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# Carbon composite beads for immobilization of carbonic anhydrase

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ABSTRACT

Fixation of anthropogenic $CO_2$ into calcium carbonate using carbonic anhydrase (CA) as a bio-catalyst appears to be a promising option of $CO_2$ sequestration. In the present study, carbonic anhydrase (CA) has been immobilized on chitosan based activated alumina–carbon composite beads. Synthesized adsorbent was thoroughly characterized using XRD, FTIR, SEM and BET-SA. Maximum adsorption capacities were determined using Langmuir model and it was observed to be 172.41 mg/g. Mechanistic and kinetic aspects have been addressed for immobilized as well as free enzyme. The $K_m$ and $V_{max}$ for immobilized enzyme was 10.35 mM and 0.99 μmol/ml/min, whereas for free enzyme it was 1.89 mM and 0.99 μmol/ml/min respectively. Proof of concept has been established for carbonation reaction. The carbonate has been characterized using various tools such as XRD and SEM which confirms the calcite nature of calcium carbonate. The $CO_2$ sequestration capacity in terms of conversion of $CO_2$ to carbonate was quantified by gas chromatography (GC). The $CO_2$ sequestration capacity of immobilized beads was found to be 19.22 mg of $CaCO_3$/mg of enzyme as compared to 33.06 mg of $CaCO_3$/mg of enzyme for free enzyme.



## 1. Introduction

Biological carbon sequestration might be the most promising, environmentally friendly and cost-effective means of reducing anthropogenic carbon dioxide from the atmosphere. In this connection, carbonic anhydrase is being used to sequester $CO_2$ by converting it into carbonate which is further precipitated as calcium carbonate by addition of appropriate calcium sources. Carbonic anhydrase (CA, E.C. 4.2.1.1) is a Zn-metalloenzyme which catalyzes the reversible hydration of $CO_2$ into bicarbonate. It is ubiquitously found in nature from prokaryotes to eukaryotes. CA has multiple functions, including pH homeostasis, facilitated diffusion of $CO_2$; inter conversion of $CO_2$ and $HCO_3^-$ and ion transport. The turnover number of this enzyme is very high which plays a major role in the carbon concentrating mechanism of aquatic photosynthetic microorganisms, which in turn, results in the absorption/utilization of atmospheric carbon dioxide. The catalytic active sites of CA consist of a $Zn^{2+}$ ion, which forms coordination bonds with the nitrogen atoms of three histidine residues. The mechanism of the hydration of $CO_2$ by the CA enzyme is initiated by a nucleophilic attack on the carbon atom of $CO_2$ by the zinc-bound –OH group to produce bicarbonate, which is then displaced from zinc by a water molecule.

Recently, carbonic anhydrase has been used extensively for sequestration of carbon dioxide. Bond et al. [1] developed an integrated system in which they used CA to accelerate the hydration of $CO_2$ for converting it into mineral carbonate. Mirjafari et al. [2] investigated the application of the CA to enhance the hydration of $CO_2$ in the solution. Liu et al. [3] studied the precipitation of $CaCO_3$ from produced waters in the presence of the CA enzyme. Favre et al. [4] reported the detailed study of CA for calcium carbonate formation.

However, there exist a number of practical problems in the use of free CA in solution such as short operational time and reuse. Several methods have been proposed to overcome these problems, one of the most successful being enzyme immobilization. Immobilization is achieved by fixing enzymes to or within solid supports, as a result of which heterogenous immobilized enzyme systems are obtained. There are various immobilization techniques, such as entrapment in matrices, adsorption on the solid surfaces, covalent bonding, and cross-linking within polymeric networks. Rayalu et al. [5] reported the immobilization of CA enriched whole cells as well as partially purified CA on different biopolymer based materials. Bond et al. [6] immobilized carbonic anhydrase in 3 different matrices: acrylamide, alginate and chitosan–alginate and illustrated improved CA stability at elevated temperatures by immobilization. Simsek-Ege et al. [7] immobilized carbonic anhydrase in an

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environmentally friendly matrix based on porous alginate beads with a relatively hard chitosan coating for biomimetic sequestration. Bhattacharya et al. [8] reported the conversion of $CO_2$ from ICE exhausts by fixation. They also developed a novel spray reactor with immobilized carbonic anhydrase for solubilisation and concentration of carbon dioxide [9]. Similarly, CA has also immobilized on poly (acrylic acidco-acrylamide) hydrogel by entrapment [10], sepharose 4B by entrapment [11], polyurethane foam [12] and surfactant modified silylated chitosan [13].

In the present study, we have synthesized low cost nitrogen containing biogenic carbon composite from the biopolymer chitosan for the immobilization of CA. This has been done with the interest of developing a suitable material for the sequestration of $CO_2$ and to mimic the reaction for the fixation of anthropogenic $CO_2$ using carbonic anhydrase (CA) as a biocatalyst. Detailed study with respect to kinetic parameters like pH, temperature and substrate, on the free and immobilized enzyme has also been done. We have also carried out carbonation study to establish proof of concept that the immobilized CA can be effectively used for its targeted application i.e. $CO_2$ sequestration through mineral carbonation.

## 2. Materials and methods

Chitosan with the deacetylation degree (DD) of 95% and the molecular weight (MW) of 360 kDa was purchased from Panvo Organics India Limited, Chennai. All the chemicals used in this study were of analytical grade and purchased from Merck, India Ltd. Tris buffer used in the carbonation study has been purchased from Calbiochem, USA. The substrate, para nitro phenyl acetate (pNPA) was purchased from Sigma Co, St. Louis, MO, USA. The partially purified extract of carbonic anhydrase (CA) from *Bacillus pumilus* was provided by Department of Microbiology, University of Delhi South Campus, New Delhi. A gram of the lyophilized powder contained 6840 U of CA.

### 2.1. Synthesis of materials

#### 2.1.1. Mesoalumina from chitosan

In order to synthesize macrosphere of meso alumina, initially 3 g of chitosan was dissolved in 100 ml of 5% (v/v) acetic acid. An aqueous aluminium nitrate solution was subsequently added to the chitosan slurry and stirred in a laboratory stirrer for 1 h at room temperature. Molar ratio of chitosan to aluminium nitrate in each synthesis was maintained as 1:5. The resultant aluminium chitosan solution was added to 50% ammonium hydroxide solution under stirring in the form of drops. Gel macrospheres thus formed were separated from $NH_4OH$ solution and was dried at ambient temperature. Dried spheres were calcined at 550 °C in air flow with heating rate of 5 °C/min which was held for 4 h at 550 °C.

#### 2.1.2. Carbon composite Beads from chitosan

6.67 g aluminium nitrate was dissolved in 20 ml of distilled water. This solution was added to chitosan slurry which was prepared by dissolving 1.5 g chitosan to 50 ml of 5% acetic acid solution with constant stirring at room temperature. The chitosan–Al molar ratio was 1.5–2. The chitosan–Al solution was added drop wise to 50% (v/v) $NH_4OH$ solution with vigorous stirring resulting in the precipitation of spherically uniform beads. After addition, it was further stirred for 30 min. The gel macrospheres were taken out, washed with distilled water and dried at 50 °C for 72 h. The dried beads were carbonized in an inert atmosphere for 2 h at 660 °C, with the heating rate of 5 °C/min. Nitrogen flow of the furnace was then stopped and $CO_2$ was introduced in a controlled rate of 100 ml/min for subjecting carbonized products to physical activation for 2.5 h. The furnace was cooled subsequently to ambient temperature in the flow of nitrogen.

### 2.2. Characterization of materials

The low angle XRD patterns were recorded on a Rigaku Miniflex II instrument using Cu Kα radiation (λ = 0.15406 nm) operated at 30 kV and 15 mA. The samples were scanned for $2\theta$ ranges from 10° to 80° with scanning speed of 5°/min. Diffraction peaks were compared with standard database reported in the Joint Committee on Powder Diffraction Standards (JCPDS) cards. The surface morphology of the material was studied by performing the scanning electron microscopy (SEM) of the materials using a JEOL6380A instrument. FTIR spectra of beads (1 wt%) mixed with KBr pellets were recorded on a Bruker Vertex-70 apparatus by diffused reflectance accessory technique. Spectra of the materials were scanned in the range 400–4000 $cm^{-1}$. Surface area and pore volume of the synthesized carbon composite was determined by the adsorption of $N_2$ at 77 K using an Autosorb instrument (Quantachrome, USA).

### 2.3. Immobilization method

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. The supernatant was centrifuged at 5000 rpm for 10 min. The pellet was discarded and the clear supernatant has been used for the assay. Esterase activity of the enzyme (CA) was measured spectrophotometrically using p-nitro phenyl acetate as a substrate according to the method described by Armstrong et al. [14] with a slight modification. 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-nitro phenyl 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-nitro phenol released per minute at room temperature. Blank experiments were also conducted to estimate the self-dissociation of p-NPA in each assay solution. All the experiments have been done in triplicate and the reported values are the mean of three replicates.

### 2.4. Immobilization study

Various parameters such as optimum time study, enzyme dose study, and material dose study have been done before kinetic study. For all the above study the experimental set up is similar as described in Section 2.3.

#### 2.4.1. Determination of optimum pH

Free CA and immobilized CA were incubated in phosphate buffer solution at different pH (6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10) for 4 h. Enzyme activity of free and immobilized enzyme was calculated as per the method described in Section 2.3.

#### 2.4.2. Determination of optimum temperature

Free and immobilized enzymes were incubated in phosphate buffer (100 mM, pH 7.0) at different temperatures (15–55 °C) for 4 h. Enzyme activity of free and immobilized enzyme was calculated as per the method described in Section 2.3.

**Table 1**
Surface area and pore characteristics result.

| S. no. | Materials | BET surface area ($m^2/g$) | Avg. pore diameter (Å) | Pore volume ($\times 10^{-6}$ $m^3/g$) |
|---|---|---|---|---|
| 1. | Carbon composite beads | 167.3 | 31 | 0.066792 ($cm^3/g$) |

### 2.5. Kinetic study

#### 2.5.1. Determination of Michaelis constant

Immobilized enzyme has been assayed at different concentrations of substrate i.e. p-nitro phenyl acetate (1, 2, 3, 4 and 5 mM). A Michaelis–Menten and Lineweaver–Burk plot has been used to calculate $K_m$ and $V_{max}$ of the enzyme.

### 2.6. Operational study

For storage stability study samples were incubated with 1 mg/ml enzyme loading in 4.8 ml phosphate buffer (0.1 M, pH 7.0) at 4 °C for a period of 30 days and relative activity was determined after every 5 days interval. Half life have been determined experimentally by monitoring 50% decline in activity.

For reuse study, the immobilized enzyme was assayed continuously in a batch process by adding buffer and substrate. Before each cycle, the used sample was washed by deionized water to remove the pNP produced by hydrolysis of substrate, pNPA.

### 2.7. Protein estimation

The concentration of protein was assayed according to the method of Lowry with bovine serum albumin (BSA) as the standard protein [15].

### 2.8. Adsorption studies

For adsorption studies, the material dose was kept constant and the enzyme concentration was varied from 1 to 5 mg/5 ml. The experimental set up was similar as described in Section 2.3. The Langmuir adsorption model was used to obtain various adsorption parameters and to get an insight into the adsorption mechanism,

### 2.9. Carbonation study

The carbonation study was done by method reported in our previous study [13]. In brief, 1 ml of Tris buffer (1 M, pH 8.3) was added to the tube containing immobilized beads + 10 ml of $CO_2$ saturated water (pH 3.57). The mixture was shaken at 25 °C and then 10 ml of 2% $CaCl_2$ was added. The time required for formation of carbonate with respect to onset of reaction was monitored in the sample as well as control (without enzyme) by turbidometric method. The precipitate was filtered using Whatmann filter paper-42 and dried at room temperature.

The precipitated material was tested for $CO_2$ evolution by gas chromatographic method, using a thermal conductivity detector (TCD). This eliminates the interference of other precipitates, such as calcium phosphate. The reaction was carried out in a borosilicate glass reactor in which carbonate precipitate was taken and 0.5 M HCl was added. The evolved gas was collected in the collector and then analyzed in GC/TCD using Porapak Q column. The amount of carbonate was back calculated with respect to the amount of $CO_2$ evolved.

## 3. Results and discussion

### 3.1. Characterization of material

#### 3.1.1. Surface area

The specific surface area and pore diameter of the materials have been measured using BET method and the observed values are given in Table 1. The study revealed that the synthesized material has high surface area with large pores.

#### 3.1.2. FTIR

The presence of nitrogen functionalities in the carbon composite beads was confirmed by Fourier transform infrared (FTIR) spectroscopy shown in Fig. 1. The peak at 1353 $cm^{-1}$ and 3055 $cm^{-1}$ corresponds to secondary aromatic amines and Ar–H stretching vibrations respectively. The peak at 2224 $cm^{-1}$ reveals the presence of –C≡N bond in the carbon composite.

#### 3.1.3. XRD

X-ray diffraction (XRD) analysis of carbon composite beads reveals the presence of graphite carbon (00-026-1077), aluminium in cubic phase (00-003-0932), and $Al_4C_3$ phase (000-011-0629) identified by JCPDS database. This can be observed in the XRD pattern in Fig. 2. In addition, there is no detection of aluminium oxide. This XRD does not show any peaks of chitosan due to calcination.

#### 3.1.4. SEM image

Scanning electron microscopy images of carbon composite beads (Fig. 3) show the development of irregular pores on the surface. Flowery pattern can be observed which indicates the porous nature of the sample.

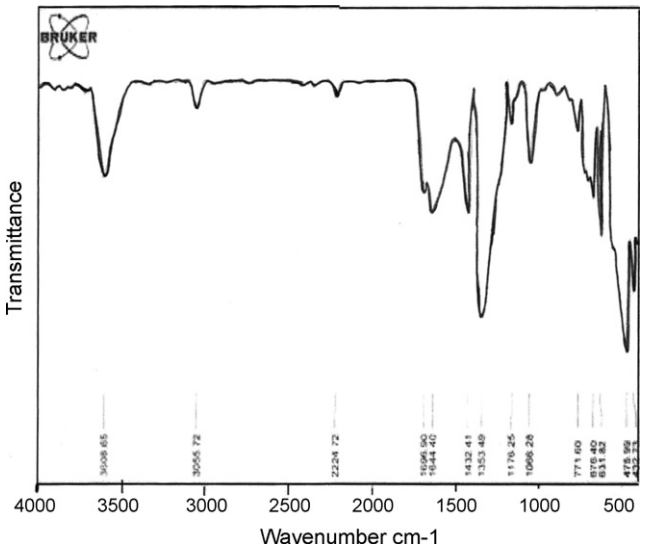



**Fig. 1.** FTIR spectra of carbon composite beads.

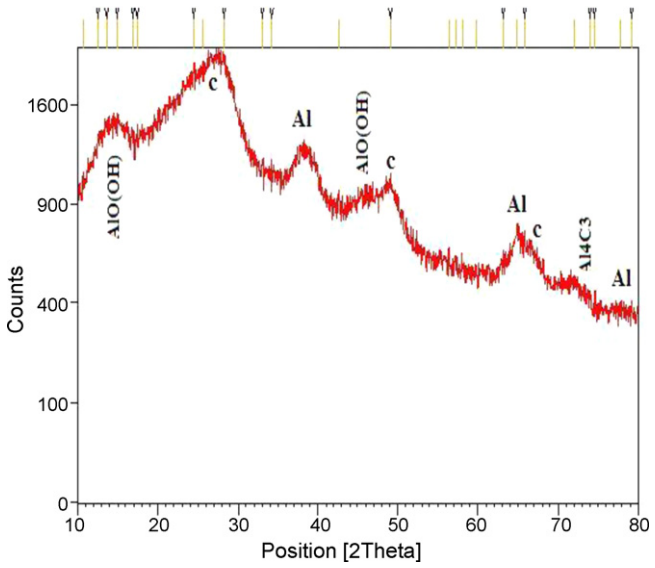



**Fig. 2.** XRD of carbon composite beads.

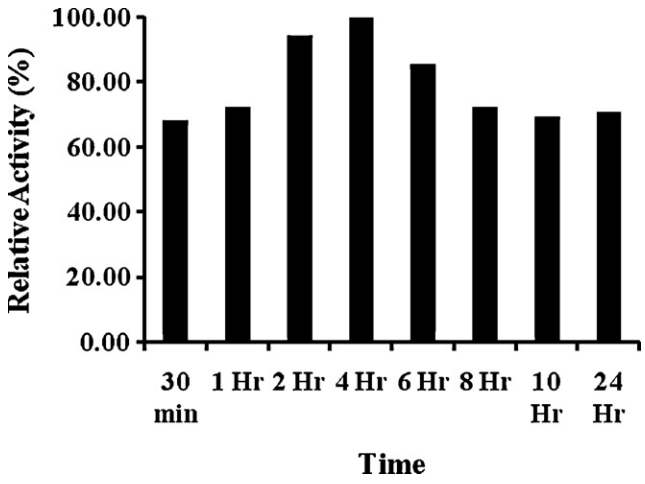



**Fig. 4.** Effect of time on immobilization of carbon composite beads.

**Table 2**
Screening of materials.

| | Mesoalumina from chitosan | Carbon composite beads from chitosan |
|---|---|---|
| Materials (mg) | 10 | 10 |
| CA used for immobilization (mg) | 0.5 | 0.5 |
| % of enzyme immobilized | 5.33 | 61.2 |
| Enzyme activity on materials (U/mg of materials) | 0.32 | 1.95 |

### 3.2. Immobilization study

#### 3.2.1. Screening of materials for immobilization of partially purified CA

Table 2 shows the screening results of the materials using pNPA with respect to partially purified enzyme. The immobilized CA shows reasonably good esterase activity. Esterase activity has been selected for screening considering the fact that active site of the enzyme is similar for the acceleration of $CO_2$ hydration as well as for the hydrolysis of esters. This has been substantiated by the study of carbonation precipitation reaction. In this study, partially purified CA has been immobilized on different materials i.e. Mesoalumina from chitosan, and carbon from chitosan composite beads.

The table shows the maximum activity for enzyme immobilization in carbon composite beads. This may be because during carbonization, nitrogen present in chitosan is retained in situ in the carbon matrix in the form of different nitrogen functionalities, resulting in nitrogen enrichment of the synthesized carbon composite. This synthesized carbon composite exhibited relatively high surface area of 167.3 $m^2/g$. The presence of nitrogen group in the carbon matrix along with the basic alumina facilitates the adsorption of enzymes on its surface. On the basis of their screening results, carbon composite bead had been selected for the further detailed studies.

#### 3.2.2. Effect of variation of time on immobilization of enzyme

The effect of time on immobilization of enzyme is presented in Fig. 4. The amount of enzyme loaded/absorbed increases up to 4 h and subsequently decreases. The decrease in activity may be attributed to leaching of enzyme or denaturation of enzyme due to stirring beyond 4 h.

#### 3.2.3. Effect of variation of material dose on immobilization of enzyme

The material dose was varied from 1 mg/5 ml to 20 mg/5 ml and the optimal dose appears to be 10 mg/5 ml as shown in Fig. 5. Further increase in dose shows constant activity of enzyme.

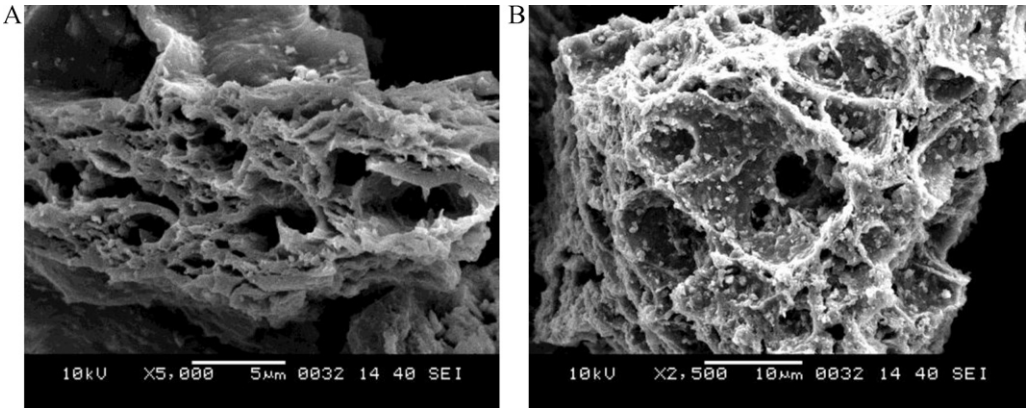



**Fig. 3.** SEM image of carbon composite beads.

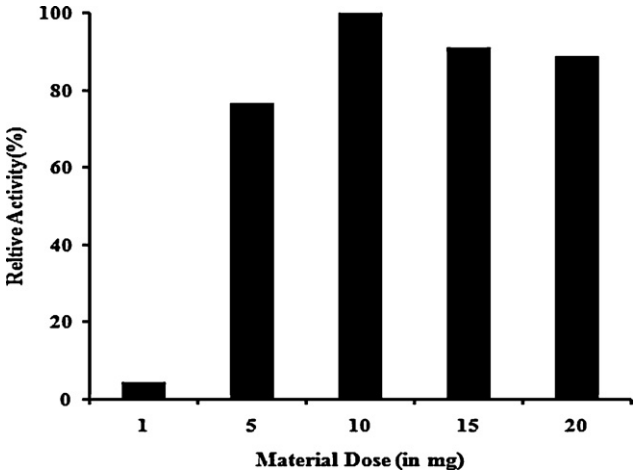



**Fig. 5.** Effect of material dose on immobilization of carbon composite beads.

### 3.2.4. Effect of variation of enzyme concentration on immobilization

The effect of enzyme concentration on immobilization of enzyme for carbon composite beads is presented in Fig. 6. In carbon composite beads, the maximum activity of enzyme was obtained at 1 mg/5 ml concentration. Beyond optimum enzyme concentration there is decrease in the enzyme activity has been observed. This may be due to overcrowding at the high concentration of enzyme on the external surface. Therefore, to avoid the overcrowding, we have selected the enzyme loading of 1 mg/ml for further studies.

### 3.2.5. Effect of temperature

Fig. 7 shows the activity of immobilized enzyme in the temperature range of 15–55 °C. It was observed that activity increases from 25 to 45 °C and then sharply decreases. Maximum activity was observed at 45 °C in immobilized enzyme while free enzyme shows maximum activity at 35 °C. This indicates that at higher temperature the support protects the enzyme from denaturation. It has been also reported that the immobilization of enzyme causes an increase in enzyme rigidity, which increases the stability towards denaturation by raising the temperature compared to free enzyme in solution [16]. From the above, it has been concluded that, at higher temperature immobilized enzyme are more stable as compared to free enzyme.

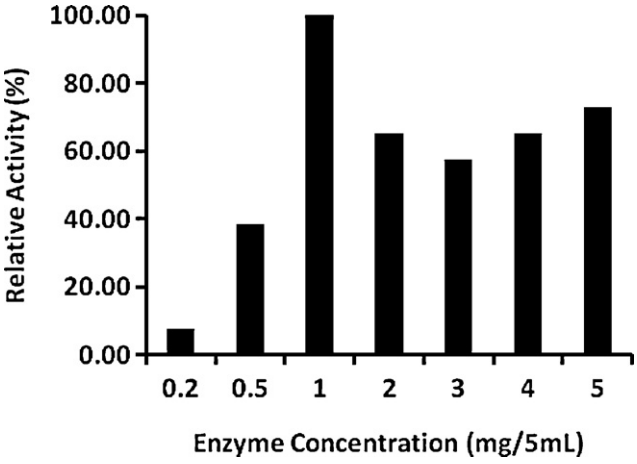



**Fig. 6.** Effect of enzyme concentration on carbon composite beads.

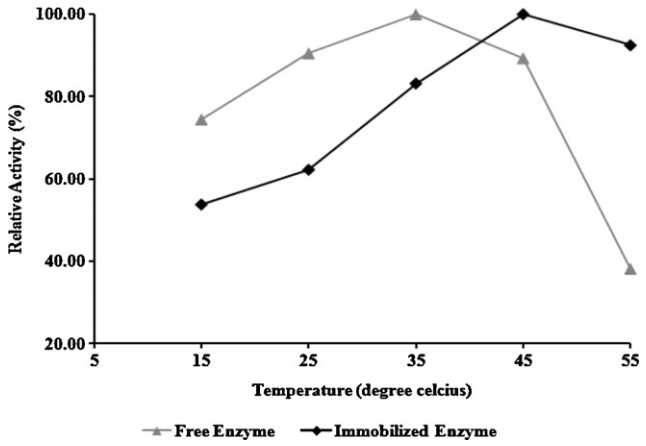



**Fig. 7.** Temperature profile of immobilized and free enzyme.

**Table 3**
Langmuir parameters of materials.

| S. no. | Material | $Q_{max}$ (mg/g) | $K$ (mg$^{-1}$) | $r$ |
|---|---|---|---|---|
| 1. | Carbon composite beads | 172.41 | 3.63 | 0.12 |

### 3.2.6. Effect of pH

pH is one of the most important parameter altering enzyme activity in an aqueous medium. The stability of immobilized enzyme at various pH is observed in Fig. 8. The effect of pH was restricted from pH 6 to 10, because CA is not stable below pH 6.0 because of the possible denaturation of the enzyme envisaged [11]. Maximum activity of free as well as immobilized enzyme was observed at pH 9.5 which suggest that at higher pH both are stable which is required for our targeted application i.e. formation of calcium carbonate which occurs generally at higher pH.

### 3.3. Adsorption study

The Langmuir adsorption equation which is valid for monolayer sorption onto a surface is given below [17];

$$\frac{1}{q_e} = \frac{1}{q_{max}k} \times \frac{1}{C_e} + \frac{1}{q_{max}} \quad (1)$$

where $q_{max}$ is the maximum amount of enzyme per unit weight of materials to form a complex monolayer on the surface bound at high $C_e$ and $K$ is a constant related to the affinity of the bind-

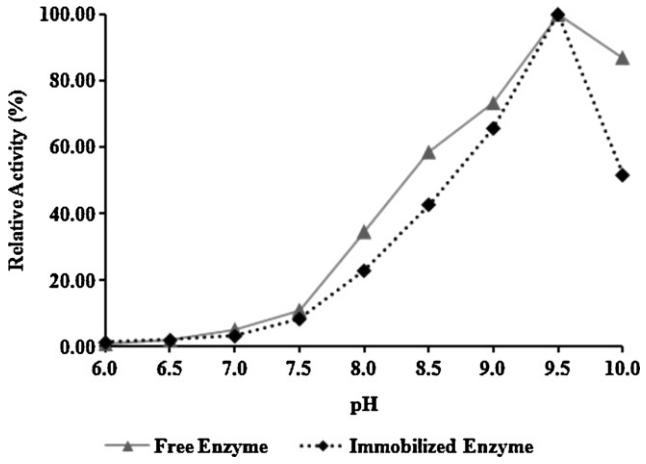



**Fig. 8.** Effect of pH on free enzyme and immobilized enzyme.

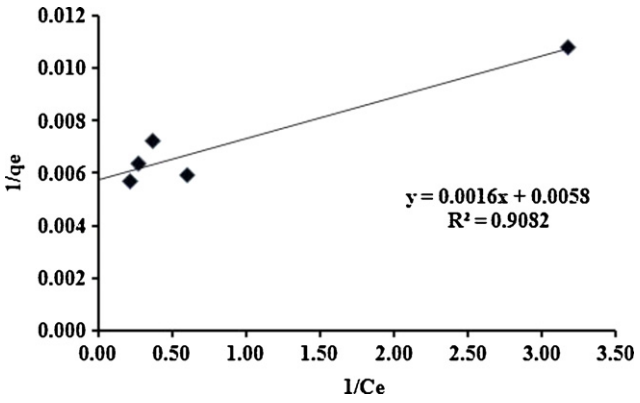



**Fig. 9.** Adsorption isotherm of carbon composite beads.

ing sites. $q_e$ represents the adsorption capacity of enzyme on the surface which was calculated using following equation;

$$q_e = (C_0 - C_e) \times \frac{V}{W} \quad (2)$$

where $q_e$ is the adsorption capacity (mg/g) in the solid at equilibrium; $C_0$, $C_e$ are initial and equilibrium concentrations of CA (mg/ml), respectively; $V$ is the volume of the aqueous solution and $W$ is the mass (g) of material used in the experiments. The values of Langmuir parameters, $q_{max}$ and $K$ were calculated from the slope and intercepts of the linear plots $1/q_e$ vs $1/C_e$. In order to predict the adsorption efficiency of the process, the dimensionless quantity ($r$) was calculated using the following equation.

$$r = \frac{1}{1 + KC_0} \quad (3)$$

where $C_0$ and $K$ are the initial concentration of CA and Langmuir isotherm constant. If the value of $r < 1$, it represents favorable adsorption if $r > 1$, it represents unfavorable adsorption. Fig. 9 shows the Langmuir plots for activated alumina carbon composite beads and Langmuir parameters were calculated. The values are given in Table 3. The experimental data for the immobilized material satisfies the Langmuir equation. Favorable adsorption was observed for the material with the value of $r$ being less than 1.

### 3.4. Kinetic study

#### 3.4.1. Determination of $K_m$ and $V_{max}$

The kinetic parameters of the hydrolytic reaction of para-nitrophenol acetate using the free and immobilized carbonic anhydrase were determined. Using Lineweaver–Burk method (Fig. 10), the apparent Michaelis constants $K_m$ and $V_{max}$ of the free enzyme and immobilized enzyme have been calculated. The values are given in Table 4. The $K_m$ of immobilized enzyme was higher as compared to free enzyme. This means that the affinity of the enzyme for its substrate has decreased. This may attributed to the lower accessibility of the substrate to the active site of the immobilized enzyme and lower transporting of the substrate and products into and out the supporting matrix [18,19].

**Table 4**
$K_m$ and $V_{max}$ of materials.

| S. no. | Materials | $V_{max}$ (μmol/min/ml) | $K_m$ (mM) |
|---|---|---|---|
| 1 | Immobilized enzyme | 0.99 | 10.35 |
| 3 | Free enzyme | 0.99 | 1.89 |

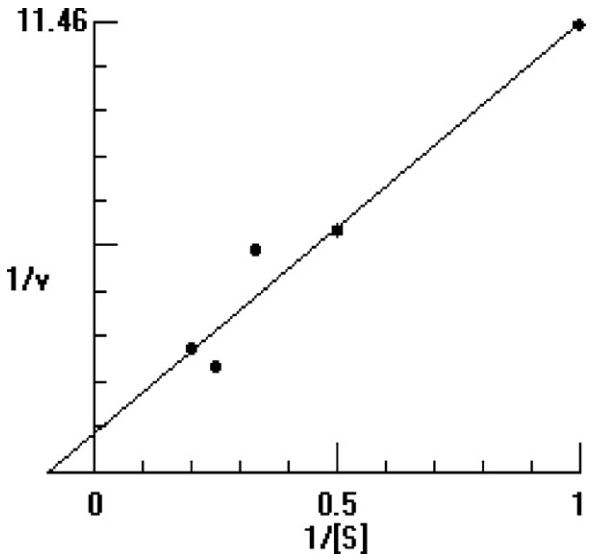



**Fig. 10.** Lineweaver–Burk plot of immobilized enzyme.

### 3.5. Operational stability

Fig. 11 shows the storage stability of free and immobilized enzyme at 4 °C. It was observed that up to 10 days, the free enzyme lost 20% its initial activity while immobilized enzyme lost 25%. But after 10th day, there is a gradual decrease in the activity of free enzyme while immobilized enzyme activity decrease is minimal. On 20th day, the immobilized enzyme shows better activity and retained 50% activity compared to free enzyme. On 25th day, free and immobilized catalyst show similar activity. From the above, we conclude that the stability of immobilized enzyme has improved and retained its 50% initial activity up to 20 days as compared to free enzyme.

The Half Life Period (HLP) of free and immobilized enzyme was observed to be 384 h and 528 h respectively. The results indicated improved half life of immobilized enzyme compared to free enzyme.

The reusability of the immobilized enzyme was evaluated in repeated batch process and 50% of the initial activity was retained after 4 cycles. The main reason for the loss of activity was the leakage of protein during rinsing of the immobilized beads with deionized water for removing the p-NP produced in the hydrolysis of p-NPA. This was confirmed by assaying the leachate. This could be improved by functionalization of materials using suitable crosslinking reagent.

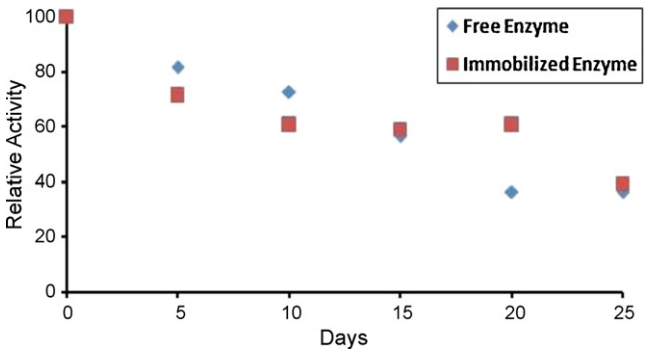



**Fig. 11.** Storage stability of free and immobilized enzyme at 4 °C.

**Table 5**
Precipitation time for calcium carbonate formation by immobilized CA.

| S. no. | Samples | Precipitation time (s) |
|---|---|---|
| 1 | Partially purified CA | 20 |
| 2 | Immobilized CA | 55 |
| 3 | Control (material) | 100 |

### 3.6. Carbonation study

Table 5 shows the time required for the formation of calcium carbonate precipitate by immobilized catalyst as well as control (only material). It was observed that the sample with immobilized material as well as reagent blank (the material) forms the precipitate. Free enzyme formed the calcium carbonate precipitation in 20 s; however, the formation of precipitate was rapid in immobilized material (55 s) because of the presence of enzyme on its surface, which helps to accelerate the carbonation reaction. However, in the blank (only material) the time taken for precipitation was observed to be 100 s, which is approximately 2 times higher compared to carbonation reaction in the presence of immobilized enzyme. Further SEM analysis and XRD confirmed the morphology of calcium carbonate.

The carbonate formed was estimated through evolution of carbon dioxide after the carbonation reaction using GC. The carbonation capacity of immobilized carbon composite beads was found to be 19.22 mg of $CaCO_3$/mg of enzyme as compared to 33.06 mg of $CaCO_3$/mg of enzyme for free enzyme. This may be due to easy access of the substrate to the free enzyme as compared to immobilized enzyme.

### 3.7. Characterization of carbonate precipitate

#### 3.7.1. XRD

Fig. 12 shows the XRD spectra of calcium carbonate obtained from immobilized catalyst. Calcium carbonate ($CaCO_3$) has three crystal phases (calcite, aragonite and vaterite). The calcite phase is thermodynamically most stable phase under the ambient conditions. The XRD patterns of precipitates show major peaks at $2\theta = 29^\circ$, $39^\circ$ and $48^\circ$ that will match with the JCPDS data (JCPDS card no. 86-2334) observed for calcite [20]. This confirms that the enzymatically formed calcium carbonate precipitates are calcite.

#### 3.7.2. SEM image

The scanning electron micrographs of precipitated calcium carbonate formed by the immobilized CA are shown in Fig. 13. The image displayed rhombohedra and rectangle shaped crystals of $CaCO_3$ formed by the immobilized CA which confirms that the crystals so formed are calcite. The image also displayed a spherical vaterite particle. These findings corroborate with those reported in the literature [4].

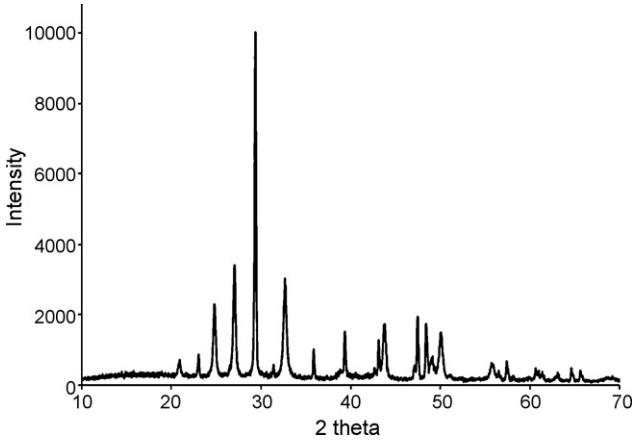
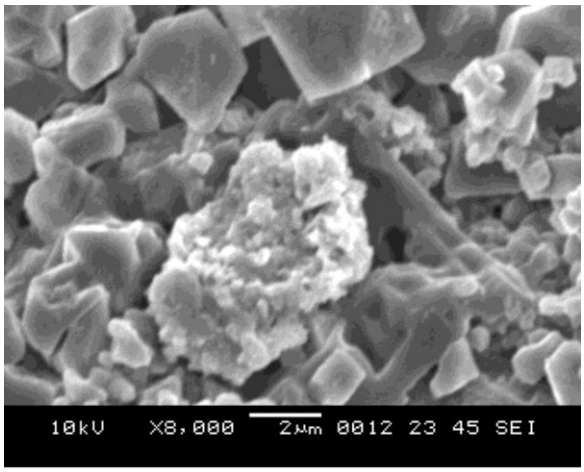



**Fig. 12.** XRD spectra of calcium carbonate formed by immobilized catalyst.




A

**Fig. 13.** SEM image of calcium carbonate formed by immobilized catalyst.

## 4. Conclusion

Low cost nitrogen containing biogenic carbon composite was synthesized from the biopolymer chitosan. The presence of nitrogen functionality in the carbon was confirmed by FTIR. This material has been further used as a matrix for the immobilization of enzyme, carbonic anhydrase. From the adsorption isotherms data, favorable adsorption was observed for the materials. This may be attributed to the high surface area of material. The kinetic parameter $K_m$ and $V_{max}$ values of immobilized enzyme and free enzyme were calculated from Lineweaver–Burk plot. The increase of $K_m$ value in the immobilized enzyme as compared to free enzyme $K_m$ value may be attributed to a possible change in the enzyme active site resulting in lowering the accessibility of the active site to the substrate. Reuse and storage study also show promising results for immobilization enzyme. This could be further improved by the functionalization of materials using suitable crosslinking reagent. Carbonate formation on the immobilized materials confirmed the potential of these materials for $CO_2$ sequestration. It was concluded that the immobilized CA could be used to accelerate the hydration of $CO_2$ in biomimetic $CO_2$ sequestration in an aqueous solution.

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