

Kinetic of carbonic anhydrase immobilized onto amberlite xad 7 and it application in sequestration of CO_2 into CaCO_3 precipitate

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Abstract. Carbonic Anhydrase (CA) was immobilized onto Amberlite XAD 7 and was used in carbon dioxide sequestration purposes. The catalytic activities for free and immobilized CA were estimated by using para-Nitrophenyl Acetate (p-NPA) as the substrate in Tris-buffer containing 10% of acetonitrile. Lineweaver-Burk plot was employed to estimate the Michaelis-Menten kinetic parameters for both free and immobilized CA. K_m value of free and immobilized CA were 2.92 mM and 5.7 mM respectively. Meanwhile the V_{max} value of free and immobilized CA were 5.95 $\mu\text{moles/min/ml}$ and 2.67 $\mu\text{moles/min/ml}$ respectively. The kinetic value obtained in the present study shown that the immobilized CA has high affinity for its substrate. On the other hand, activity and stability study at various pH and temperature indicates that the optimum pH for free CA was found to be at pH 9 while for immobilized CA was at pH 10. For optimum temperature, a free CA was performed optimally at temperature 25°C and immobilized CA was working effectively at temperature 50°C. The immobilized CA onto Amberlite was tested in the CO_2 sequestration process and the formation of the white CaCO_3 precipitate was observed during the process. CaCO_3 powder obtained during the process was validated with the XRD analysis. The finding indicated that immobilized CA onto Amberlite XAD7 retained its enzymatic activity and stability and thus perform well in the CO_2 sequestration which gave the white CaCO_3 precipitate.

1. Introduction

Nowadays, global warming becomes one of the major problems to the earth. One of the major contributors of the greenhouse gases is carbon dioxide. These happen due to the rising of anthropogenic CO_2 that has been emitted by the industrial sector such as ammonia production, natural gas processing, cement manufacturer and many others. According to Calleja et al, [1] the emission of carbon dioxide from fossil fuel plants is the main contributor to the problem arises. In 2007, the Intergovernmental Panel on Climate Change (IPCC) has established Carbon Capture and Storage (CCS) as an option for stabilization of greenhouse gas concentration while allowing for continuation of using fossil fuel [2]. CCS concept is based on the reduction of CO_2 emissions to the atmosphere produce by industrial processes. There are three major stages in CCS which are; i) capture of CO_2 gas in atmosphere, ii) transport of CO_2 storage place and iii) storage of captured CO_2 . There are three major techniques of CCS which categorized as post-combustion, oxy-combustion and pre-combustion.



Technology of CCS involves several processes such as physisorption/chemisorption, membrane separation/molecular sieve, carbamation, amine physical absorption, amine dry scrubbing, mineral carbonation. Unfortunately, the existing technology has several drawbacks such as in amine technology, there are limitations on insufficient carbon dioxide storage, equipment corrosion and many others [3].

Moreover, investigation on expenses of conventional CCS technologies has found that most of the system is costly. The estimated cost of the process shown in Figure 1 is around \$41-\$72 per ton of CO₂ sequestered. This cost can be reduced if the captured CO₂ can directly convert into valuable product without bothering any further compression, transportation and injection processes. Thus, a study on enzymatic carbon capture becomes one of the alternatives to tackle this limitation. Muradov (2012) has suggested the reaction between carbonic anhydrase and CO₂ to form bicarbonate product which is more practical and valuable in the market. Carbonic anhydrase (CA) catalyzes the conversion of carbon dioxide and water into bicarbonate via a hydration mechanism. Biocatalyst offers a good opportunity due to its high level of catalytic efficiency and a high degree of selectivity. Unfortunately, there are some limitations with biocatalyst such as high cost, limited stability, difficulty in handling, and so on. Thus, to overcome the problems, enzyme needs to be immobilized into the support material to enhance its usability and reaction [5], [6]. Many techniques of enzyme immobilization have been developed wherein adsorption on solid support material is the easiest and the conventional method. In this paper, Amberlite XAD-7 is being extensively studied for enzyme immobilization by virtue of its non-toxic.

Amberlite XAD 7 is a porous resin that is used to immobilize enzymes via simple adsorption. To help the attachment of the enzyme into the Amberlite XAD 7, glutaraldehyde cross-linking was used to bind the enzyme via covalent bond. To the best of our knowledge, detailed studies on immobilization of carbonic anhydrase on Amberlite XAD-7 have not been reported so far. In this paper, CA was immobilized onto Amberlite XAD7 and detailed kinetic parameters like pH, temperature of free and immobilized was investigated. Characterization of immobilized enzyme was done using SEM and FTIR. While calcium carbonate precipitate was characterized using XRD for the validation process.

2. Methodology

2.1. pH and Thermal stability

The immobilized enzyme was prepared according to the Wanjuri et al [10] and 0.2g immobilized enzyme was weighed for thermal and pH stability study. 13.5 ml buffer pH 7 and 1.5 ml p-NPA solution were added into the beaker. The experiment for the pH stability was carried out for 2 hours and 15 minute interval for absorbance analysis at 400 nm. The similar step was repeated with pH 6, 8, 9 and 10. For thermal stability, the temperature of the reaction was set at six different temperatures, which are 30 °C, 40 °C, 50 °C, 60 °C and 70 °C. Similar with pH stability, 15 ml of reaction volume was used with 13.5 ml of pH buffer, and 1.5 ml p-NPA solution as well as 0.2 g of immobilized enzyme was used. The absorbance was taken for every 15 minutes of interval for 2 hour duration. The absorbance reading was taken at 400 nm wavelength.

2.2. Kinetic study

For immobilized CA the buffer volume reaction used was 15 ml with reaction temperature set at 25 °C and pH 7.5. p-NPA solution with different concentration from 2.5 mM, 2.0 mM, 1.5 mM, 1.0 mM and 0.5 mM was prepared. The absorbance reading was taken for 2 hour with 15 minute interval. The absorbance reading was taken at 400 nm.

2.3. CaCO₃ Precipitation

The main experiment was started with the preparation of 200 ml of Ca(OH)₂ solution with concentration of 30 mM. During the precipitation reaction, the reading of pH was taken for every 15 seconds with constant flow rate and rpm (1L/min and 200 rpm). The reaction was stop when the pH

achieved 7. The carbonation reaction was tested for variation of temperature from 30 °C, 50 °C, 70 °C and 85 °C. For the characterization of the precipitation, the solution was filtered and dried overnight. The dried precipitation was examined using XRD.

3. Results and discussion

3.1. pH and temperature stability

Knowing the optimum pH is an important factor as it is a crucial parameter for an enzyme to work at its best performance. Besides, pH also plays a part in altering enzyme activity in aqueous solution. Figure 1(a) shows the effect of pH of free and immobilized CA to its activity. For free enzyme, the optimum pH of CA was obtained at pH 9 while for immobilized CA, the optimum pH was at 10. From the study, the optimum pH was changed from free cell to the immobilized cell. The optimum pH of many enzymes is shifted to higher pH if carrier used is anionic and towards low pH if it is cationic. In this study, the carrier used for immobilization were anionic thus it changes the degree of ionization of amino acid residues at the active site which lead to change in electrostatic potential and shift the pH towards alkaline [8] [7]. Figure 1(b) presents the thermal stability profile of both free and immobilized CA. The results clearly showed that the activity of free enzyme was optimum at temperature 25 °C and gradually decreases as the temperatures approach 70 °C. Meanwhile, the activity of immobilized CA was increased and optimum at temperature 50 °C before the activity was dropped when temperature 60 °C and 70 °C was used in the system. From results, it was observed that the loss of activity for immobilized enzyme was lower compared to the free enzyme. This phenomenon happens due to the presence of carrier (Amberlite) that has protected the enzyme when the temperature was increased and eliminated the potential of enzyme deactivation to occur. Immobilization is the reason for the conformational flexibility of the enzyme thus leading to the increase in enzyme rigidity which commonly directly increases the enzyme stability towards high temperatures [9] [8]. Moreover, it is often observed that immobilized enzyme has higher thermal stability than free enzyme due to the conformational flexibility in immobilized enzyme [10] [9].

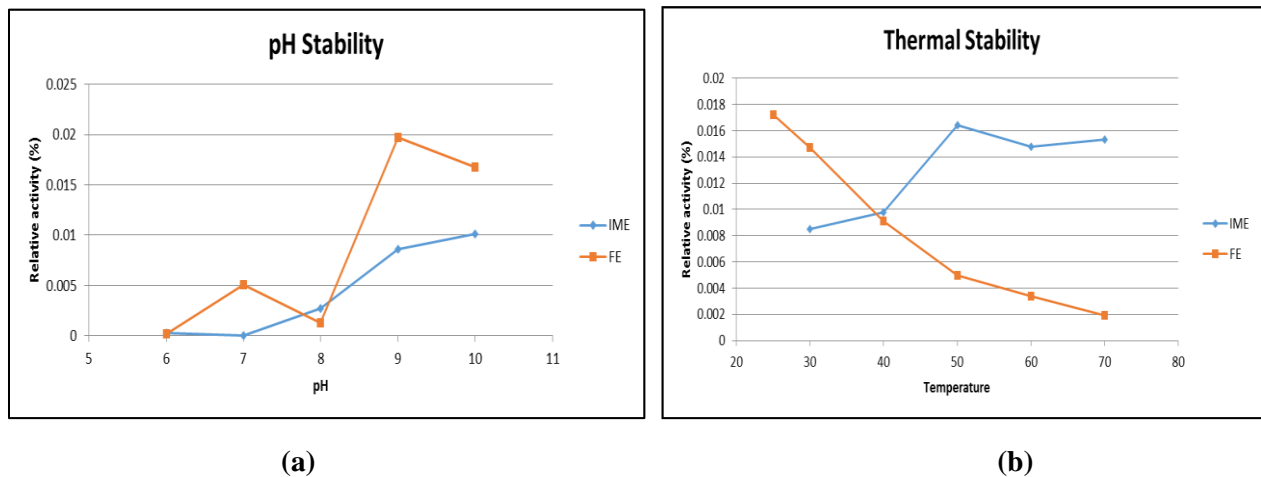


Figure 1. (a) pH stability profile for free CA and immobilized CA (b) Temperature stability profile for free CA and immobilized CA

3.2. Kinetic study

The kinetic constants (K_m and V_{max}) for free and immobilized enzyme were determined by using Lineweaver-Burk plots. The values of K_m and V_{max} of free and immobilized CA were calculated from the intercepts of x and y axes of the Lineweaver-Burk plot respectively. The values of K_m calculated for both free and immobilized CA were 2.919 mM and 5.711 mM respectively while V_{max} for free and

immobilized CA were 5.952 $\mu\text{moles}/\text{min}/\text{ml}$ and 2.666 $\mu\text{moles}/\text{min}/\text{ml}$. Note that the value of K_m for free is always lower than the immobilized enzyme. The experiment conducted by S. Wanjari et al [7] [10], indicated that the value of K_m for free enzyme is much lower compared to the K_m value of immobilized enzyme. K_m is defined as the substrate concentration at which the reaction rate is half of its maximum value. Therefore, in other words, smaller value of K_m indicates that the enzyme has achieves its maximum catalytic efficiency at low substrate concentrations. Thus, the smaller the K_m , the more efficient the enzyme works [11]. From the results, free CA has lower value of K_m as compared to immobilize CA. From the statement, it can be concluded that free CA has higher catalytic efficiency compared to immobilize CA. There are several factors that contribute to the change of kinetic parameter of immobilize enzyme such as conformational changes and steric effect [12].

3.3. CaCO_3 precipitation

In order to test the capability of immobilized enzyme in carbonation process, studies were carried out using a buffering process. In the carbonation reaction, it was observed that immobilized CA react with CO_2 to gave white precipitate. The XRD analysis conducted show that the white precipitate formed was CaCO_3 . The XRD pattern of precipitate products indicated that all three calcium carbonate precipitated are indeed calcite. The regions of peaks show that atomic arrangements are the same for the three precipitated calcium carbonate. This result indicates that the transformation of CO_2 to CaCO_3 was possible by the involvement of immobilized CA which catalyzes the formation of bicarbonate ion, HCO_3^- . Similar mechanism of carbonation by carbonic anhydrase was reported by Favre et al [13].

4. Conclusion

The Carbonic anhydrase (CA) enzyme immobilized onto Amberlite XAD 7 has shown a potential to be used in hydration of CO_2 based on its activity and stability on various pH and temperature. The study indicated that the optimum temperature for immobilized CA was at 50°C and the optimum pH was at 9. The value obtained was higher compared to stability of free CA. The catalytic activity of immobilized enzyme calculated using Michaelis Menten was 5.7mM and 2.67 $\mu\text{moles}/\text{min}/\text{ml}$ for K_m and V_m value respectively. The kinetic value obtained in the present study shown the capability of the immobilized CA to be used in the reaction process. The immobilized CA was used in CO_2 hydration process and the results show that CaCO_3 precipitation was formed as a product of the reaction. The formation of CaCO_3 validated the present of CA onto Amberlite and its capability to be used as a catalyst in CO_2 sequestration process.

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References

- [1] E. Torralba-Calleja, J. Skinner, and D. Gutiérrez-Tauste, "CO₂ Capture in Ionic Liquids: A Review of Solubilities and Experimental Methods," J. Chem., vol. 2013, pp. 1–16, 2013.
- [2] T. Barker, "Climate Change 2007 : An Assessment of the Intergovernmental Panel on Climate Change," Change, vol. 446, no. November, pp. 12–17, 2007.
- [3] O. F. Dawodu and A. Meisen, "Degradation of alkanolamine blends by carbon dioxide," Can. J. Chem. Eng., vol. 74, no. 6, pp. 960–966, 1996.
- [4] N. Z. Muradov, "Fossil fuel decarbonization: in the quest for clean and lasting fossil energy.," in Carbon-Neutral Fuels Energy Carriers, 2012, pp. 667–786.
- [5] S. Hosseinkhani and M. Nemat-Gorgani, "Partial unfolding of carbonic anhydrase provides a method for its immobilization on hydrophobic adsorbents and protects it against irreversible

- thermo-inactivation,” *Enzyme Microb. Technol.*, vol. 33, no. 2–3, pp. 179–184, 2003.
- [6] F. Azari, S. Hosseinkhani, and M. Nemat-Gorgani, “Use of reversible denaturation for adsorptive immobilization of urease,” *Appl Biochem Biotechnol*, vol. 94, no. 3, pp. 265–277, 2001.
- [7] S. Wanjari, C. Prabhu, T. Satyanarayana, A. Vinu, and S. Rayalu, “Immobilization of carbonic anhydrase on mesoporous aluminosilicate for carbonation reaction,” *Microporous Mesoporous Mater.*, vol. 160, no. 4, pp. 151–158, 2012.
- [8] S. Kumar, R. K. Yadav, and S. Negi, “A comparative study of immobilized lipase produced from *Penicillium chrysogenum* SNP5 on two different anionic carriers for its pH and thermostability,” *Indian J. Biotechnol.*, vol. 13, no. 3, pp. 301–305, 2014.
- [9] M. A. Abdelnaby, “Immobilization of *Aspergillus-Niger* Nrc-107 Xylanase and Beta-Xylosidase, and Properties of the Immobilized Enzymes,” *Appl. Biochem. Biotechnol.*, vol. 38, no. 1–2, pp. 69–81, 1993.
- [10] Ş. Akkuş Çetinus and H. Nursevin Öztop, “Immobilization of catalase into chemically crosslinked chitosan beads,” *Enzyme Microb. Technol.*, vol. 32, no. 7, pp. 889–894, 2003.
- [11] W. Cleland, “Enzyme kinetics 1,” *Wiley Encycl. Chem. Biol.*, vol. 6, no. 2, pp. 1–11, 1967.
- [12] J. F. Kennedy, *Immobilized Enzyme Principles (Applied Biochemistry and Bioengineering, Volume 1)*, vol. 67, no. 2. Elsevier, 1978.
- [13] N. Favre, M. L. Christ, and A. C. Pierre, “Biocatalytic capture of CO₂ with carbonic anhydrase and its transformation to solid carbonate,” *J. Mol. Catal. B Enzym.*, vol. 60, no. 3–4, pp. 163–170, 2009.