Bioresource Technology 102 (2011) 10194–10201

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Activity and stability of immobilized carbonic anhydrase for promoting CO_2 absorption into a carbonate solution for post-combustion CO_2 capture

Shihan Zhang [a], Zhaohui Zhang [a,1], Yongqi Lu [a,*], Massoud Rostam-Abadi [a,b], Andrew Jones [c]

[a] Illinois State Geological Survey, Prairie Research Institute, University of Illinois at Urbana-Champaign, United States
[b] Department of Civil & Environmental Engineering, University of Illinois at Urbana-Champaign, United States
[c] US Department of Energy, National Energy Technology Laboratory, United States

ARTICLE INFO

Article history:
Received 30 July 2011
Received in revised form 12 September 2011
Accepted 12 September 2011
Available online 17 September 2011

Keywords:
CO_2 capture
Enzyme immobilization
Stability
Carbonic anhydrase
Carbonate

ABSTRACT

An Integrated Vacuum Carbonate Absorption Process (IVCAP) currently under development could significantly reduce the energy consumed when capturing CO_2 from the flue gases of coal-fired power plants. The biocatalyst *carbonic anhydrase* (CA) has been found to effectively promote the absorption of CO_2 into the potassium carbonate solution that would be used in the IVCAP. Two CA enzymes were immobilized onto three selected support materials having different pore structures. The thermal stability of the immobilized CA enzymes was significantly greater than their free counterparts. For example, the immobilized enzymes retained at least 60% of their initial activities after 90 days at 50 °C compared to about 30% for their free counterparts under the same conditions. The immobilized CA also had significantly improved resistance to concentrations of sulfate (0.4 M), nitrate (0.05 M) and chloride (0.3 M) typically found in flue gas scrubbing liquids than their free counterparts.

1. Introduction

Carbon dioxide (CO_2) is a greenhouse gas that has been shown to be a major contributor to global warming. In the US, fossil fuel-fired power plants emit about 2.4 billion metric tons of CO_2 each year, or about 40% of total US emissions. Various technologies, including absorption, adsorption and membranes, have been proposed for capturing the CO_2 emitted from fossil fuel-fired power plants. However, only mono-ethanol-amine (MEA)-based absorption processes are currently available for post-combustion CO_2 capture, but they are expensive. A major part of the capture cost using MEA processes, amounting to about 60%, is the parasitic power loss due to the extraction of power plant steam for stripping the CO_2 from the MEA (Singh et al., 2003). Recently, a novel Integrated Vacuum Carbonate Absorption Process (IVCAP) has been proposed to reduce the energy usage for CO_2 stripping (Chen et al., 2007). The process employs a potassium carbonate (PC, K_2CO_3) aqueous solution as a solvent for CO_2 absorption. The weak affinity of CO_2 for K_2CO_3 allows the CO_2 to be stripped from the CO_2-rich solution at a low temperature (50–70 °C) and vacuum condition (2–8 psia). These temperate/pressure conditions enable the use of either waste steam or low-quality steam from the power plant's steam cycle system to provide the heat required for CO_2 stripping and this significantly reduces the parasitic power loss. However, K_2CO_3 is only weakly alkaline and thus a K_2CO_3-based system has a much slower CO_2 absorption rate than MEA-based systems. A conventional approach for increasing the rate of CO_2 absorption into a weak solvent is to mix one or more solvents with stronger CO_2 affinities into the prime solvent (Cullinane and Rochelle, 2004, 2005). This approach, however, will increase the heat of absorption and change the phase equilibrium of the system. As a result, a higher temperature, or a higher level of vacuum, will be required for CO_2 stripping. This study suggests that the *carbonic anhydrase* (CA) enzyme can be employed as a catalyst to accelerate the absorption rate in the IVCAP without changing the heat of absorption and phase equilibrium of the system.

Carbonic anhydrase is a zinc metalloenzyme that can efficiently catalyze the hydration of CO_2 to form bicarbonate. The turnover rate of the CA enzyme can be as great as $10^6\ s^{-1}$ (Kernohan, 1965). A free CA enzyme homogeneously dissolved in a solution may offer a greater specific activity compared to an immobilized enzyme. However, immobilization can improve the stability of the enzyme, which is important for the CO_2 capture application in a power plant. The enzyme can either be immobilized onto packing materials, or onto fine particle carriers that are suspended in

* Corresponding author. Tel.: +1 217 244 4985.
E-mail address: lu@isgs.illinois.edu (Y. Lu).
[1] Visiting scholar from the College of Biological & Environmental Engineering, Zhejiang University of Technology, China.

0960-8524/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2011.09.043

the solvent and if the carrier is not stable at the elevated stripping temperature, the carrier can be separated from the solvent prior to stripping. The CA enzyme commonly has been immobilized using covalent coupling methods (Crumbliss et al., 1988; Bhattacharya et al., 2004). Other methods have included enzyme entrapment in capsules (Drevon et al., 2003; Ozdemir, 2009) and enzyme adsorption (Azari and Nemat-Gorgani, 1999). A variety of support materials, such as iron filings, methacylic acid polymer, silica, graphite, controlled pore glass, alginate, and polyurethane foam have been investigated for the CA enzyme immobilization (Crumbliss et al., 1988; Bhattacharya et al., 2003; Drevon et al., 2003; Ozdemir, 2009). The reported studies showed that immobilization generally resulted in an activity reduction but increased the stability of the CA enzyme over time. For example, one study showed that the initial activity of a bovine CA immobilized on silica materials was 30% less, and on graphite materials 60% less than its free counterpart; however, the immobilizations resulted in the CA enzymes retaining more than 70% of those initial activity levels during 500 days of storage at 4 °C (Crumbliss et al., 1988). Another study showed that the enzyme unfolding temperature of a single CA molecule encapsulated onto a spherical nano-gel increased from 64 to 81 °C (Badjic and Kostic, 1999). Several processes employing immobilized CA enzyme as a catalyst for CO_2 capture from flue gases also have been reported, including a spray reactor process employing the CA enzyme immobilized onto iron filing matrices (Bhattacharya et al., 2004), a hollow fiber contained liquid membrane (CLM) system with the CA immobilized in a liquid membrane (Bao and Trachtenberg, 2006), and a packed-bed reactor with the CA immobilized onto inorganic or organic supports (Rejean and Peter, 2003).

The above mentioned studies demonstrate that immobilizing the CA enzyme could effectively improve the enzyme's stability and promote the absorption of CO_2 into various solvents. However, most of these studies were performed at low or room temperatures and at solution conditions (pH, composition, etc.) much different from those proposed in the IVCAP. In addition, the flue gases emitted by coal combustion contain significant amounts of SO_2, NO_x, HCl and other gases. As a result, sulfates, nitrates, and chlorides tend to be major impurities found in the scrubbing liquids at power plants. In the IVCAP, the immobilized CA would be subjected to temperatures ranging from 40 to 60 °C, pH ranging from 9 to 12, and the flue gas impurities. It should also be noted that the enzymes used in the previous studies represented almost exclusively the type of bovine CA enzymes produced in a laboratory environment at a small scale only suitable for research purposes.

For our study, a new CA enzyme manufactured by a leading enzyme company in a pilot-scale unit that is scalable for large-scale capture applications was employed. A commercially available bovine CA enzyme was used as a reference for comparison. The two CA enzymes were covalently immobilized onto two different controlled pore glass (CPG) materials and an activated carbon. The CPG was selected as a support material because: (1) it has a uniform physical structure (pore size and surface) and thus is ideal for the immobilization study, and (2) the results can be applied to less expensive ceramic materials that possess similar surface functionalities. The activated carbon support material was selected because it is widely available and its surface chemistry and pore structure can be easily tailored for enzyme immobilization. The activities of the immobilized CA enzymes were tested and compared with those of their free counterparts. The thermal stability and resistance to chemical impurities of the immobilized enzymes were investigated over a 3 month period under the temperature and solution conditions expected in the IVCAP.

2. Experimental methodology

2.1. Materials

A leading enzyme manufacturer supplied a technical-grade CA enzyme (ACA). The ACA was produced in a pilot-scale manufacturing unit. The as-received sample was in the form of concentrated enzyme (~3 g/L) in an aqueous solution. According to the manufacturer, the liquid sample contained small amounts of impurities such as low molecular weight fermentation residues, processing aids, salts, and other proteins. The reference CA enzyme (SCA), a bovine CA enzyme, was purchased from Sigma–Aldrich Co.

The activated carbon (AC), CAL-AC was supplied by Calgon Carbon Corporation. The AC had a particle size of 40–60 mesh (250–400 μm). Two controlled pore glass materials with pore sizes of 38 nm (CPG38) and 100 nm (CPG100) were purchased from Sigma–Aldrich. The CPG samples had a particle size of 200–400 mesh (38–75 μm). The following additional chemicals needed for our experiments also were purchased from Sigma–Aldrich Co.: γ-aminopropyl triethoxysilane (⩾98%); glutaraldehyde solution (25%, w/w); *N*-hydroxy succinimide (⩾97%); *N*-cyclohexyl-*N*-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (⩾95%); 1,4-dioxane (⩾99.8%); methanol (⩾99.8%) and H_4BNa (⩾98%). The reagents HNO_3, NaH_2PO_4, Na_2HPO_4, $KHCO_3$, K_2CO_3, Tris–hydroxymethyl aminomethane, toluene and acetone needed for the experiments were obtained from Fisher Scientific Inc.

2.2. Enzyme purification

The ACA enzyme was purified before immobilization. The as-received ACA liquid sample was mixed with $(NH_4)_2SO_4$ to 80% saturation of the sulfate and refrigerated at 4 °C overnight to precipitate the protein content. The solution was then centrifuged at 4 °C and 10,000 rpm and the enzyme precipitant was dissolved in a Na_2HPO_4–NaH_2PO_4 buffer (30 mM, pH 8.0). The resulting solution was dialyzed twice against 10 mM K_2CO_3 solution (pH 10) for 8 h with Fisherbrand regenerated cellulose dialysis tubing (flat width: 33 mm). No purification process was applied to the SCA reference enzyme because it was pure in its as-received condition.

2.3. Enzyme immobilization

A covalent coupling method based on the one developed by Weetall and Lee (1989) was used for immobilization of the CA onto the CPG support (CA-CPG). It involved activation of the CPG surface with a silane and an aldehyde, followed by covalent coupling between the CA enzyme and the activated CPG. The procedure involved cleaning the CPG by boiling it in a 5% nitric acid solution for 45 min, washing with deionized (DI) water, mixing 0.1–0.2 g of the cleaned CPG material with 10 mL of 10% (v/v) γ-aminopropyl triethoxysilane dissolved in toluene, stirring the mixture mildly for 24 h at room temperature, filtering and washing with toluene and acetone, and air drying the filtrate at 110 °C for 4 h. The silanized CPG was mixed with 10 mL of 2.5 wt.% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.0) and stirred mildly for 1 h at room temperature. The resulting CPG then was washed with a 0.05 M phosphate buffer (pH 8.0) solution, stirred in 10–20 mL of CA solution (400 mg/L) in 0.1 M phosphate buffer (pH 8.0) for 3 h at room temperature, and washed thoroughly with the same phosphate buffer (pH 8.0) to remove unbound enzyme. The extra aldehyde on the CPG was quenched by immersing the CPG in a 0.1 M Tris–H_2SO_4 (pH 8.1) solution for 1 h. The now immobilized CA-CPG was further mixed in 0.2% $NaBH_4$ in a Na_2HPO_4–NaH_2PO_4 buffer (0.1 M, pH 8.0) for 30 min to reduce the Schiff-base formed during the coupling reaction between the