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## Reducing the Permeability of Sandstone Porous Media to Water and CO<sub>2</sub>: Application of Bovine Carbonic Anhydrase Enzyme

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### Abstract

This paper presents the results of an investigation on the application of the enzyme bovine carbonic anhydrase for reducing the permeability of a sandstone porous medium during CO<sub>2</sub> flooding. This process provides a method for improving conformance of injected water and CO<sub>2</sub> in an absolutely environmentally friendly manner. Carbonic anhydrase enzyme accelerates the hydration reaction of CO<sub>2</sub> with water. Hence, in presence of carbon dioxide and divalent ions, such as calcium, this enzyme leads to rapid precipitation of calcium carbonate. The precipitation reaction causes reduction in the permeability of the flooded regions of the reservoir. Therefore, upon subsequent injection of CO<sub>2</sub> and water, injected fluids would flow through the unswept parts of reservoir, improving the conformance of the injected CO<sub>2</sub> and water.

Experiments were carried out to investigate the effect of various enzyme concentrations, temperature and pH on the precipitation reaction. Enzyme concentrations at 2, 4 and 8 micro mole per liter were tested. Also, effect of temperature was studied by performing experiments at room temperature, 30 °C and 55 °C. A mathematical model was developed to predict the extent of precipitation of calcium carbonate. Subsequently, coreflood experiments, using Berea sandstone cores, were conducted and the degree of reduction in the permeability of the cores was measured. Also, in order to investigate the effect of injection scheme on permeability reduction, two different methods of injection were tested. For all sets of flow experiments, the permeability of the core was reduced to less than half of its initial value.

### Introduction

Carbon dioxide miscible flooding is one of the most promising enhanced oil recovery (EOR) methods for light or medium oil reservoirs[1]. Recently, the interest in this method has been boosted because of the higher oil prices. If the reservoir pressure is at or beyond the minimum miscibility pressure (MMP) of the injected stream of CO<sub>2</sub>, multiple contact miscibility will be achieved in the reservoir. The ability to achieve dynamic miscibility at attainable pressures in a wide range of reservoirs is a major advantage of the CO<sub>2</sub> miscible process. This makes CO<sub>2</sub> an ideal displacement fluid for many crude oils. However, even when pressure conditions for miscibility are met, this high microscopic sweep efficiency is not often approached in reservoir operations, due principally to the non-uniformity of the flow patterns and unfavorable mobility ratio between injected CO<sub>2</sub> and oil. Large-scale reservoir heterogeneities, such as fractures or high-permeability streaks can intensify viscous fingering of CO<sub>2</sub> and cause early breakthrough of injected carbon dioxide, which will reduce oil recovery efficiency [2].

In order to improve the sweep efficiency of CO<sub>2</sub> flooding projects, various methods have been proposed and tested via laboratory and field tests. In some of these studies, attempts have been made to achieve a more favorable CO<sub>2</sub> mobility by changing the CO<sub>2</sub> relative permeability. WAG (Water Alternating Gas) process is an example of these methods. In this process, CO<sub>2</sub> mobility is reduced by reducing the relative permeability to CO<sub>2</sub> via increased water saturation [3].

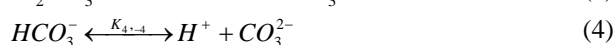
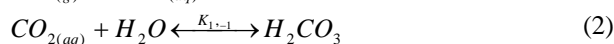
Other methods, such as CO<sub>2</sub>-foam and viscosified carbon dioxide process aim at increasing the viscosity of the injected gas phase, e.g. carbon dioxide, by using a surfactants or chemical thickening agents [4, 5].

A more common alternative to improve the problem of poor sweep efficiency of CO<sub>2</sub> in oil reservoirs is by blocking the high permeability streaks, and/or fractures, in reservoirs. This can be achieved through in-depth placement of polymer gels. In in-depth gel placement technique, a gelling solution is injected into the reservoir, where the gelation takes place and the liquid solution changes form to a gel which blocks the high permeability regions of reservoir. In-depth gel placement has been used for water shut-off, as well as improving CO<sub>2</sub> conformance, purposes extensively.

The process presented in this paper is based on enhancing the chemical reaction between injected carbon dioxide and calcium ions present in reservoir in order to precipitate

calcium carbonate in the reservoir and along the path of injected CO<sub>2</sub>. Once precipitation occurs, the permeability of porous media in that region will be reduced and subsequent CO<sub>2</sub> injected will be diverted towards unswept parts of reservoir. The approach tested for enhancing the reaction between CO<sub>2</sub> and calcium ions is through addition of carbonic bovine anhydrase enzyme[6].

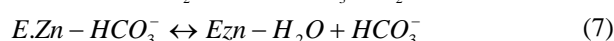
The natural precipitation of CO<sub>2</sub> in the form of minerals takes place very slowly over geological times, as presented in the following steps:



Among these reactions, reaction 2 is the slowest one with the rate constants of  $K_1 = 6.2 \times 10^{-2} s^{-1}$  and  $K_{-1} = 23.7 s^{-1}$  [7, 8].

To promote the rate of reaction 2, an enzyme could be used. The suitable enzyme for this reaction is enzyme carbonic anhydrase. This enzyme acts as a catalyst and increases the rate of this reaction by decreasing its activation energy.

Using enzyme carbonic anhydrase changes the mechanism of hydration of CO<sub>2</sub>. The new mechanism is as follows:



In this mechanism, the hydration reaction (reaction 2) will be eliminated and the problem of slow rate of CO<sub>2</sub> hydration will be resolved.

In the present work the feasibility of using this enzyme as a catalyst for hydration of CO<sub>2</sub> during carbon dioxide injection in porous media, as well as its precipitation in the form of calcium carbonate was studied through both batch and flow experiments. The effect of enzyme concentration, temperature and buffer on the hydration of CO<sub>2</sub> and formation of calcium carbonate were investigated in batch experiments. While, flow experiments were conducted in a core samples to study the possibility of applying this biomimetic precipitation in porous media.

## Materials and Methods

### Enzymatic Hydration of CO<sub>2</sub>

Enzyme bovine carbonic anhydrase was purchased from Sigma-Aldrich Company, and was used as received. As carbonic anhydrase catalyzes the hydration reaction of CO<sub>2</sub>, it transfers hydrogen ions between the active sites of the enzyme and the surrounding buffer. This results in a change in pH. Therefore, measuring pH through delta pH method is a good way of monitoring the progress of this enzymatic reaction [9]. For measuring pH, the Beetrode electrodes, World Precision Instruments Inc. (WPI), were used. These electrodes must be used in combination with a reference electrode. The reference electrode used in these experiments was the Dri-Ref electrode, which was provided with WPI.

The effect of the enzyme on the hydration of carbon dioxide was studied using a reaction mixture containing: 15 ml

of phosphate buffer (Potassium dehydrate phosphate/ Sodium hydroxide buffer with a pH of 6.86, Fisher Scientific) and 5 ml of the enzyme solution at the concentrations 0.2, 0.4, 0.8, 2 and 6 μM. Each reaction mixture containing a particular concentration of the enzyme was transferred to a beaker and its temperature was maintained at 0°C. The mixture was stirred with a magnetic stirrer.

CO<sub>2</sub> solution was prepared by bubbling deionized water with gaseous CO<sub>2</sub> and then at the start of the experiments, 20 ml of this solution was introduced to the mixture of the enzyme and buffer. Progress of the reaction was monitored by measuring pH of the mixture. The experiment was stopped when there was no further change in pH.

A *control reaction mixture* was prepared by replacing the enzyme solution with 5 ml of deionized water. This was to compare the reaction progress with and without the enzyme. Figure 1 demonstrates the pH change at 0°C. Addition of CO<sub>2</sub> to the reaction mixture at time zero tended to drop pH of the mixture to the low values, but presence of the buffer balanced this effect and pH leveled off at a value a little lower than pH of the buffered solution.

Since the initial rate of pH drop (-r<sub>A</sub>) is equal to the slope of the curves at the first few seconds of the reaction, to compare the rate of pH drop at different concentrations, the slope of the curves in Figure 1 are calculated for the early part of the reaction and -1/slope, which is equivalent to -1/(-r<sub>A</sub>) for each enzyme concentration is measured and shown in Figure 2. It is clear that rate of drop in pH depends on the concentration of the enzyme. At higher enzyme concentrations, pH drops faster. Also, the results show that in the absence of the enzyme (control run) the rate of decrease of pH is much slower compared to the rate of pH-drop in the presence of the enzyme. Even when concentration of the enzyme is very low, for example 0.2 μM, pH-drop is much faster than the control run. These results indicate that enzyme carbonic anhydrase increases the rate of hydration of CO<sub>2</sub> even at low concentrations.

Next, the effect of temperature on the enzymatic hydration of CO<sub>2</sub> was studied by changing the temperature of the reaction mixture from 0°C to 30°C. The results of the experiments at 30°C are shown in Figure 3. The same trend in the change of pH can be seen in this figure. But this time the rate of change in pH was faster at 30 °C than at 0°C. Also, the rates at concentrations 0.4 and 0.8 μM were closer (Figure 4). The comparison of rates at different concentrations at 30°C is illustrated in Figure 4. These results imply that at high temperatures the concentration of the enzyme is not as significant as it was in low temperatures. This is beneficial because in warm environments a low concentration of the enzyme will result in a higher rate of hydration and subsequent precipitation.

To observe the effect of buffer on the reaction, all the above experiments were repeated in the absence of phosphate buffer. In these experiments, 15 ml of phosphate buffer was replaced by 15 ml of deionized water in the reaction mixture. Figures 5 and 6 illustrate these results. Since there was no buffer present in these experiments, there was no control on pH and pH decreased to low values around 4. The pH values

were a little lower at 30°C, which is because of the effect of temperature on pH of the solution.

### Enzymatic precipitation of calcium carbonate

In order to study the effect of various parameters on the precipitation reaction several sets of experiments were conducted as presented in Table 1.

The effect of bovine enzyme on the precipitation of carbon dioxide in form of calcium carbonate ( $\text{CaCO}_3$ ) was studied using a reaction mixture containing: 15 ml of the enzyme solution with concentrations of 3 and 6  $\mu\text{M}$ , 0.9 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  and 2.52 g of Tris buffer (Hydroxymethyl-Aminomethane provided by EM Science) in 15 ml of deionized water. To start the reaction, 60 ml of  $\text{CO}_2$  solution was added to this mixture. The reaction mixture was maintained at 0°C.

In the first set of experiments, after adding  $\text{CO}_2$ , the mixture was left for 2 hours and then it was filtered and dried to measure the weight of  $\text{CaCO}_3$  that was precipitated. Additionally, the effect of temperature on the enzymatic formation of  $\text{CaCO}_3$  was studied by changing the temperature from 0°C to 30°C and 50°C.

To compare the rate of enzymatic formation of  $\text{CaCO}_3$  with the non-enzymatic reaction, three samples of the original reaction mixture with the enzyme concentration of 6  $\mu\text{M}$  were prepared (samples 1, 2 and 3). Also, three other samples (4, 5 and 6) were prepared by replacing 15 ml of the enzyme with 15 ml of deionized water (non-enzymatic precipitation). For each series of samples, the experiment was initiated by adding  $\text{CO}_2$  solution to the first sample of that series. After 5 minutes the sample was filtered. Then,  $\text{CO}_2$  was added to the second sample. After 10 minutes the second sample was filtered. The third sample was filtered after 15 minutes. This gave the change in the amount of  $\text{CaCO}_3$  in 5-minute intervals.

The results of the precipitation experiments are tabulated in Table 2. All these results showed that the enzymatic precipitation of  $\text{CaCO}_3$  was not dependent on the concentration of bovine enzyme. With bovine enzyme at concentrations of 3 and 6  $\mu\text{M}$ , the weight of  $\text{CaCO}_3$  was respectively 0.2098 and 0.2106 g at 0°C and 0.1280 and 0.1283 g at 30°C. These numbers are very close and the difference is negligible.

The amount of  $\text{CaCO}_3$  decreased as the temperature increased. The weight of  $\text{CaCO}_3$  at 0°C, 30°C and 50°C was 0.2098, 0.1283 and 0.096 g respectively. This is because the solubility of carbon dioxide in water decreases with temperature [10].

In the experiments that the Tris buffer was eliminated from the reaction mixture, there was no precipitation at all. This result did not depend on the temperature or concentration of the enzyme. When the buffer is absent from the reaction mixture, addition of  $\text{CO}_2$  to the mixture drives the pH down to low values (around 4). The chemistry of  $\text{CO}_2$  hydration and bicarbonate dissociation shows that in low pH, there is not enough carbonate ion present [11]. As a result, the solution does not get saturated with  $\text{CaCO}_3$ . This is the reason that precipitation is not observed under these conditions.

In the absence of the enzyme, the precipitation was observed and after 2 hours the amount of  $\text{CaCO}_3$  was almost

the same as its amount at the presence of the enzyme (Table 2). Figure 7 indicates that precipitation of calcium carbonate was faster in the presence of bovine enzyme. This figure exhibits a comparison between the precipitation in the presence and absence of the enzyme. It is clearly seen that in the presence of the enzyme,  $\text{CaCO}_3$  reached to its maximum value in less than 10 minutes; however when no enzyme was added to the reaction mixture the formation of calcium carbonate took place very slowly.

Turbidity experiments were also conducted to observe the rate of formation and settlement of  $\text{CaCO}_3$  at different conditions. To observe the effect of the enzyme on the precipitation, 6 ml of the original reaction mixture with enzyme concentration of 6  $\mu\text{M}$  was transferred to a test cell, and then 13.5 ml of  $\text{CO}_2$  was injected into the cell. The change in the turbidity of the sample was monitored using the Orbeco-Hellige digital turbidimeter (Model 965-10A).

To investigate the precipitation in the absence of the enzyme, the enzyme was replaced by deionized water. In another experiment, Tris buffer was eliminated from the reaction mixture.

The results from the precipitation experiments were in a very good agreement with the turbidity experiments. The turbidity measurements showed that in the presence of the enzyme, the precipitation started momentarily after injection of  $\text{CO}_2$  and jumped to its highest extent, which is in agreement with results shown in Figure 7. Also, the turbidity measurements showed that  $\text{CaCO}_3$  settled down much faster at the presence of enzyme as presented in Figure 8. This figure shows that although  $\text{CaCO}_3$  was formed in the absence of the enzyme too, the rate of formation was slow and it settled down slowly. Also, this figure shows that precipitation of  $\text{CaCO}_3$  did not take place when the buffer was not added to the mixture.

### Flow Experiments

The flow experiments were carried out in two Berea sandstone cores. These cores were 10 cm (4 inches) long and 2.5 cm (one inch) in diameter. Various fluids were injected through an ISCO 500 D syringe pump and a Fisher infusion syringe pump. Validyne pressure transducers and data gathering system were used for monitoring the pressure drop across the cores at various stages of flow experiment.

The goal of the flow experiments was to study the change in the permeability of a porous medium as a result of the enzymatic precipitation of  $\text{CO}_2$ . The initial permeability of the cores was measured by injecting 1% brine into and measuring the pressure drop across the cores.

Next, in order to investigate the onset and extent of precipitation of  $\text{CaCO}_3$  in porous media during the flow experiments, the following enzyme solution was prepared: 0.5 grams of calcium chloride and 1.4 grams of Tris buffer in 50 ml of 6- $\mu\text{M}$  enzyme solution.

Two flow experiments were designed and conducted. In the first experiment,  $\text{CO}_2$  solution and the mixture of the enzyme, buffer and calcium chloride were injected into the core alternately. But, in the second experiment, the  $\text{CO}_2$  solution and the enzyme solution were injected simultaneously in the second core.

After each injection, the cores were left for one hour. Then, for each core, 1% brine was injected and the pressure-drop

across the core was measured. The post-precipitation permeability to brine was measured and compared with the initial permeability to brine.

For the first flow experiment, a brine solution was saturated with CO<sub>2</sub> by bubbling CO<sub>2</sub> in brine solution for few hours. Then a 50 mL syringe was filled with CO<sub>2</sub> saturated solution. A second 50 mL syringe was filled with the enzyme solution. The solutions contained in these two syringes were injected into the first core alternately in 4 mL volumes. Then, the inlet and outlet of the core were shut for one hour before resuming brine injection. Figures 9 and 10 demonstrate the pressure-drop for brine injection observed for this experiment before and after the alternate injection of the chemicals. Comparison of these two figures shows that the pressure-drop during the brine injection after the injection of chemicals is almost twice the initial pressure-drop. This indicates that after injection of CO<sub>2</sub> saturated solution and the mixture of enzyme solution, Tris buffer and calcium chloride, these chemicals have come into contact with each other and a precipitation reaction has occurred. As the result of the reaction, some of the calcium has precipitated in the form of calcium carbonate. The precipitate has filled some of the pores of the porous medium and thus has reduced the permeability of the core. The calculations show that the alternate injection of CO<sub>2</sub> and the enzyme mixture reduced the permeability of the core from 8.24 md to 4.38 md.

In the second experiment, the two 50 mL syringes were filled with solutions similar to the ones used for the first experiment. However, in this experiment the two solutions were injected in the core simultaneously, rather than alternately. Figures 11 and 12 show the pressure-drop during the brine injection before and after the simultaneous injection of the chemicals into the second core. These figures clearly show that the pressure-drop across the core is much higher after the injection of the chemicals. The pressure-drop is raised by a factor of 4, from about 11 psi to almost 44 psi. In correspondence to this rise in the pressure-drop, permeability has decreased from the initial value of 9.81 md to the final value of 2.49 md.

Comparing the results for the alternate and simultaneous injection schemes indicates that the permeability reduction in the simultaneous injection is much higher. This means that co-injection of two solutions is more effective in terms of precipitating more calcium carbonate and reducing permeability of the porous medium.

## Conclusions

The following conclusions have been made based on the experiments conducted throughout this study:

1. The effect of enzyme concentration, temperature and buffer on the hydration reaction and precipitation were studied. The results show that this enzyme is a very effective catalyst. It promoted the hydration of CO<sub>2</sub> and consequently the precipitation of CaCO<sub>3</sub>.
2. The rate of hydration reaction increased with temperature at constant concentration. At a constant temperature, the higher rate was obtained with higher enzyme concentrations. But the concentration effect faded at higher temperatures.

3. Enzyme carbonic anhydrase not only enhanced the hydration reaction of carbon dioxide, but it also promoted the formation of CaCO<sub>3</sub>. The rate of precipitation of carbon dioxide was higher in the presence of the enzyme.
4. The results of the flow experiments suggest that enzymatic precipitation of carbon dioxide can cause reduction in the permeability of the porous medium.
5. The change in the permeability of the porous media is affected by the method of injection of the enzyme and CO<sub>2</sub> in the porous medium. The simultaneous injection of the enzyme and carbon dioxide results in a higher reduction of permeability.

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Set	Temp.	Enzyme Concentration	Buffer
1	0°C	3 μM	Yes
2	0°C	6 μM	Yes
3	0°C	6 μM	No
4	0°C	No Enzyme	Yes
4	30°C	3μM	Yes
5	30°C	6μM	Yes
6	50°C	6μM	Yes
7	50°C	No Enzyme	Yes
8	50°C	6μM	No

Table 1: Summary of precipitation experiments

Set	Weight of Precipitate (grams)	Number of Runs
1	0.2098	4
2	0.2106	4
3	0.19	
4	0	2
5	0.1280	4
6	0.1283	4
7	0.096	3
8	0.0941	2
9	0	2

Table 2: Results of precipitation experiments

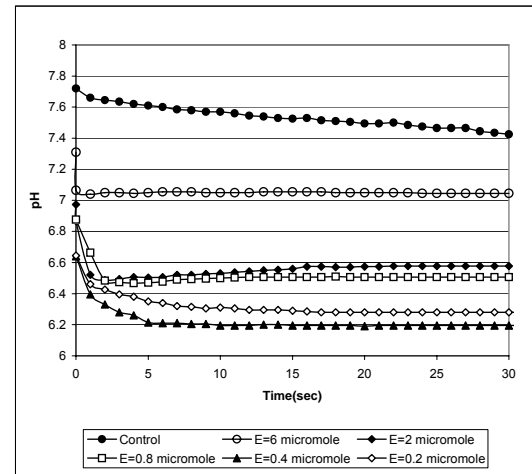


Figure 1: Results of pH experiment at T = 0°C and in the presence of buffer

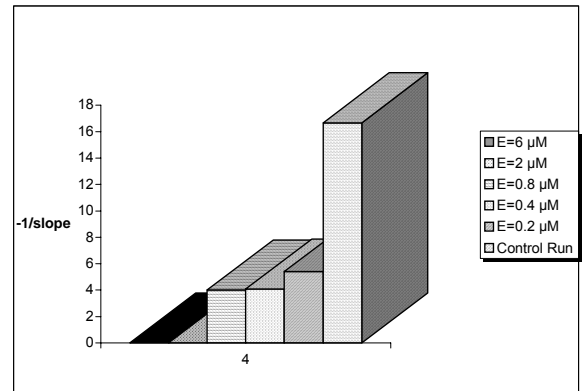


Figure 2: Comparison of the rate of pH drop for different enzyme concentrations at T = 0°C

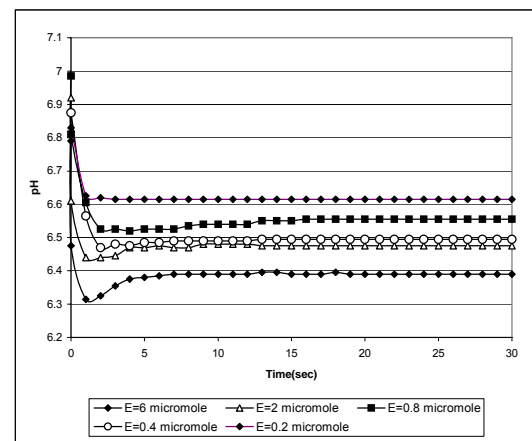


Figure 3: Results of pH experiment at T = 30°C and in the presence of buffer

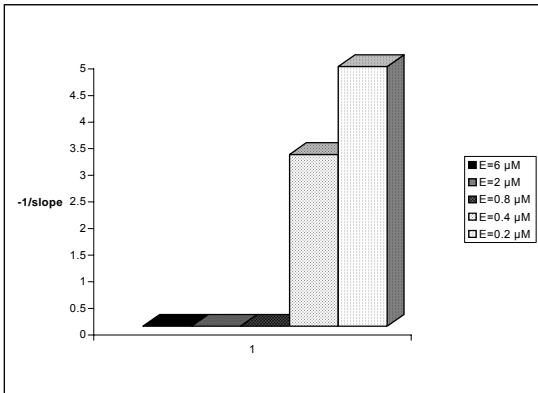


Figure 4: Comparison of the rate of pH-drop for different enzyme concentrations at 30°C

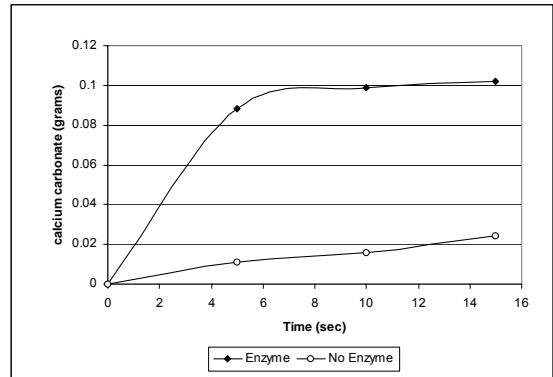


Figure 7: Comparison of precipitation with and without enzyme

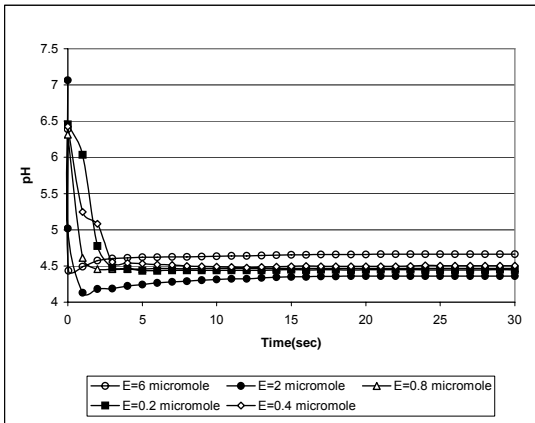


Figure 5: Results of pH experiment at T = 0°C, No buffer

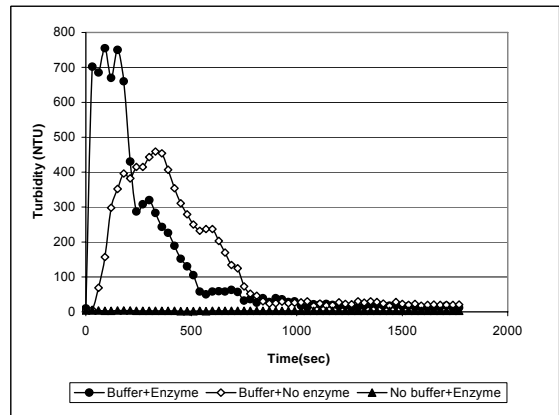


Figure 8: Results of the turbidity experiment

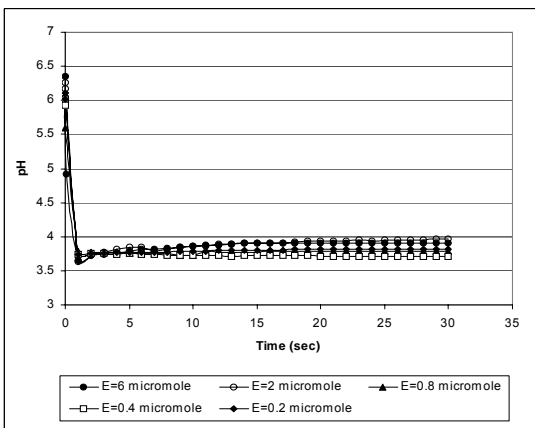


Figure 6: Results of pH experiment at T = 30°C, No buffer

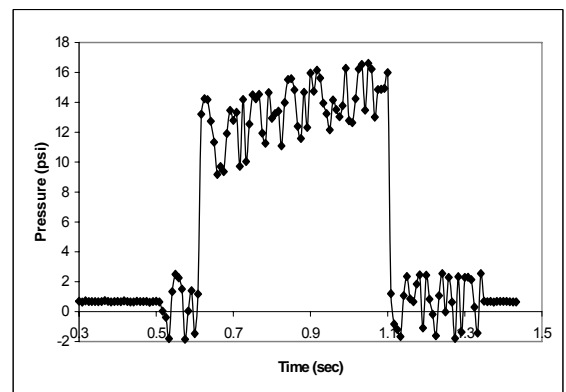


Figure 9: Pressure drop during brine injection for measuring the initial brine permeability for the first flow experiment

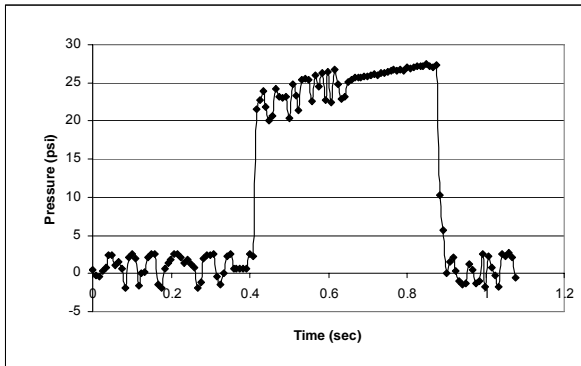


Figure 10: Pressure drop during brine injection for the first experiment, after alternate injecting of  $\text{CO}_2$  and enzyme solutions

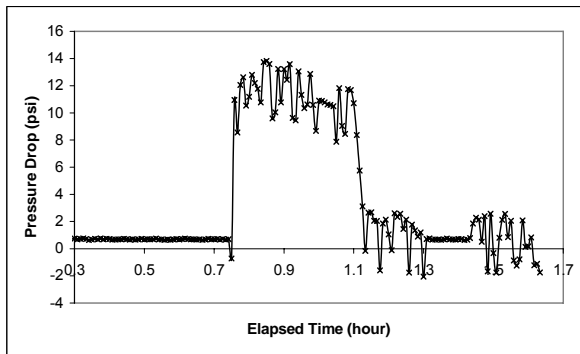


Figure 11: Pressure drop during brine injection for musearing the initial permeability to brine for the the second flow experiment

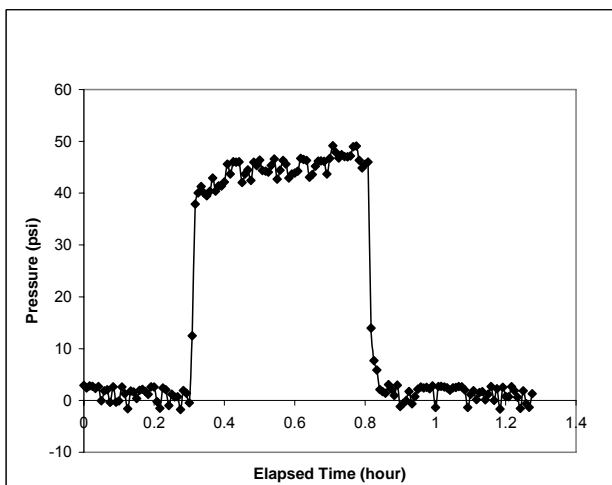


Figure 12: Pressurer drop during brinn einjection for the second flow experiment, after co-injection of  $\text{CO}_2$  and enzyme solutions