However, the pressure drop with the 0.5-μm matrix pore was only about 10.5% less than without any reactor core control (Fig. 8). The average matrix pore size of 2 μm offered only a 5% decrease in pressure in the outlet. A decrease in pore size led to an increased drop in pressure in the outlet and increased pressure in the inlet. However, the pressure drop in the outlet was <11% with the moderate pore size (Fig. 8).

Efficiency of Single Versus Multiple Reactors for CO_2 Reduction

A single reactor with an equal combined volume was inferior with regard to minimizing CO_2 from the emission stream than the multiple reactors with lower volumes (Fig. 9) that adds up to the volume of the single reactor (Fig. 9). Using

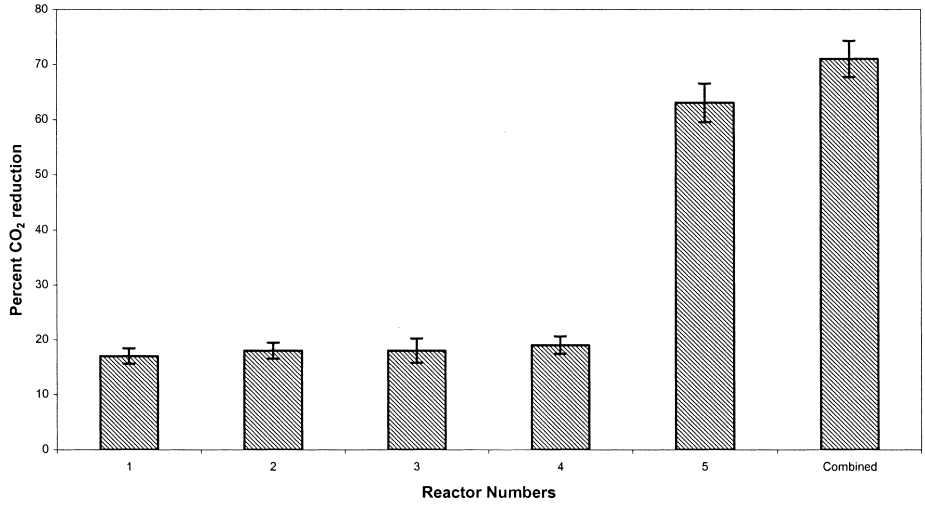

Figure 9. A comparison of CO_2 reduction using multiple reactors and a single reactor with comparable volume of combined multiple reactors. These reactors had an enzyme load of about 0.5 mg/mL. The single reactor (core volume 1000 mL) had the combined volume of four small reactors (core volume 250 mL), and was used for CO_2 solubilization/extraction from the emission stream. The percent CO_2 measured correlated with the pH-based measurement of CO_2 solubilization in aqueous phase per 100 mL of solution extracted. The bars indicate percent CO_2 reduction (mean ± standard deviation) of three independent measurements.

four reactors of 250 mL and a single reactor of 1000 mL, it was found that the multiple reactors provided better extraction/reduction of CO_2 (Fig. 9). Using these enzymatic reactors we found that CO_2 could be extracted from the emission stream in much the same fashion as in solvent extraction for organics. That is, the presence of multiple incremental volumes of extractant results in better extraction than a single extraction with equal combined volume. Thus, using multiple reactors, reduction of CO_2 roughly obeys the equation:

$$A_{mr} = A[KV_1(KV_1 + V_2)]^n$$

where K is the distribution coefficient for CO_2 ($K = C^{gas}/C^{solution}$), A_{mr} is the amount of CO_2 left in the emission stream after n reactors, A is the amount of CO_2 in the stream without any reactor, V_1 is the volume of emission gas used, and V_2 is the volume of water used for solvation of CO_2 in each reactor.

DISCUSSION

Abatement of pollution at the source is always the best approach. Biotechnological means to reduce CO_2 pollution have the potential to generate high-value products of commerce as byproducts of fixation, rendering the overall process economically advantageous. Available nonbiotechnological CO_2 reduction strategies are not designed to work for pollution control at the source. However, high volume and weight should be requirements for biotechnological CO_2 control devices because the fixation and biocatalysts need aqueous solubilization. The stationary emission sites are attractive for attempting biotechnological control/conversion of CO_2 owing to these requirements. However, for both chemical or biochemical conversion technologies, the CO_2 concentration step/device is a key requirement. Although the CCM strategy is used for a variety of organisms (Kaplan et al., 1990), no biotechnological process has been described for employing such strategies for CO_2 capture. Using CA derived from a thermophilic organism we have attempted to achieve efficient CO_2 capture directly from the emission exhaust stream, which could be fed to other coupled reactors for further conversion or fixation. In the present studies, CA was immobilized in different porous matrices and water spray, instead of solution phase, was applied to enhance the solubility of CO_2 and, therefore, enhanced capture without any significant pressure drop or backpressure in the emission stream with facilitated mass transfer to aqueous phase. The novelty of this biotechnological process lies in using the immobilized enzyme in porous matrix and using water spray instead of a solution phase enzyme so that mass transfer resistance to the emission gas is negligible. Prior to entry in the reactor, a highly porous filter was designed, offering negligible resistance that holds macroscopic soot and countercurrent water flow across the connector tube bringing emission gas to the reactor. With some gas emissions, where oxygen and sulfur contents are high, use of an alkaline pyrogallol spray column, becomes necessary. These treatments are needed before the actual emission stream entry to the CA reactor cores with most actual emissions. Prior treatment renders actual emission gas free of soot (and, if necessary, other contaminating gases) and brings the temperature to between 60° and 80°C, suitable for operation of this biotechnological process. In all experiments reported herein, the average temperature of the emission gas was kept at 60°C. The coupled multiple immobilized reactors will allow controlled release of soluble CO_2 near the active site of Rubisco (Chakrabarti et al., 2003a, 2003b), and therefore conversion of the captured CO_2 into a fixed or concatenated state. The investigations in this study were performed using thermophilic CA; however, by employing a reactor design such as that shown in Figure 1a some experiments were also repeated with purified recombinant human carbonic anhydrase IV (Waheed et al., 1997). The recombinant CA showed a very similar profile but had less CO_2 reduction ability and inferior stability (data not shown).

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