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Immobilization of carbonic anhydrase on mesoporous aluminosilicate for carbonation reaction

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ABSTRACT

Mesoporous aluminosilicates synthesized using a template of Pluronic F127 with high surface area and pore diameter appears to be tailor made for enzyme adsorption. Carbonic anhydrase (CA) with diameter of 35 Å has been immobilized in these crystalline, ordered mesoporous materials and tested for mechanistic and kinetic aspects using *p*-NPA assay. K_m, V_{max} and K_{cat} of the CAImAlK was observed to be 0.158 mM, 2.307 μmol/min/ml and 1.9 s^{-1}, respectively compared to K_m, V_{max} and K_{cat} of 0.876 mM, 0.936 μmol/min/ml and 2.3 s^{-1}, respectively for free CA. Storage stability test was conducted up to 25 days and it was observed that the HLP at 25 °C improved by a factor of 1.6 for CAImAlK (600 h) compared to 360 h for free CA. Proof of concept has been established for carbonation reaction. The CO_2 sequestration capacity in terms of conversion of CO_2 to calcium carbonate was quantified by gas chromatography (GC). The CO_2 sequestration capacity of CAImAlK was determined to be 16.14 mg of $CaCO_3$/mg of CA compared to 33.08 mg of $CaCO_3$/mg of CA for free CA.

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1. Introduction

Atmospheric concentrations of greenhouse gases (GHGs) such as carbon dioxide (CO_2), methane, chlorofluorocarbons, and nitrous oxides are increasing due to anthropogenic activities. CO_2 is one of the most abundant greenhouse gases, which is produced mainly by burning of fossil fuels such as coal, oil, and natural gas. The CO_2 concentration in the atmosphere has increased from 280 ppm during the preindustrial era to about 380 ppm in 2007 with an accumulation rate of about 1.5 ppm v per year [1]. Extensive R&D efforts are therefore, underway to develop new approaches to capture and store or sequester the CO_2 to avoid its release into the atmosphere. Oceanic, terrestrial and geological carbon sequestration processes are relatively well explored and already finding some practical applications. The fixation of CO_2 in the form of inorganic carbonates, commonly known as "*Mineral Carbonation*" is emerging as an attractive option for carbon capture and storage [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 overall objective of the present research is to develop a system resembling a CO_2 scrubber that can be used to reduce CO_2 emissions from, for example, fossil-fuel-burning power plants and petrochemical industries [3]. In the system envisaged, an enzyme serves to catalyze the rate of CO_2 hydration for subsequent fixation into stable mineral carbonates. Carbonic anhydrase (CA) is the biological catalyst responsible for the inter conversion of carbon dioxide and bicarbonate in living organisms [4]. CA is a major zinc-based metalloenzyme, which is ubiquitous in nature and found in the prokaryotic as well as eukaryotic domains. Each molecule of the CA isoenzyme can catalyze 1.4×10^6 molecules of CO_2 in 1 s.

Gaseous CO_2 dissolves rapidly in water to produce a loosely hydrated aqueous form.

$$CO_2\ (\text{gaseous}) \leftrightarrow CO_2\ (\text{aqueous}) \quad (1)$$

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

$$CO_2\ (\text{aqueous}) + H_2O \overset{CA}{\leftrightarrow} H_2CO_3 \quad (2)$$

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \quad (3)$$

Once bicarbonate ions are present in solution, carbonate ions can be produced by the following reaction [5]. This carbonate ion reacts with calcium chloride to form calcium carbonate.

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \quad (4)$$

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3 \downarrow \quad (5)$$

There are some disadvantages associated with usage of free CA in solution including low stability, limited repeatable usage and recovery problem. These disadvantages can be eliminated by

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immobilizing the enzyme. The advantages of using immobilized enzyme are as follows; firstly, it allows separation of enzyme from the reaction mixture and hence it lowers the cost of down-streaming processing of the product by reusing it several times. Secondly, the stability of enzyme improved i.e. enzyme can active at harsh condition such as at high temperature and pH [5].

Mesoporous molecular sieves of the zeolites and zeotype molecular sieves with highly ordered mesopores and an extremely high surface area are very suitable for enzyme immobilization [6–10]. The ordered mesoporous silica materials have received considerable attention because of their unique structures with organized porosity, high specific surface area and pore volume, and well-ordered mesopores that are considerably larger than zeolites and zeotype molecular sieves with potential applications mainly in the field of catalysis, adsorption, separation, sensors, and fuel cells [11]. These materials can be prepared by using either a cationic or an anionic or a neutral surfactant as a structure directing agent [12]. There are numerous reports which deal with the preparation of various types of one and three dimensional mesoporous materials, such as MCM-41, MCM-48, SBA-1, SBA-15, AMS, HMS, MSU, etc. [13]. The micelle formed during the self assembly consists of a core of hydrophobic block and a shell of hydrophilic block. Under acidic conditions, the self assembly is followed by the hydrolysis and condensation of silica source which results in the formation of inorganic (I^+) network of silica.

This paper reported the synthesis and characterization of mesoporous aluminosilicates with high structural order by using non ionic surfactant as a template in a highly acidic medium. The effect of contact time, material dose, enzyme dose, pH and temperature was investigated for identifying optimal conditions for immobilizing CA. Elucidation of kinetic and mechanistic aspects was studied using *p*-Nitrophenyl acetate (*p*-NPA) and carbonation reaction. To the best of our knowledge the immobilization of CA on mesoporous aluminosilicates has not been attempted in the past.

2. Materials and methods

2.1. Materials

All chemicals used in the study were of analytical grade and purchased from Merck, India Ltd.; Loba Chemicals, India; BDH, Germany; Sigma Chemicals Co., USA; BASF, Wako. *p*-NPA was purchased from Sigma Aldrich, US. The crude extract of partially purified CA has been given by Department of Microbiology, University of Delhi South campus, New Delhi. One gram of lyophilized powder has 6840 units of CA. One unit of enzyme activity was expressed as 1 μmol *p*-Nitrophenol released per minute at room temperature.

2.2. Experimental

2.2.1. Synthesis of mesoporous aluminosilicates (AlKIT-5)

In a typical synthesis procedure, 5 g of Pluronic F127 is stirred with 240 ml of double distilled water in the presence of 3 ml of concentrated HCl for 4 h at 45 °C. This is followed by the slow addition of 24 g of triethoxy silane (TEOS) with continued stirring and addition of 3.3 g of aluminum isopropoxide to the suspension. The entire mixture is stirred for 24 h at 45 °C and kept in static condition at 100 °C for 24 h. The material was then filtered and allowed to dry at 100 °C followed by calcination at 540 °C for 24 h.

2.2.2. Characterization of materials

The mesoporous aluminosilicates was scanned for powder X-ray diffraction (XRD) (Model no. TW 3660/50), with Cu Kα radiation (λ = 1.540 Å) at 45 kV and 40 mA and scanned in the range of 2Θ = 1–5°. Transmittance Electron Microscopy (TEM) image of Al-KIT-5 was obtained using a TEM instrument (Model no. JEM-2100). Scanning electron microscopy (SEM) images of mesoporous material before and after carbonation reaction were obtained using a JEOL JED-2300 SEM. The specific surface area and pore volume of mesoporous materials was measured using Micromeritics, USA ASAP 2000 by N_2 gas adsorption–desorption according to the Brunauer, Emmet and Teller (BET) method. Fourier Transform Infrared Spectroscopy (FTIR) spectra of the calcium carbonate from immobilized materials (1 wt.%) mixed with KBr pellets were recorded on Bruker spectroscopy. Spectra of all the materials were scanned in the range of 500–4000 cm^{-1}.

2.2.3. Immobilization of CA

About 10 mg material has been weighed and washed with deionized water. After washing, 4.8 ml phosphate buffer (100 mM, pH = 7) and 0.2 ml enzyme (1 mg/ml) has been added. The sample has been kept in the shaker for 6 h at 120 rpm. After shaking, the material and supernatant were separated. Then the supernatant has been centrifuged at 5000 rpm for 10 min. Pellet has been discarded and the clear supernatant has been used for the assay. Esterase activity of the enzyme (CA) was measured spectrophotometrically using *p*-Nitrophenyl acetate as a substrate according to the method described by Armstrong et al. with a slight modification [14,15]. The assay system consisted of 0.2 ml enzyme in a 1 cm spectrophotometric cell containing 1.8 ml of 100 mM phosphate buffer (pH = 7) and 1 ml of 3 mM *p*-Nitrophenyl acetate. The change in absorbance at 348 nm was measured over 5 min, before and after adding enzyme. One unit of enzyme activity was expressed as 1 μmol *p*-Nitrophenol released per minute at room temperature. The concentration of protein was assayed according to the method of Lowry with BSA as the standard protein [16].

2.2.4. Wilbur–Anderson assay (W–A method)

Wilbur–Anderson assay [17,18] was performed in vessel maintained at 4 °C with water-jacket and constant-temperature circulator by using crushed ice. The vessel was sealed with a rubber-stopper fitted with a pH electrode. Fifty microliters of sample was added to 3 ml of 20 mM Tris buffer solution of pH 8.3. The reaction was started by addition of 2 ml of water saturated with CO_2 at about 4 °C. CA activity was estimated by monitoring the time required for pH to change from 8.3 to 6.3.

2.2.5. Adsorption studies

The Langmuir and Freundlich models are most commonly used isotherms to represent the equilibrium distribution of adsorbate from a liquid phase onto a solid phase. To determine the equilibrium isotherms, enzyme concentration were varied, while the material dose was kept constant. The Freundlich model, which is indicative of surface heterogeneity of the sorbent, is given by the following linearized equation:

$$\log(q_e) = \log K_F + 1/n \log(C_e).$$

here K_F and $1/n$ are Freundlich constants, related to adsorption capacity and adsorption intensity (heterogeneity factor), respectively.

2.2.6. Protocol for mineralization of CO_2

Carbonation reaction was studied by the method reported by Favre et al. with slight modification [19]. In a typical procedure, 1 ml of Tris buffer (1 M, pH 8.0) was added to 10 ml of CO_2 saturated water. The mixture was shaken at 25 °C and then 10 ml of 2% $CaCl_2$ was added along with 1 ml (1 mg/ml loading) of the enzyme in phosphate buffer (0.1 M, pH 7.0). The time required for formation of carbonate with respect to onset of reaction (precipitate formed) was monitored in the sample as well as control (without CA). The carbonate obtained after 10 min was filtered

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