Dispersion of bacterial cells during microbially induced calcium carbonate precipitation in fracture sealing

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Abstract. Fluid leakage through fracture networks is one of the primary environmental issues in water reservoir management, oil extraction, and geological carbon sequestration. Microbially induced calcium carbonate precipitation (MICP) offers a promising alternative to seal fractures and improve the performance of those relevant engineering over their service life. The dispersion of bacterial cells plays an important role in the growth and distribution of CaCO$_3$ during the MICP process. The effect of calcium ion concentration, and flow rate on the diffusion behaviors of bacterial cells are studied by directly observing the mixing of two streams of solution, i.e., bacterial solution and cementation solution, in a microfluidic chip. Micromodel experiments show that the high concentration of calcium ion results in a significant retarding effect in the diffusion of bacterial cells given the chemotaxis. The transverse diffusion of motile bacteria might be suppressed by high-rate fluid flow or promoted by low-rate flow. Our work illustrates the significant impacts of solute concentrations and fluid flow rate on the dispersion of bacterial cells and deepens the understanding of the delivery of bacterial suspensions in fracture sealing with MICP.

1. Introduction
Leakage through fractured rocks is one of the primary environmental issues for the subsurface reservoir systems [1-3]. Fracture grouting can seal the leakage passages and increase the strength of the rock mass, hence is widely used in the leakage prevention of underground engineering. Traditional cement-based grouting materials are typically highly viscous [4], while chemical-based grouting might be harmful to the environment [5, 6]. Microbially induced calcium carbonate precipitation (MICP) offers a promising alternative with its low-viscosity grout and long-term environmental compatibility [7-9]. Minto et al [9] examined the distribution of calcium carbonate (CaCO$_3$) in an artificial fracture and investigated the influence of injection strategies on the sealing efficiency. Phillips et al [8] performed a field MICP injection test with conventional oilfield fluid delivery technologies. The significant decrease in permeability indicated the ability in leakage prevention of MICP sealing technology.

Although the feasibility of MICP in fracture grouting has been proved through laboratory and field studies, the practical application of the MICP sealing technology is still a challenge due to the limited understanding of precipitation kinetics and bacterial hydrodynamics [7, 10, 11]. Several previous studies have investigated the precipitation kinetics through macroscale and microscale tests [12-15]. However, few studies have been carried out to analyze the hydrodynamics of bacterial cells in the MICP process, which plays an important role in the transport of bacteria and directly affect the growth of CaCO$_3$ [16-18]. Owing to the lack of direct visualization of ureolytic bacterial diffusion, the key hydraulic parameters, e.g. bacterial diffusion, are referred to the experimental observations of model
microorganisms such as Escherichia coli [19, 20], which may cause variations of several orders of magnitude among different researches [17, 18].

This study aims to investigate the diffusion behavior of ureolytic bacteria in cementation solutions (i.e. solution of urea and calcium chloride (CaCl$_2$)) of different concentrations at different flow rates. A microfluidic chip with two arms is designed to inject bacterial suspension and cementation solution simultaneously. The bacterial cells are tracked using fluorescence staining and the concentration of the bacteria is evaluated based on the fluorescence intensity. The diffusion behavior could then be analyzed based on the distribution of the bacteria. The results will advance our understanding on the hydrodynamics of the ureolytic bacteria during MICP process.

2. Materials and Methods

2.1. Microchip

The microfluidic chip consists of a PDMS (polydimethylsiloxane) microchannel and a glass slide covered by PDMS layer as shown in figure 1a. The PDMS microchannel is fabricated using a silicon master sheet. The silicon master sheet is produced with photolithography techniques. The microchannel starts with a structure of “Y” shape, and the bacterial suspension and cementation solution are injected into a main channel through the two arms with a syringe pump with two tubes. The main channel is 943 μm wide and 2.68 cm long. Its depths could be either 17 μm or 120 μm in different tests as shown in figure 1b.

2.2. Bacterial Suspension and Cementation Solution

A widely-used ureolytic bacteria, *Sporosarcina pasteurii*, is adopted in this study. The cultivation details including the medium composition are provided in previous studies, e.g. [21, 22]. The bacteria were harvested by centrifuging the cultured medium at 10000g for 15 mins at 4 °C twice. Pellets after centrifugation are resuspended in 0.85% NaCl solution to obtain the bacterial suspension. Fluorescence
staining procedures are performed to dye the bacterial cells using Live/Dead Baclight Bacterial Viability kit (Molecular Probes, USA). Two cementation solutions with the same urea concentration (0.5 mol/L) and different concentrations of CaCl₂, i.e. 0.05 and 0.5 mol/L, are prepared to examine the effects of calcium ion concentrations on the diffusion behaviors of ureolytic bacteria cells.

2.3. Experimental Procedures

The bacterial suspension and cementation solution are injected through the two arms using syringe pumps (Harvard Elite 11, USA) and converged in the main microchannel. Before injection, the microchannel is filled with deionized water in a vacuum. Three flow rates, i.e. 40, 10, and 5μL/h, are tested in this study. A parabolic velocity profile is expected between the bottom and the top surfaces due to the wall effect [23] as illustrated in figure 1b. Mean fluid velocity \( U \) is calculated from the flow rate. The mean shear rate \( S \) could then be calculated from the mean fluid velocity, \( S = 3U / h \). The experimental setup and corresponding mean fluid velocity \( U \) in the main channel are provided in Table 1. The labeled bacterial cells are observed under an inverted fluorescence microscope with 425 nm excitation light. Fluorescence images are captured with a CMOS camera (Olympus DP74, Japan) through a 10× objective after 10 min of injection. To alleviate the effects of fluorescence quenching, the excitation light is cut off immediately after the image is captured.

| No.   | Concentration of CaCl₂ (mol/L) | Flow rate (μL/L) | Mean fluid velocity (m/s) | Mean shear rate (s⁻¹) |
|-------|--------------------------------|-----------------|---------------------------|------------------------|
| T-H-17-40 | 0.5                            | 40              | \( 6.9 \times 10^{-4} \) | \( 122 \)               |
| T-L-17-40 | 0.05                           | 40              | \( 6.9 \times 10^{-4} \) | \( 122 \)               |
| T-H-17-10 | 0.5                            | 10              | \( 1.7 \times 10^{-4} \) | \( 30 \)                |
| T-L-17-10 | 0.05                           | 10              | \( 1.7 \times 10^{-4} \) | \( 30 \)                |
| T-H-17-5  | 0.5                            | 5               | \( 8.7 \times 10^{-5} \) | \( 15 \)                |
| T-L-17-5  | 0.05                           | 5               | \( 8.7 \times 10^{-5} \) | \( 15 \)                |
| T-H-120-40| 0.5                            | 40              | \( 9.8 \times 10^{-5} \) | \( 2.5 \)               |
| T-L-120-40| 0.05                           | 40              | \( 9.8 \times 10^{-5} \) | \( 2.5 \)               |
| T-H-120-10| 0.5                            | 10              | \( 2.5 \times 10^{-5} \) | \( 0.6 \)               |
| T-L-120-10| 0.05                           | 10              | \( 2.5 \times 10^{-5} \) | \( 0.6 \)               |
| T-H-120-5 | 0.5                            | 5               | \( 1.2 \times 10^{-5} \) | \( 0.3 \)               |
| T-L-120-5 | 0.05                           | 5               | \( 1.2 \times 10^{-5} \) | \( 0.3 \)               |

3. Test Results and Discussion

Bacteria could disperse in fluid environments due to fluid flow and bacteria swimming [24]. The bacterial motility varies among different species and even different individuals [25]. The motile behavior of *Sporosarcina pasteurii* will first be investigated under steady condition herein. The bacterial suspension and cementation solution are injected into the main channel and then maintained still for 10 mins allowing bacteria dispersion under static condition. Figure 2 shows the distributions of bacteria in different cases with different channel depths and CaCl₂ concentrations. The results show that the bacteria present significant dispersion in the case with 120 μm depth and low concentration of CaCl₂ (0.05 mol/L), while the dispersion is not obvious in other cases with lower depth or higher concentration (0.5 mol/L). This phenomenon indicates that the presence of high concentration of CaCl₂ has retarding effect on the
Figure 2. Distributions of bacteria in the static condition with different concentrations and channel depths. a, \( h = 17 \mu m, 0.05 \text{ mol/L CaCl}_2 \); b, \( h = 17 \mu m, 0.5 \text{ mol/L CaCl}_2 \); c, \( h = 120 \mu m, 0.05 \text{ mol/L CaCl}_2 \); d, \( h = 120 \mu m, 0.5 \text{ mol/L CaCl}_2 \).

random walk of bacterial cells, which might be caused by the calcium-induced flocculation or the increase in fluid viscosity due to the formation of amorphous calcium carbonate [26, 27]. As for the cases with low depth, the restraint behavior might result from the confined space as well as the adsorption of PDMS plates [28].

Fluid flow may change the dispersion behavior of bacterial cells [29]. Figures 3 and 4 show the distributions of bacteria under flow conditions with different concentrations of CaCl₂ and different depths. For the cases with depths of 17 μm, the distributions of bacteria at different subsections exhibit few differences from the static cases, showing no obvious dispersion, regardless of the concentrations of CaCl₂ or the locations of the subareas (see Figure 3). While for the cases with depths of 120 μm, different bacteria dispersion behaviors could be clearly noted (see figure 4). The dispersion in the case with a flow rate of 5 μL/h is even more obvious than that in the static state. Nevertheless, the dispersion is less obvious in the case of a flow rate of 10 μL/h and hardly notable in the case of a flow rate of 40 μL/h. Due to the velocity gradients, the fluid in the main channel is in a shearing state, which may promote or inhibit the dispersion of the rod-like shaped *Sporosarcina pasteurii* depending on the mean shear rate [25]. A proper mean shear rate, which is related to the intrinsic rotary diffusivity, may enhance the tumbling of the bacterial cells and increase the probability to disperse in the transverse direction. Meanwhile, a much larger mean shear rate may promote the cell alignment with the flow direction and hamper the stochastic swimming of the cells.
Figure 3. Distributions of bacteria in the flow condition with different concentrations and flow rates in the microchannel of 17 μm depth. a, 40 μL/h and 0.5 mol/L CaCl₂; b, 40 μL/h and 0.05 mol/L CaCl₂; c, 5 μL/h and 0.5 mol/L CaCl₂; d, 5 μL/h and 0.05 mol/L CaCl₂ (the images from top to bottom are the subareas located at 0, 1.34, 2.68 cm of the main channel as shown in Figure 1).

Figure 4. Distributions of bacteria in the flow condition with different flow rates and channel depths for the 0.05 mol/L solutions. a, h =17 μm; b, h =120 μm.

4. Conclusions
This study investigates the diffusion behaviors of *Sporosarcina pasteurii* in static and flow conditions using the microfluidic chips. Results show that the dispersion capability of the bacteria varies with flow rate, concentration of CaCl₂ and depth of the main channel. In static conditions, a deeper main channel and a lower concentration of CaCl₂ solution will promote the transvers diffusion of the bacterial cells. In flow conditions, low-rate fluid shear may promote the transverse transport of motile bacteria, while higher-rate shear may hamper the transverse diffusion.
References

[1] Akbarabadi M and Piri M 2013 Adv Water Resour 52 190-206
[2] Chen Y-F, Hu S-H, Hu R and Zhou C-B 2015. Water Resour Res 51 2096-118
[3] Jackson R B, Vengosh A and Carey J W, et, al. Annu. Rev. Fluid Mech.(vol 39) ed. by A. Gadgil and D. M. Liverman pp. 327-62
[4] El Tani M 2012 Rock Mech Rock Eng 45 547-61
[5] Sui W H, Liu J Y, Hu W, Qi J F and Zhan K Y 2015 Tunn. Undergr. Space Technol. 50 239-49
[6] Klepikova M V, Roques C, Loew S and Selker J 2018 Water Resour Res 54 1410-19
[7] Cuthbert M O, Mcmillan L A, Handley-Sidhu S, Riley M S, Tobler D J and Phoenix V R 2013 Environ Sci Technol 47 13637-43
[8] Minto J M, Maclachlan E, El Mountassir G and Lunn R J 2016 Water Resour Res 52 8810-27
[9] Phillips A J, Cunningham A B, Gerlach R, Hiebert R, Hwang C, Lomans B P, Westrich J, Mantilla C, Kirksey J, Esposito R and Spangler L 2016 Environ Sci Technol 50 4111-17
[10] El Mountassir G, Lunn R J, Moir H and Maclachlan E 2014 Water Resour Res 50 1-16
[11] Minto J M, Lunn R J and El Mountassir G 2019 Water Resour Res 55 7229-45
[12] Stocks-Fischer S, Galinat J K and Bang S S 1999 Soil Biol. Biochem. 31 1563-71
[13] Ferris F G, Phoenix V, Fujita Y and Smith R W 2004 Geochim. Cosmochim. Acta 68 1701-10
[14] Wang Y Z, Soga K, Dejong J T and Kabla A 2019 Geotechnique 69 1086-94
[15] Xiao Y, He X, Wu W, Stuedlein A W, Evans T M, Chu J, Liu H, Van Paassen L A and Wu H 2021 Acta Geotech
[16] Ebibgo A, Phillips A, Gerlach R, Helmig R, Cunningham A B, Class H and Spangler L H 2012 Water Resour Res 48 17
[17] Hommel J, Lauchnor E, Phillips A, Gerlach R, Cunningham A B, Helmig R, Ebibgo A and Class H 2015 Water Resour Res 51 3695-3715
[18] Hommel J, Lauchnor E, Gerlach R, Cunningham A B, Ebibgo A, Helmig R and Class H 2016 Transport Porous Med 114 557-79
[19] Licata N A, Mohari B, Fuqua C and Setayeshgar S 2016 Biophys. J. 110 247-57
[20] Rossy T, Nadell C D and Persat A 2019 Nat Commun 10 9
[21] Liu L, Liu H, Stuedlein A W, Evans T M and Xiao Y 2019 Can Geotech J 56 1502-13
[22] Xiao Y, Zhao C, Sun Y, Wang S, Wu H, Chen H and Liu H 2020 Acta Geotech 16 1391-400
[23] Di Carlo D 2009 Lab Chip 9 3038-46
[24] Lauga E. Annu. Rev. Fluid Mech.(vol 48) ed S. H. Davis and P. Moin pp 105-130
[25] Rusconi R, Guasto J S and Stocker R 2014 Nat. Phys 10 212-17
[26] Li S, He X, Xiao Y and Xu Y 2018 22nd Int. Conf. on Miniaturized Systems for Chemistry and Life Sciences 914-17
[27] Wang Y Z, Soga K, Dejong J T and Kabla A J 2019 J Geotech Geoenviron 145 04021036
[28] Kou S Z, Cheng D H, Sun F and Hsing I M 2016 Lab Chip 16 432-46
[29] Dehkharghani A, Waisbord N, Dunkel J and Guasto J S 2019 P Natl Acad Sci USA 116 11119-24

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