

## Effect of Different CO<sub>2</sub> Flow Rate and Calcium Chloride Concentration on CO<sub>2</sub> Sequestration using Immobilized Carbonic Anhydrase

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### ABSTRACT

The present investigation deals with the development of sequestration process of CO<sub>2</sub> in carbonic solution accelerated by immobilized Carbonic anhydrase (CA). Sequestration process of the CO<sub>2</sub> using biocatalyst has become one of the green and promising approaches as compared to the chemical-based method that cause environmental pollution issue, difficult of the separation and recovery of the solution and also cost ineffective. The present study is focused on the effect of the CO<sub>2</sub> flow rate and Carbonic solution concentration on the sequestration of CO<sub>2</sub> using immobilized CA. The CO<sub>2</sub> flow rate was varied from 200 to 800 L/min while, Carbonic solution was prepared using Calcium chloride at 12, 36 and 48 g/L. The results indicate that increase in CO<sub>2</sub> flow rate results in a proportional increment of the CaCO<sub>3</sub> precipitate. At 200, 500 and 800 L/min of CO<sub>2</sub> feeding, amount of CaCO<sub>3</sub> precipitate was 0.23, 0.26 and 0.30g respectively. On the other hand, the optimum concentration of the carbonic solution was obtained at 36 g/L. The results reveal that at higher CO<sub>2</sub> flow rate enter the system, the fastest of the equilibrium process can be achieved whereas for carbonic solution concentration, higher concentration of the solution did not significantly affect time for the process to achieve the equilibrium. Time for the process to reach equilibrium at CO<sub>2</sub> flow rate of 200, 500 and 800 was 14 minutes, 6 minute and 4 minutes respectively. While at different Carbonic solution concentration, the time to reach equilibrium was constantly achieved at 4 minutes for each concentration. The precipitate CaCO<sub>3</sub> was validated with XRD analysis and the results indicate a sharp peak that represent the crystal structure of CaCO<sub>3</sub> at  $2\theta = 23.07, 29.42, 36.0, 39.43, 43.3, 47.53, \text{ and } 48.53$ . The morphology of the precipitate observed using scanning electron microscope showed a rectangular shape of the CaCO<sub>3</sub>. From the study, the optimum carbonic solution concentration was 36 g/L and the CO<sub>2</sub> loading can be speed up to 800 L/min to achieve a faster sequestration process.

#### Keywords:

Sequestration; immobilized; Carbonic anhydrase; CO<sub>2</sub>; CaCO<sub>3</sub>; Calcium chloride

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## 1. Introduction

The percentage of carbon dioxide (CO<sub>2</sub>) in the environment has been increased due to the industrial growth. CO<sub>2</sub> emission is mainly caused by the combustion of fossil fuels and the industrial growth itself. The CO<sub>2</sub> is known as greenhouse gases that contribute to the greenhouse effect and climate change around the world. The carbon capture and storage techniques (CCS) have been introduced to capture CO<sub>2</sub> and prevent it from being emitted to the atmosphere. Hence, the amount of the greenhouse gases including CO<sub>2</sub> could be reduced. This technologies chain are consists of three part which are capturing the CO<sub>2</sub>, transporting the CO<sub>2</sub> and storing the CO<sub>2</sub> in underground well like in the depleted oil and gas wells or in deep saline aquifer formation. In capturing the CO<sub>2</sub>, there are several technologies has been employed such as post combustion capture, pre-combustion capture and oxygen fuel combustion. For the storage of carbon dioxide, several options have been highlighted like geological storage, ocean storage and mineralization [1,2]. In the ocean storage, CO<sub>2</sub> was injected at a deep depth in the ocean. The injected carbon dioxide was dissolved and formed the hydrate which heavier than water plume. The hydrate is settles down in the bottom of the sea. While in geological storage, CO<sub>2</sub> are stored in the place like depleted oil and gas reservoir, saline aquifer and unmineable coal. However, there are limitations for both geological and ocean storage. For example, in the ocean storage, the CO<sub>2</sub> concentration will be increased and as a consequence will cause the acidification condition that can affect the marine life and interrupt to the coral growth rate. Meanwhile, leaking is the main problem in the geological storage. CO<sub>2</sub> leaking can cause the changes of the water pH, affect the human health and bring death to the animal. Besides, the injection of CO<sub>2</sub> near to the water source can affect the potable ground water resource.

Another option for the CO<sub>2</sub> storage is carbon sequestration. It is so-called long-term CO<sub>2</sub> storage which could reduce the amount of CO<sub>2</sub> in the atmosphere. One of the methods in CO<sub>2</sub> sequestration is by mineral carbonation where this technique is producing carbonate from the reaction of calcium and magnesium with CO<sub>2</sub> [3]. There several types of stable carbonate solution has been explored for this purpose such as calcite (CaCO<sub>3</sub>), dolomite (CaO.5MgO.5CO<sub>3</sub>), magnesite (MgCO<sub>3</sub>) and siderite (FeCO<sub>3</sub>). There are two type mineral carbonation which are in-situ carbonation and ex-situ carbonation. In-situ carbonation occurs when underground magnesium and calcium mineral was reacted with injected carbon dioxide. While ex-situ carbonation reaction is same as in-situ carbonation but it takes place above the ground. The formation of calcium carbonate has been lot of interest in this field since it is a simple process and widely used techniques in polymer industry [4]. While the precipitate obtained during the process in form of calcium or magnesium carbonate has a commercial value. Thus, in this research the performed of CO<sub>2</sub> sequestration using mineral carbonization with the present of *Carbonic anhydrase* was investigated. The study explored the effect of CO<sub>2</sub> flowrate and carbonic ion concentration on the equilibrium condition and amount of the precipitated form during the process. The morphology of calcium carbonate precipitate obtained from the study was characterized using Scanning Electron Microscope (SEM), X-Ray Diffractometer (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) analysis.

## 2. Method

Chloride solution at different concentration for this study was prepared using analytical grade calcium chloride purchase from System. While the bovine *Carbonic anhydrase* (CA) enzyme 35 KDA unit/mg protein (3.5 mm in size) was purchased from Sigma Aldrich. CO<sub>2</sub> gas with 100% purity was purchased from Polygas. For the immobilization of enzyme, 50mM Tris-buffer at pH 8 was purchased from Sigma Aldrich and glutaraldehyde for the crosslinking agent was purchased from Merck. CA was

immobilized using 1% glutaraldehyde onto Polyvinylidene fluoride (PVDF). Immobilization method was conducted as elaborate in [5]. Sequestration of CO<sub>2</sub> was conducted using gas rig membrane reactor as illustrated in Figure 1. The setting was made for flow rate and temperature using the controller at membrane CO<sub>2</sub> rig as tabulated in Table 1.

**Table 1**

Parameter set up for CO<sub>2</sub> sequestration in membrane reactor

Settings	Value
Flowrate, L/min	200, 500 and 800
Temperature, °C	30
Carbonic solution concentration, g/L	12, 36, 48

Next the tris-buffer was added with different mass of calcium chloride and was used as a carbonic solution in the process. The pH reduction during the process was observed using the build in pH meter. The reaction was stopped when pH 7 was achieved and time to reach the targeted pH was recorded. The cloudy solution which contains calcium carbonate precipitate was filtered using the filter paper. The precipitate was dried using oven (Memmet) for further analysis.



**Fig. 1.** The arrangement of CO<sub>2</sub> sequestration rig

There are three analyses was performed for the characterization of the calcium carbonate powder which are X-ray Diffraction (XRD, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM). X-ray Diffractometer (XRD, PANalytical) model X'Pert PRO that equipped with the 2 $\theta$  range of 20° to 80° with step size 0.02 was performed. While FTIR from Perkin Elmer, model Spectrum One, USA was used to characterize the functional group of the precipitate. Two scans were performed between the wavenumber 4000 to 515 cm<sup>-1</sup> for each spectrum. The crystallographic property of the Precipitate Calcium Carbonate (PCC) was performed using the Scanning Electron Microscope.

### 3. Results and Discussion

#### 3.1 The Effects of CO<sub>2</sub> Flowrates on the CO<sub>2</sub> Hydration

CO<sub>2</sub> hydration and precipitation of calcium carbonate in this process was evaluated for different CO<sub>2</sub> loading and concentration of the calcium chloride. Time taken to reach pH 7 for this process was measured. The CO<sub>2</sub> loading was set at 200, 500 and 800 L/min and concentration of calcium chloride was varied at 12.5 g/L, 36 g/L and 48 g/L. Figure 2 shows the relation between CO<sub>2</sub> loading and mass of precipitate CaCO<sub>3</sub> in *Carbonic anhydrase* (CA) catalyzed CO<sub>2</sub> hydration process. Based on the result, mass of precipitate was increased proportionally with the increment of the CO<sub>2</sub> loading. At 800 L/min, 0.33 g of precipitate CaCO<sub>3</sub> was obtained while at 200 and 500 L/min of CO<sub>2</sub> loading, the amount of precipitate was measured as 0.24 and 0.288 g respectively. Theoretically at higher CO<sub>2</sub> loading, high concentration of the CO<sub>2</sub> was supplied into the system and this will encourage more formation of HCO<sub>3</sub><sup>-</sup> ion for the reaction. Similar finding has been reported by Altiner who indicated that the increase of CO<sub>2</sub> flowrates produces high amount of precipitate CaCO<sub>3</sub> due to the high CO<sub>2</sub> as feedstocks [6]. The formation of precipitate CaCO<sub>3</sub> from the CO<sub>2</sub> gas can be summarized in Eq. (1)-(5) which involve with dissolution process, carbonic acid reaction, bicarbonate reaction, carbonate reaction and formation of CaCO<sub>3</sub> precipitate.

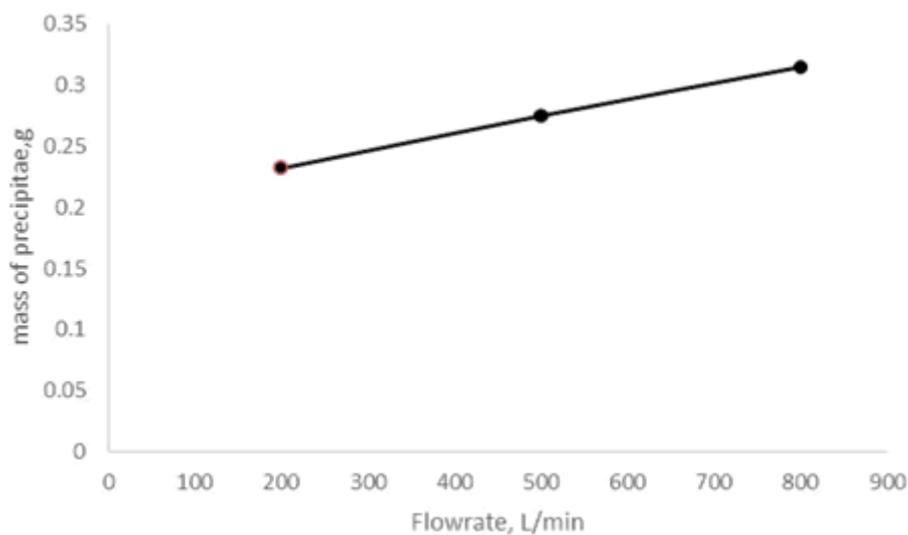


Fig. 2. Effect of flowrate on mass precipitate produced

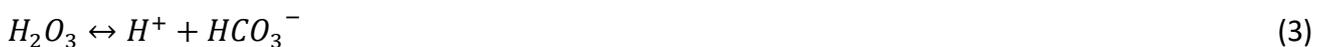
Dissolution



Carbonic acid reaction



Bicarbonate reaction



Carbonate reaction



Calcium carbonate reaction



This process is accelerated by *Carbonic anhydrase* (CA) enzymes to increase CO<sub>2</sub> sequestration process by conversion and mineralization of CO<sub>2</sub> into CaCO<sub>3</sub>. According to the results, 800 L/min of CO<sub>2</sub> gas is not a substrate saturation point for the CA activity and penetration of CO<sub>2</sub> into the aqueous solution of calcium ions is not limited. According to the experimental data on the time for each concentration to reach pH 7.0 as tabulated in Table 2, higher CO<sub>2</sub> flowrate encourage a faster reaction where at 800 L/min, time taken for the solution to reach pH 7.0 was 4.30 min. Whereas at lower CO<sub>2</sub> flowrate of 200 and 500 L/min, the time to reach pH 7 was 17.8 and 6.37 min respectively. Altiner also claim that the carbonation reaction was stopped at pH 7.5 due to the dissolution of calcium carbonate [6]. CaCO<sub>3</sub> precipitate (PCC) particle which dispersed in the carbonate solution was no longer dissolved which caused by the high concentration of  $HCO_3^-$ . While Ruiz-Agudo *et al.*, claim that the calcite growth rate decreased with increasing pH due to the calcite growth and the effect of OH ions on solute hydration [7]. Thus, the present study was investigated the time of the solution to reach pH 7 at different CO<sub>2</sub> flowrate in order to determine the saturation time for each CO<sub>2</sub> loading in the system. The finding indicates that CO<sub>2</sub> loading can be further increased higher than 800 L/min until an optimum loading of CO<sub>2</sub> can be achieved for this catalysis.

**Table 2**

Effect of different CO<sub>2</sub> flowrate on time for solution to reach pH 7

Flowrate (mL/min) at temp 30°C	Time taken to reach pH 7, min
200	17.8 ± 4.91
500	6.37 ± 0.24
800	4.30 ± 0.09

### 3.2 The Effects of Calcium Chloride Concentration on the CO<sub>2</sub> Hydration

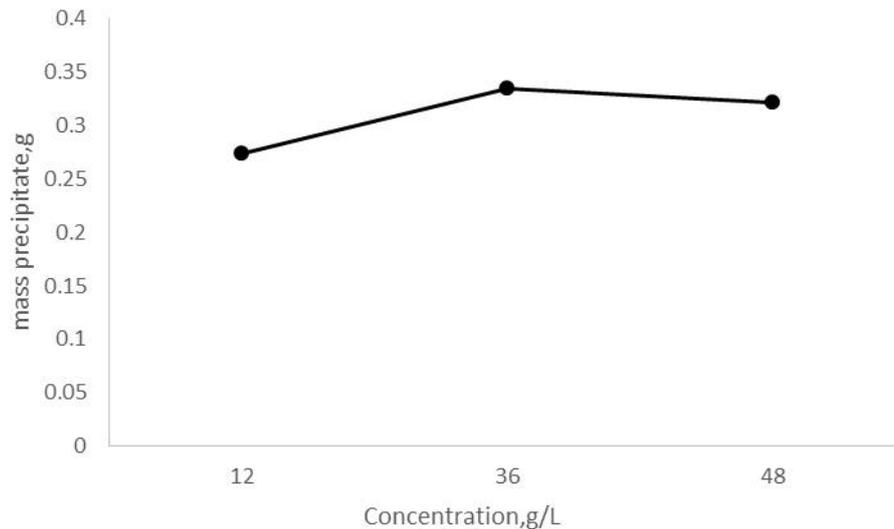
Concentration of the calcium chloride is another factor that needs to be investigated for the CO<sub>2</sub> sequestration performance. Figure 3 shows the plot of calcium chloride concentration versus mass of CaCO<sub>3</sub> precipitated formed. From the result in Figure 3, it shows that when the concentration of calcium chloride was increased, the mass of precipitated was higher. Higher concentration of calcium chloride provides more  $Ca^{2+}$  ions which produce high amount of calcium carbonate.

The study indicate that 36 g/L was the optimum concentration of calcium chloride that produced 0.345 g of CaCO<sub>3</sub> precipitate. The results also in line with the experimental data on the time for the solution to reach pH 7 as tabulated in Table 3. The results show that using 36 g/L calcium chloride solution, time required to reach pH 7 was 4.3 min, whereas for 12 g/L and 48 g/L of calcium chloride solution, time to reach pH 7 was 4.78 and 4.73 min respectively.

**Table 3**

Effect of different calcium chloride concentration on time for solution to reach pH 7

Concentration (g/L) at temp 30°C	Time taken to reach pH 7, min
12.15	4.78 ± 0.31
36	4.3 ± 0.14
48	4.73 ± 0.64

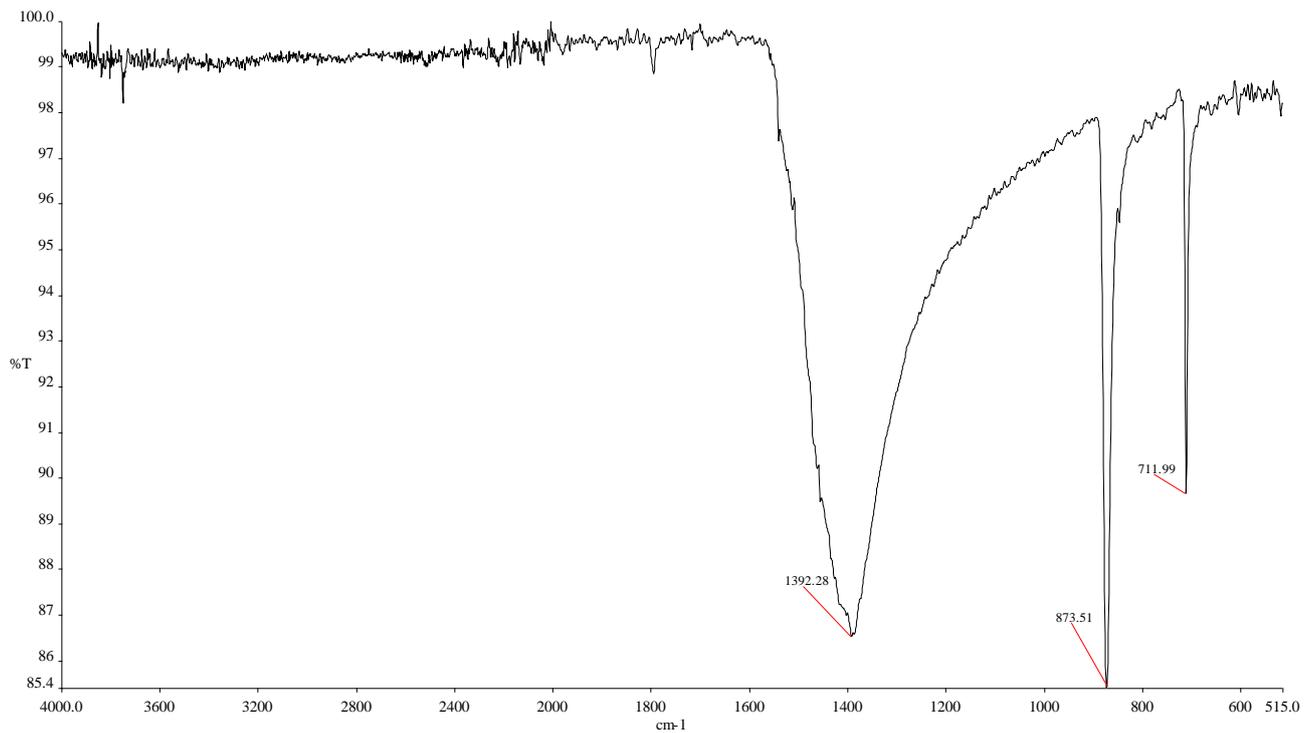


**Fig. 3.** The relation between calcium chloride concentration and mass of precipitate

### 3.3 The Analysis of Calcium Carbonate Precipitate

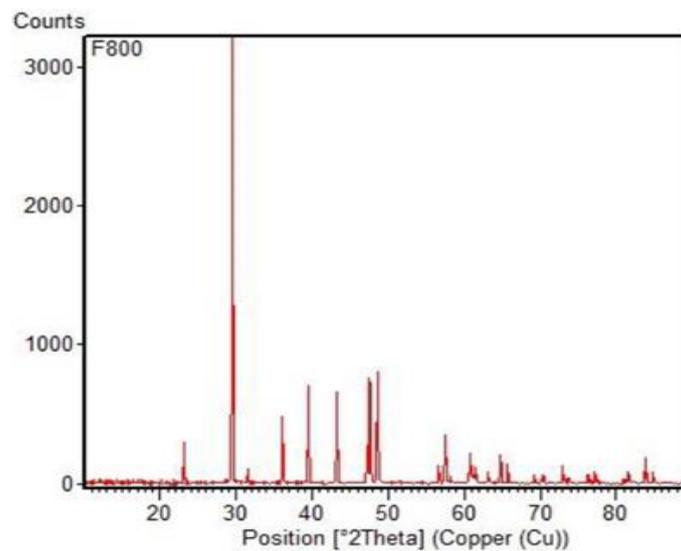
Figure 4 show the FTIR spectrum analysis of the  $\text{CaCO}_3$  precipitate obtained from the study. Based on the spectrum in Figure 4, the peaks were indicated at  $1392.28 \text{ cm}^{-1}$ ,  $873.51 \text{ cm}^{-1}$  and  $711.99 \text{ cm}^{-1}$  which represent the functional group of the  $\text{CaCO}_3$ . Peak at  $1392.28 \text{ cm}^{-1}$  in this study was represented the C-O stretching. The FTIR spectrum of calcium carbonate at  $875$  and  $712 \text{ cm}^{-1}$  has been reported [8]. While Bao *et al.*, claim that the asymmetric stretching and bending vibration of  $\text{CO}_3^{2-}$  occur at absorption peaks at  $2514 \text{ cm}^{-1}$ ,  $1423, 876 \text{ cm}^{-1}$  and  $712 \text{ cm}^{-1}$  [9]. Jimoh *et al.*, also reported that the typical FTIR spectrum for calcite is around  $1433 \text{ cm}^{-1}$ ,  $874 \text{ cm}^{-1}$  and  $713 \text{ cm}^{-1}$  which is closer to the spectrum that was obtained in this study [10].

XRD analysis of calcium carbonate precipitate obtained in this study is illustrated in Figure 5. Based on the result in Figure 5, the peak shows a needle peak which represent a crystal structure of calcite. According to the Daskalakis *et al.*, the calcite XRD spectrum corresponding to 2 theta was 23.05, 29.40 and 35.96 as shown in the result [11]. While Kim *et al.*, claimed that there are two type of calcium carbonate crystal which are calcite and vaterite [12]. Both type of crystal have their own peaks pattern. The calcite has the diffraction peaks at  $2\theta = 23.07, 29.42, 36.0, 39.43, 43.3, 47.53,$  and  $48.53$ . Whereas diffraction peaks for vaterite can be obtained at  $2\theta = 24.89, 27.05, 32.74,$  and  $43.84$ . According to the Sharma *et al.*, the major form of calcium carbonate that produce through the biomimetic  $\text{CO}_2$  sequestration was the calcite phase which showed the diffraction peaks at  $2\theta = 29.4^\circ$  and  $43.8^\circ$  [13]. This finding is consistence with the results obtained from the previous researcher.

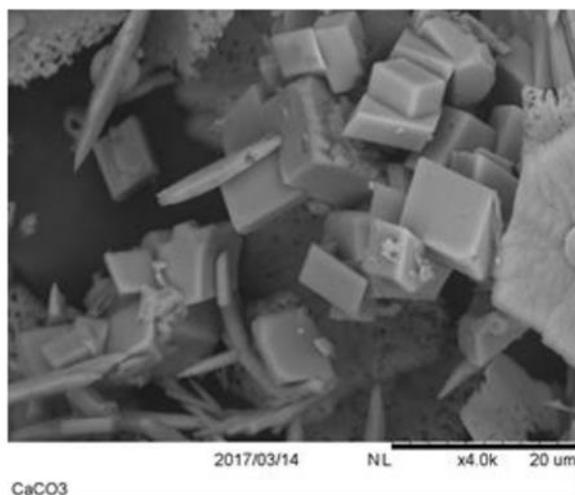


**Fig. 4.** The FTIR spectrum analysis of calcium carbonate

SEM analysis of  $\text{CaCO}_3$  precipitate obtained in this study is illustrated in the Figure 6. The image reveals that the surface morphology of  $\text{CaCO}_3$  precipitate obtained in this study was in a rectangular shape and smooth surface. Li *et al.*, has also reported a similar structure as obtained through this study [14]. The research by López-Periago *et al.*, indicated that the morphology of the calcite is a rhombohedral crystal structure with  $\{1\ 0\ 4\}$  face for a stable surface [15]. Moreover, it has a smooth surface and consist a regular crystal size.



**Fig. 5.** The XRD spectrum for  $\text{CaCO}_3$



**Fig. 6.** Surface morphology of CaCO<sub>3</sub> precipitate

#### 4. Conclusion

The study indicated that hydration and precipitation process can be accelerated using the *Carbonic anhydrase* enzyme. The mass of precipitate CaCO<sub>3</sub> and time taken for the solution to reach pH 7 was depended on the CO<sub>2</sub> flowrates and concentration of calcium chloride solution. Based on the results, higher CO<sub>2</sub> flowrate produced higher mass of precipitate CaCO<sub>3</sub> and shorter time was taken for the solution to reach pH 7.0. The study indicated that 800 L/min of CO<sub>2</sub> results in only 4.30 minute to precipitate about 0.33 g of CaCO<sub>3</sub>. For the effect of the CaCl concentration, the optimum CaCl concentration was obtained at 36g/L. At this concentration, time taken to reach pH 7.0 was 4.3 min with 0.345g of precipitate calcium carbonate precipitate was recorded. This study indicated that higher concentration of CaCl was not significantly affect time taken for the solution to reach pH 7.0 and mass of the precipitate obtained.

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