Plugging High-Permeability Zones of Oil Reservoirs by Microbially Mediated Calcium Carbonate Precipitation

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ABSTRACT: Simple plugging of the high-permeability “thief zones” of oil reservoirs is the most plausible and also the most straightforwardly achievable approach to enhance sweep efficiency and oil recovery. Sporosarcina pasteurii is a representative microorganism with the ability to precipitate calcium carbonate (CaCO₃) via enzymatic hydrolysis of urea in the presence of calcium ions. Microbially induced calcium carbonate precipitation (MICP) can cement and seal the granular and fractured media and thus can be used as a potential microbial plugging agent for the high-permeability zones of oil reservoirs. The following investigated the microscopic characteristics of MICP plugging and its efficacy in permeability reduction. The columns of near-spherical silica sand and angular silica sand with three separate granularities (40/60, 60/80, and 80/120 mesh) were used as artificial rock cores representing distinct pore sizes and pore characteristics to investigate the efficacy and microprocess of MICP plugging with different biotreatment periods. The results indicated that permeability is reduced significantly after only short periods of biotreatment. After eight cycles of MICP treatments, the permeability for each type of cores dropped by 54−90% of individual initial permeabilities. The measured CaCO₃ content indicated that the decreasing rate in permeability with the increasing CaCO₃ content experiences three contrasting stages, namely, slow decline, speedy decline, and plateauing. X-ray diffraction indicated that most of the generated CaCO₃ crystals occur as vaterite with only a small amount of calcite. Imaging by scanning electron microscopy further revealed the microprocess of MICP plugging. Microorganisms first concentrate on the pore wall to secrete CaCO₃, forming a thin and large uniform layer of CaCO₃. Then, some nucleation sites of CaCO₃ crystals will experience further preferential growth, resulting in large, dominant crystals that act as a plugging agent within the pore space. Compared to extracellular polymeric substances, which are currently the primary microbial plugging agent used to enhance sweep efficiency of oil reservoirs, bio-CaCO₃ appears more effective in plugging in terms of its morphology, size, and growth characteristics.

1. INTRODUCTION

Crude oil plays a predominant role in global energy systems and chemical industry.¹ The growing global demand for crude oil and recoverable reserve collapse have driven advances in enhancing oil recovery from existing oil fields around the world.² Enhanced oil recovery (EOR), also termed tertiary recovery, uses sophisticated techniques to recover oil, which is locked within reservoirs and cannot be extracted by primary and secondary recoveries.³ Based on the report of U.S. Geological Survey World Petroleum Assessment 2000,⁴ the largest 54 oil basins in the world have produced 687 billion barrels (as of the year 2000) and reported 845 billion barrels of proved reserves, giving an estimated ultimate recovery of 1,532 billion barrels, for an overall recovery efficiency of 33%.⁵ This indicates that up to 67% of the total petroleum reserves (3090 billion barrels of crude oil) will be left in these oil basins. Thus, it is necessary to develop and improve cost-effective EOR techniques to enhance recovery efficiency. The purpose of EOR is not only to restore formation pressure but more importantly to improve oil displacement and sweep efficiency in the reservoirs.⁶,⁷

Received: February 28, 2020
Accepted: May 29, 2020
Published: June 9, 2020

http://pubs.acs.org/journal/acsofre000902
ACS Omega 2020, 5, 14376−14383

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Sweep efficiency is a term used to measure the extent to which the displacing fluid is in contact with the oil-bearing parts of the reservoir and is one of the critical indicators affecting oil recovery. Unfavorable reservoir heterogeneity can negatively impact the sweep efficiency because waterflood fluids (the fluids i.e., injected into the reservoir formation to displace residual oil) might preferentially flow through high-permeability “thief zones” without sweeping through low-permeability oil-saturated zones. Therefore, simple plugging of the high-permeability “thief zones” is the most plausible and also the most straightforward, achievable approach to enhance sweep efficiency and oil recovery. The commonly used plugging agents for the high-permeability zones of the oil reservoir include chemically cross-linked polymers (e.g., hydrogel, polyacrylamide, hydrolyzed polyacrylamide, and Xanthan gum) and microbiologically mediated products (e.g., biofilm, flocks, and biopolymer). Polymers are available for use but are expensive with an additional cost of between US$8 and US$16 per incremental barrel and the potential risk of contamination to the aquifers. Microbial plugging is a biologically based technology that usually involves insoluble biopolymers and biomass generated by injected bacteria or by indigenous microorganisms to occupy and plug pore spaces of the high-permeability zones. This in turn results in permeability reduction of high-permeability zones. As a consequence, plugging of high-permeability “thief zones” diverts waterflood fluids toward the lower permeability oil-saturated zones within the reservoir. Ultimately, this causes the flow to be equally divided between high- and low-permeability formations, that is, improvement of sweep efficiency. Microbial plugging has several unique advantages, such as less-expensive setup, less environmental contamination, less dependence on crude oil prices, lower energy input requirement, and more accessible applications. In addition, some microbial metabolites can also interact with crude oil to reduce interfacial tension between oil and water and alter reservoir rock wettability.

Extracellular polymeric substances (EPSs) are the current primary microbial plugging agent for enhancing sweep efficiency. EPSs are high-molecular weight polymers composed of sugar residues secreted and usually remain attached to the outer surface of the cell. EPSs generally maintain a large surface area but of a nanoscale thickness of 200—1000 nm. When ESP plug large pores, microorganism needs to consume more nutrient and reaction time to secrete sufficient mass of EPSs within its metabolism process, thereby yielding a considerable thickness of biofilm and achieving effective plugging. This undoubtedly limits both the efficiency and cost of EPSs as the plugging agent and also drives the searches of more potential microorganisms and their mediated products as reservoir plugging agents.

Research in biomineralization has indicated that certain strains of microorganisms are able to induce calcium carbonate (CaCO₃) precipitation by enzymatic hydrolysis of urea and bioavailable calcium ions. This bioprocess is often referred to as microbially induced carbonate precipitation (MICP). *Sporosarcina Pasteurii* is a ureolytic bacillus that is a representative microorganism capable of precipitating CaCO₃. Unlike chemically synthesized CaCO₃, the CaCO₃ induced by microbial hydrolysis of urea exhibits high cohesiveness and mechanical strength and thus has the potential applications as a bioclogging agent to reduce the permeability of permeable medium. Especially in terms of the micromorphology between EPSs and microbially mediated CaCO₃, the latter individual crystal can grow to tens of microns, thus exhibiting stronger efficacy as the plugging agent.

*S. Pasteurii* can catalyze the hydrolysis of urea into ammonia (NH₃) and carbon dioxide (CO₂) via secreting urease enzyme in its metabolism, eq 1. These hydrolyzed products then diffuse into the solution around the cells and promptly hydrolyze to ammonium (NH₄⁺), eq 2, and carbonate (CO₃²⁻), eq 3. When this hydrolysis reaction occurs in the presence of dissolved calcium ions surrounding, the carbonate reacts with calcium ions to form CaCO₃. The hydroxide (OH⁻) generated from urea hydrolysis leads to a pH increase, which provides favorable conditions for CaCO₃ precipitation. As the concentration of CaCO₃ around the bacteria exceeds its saturation point, supersaturated CaCO₃ can first transform into amorphous precipitation (CaCO₃·H₂O) and then turned into vaterite or calcite with microorganisms as the nucleation sites, eq 4.

\[
\text{CO(NH}_2\text{)}_2 + \text{H}_2\text{O} \xrightarrow{\text{bacterial urease}} \text{NH}_2\text{COOH + NH}_3 \\
\rightarrow 2\text{NH}_3 + \text{CO}_2 \text{ urea hydrolysis} \\
\]

\[
2\text{NH}_3 + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + 2\text{OH}^- \text{ pH increase} \\
\]

\[
2\text{OH}^- + \text{CO}_2 \rightarrow \text{CO}_3^{2-} + \text{H}_2\text{O carbonate formation} \\
\]

\[
\text{cell} - \text{Ca}^{2+} + \text{CO}_3^{2-} \\
\rightarrow \text{Ca}^{2+} + \text{cell} \rightarrow \text{cell} - \text{Ca}^{2+} \\
\text{amorphous CaCO}_3 \rightarrow \text{vaterite} \rightarrow \text{calcite} \\
\text{precipitation and crystallization} \\
\]

In this study, we tried to investigate the microscopic characteristics of MICP plugging and its effectiveness in permeability reduction by preparing artificial rock cores. Specifically, *S. pasteurii* was used as the microbial stock for plugging. The aggregates of near-spherical silica sand and angular silica sand with three separate size fractions (40/60 mesh (0.250—0.250 mm), 60/80 mesh (0.250—0.180 mm), and 80/120 mesh (0.180—0.125 mm)) aggregates were used as two series of permeating columns with contrasting pore characteristics to investigate the efficacy and microprocess of MICP plugging with different biotreatment periods. The effectiveness of MICP plugging was examined through measurements of the permeability reduction with different biotreated durations. The micromorphology of generated CaCO₃ as well as its distribution within the intergranular space was examined by scanning electron microscopy (SEM) to define microscopic processes of MICP plugging.

### 2. RESULTS

Each MICP-treated sand column was dissected transversely into five equal length sections (50 mm length) for each permeability and CaCO₃ content test. The schematic of the experimental apparatus for the permeability test is shown in Figure 1. The MICP-treated sand column with a wrapped PVC holder was dissected transversely into five equal length sections (50 mm length). The two ends of PVC tube were glued with a PVC tube with the same diameter and 25 mm in length to fix the rubber plug. One end of the section was connected to a peristaltic pump, and the other end is connected to the atmosphere. A
digital manometer (range: \(\pm 517 \text{ kPa}\); resolution: \(0.25 \text{ kPa}\)) was connected between the inlet of the section and the peristaltic pump to measure the inlet flow pressure. The permeability was determined by constant flow rate measurement and calculated using Darcy’s law

\[
k = \frac{QL\mu}{\Delta PA}
\]

where \(k\) is the permeability \((\text{m}^2)\), \(Q\) is the volumetric flow rate \((\text{m}^3/\text{s})\), \(L\) is the length of the sample \((\text{m})\), \(\mu\) is the dynamic viscosity of the fluid \((\text{Pa}\cdot\text{s})\), \(\Delta P\) is the pressure difference from the inlet to the outlet \((\text{Pa})\), and \(A\) is the cross-sectional area of the sample \((\text{m}^2)\).

Measured permeabilities are shown in Figure 2. Overall, the permeabilities of the biotreated cores obviously decrease with an increase in cumulative MICP treatments. Specifically, after eight cycles of MICP treatments, the permeabilities of the cores composed of intact Ottawa sand with the size distributions of 40/60, 60/80, and 80/120 mesh maximally drop to 7.37 D (1 Darcy = \(9.87 \times 10^{-13} \text{ m}^2\)), 0.81, and 0.18 D, respectively. Also, the permeabilities of the cores composed of crushed Ottawa sand with the size distributions of 40/60 mesh, 60/80 mesh, and 80/120 mesh maximally drop to 1.92, 0.13 and 0.10 D, respectively. Compared with the cores of the identical particle size distribution, the permeability reduction of the crushed Ottawa sand aggregates is more severe than that of intact Ottawa sand at the identical durations of biotreatment. This is consistent with a greater reduction in pore throat diameter occurring for the narrower pore throats of crushed Ottawa sand. Following the permeability measurement, the CaCO3 content of each segment was examined by soaking \(\sim 10 \text{ g}\) of each segment into 5.0 mol/L HCl to fully dissolve CaCO3 and then measuring the dry weight difference before and after soaking. According to biochemical eqs 1–4, it can be seen that CaCO3 is the only substance that is produced through the MICP process, and additionally, the pre-existing acid-soluble impurities attached to sand particles were removed by hydrochloric acid solution before tests. Thus, the CaCO3 content can be expressed as the ratio of the difference in dry weight before and after soaking in HCl to the remaining dry weight after immersing. The correspondence between permeability reduction of each segment and its CaCO3 content is

![Figure 1. Schematic of the experimental apparatus for the permeability test.](https://pubs.acs.org/doi/10.1021/acsomega.0c00902/acsomega.0c00902.s001)

![Figure 2. Variation in permeability for the treated artificial cores representing (a) 40/60 mesh aggregates, (b) 60/80 mesh aggregates, and (c) 80/120 mesh aggregates for 4, 6, and 8 cycles of nutrient circulation.](https://pubs.acs.org/doi/10.1021/acsomega.0c00902/acsomega.0c00902.s002)
shown in Figure 3. The dimensionless relative permeability (ratio of the measured permeability to initial permeability) is used to compare the normalized efficacy of bio-CaCO3 plugging among these cores with distinct pore features and pore sizes. The decline rate of permeability with the accumulation of CaCO3 can be roughly divided into three contrasting stages, as shown in Figure 4. At the initial stage of CaCO3 accumulation, the decline rate in permeability is relatively slow with the increase in CaCO3 content. When the CaCO3 content accumulates to a certain level, the permeability reduction shows greater sensitivity to the increase in CaCO3 content, which is the stage of rapid decline in permeability. As CaCO3 accumulates to a certain level, the decline rate of permeability tends to be slow. This macroscopic behavior of the permeability reduction with the accumulation of CaCO3 can be explained by investigating the microscopic characteristics of the CaCO3 distribution contributing to plugging, which is discussed in the following section.

3. MICROSCOPIC ANALYSIS

We determined the mineral compositions of microbially mediated CaCO3 through X-ray diffraction (XRD) measurements. The microstructure of the deposited CaCO3 as well as its distribution within the pore space was examined by SEM to describe the microscopic processes of microbially mediated plugging.

3.1. Crystal Structure of Microbially Induced Calcium Carbonate and Its Distribution. The cores composed of 60/80 mesh aggregates with 6 cycles of MICP treatment were used for mineral composition analysis by XRD. The XRD results suggested that the mineral compositions of these two types of biotreated aggregates are identical, which both include calcite, vaterite, and quartz (Figure 5). The detected Quartz is the mineral component of silica sand. Vaterite and calcite, represented by two polymorphs of CaCO3, are the microbially produced components. The XRD results also implied that more than 90% of CaCO3 induced by microbial hydrolysis of urea is vaterite. The micromorphology of the generated CaCO3 crystals as well as its distribution within the intergranular spaces was further examined by SEM. As shown in Figure 6, the particle surfaces were covered with irregularly distributed cubic and spherical CaCO3 crystals, which are calcite and vaterite. In addition, smaller CaCO3 crystals formed a film-like form covering the surface of the particles. In terms of morphology and size, the microbially mediated CaCO3 and EPSs are significantly different. EPSs generally maintain a larger surface area but of a nanoscale thickness (200–1000 nm) while the single microbial CaCO3 crystal can grow to tens of microns. The latter suggested a stronger potency in plugging the pore space.

3.2. Mechanism and Process of Microbially Mediated Plugging. We observed the cumulative microscopic process of microbially mediated CaCO3 via comparison to the SEM images of the cores of 60/80 mesh crushed Ottawa sand with biotreated 4, 6, and 8 cycles [Figure 7a–c]. The precipitate of CaCO3
irregularly distributes on the pore surfaces in the form of granular crystal. As the biotreated durations increase, the quantity and size of the generated CaCO₃ crystals increase significantly. Based on the SEM images, the mechanisms promoting the deposition of biogenerated CaCO₃ in the pore space are illustrated by Figure 8. Microorganisms first adhere to the surface of the particles and gradually induce the production of CaCO₃ with the supply of urea and calcium ions. As the concentration of CaCO₃ around the bacteria exceeds chemical saturation, supersaturated CaCO₃ will transform into amorphous precipitation products, which then crystallize with microorganisms as crystal nuclei and form thin layers of CaCO₃ (the process (a) in Figure 8). With microbially derived CaCO₃ gradually accumulating, some nucleation sites of CaCO₃ crystals will experience further preferential growth, resulting in large, dominant crystals (the process (b) in Figure 8). As this mass continues to accumulate, the pore spaces are occluded, resulting in a significant and continuous decrease in the penetrability of the ensemble medium (the process (c) in Figure 8). As the potential flow channels are almost occupied and plugged by CaCO₃, the change in permeability slows and then halts, as nutrients are expended and further supply is limited. This microcosmic explanation is consistent with the observation that the change rate in permeability with the increasing CaCO₃ content experiences three contrasting stages, namely, slow decline, speedy decline, and plateauing.

4. DISCUSSION

A series of permeating column experiments and SEM analysis examined the efficacy and microprocess of microbial-mediated CaCO₃ clogging. Compared to EPSs, which are currently the primary microbial plugging agent used to enhance sweep efficiency, bio-CaCO₃ is more effective in plugging in terms of its morphology, size, and growth characteristics. Further research should concentrate on the optimizing of input nutrient fluxes (urea and calcium ion) and on MICP injection strategies. When the reaction rates of urea hydrolysis and CaCO₃ precipitation are faster than the input rate, cementation is immediately adjacent to the injection source. The urea and calcium ion are depleted before they reach biological communities farther along the flow path. Conversely, increasing the flow rate to exceed the rate of urea hydrolysis and CaCO₃ precipitation allows for a more uniform distribution of chemicals along the entire flow path direction, thus resulting in a homogeneous clogging in the field scale. However, an excessive input flow rate is able to cause the fluid shearing force to exceed the adhesion of the microorganism on the solid surface, thereby causing the microorganism to be carried away by the fluid.

When MICP is implemented on an engineering scale, certain factors, which are not fully considered in the laboratory scale, should be taken into account. First, the volume of the target formation area should be clarified to determine the dosage of bacterium and transport time, so that the bacteria solution can completely sweep through the treatment area. The second part would be the matching relationship of dosages of bacterium and urea-Ca²⁺ at MICP treatment. S. pasteurii in the MICP process provides urease for urea hydrolysis and also acts as nucleation sites for the crystallization of CaCO₃. The injection of the nutrient solution potentially causes a portion of the microorganisms that have previously adhered to the pore surface to be washed away. In addition, the bacteria act as nuclei and are gradually surrounded during crystallization. This impedes the transmission of nutrient ultimately rendering the bacterium...
inactive. The scenarios mentioned above could result in a decrease both in the number of microorganisms capable of secreting urease and in the precipitation rate of CaCO₃ during the MICP anaphase. Therefore, a rational matching of the injected urea volume with the timing of bacterial supplementation is critical to improving the efficiency of the reactants and ensemble of MICP plugging. Although the cost of MICP is relatively low based on the current experimental data, the following research studies should also consider alternative urea sources and calcium ion sources, such as urea fertilizer and CaCl₂ ice melting products, to reduce both the cost and economic feasibility of MICP plugging technology.

5. CONCLUSIONS

One potential method for plugging high-permeability zones of oil reservoirs has been proposed via microbially mediated CaCO₃ precipitation. A series of artificial rock cores with distinct pore sizes and pore characteristics were used to investigate their efficacy in permeability reduction. After eight cycles of microbial treatments, the permeability for each type of cores maximally drops by 54–90% of initial permeabilities. Specifically, given a fixed duration of treatment, the decline in permeability is the fastest for small (80/120 mesh) particle size samples. Moreover, the decline rate in permeability for nonspherical aggregates (crushed Ottawa sand) is faster than the near-spherical aggregates (spherical aggregates) for the identical pore size. The change rate in permeability with the increasing CaCO₃ content experienced three contrasting stages, namely, slow decline, speedy decline, and plateauing.

XRD indicated that most of the generated CaCO₃ crystals occur as vaterite with only a small amount of calcite. Imaging by SEM further defined the microprocess of MICP plugging. Microorganisms first adhere to the pore surface and gradually induce the precipitation of CaCO₃ in the form of thin film of CaCO₃. With microbially derived CaCO₃ gradually accumulating, some nucleation sites of CaCO₃ crystals will experience further preferential growth, resulting in large, dominant crystals. As this mass continues to accumulate, the pore spaces are occluded, resulting in a significant and continuous decrease in the penetrability of the ensemble medium. As the potential flow channels are almost occupied by CaCO₃, the change in permeability slows and then halts, as nutrients are expended and further supply is limited.

6. EXPERIMENTAL METHODS

The sand columns were used as artificial rock cores, which are composed of crushed Ottawa sand and intact Ottawa sand with three separate size fractions (40/60, 60/80, and 80/120 mesh) representing variable pore characteristics and pore size. They were treated for different MICP treatment periods, and the levels of permeability reduction were then measured.

6.1. Characteristics of Particle and Pore Space Formed by Particle Packing. Crushed Ottawa sand and intact Ottawa sand were assembled as two series of artificial rock cores to reflect distinct pore morphologies. Compared with natural sandstone cores, the initial permeability and porosity of the artificial bead-pack could be controlled and restrained within a narrower range. Also thus, using the artificial bead-pack as the substrate samples could reduce the influence of the difference in the initial properties of the samples on the experimental results. In addition, the main mineral composition of sandstone is quartz, which is also the mineral composition of Ottawa sand. These two types of silica sand have the same mineral composition (quartz) but contrasting morphological characteristics. The morphology of each aggregate type was characterized using a Morphologi G3 analyzer (Malvern Panalytical Company). Typical 2D projections of the two types of particle shapes are shown in Figure 9. The intact Ottawa sand particles showed the near-spherical shape while the crushed Ottawa sand particles showed the angular shape.

Each type of sand was sieved into three grain-size classifications: 80/120 mesh (0.180–0.125 mm), 40/60 mesh (0.425–0.250 mm), and 60/80 mesh (0.250–0.180 mm). Please note that the crushed sand was sieved after they were crushed. The sieved sands were soaked in hydrochloric acid solution (5 mol/L) for 12 h to remove the acid-soluble impurities and then washed in ultrapure water and completely dried before MICP treatment. Subsequently, the porosity and permeability for the granular packing of each size fraction were determined by helium porosimetry and constant head permeability test, respectively. The sample/particle assembly process for the porosity and permeability measurement was the same as that for the subsequent MICP experiment, which was to cover the sample with a 100 g balance weight and manually vibrate for 1 min. The porosity of the granular packing for each particle size is similar while the permeability declines sharply as the particle size decreases (Table 1). Moreover, the permeability of nonspherical aggregates (ground Ottawa sand) is lower than that of the near-spherical aggregates (intact Ottawa sand) for the identical particle size. This is because of the irregularity shape of the ground Ottawa sand, which results in a heterogeneous distribution of pore size and morphology between the particles. This irregular pore size distribution and morphology spontaneously produce narrower pore throats in polyhedral aggregates compared to near-spherical aggregates with the identical particle size. Therefore, it is suitable to use the artificial cores composed of the two types of morphologically contrasting sands to investigate the plugging mechanism and effectiveness of MICP in reservoirs with different pore characteristics.

6.2. Bacteria Cultivation. The bacterial strain of *S. Pasteurii* (ATCC no. 11859) was cultured aerobically in the ammonium–yeast extract media [20 g yeast extract, 10 g (NH₄)₂SO₄, and 1.0 L 0.13 M Tris buffer (PH 9.0)] at 30°C in a water bath shaker (200 rpm) for approximately 36 h before harvesting at OD₆₀₀ = 1.4–1.6. OD₆₀₀ is an abbreviation standing for optical density measuring the concentration of bacteria or other cells in a liquid at a wavelength of 600 nm. If the measured optical density (OD₆₀₀) is between 0.2 and 0.8, the biomass concentration (Y) is recovered from the optical density (OD₆₀₀) as Y = 8.59 × 10⁻² OD₆₀₀. If the measured optical density (OD₆₀₀) is higher than 0.8, the bacteria solution need to be diluted to a

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**Figure 9.** Morphological 2D images of these two types of silica sand: (a) Intact Ottawa sand (near-spherical shape) and (b) Crushed Ottawa sand (angular shape).
OD$_{600}$ value between 0.2 and 0.8, and the biomass concentration ($Y$) is recovered from the OD$_{600}$ value of the diluted solution and dilution factor as $Y = 8.59 \times 10^{-7} \times K \times$ OD$_{600}$.\textsuperscript{1,2,27,36} where $Y$ is the biomass concentration per mL and $K$ is the dilution factor.\textsuperscript{20,33,36} The suspended bacteria solution culture was then centrifuged twice at 4000 g for 30 min, removing the supernatant liquor and supplementing the fresh growth media after each centrifugation. After the centrifugation process, the bacteria solution was stored at 4 °Celsius prior to MICP treatments.

6.3. Experimental Apparatus and MICP-Treatment Procedure. The columniform PVC (poly vinyl chloride) tubes [300 mm in length and 25 mm in internal diameter] were used as the bead-pack holder. The aggregates placed in the PVC tube were manually vibrated for 1 min, while they were loaded with a 100 g balance weight, and finally, a sand column with a length of about 250 mm (10 in.) was assembled for the subsequent MICP treatment. Each grain-sized fraction of these two categories of silica sand was prepared into three identical columns for different exposures of MICP treatments. The experimental apparatus is illustrated in Figure 10. The S. Pasteurii suspension, stabilizing solution (0.05 mol/L CaCl$_2$), and cementation solution (1.0 mol/L urea and 1.0 mol/L CaCl$_2$) were sequentially injected into the column from the bottom entry of the core holder by a peristaltic pump. Calcium ions in the stabilizing solution can enhance the adhesion of bacteria to the surface of the sand particles as well as bacterial flocculation.\textsuperscript{31} The MICP fluid injection was one-way to mimic the transport of bacteria/nutrients in the reservoirs during the MICP plugging process. A standard injection procedure is shown in Figure 11. The sand flow-through columns for each category separately implemented four, six, and eight injection cycles, representing various biotreatment periods.

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### Notes

The authors declare no competing financial interest.

### Acknowledgments

This work was supported by the National Science Foundation of China under grant no. 51604051 and the Natural Science Foundation of Chongqing under grant no stc2018jcyjA2664. This support is gratefully acknowledged.

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