

Effect of Temperature and CO₂ Flowrate on the Formation of CaCO₃ in the Hydration Reaction of CO₂ Catalyzed by Immobilized *Carbonic anhydrase* into PVDF Membrane

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CO₂ conversion to mitigate CO₂ emissions is of great importance to reduce the negative impact of CO₂ on climate change. Different technologies have been developed for CO₂ conversion, including chemical, photocatalytic, electrochemical, and biological transformation. Among them, biological conversion by using *Carbonic anhydrase* (CA) to efficiently convert CO₂ to HCO₃⁻ is a promising one, owing to its high specificity and selectivity under the mild condition and presenting environmentally friendly nature. The production of CO₂ hydrates requires relatively high pressures and low temperatures, to make these applications feasible. However, free CA unable to withstand with too extreme temperature and pressure. Thus, the present work was conducted with the aim to determine the optimum parameter in the CO₂ hydration using immobilized CA. In this research, CO₂ hydrate were formed using CaCl biomimetic solution with an immobilized CA into PDVF membrane. Temperature variation from 30 to 85 °C and CO₂ flowrate from 100 – 800 mL/min were evaluated for the CO₂ hydration process. Time taken to reach pH 7 and the amount of CaCO₃ precipitate formed during the process were used to elucidate the performance of the CO₂ hydration process. The finding indicated that temperature plays an important role in reducing time taken for pH 7 to be reached. At 30 °C, 16 min was required for the CO₂ hydration process and reaction time was decreased with the increasing of the temperature. At higher temperature of 85 °C, 3.4 min was recorded as an optimum time for the pH to turn to 7. Similar trend was observed with increasing of CO₂ flowrate. Higher CO₂ flowrate has shorter the time for the reaction to occur and 3 to 4 min was recorded as an optimum time for CO₂ hydration process in the CO₂ membrane reactor. This work might help comprehend CO₂ hydrate storage technology, in separation of CO₂ from methane gas and could give theoretical basis for subsequent applications in the sector.

1. Introduction

Carbon dioxide is one of the greenhouse gasses (GHG) that contributed to the environment impact. CO₂ emission can be classified as point source emission and mobile emission. Industries and power plant are the major point source emission while mobile emission includes that by automobiles. With the increment of human population and demand of the energy, the concentration of CO₂ gas in the atmosphere has been increased yearly. The united Nation's Intergovernment Panel on Climate Change (IPCC) has suggested the threshold for maximum global temperature rise of 2 °C (Verma et al. 2021). This indicate that the significant control of CO₂ emission needs to be done to reduce the CO₂ concentration at the environment. Considering effort has been directed toward developing technology that would be effective and efficient in reducing global CO₂ emission. One of the approaches to beat this purpose is carbon capture technologies, to control GHG effect and the CO₂ accumulation at atmosphere. The main technologies for carbon capture are including physical and chemical absorption, solid adsorption, membrane

separation, cryogenic technology, ionic liquids and hybrid system (Dubey and Arora, 2022). A broad application of cryogenic separation and pressure swing adsorption (PSA) in carbon capture (CC) creates drawback of operations such as large energy consumption (Hasib-ur-Rahman and Larachi, 2013). While, in the absorption process by using aqueous amine based, higher absorption of CO₂ was recorded, but operation of amine required a high operating cost, a high energy requirement, degradation of amine and equipment corrosion (Wanjari et al., 2012). CO₂ conversion to mineral carbonates is the safest means for CC of CO₂, and the most effective as well as the least energy consuming method. In aqueous carbonation, CO₂ is converted to carbonic acid. Then this acid is reacted with an alkali metal ion to be precipitated as a mineral carbonate. The process mimics natural weathering of rocks and is kinetically limited.

With those limitations, biological CC approach seems to have a promising technique to overcome this issue. One of the bioprocessing technologies for CC is by using enzymatic approach. The implementation of Carbonic anhydrase enzyme in CC technology offers an environmentally friendly approach with a higher reduction and recovery of CO₂. Carbonic anhydrase (CA) is a biocatalyst which catalyse reversible hydration reaction of carbon dioxide that transform CO₂ into bicarbonate ion in aqueous solution. This natural enzyme with a high turnover rate (k_{cat}) which varies between 10^4 and 10^6 molecule CO₂/(molecule CA·s) and a specificity constant (k_{cat}/K_m) of about 10^8 M⁻¹ s⁻¹ (Rasouli et al., 2021). The process involves with the formation of calcium carbonate (CaCO₃) through carbonation reaction catalysed by CA (Hasib-ur-Rahman and Larachi, 2013). However, free CA has limitation whereby CA is non-reusability, denatured and reduction of stability at higher pressure and temperature and considerable high amounts of enzyme is required (Rasouli et al., 2022). This factor will significantly contribute to the low catalytic activity and hydration process. Thus, to overcome this limitation, immobilization of CA using solid support is preferable to improve the stability and activity of the CA in the hydration of CO₂. Among the solid support used in immobilization of CA in hydration process, membrane is the promising support to meet this purpose which offer green and affordable alternative, low operating and capital costs. Enzymatic membranes perform rapid catalysis of substrates and can exhibit superior separation properties (Luo et al. 2020). Besides, Sahoo et al. (2013) also stated that membrane had shown great advantage to overcome intrinsic difficulties of CO₂, less energy consumed and more operations flexibility. Thus, in this present work, CA was immobilized into PVDF membrane, to provide physical support and improving rheological impact to the CA. The present work is aimed to determine the optimum temperature and CO₂ flowrate and produce environmentally friendly practices in the CO₂ hydration using immobilized CA. Free and immobilized CA were used to compare the performance of CA in hydration of CO₂.

2. Methodology

2.1 Material

The support materials for the present study were polyvinylidene fluoride (PVDF) flat sheet membrane (Merck Millipore). The *Carbonic anhydrase* enzyme with the enzyme unit of 35,000 (Wilbur Anderson) was purchased from Sigma Aldrich. While glutaraldehyde (GA) as an intermolecularly cross linker reagent and Tris buffer were purchased from Sigma Aldrich without any further treatment and purification. Carbon dioxide gas (100 % purity) and calcium chloride (CaCl₂·2H₂O) from Merck were used as a solution in hydration process.

2.2 Immobilization of CA onto membrane

Enzyme immobilization onto support materials was conducted as described by Sahoo et al. (2012) with some modification on the support materials. The CA enzyme was immobilized onto PVDF membrane with GA as a crosslinkers agent. The CA solution (0.3 mg/mL) was dissolved in 50 mmol/L Tris buffer solution, pH 8. Then, 5 mL of CA solution was soaked with PVDF membrane (3 cm diameter) and the solution was stirred at 100 rpm for about 1 h at 25 °C. After that, 5 mL of GA (1 % v/w) was added, and the mixture was stirred continuously for another 1 h. Then, the PVDF membrane was separated from the solution and washed three times using distilled water. The PVDF membrane-CA was then dried at room temperature and stored at 4 °C. The CA concentration before and after immobilization onto the support materials were analyzed by using Bradford protein analysis.

2.3 Hydration of CO₂ using immobilized CA into PVDF membrane

The formation of CaCO₃ through enzymatic system was carried according to the method reported by Yadav et al. (2012) with slight modification. For this study, the CO₂ hydration set up was conducted as illustrated in Figure 1. Pure CO₂ gas was fed into the CaCl solution with the present of CA immobilized on PVDF membrane. The effect of different CO₂ feed flowrate (100, 200, 500, 700 and 800 mL/min) and different temperature (30, 40, 50, 60 and 70 °C) were investigated for CO₂ hydration. In this process, CO₂ gas was dissolved into CaCl₂ solution and formed precipitated CaCO₃. The rate of CaCO₃ formation was used to identify the amount of CO₂ that involved in hydration process. The reaction was initiated by addition of 4.87 mg of calcium chloride, CaCl₂·2H₂O into 200 mL of 50 mmol/L Tris buffer. The reaction was started when 1 L/min of CO₂ gas was allowed to dissolve

into the mixture at speed shaking of 120 rpm. During the reaction, the pH of mixture was recorded for every 15 s. The initial pH of the reaction was pH 10.60 and reaction was stopped when the pH of the mixture reaches pH 7. pH 7 is used as indicator in this study based on the finding by Enisa and Dena (2022) who stated that CaCO_3 precipitate are dissolved at lower pH due to the reversible reaction. Then, the precipitate was filtered by using filter paper, dried at 60 °C and stored at room temperature for further analysis. The effect of each parameter in the present study was conducted in triplicate and the obtained data was plotted with standard deviation.

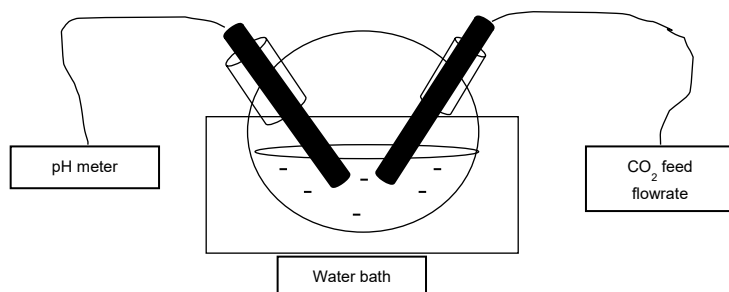


Figure 1: The schematic diagram for the carbonation experiment

3. Results and discussion

3.1 Effect of temperature and CO_2 flowrate on the hydration of CO_2 using immobilized CA into PVDF membrane

The effect of CO_2 feed flowrate and temperature on hydration of CO_2 by using immobilized CA into PVDF membrane was studied and the results is revealed in Figure 2. The comparison studies were based on the mass production of CaCO_3 and the time required of the final solution to reach pH 7 from initial pH of 10. The carbonation reaction had been stopped at pH 7 because CaCO_3 precipitate would be dissolved at lower pH. Yadav et al. (2012) also claimed that HCO_3^{2-} ion was soluble at the pH range below than pH 8. As the acidity of the solution increase, it will shift the reaction to the right and caused the CaCO_3 to dissolve. According to Kenji (2022), prolonged bubbling with CO_2 will decomposed the CaCO_3 precipitates to form $\text{Ca}(\text{HCO}_3)_2$, which is water soluble. Thus, determination of the CO_2 flowrate injected to the designed system is significant to provide the optimum precipitation of CaCO_3 in CO_2 hydration process.

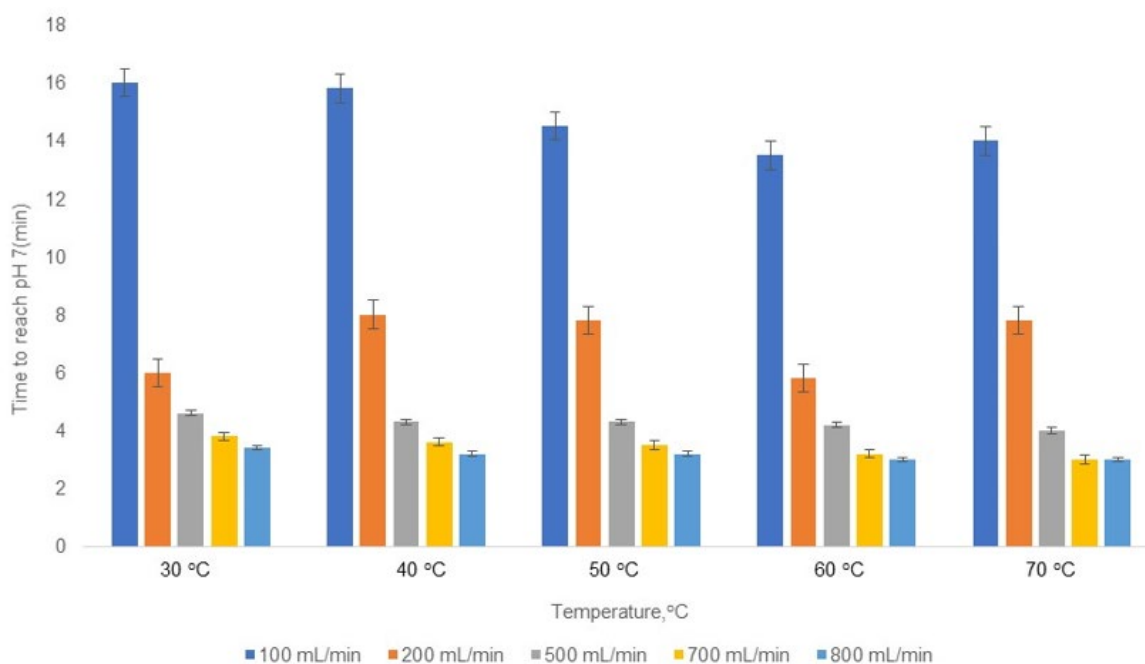


Figure 2: Effect of temperature and CO_2 flowrate on the time for reaction solution to reach pH 7

From Figure 2, it was observed that CO₂ feed flowrate gave a significant effect towards hydration of CO₂ catalyzed by immobilized CA into PVDF membrane. The results reveal that slow CO₂ flowrate required longer reaction time for the pH of the reaction solution to reach pH 7 and the fastest time was observed at high CO₂ flowrate. At 800 mL/min CO₂, less than 5 min was required for the reaction to turn pH of the solution into pH 7. While at 200 mL/min and different temperature, time between 13–16 min was recorded for the pH of the solution to be at 7. This condition might be due to the solubility of the CO₂ into the aqueous solution where the gas solubility is proportional to the pressure and flowrate. As the solubility of CO₂ increase, dissolution of CO₂ in aqueous phase is also higher. Thus, at higher gas flowrate, solubility of the CO₂ in aqueous solution is increased and consequently fast carbonization of CO₂ was recorded. Gul and Un (2022) also claimed the similar scenario where the absorption capacity of CO₂ increases as the temperature decreases and as the flow rate increases. A higher flow rate creates turbulent conditions which is favorable for mass transfer of CO₂ to immobilized CA. With increasing gas flow rates, the driving force between CO₂ and water is strengthened and more CO₂ molecules transfer from the bulk gas phase to the gas-liquid boundary layer, causing an increase in mass-transfer coefficient (Kapoor et al., 2021). The dissolution, hydration and formation of precipitate CaCO₃ during carbonation of CO₂ can be summarized as Eq(1)–Eq(5). Immobilized CA play an important role in acceleration of hydration process in Eq(2) that normally is the slowest step in hydration (Verma et al., 2021). In CO₂ hydration catalyzed by CA, catalysis will begin with water coordination with the Zn²⁺ ion bound active site of CA. Then, the adsorbed water dissociates to form a hydroxide – CA complex, followed by a nucleophilic attack of CO₂ to form the bicarbonate ion. The bicarbonate ion then rearranges the proton, followed by desorption of the bicarbonate ion. A water molecule then replaces this bicarbonate ion, followed by water dissociation leading to the initial structure of the enzyme. In this reaction, Histidine (His) is play an important role in the displacement and rearrangement of the H⁺ ion within the CA molecules which is occurred in the present of basic or buffer media.

Dissolution:



Hydration:



Dissociation:



Carbonate formation:



Precipitation:



On the other hand, there is no significant changes were observed on the time for the reaction to reach pH 7 with different reaction temperature. The time taken for reaction solution to reach pH 7 at 100, 200, 500, 700 and 800 mL/min were in range of 14 – 16, 6 – 8, 4 – 5, 3 – 4 and 3 – 4 min. The consistency of the results for the time taken at different temperature might be due to the stability of immobilized CA into polymeric membrane that provide better environment to the enzymatic hydration of CO₂. Similar results were reported by Kanbar and Ozdemir (2010) on the stability study of carbonic anhydrase (CA) immobilized within polyurethane (PU) foam. The finding claims that the immobilized CA was found to be 98% stable below 50°C, whereas a decrease in activity was seen at temperatures between 50 and 60°C. For this process, enzyme might be protected by solid support from direct exposure to extreme temperature and distribute heat away from the enzyme. The temperature stability is expected to be a consequence of the enzyme structure distortion (Glowacki et al., 2012) together to altered temperature sensitivity of substrate-enzyme, enzyme-product transitions stages, heat capacity of enzyme-bound intermediates, ionic status at the enzyme active center (DeLong et al., 2017). Then further analysis was conducted on the mass of precipitate CaCO₃ at different temperature and constant CO₂ flowrate (200 mL/min). The result is tabulated in Table 1. The finding indicates that, temperature gave a significant effect to the formation of precipitate CaCO₃ either with PVDF immobilized CA or free CA. With the changes of temperature reaction from low (30 °C) to high temperature (85 °C), formation of precipitate CaCO₃ was reduced for both reaction condition. Reduction of precipitate CaCO₃ at higher temperature may also be due

to the thermodynamics of the exothermic CO₂ absorption system that could cause reversible reactions when the temperature was too high. According to Alcantara et al. (2021), an increase in the reaction temperature would reduce the equilibrium constant and the product conversion. Thus, direct conversion of CO₂ to high-value-added products would present higher conversions at lower temperatures. The increase in temperature could also increase the CO₂ vapor pressure over the solution that leads to decrease in the physical solubility of CO₂ in the solvent (Gul and Un, 2022). Similar result also was reported by Zhang et al. (2020) who claimed that higher amount of CO₂ was dissolved in solution at lower temperature, which is beneficial for the conversion rate. This present finding shows that 30 °C is the optimum temperature in formation of precipitate CaCO₃ for PVDF immobilized CA and free CA. However, at 30 °C, reaction with free CA gave 1.8 % higher amount of CaCO₃ precipitate as compared to PVDF immobilized CA. The amount of precipitate CaCO₃ form was 0.283 ± 0.001 g and 0.278 ± 0.002 g using free CA and PVDF immobilized CA respectively. On the other hand, at higher temperature (>40 °C), precipitate CaCO₃ obtained using PVDF immobilized CA was higher than free CA. This condition was also reflected with the results from the time taken to reach pH 7 that indicate the stability of the enzyme with the immobilization into PVDF membrane. As a result, shorter time is required for the reaction to reach pH 7 and 1.9 % higher CaCO₃ was formed as compared to the condition with free CA. Thus, taking selective carbonate precipitation into consideration and range of temperature used in the present study, the reaction temperature at 30 °C was the most suitable temperature for low CO₂ flowrate of 200 mL/min.

Table 1: CaCO₃ mass at different temperature and CA enzyme

Temperature (°C)	Mass (g)		
	CA without membrane	Immobilized CA - membrane	Different CaCO ₃ (%)
30	0.283 ± 0.001	0.278 ± 0.002	1.8
40	0.249 ± 0.064	0.265 ± 0.023	6.4
50	0.229 ± 0.012	0.255 ± 0.017	11.4
60	0.225 ± 0.020	0.251 ± 0.016	11.6
70	0.219 ± 0.008	0.246 ± 0.018	12.3
85	0.213 ± 0.023	0.217 ± 0.015	1.9

4. Conclusions

Hydration of CO₂ catalysed by free and immobilized CA into PVDF membrane was conducted and precipitation of metastable forms of calcium carbonate from solution was favoured to rhombohedra shape of calcite formation of CaCO₃. At low CO₂ flowrate (200 mL/min), long reaction time was required for the reaction to reach pH 7 and the shortest time for the reaction solution to turn to pH 7 was 3–3.5 min which occurred at high CO₂ flowrate (> 700mL/min). On the other hand, for temperature range of 30 – 85 °C, a higher CaCO₃ precipitate was obtained at 30 °C. In comparison between free CA and immobilized CA into PVDF membrane, immobilized CA into PVDF membrane provided better stability to the CA and as a result, up to 12 % higher mass of CaCO₃ was recorded. The effect of temperature and CO₂ flowrate on the hydration process is related to the solubility of the gas and catalytic performance of immobilized CA which might affect the formation and polymorph of CaCO₃.

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