Iran. J. Biotechnol. January 2022;20(1): e3024 DOI: 10.30498/ijb.2021.279942.3024

Research Article

Experimental Study on Microbial Induced Calcium Carbonate Precipitation to Enhance Reservoir Recovery

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Background: Bacillus subtilis can produce urease in the presence of urea as the main carbon source and induce mineralization in the presence of precipitable cations.
Objectives: The objective of our study was to demonstrate that Bacillus subtilis catabolizes glucose first in the presence of both glucose and urea carbon sources. Using its feature of catabolizing glucose first to delay the mineralization time, it proved its potential application in enhancing the recovery of heterogeneous reservoirs.
Material and Methods: The metabolic process of Bacillus subtilis was monitored by changing the glucose content in the bacterial medium by UV spectrophotometer and pH meter. Using a non-homogeneous physical model, experiments were conducted to improve reservoir recovery by microbial mineralization after polymer oil drive.
Results: The higher the glucose content in the medium, the longer the time for the pH of the bacterial solution to reach 7 and the longer the end of the logarithmic phase of growth. the glucose content of the 48 h medium was significantly correlated with the consumption of the bacteria and the quality of the precipitation. In the oil drive experiment: the permeability of the high permeability model was reduced from 1200 md to 136 md with a reduction rate of 88.6 %, and the permeability of the low permeability model was reduced by 22 md, and the crude oil recovery was increased by 7.9 %.
Conclusions: It was demonstrated that the addition of glucose to the culture medium retarded the mineralization of bacteria. Only 0.2 times the pore volume of the bacterial solution and the cementing solution is required to form an effective seal, thus improving the recovery of crude oil.

Keywords: Environmental protection, Microorganism, MICP, Petroleum engineering

1. Background

In the process of oil field development, the oil recovery method that uses the natural energy of the reservoir is primary oil recovery. After primary oil recovery, development by supplementing formation pressure is called secondary oil recovery.

Tertiary oil recovery is the development method of oil recovery by using new technologies such as physical, chemical and biological tailings after the oil field has been extracted by using natural energy and the traditional use of artificial supplemental energy (water and gas injection). This type of oil drive is mainly

through chemical, steam, gas or microbial injection, thus changing the nature of the replacement phase and oil-water interface or the physical properties of crude oil (1). Among them, the main mechanism of microbial enhanced oil recovery is microbial metabolism to produce bio surfactants. organic solvents or biogas to modify the fluidity of crude oil or to block non-homogeneous high permeability channels by substances such as extracellular polymers secreted by microorganisms to enhance the production of hydrocarbons (2).

MICP, microbial induced carbonate precipitation, was first proposed by *Cripps* (3) at the end of the last century, and *Mitchell* (4) proposed "Bio-Earth Technology" in the field of geotechnical engineering based on the precipitation of calcium carbonate induced by microorganisms. Since then, more and more researchers (5-6) have begun to explore the mechanism and phenomenon of the technology and try to make it better applied in the engineering field by changing various conditions and parameters.

There are different mechanisms of mineralization in MICP and they are: photosynthetic algae and blue microorganisms, urea-degrading microorganisms, nitrifying/denitrifying microorganisms, iron sulfate reducing bacteria, ammonifying microorganisms and other fungi. Among them, calcium carbonate precipitation caused by microbial hydrolysis of urea has become the most important category for researchers due to its high precipitation efficiency and wide applicability. These microorganisms are able to use urea as a carbon source and produce urease to accelerate urea decomposition and combine with exogenous Ca^{2+} to form $CaCO_3$ precipitation (7).

The following is the reaction process:

$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH^- \quad (1)$$

$$HCO_3^- + H_2O + OH^- \rightarrow CO_3^{2-} + 2H_2O \quad (2)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3\,(s) \quad (3)$$

In recent reports, we found that petroleum engineers and researchers have been applying this technology to production operations: *C. I. Noshi* (8) have applied MICP-based self-healing cement to cementing operations with good results; *Adrienne J. Phillips* (9) have applied this technology to seal fractures in sandstone near the subsurface at 340.8 m to reduce fluid leakage. *Dominique J. Tobler* (10) used *Bacillus pallidus* to seal fractured granite cores and showed that the MICP-treated fractures were more resistant to shear forces.

2. Objectives

However, as indicated from a study conducted by *Cuthbert* (11), such a technology faces a problem that it is difficult to control when applied practically. This researcher suggested that the farther away from the injection end, the less calcium carbonate deposit there would be, probably attributed to the adsorption and retention of the microorganisms and the calcium carbonate crystals within the porous medium. If the area around the water injection well was blocked by the microbial-induced calcium carbonate precipitation on the oilfield site, water injection would be more difficult to achieve, and the injection equipment might be seriously damaged, which could hinder the subsequent oilfield development. Accordingly, an analysis should be conducted on how to make the microorganisms migrate to deeper positions, and how the uniformly deposited calcium carbonate is synthesized in the reservoir. *Wu* (13) employed a single-phase mixture of microorganisms and calcium ions for the injection. With the increase in the flow rate, the total calcium carbonate deposition formed by the entire system increased. As revealed from all the mentioned studies, changes in the flow rate may cause different precipitation results. In the present study, an effective urease-producing strain was isolated from the soil in the garden of Yangtze University. In addition, the time of microbial mineralization was delayed by replacing the microbial culture medium. Moreover, several pioneering experiments were performed to verify whether this technology is potentially capable of selectively plugging higher permeability reservoirs during the oil field development.

3 Materials and Methods

3.1. Screening of Microorganisms

From the soil of the garden of Yangtze University, 500 g of pretreated sandy soil was weighed. Based on a 10×10 cm area selected arbitrarily, the sandy soil was pretreated, and 1 L of 5 $moL.L^{-1}$ urea solution was added to the soil area per day for the next 5 days, as an attempt to enable the bacteria of the sandy soil to adapt to the high concentration of urea. After the 5^{th} day of the

treatment, a 200 g sample was taken at 5 cm from the ground with a sterile sampling bag to prepare a 100mL screening medium which covered peptone (Beijing Aoboxing Biotechnology Co., Ltd.) 3 $g.L^{-1}$, sodium chloride (China National Pharmaceutical Group Co., Ltd.) 5 $g.L^{-1}$, potassium dihydrogen phosphate (China National Pharmaceutical Group Co., Ltd.) 2 $g.L^{-1}$, urea (Tianjin Zhiyuan Chemical Reagent Co., Ltd.) 20 $g.L^{-1}$, glucose (Tianjin Zhiyuan Chemical Reagent Co., Ltd.) 0.05 $g.L^{-1}$, 0.2 % phenol red solution (Shanghai Maikun Chemical Co., Ltd.) 4 $mL.L^{-1}$, as well as agar (Beijing Aoboxing Biotechnology Co., Ltd.) 20 $g.L^{-1}$, pH 7. The urea and the glucose were prepared separately, and the other solutions were sterilized and then treated for the screening. Subsequently, the medium was autoclaved at 121 °C for 20 min. It was cooled to the ambient temperature on a sterile table then poured into a petri dish. With the dilution coating method, the enriched medium was diluted based on three concentration gradients (i.e., 10^{-6}, 10^{-7} and 10^{-8}) on the prepared plates and then incubated in a constant-temperature incubator at 35 °C. When colonies were growing on the plates, the individual colonies around with the medium turning red were picked out and then inoculated onto plates of urease producing strains using the scratch method; subsequently, they were incubated at 35 °C. The mentioned steps were repeated several times till a single strain free of trampy bacteria was isolated. It was identified by using 16S rRNA as a *Bacillus subtilis* strain with the landing number of MZ165021.

3.2. Microbial Growth Stage Analysis

In this study, a pH meter was adopted to monitor the metabolism of glucose and urea achieved by bacteria. The UV spectrophotometer (Shanghai Jinghua Technology Instrument Co., Ltd.,722N) was employed to monitor the bacteria's growth and metabolism. The growth and metabolism of this strain of microorganism for glucose was investigated by performing the shaking flask experiment, and the pH and bacterial concentration variations of the bacterial solution were monitored per 4h. Moreover, the monitoring was stopped when the OD_{600} value was down-regulated three times in a row, which demonstrated that the microorganism had reached the decaying stage. The experimental medium covered peptone 3 $g.L^{-1}$, NaCl 5 $g.L^{-1}$, KH_2PO_4 2 $g.L^{-1}$, urea 2 $g.L^{-1}$, glucose 0.05 $g.L^{-1}$.

3.3. Oil Repelling Experiment Preparation

The sand-filled model was prepared: the heterogeneous parallel sand box model consisted of two 5×6×30 cm visual single-layer sand boxes, filled with 40-80 mesh and 180-200 mesh quartz sand, respectively. The quality of the model was recorded after the repeated beating and filling processes. Subsequently, the next experiment was performed.

The model was vacuumed to saturate water: a vacuum pump was used to vacuum the two models for 24 h. Then, the two modes were connected to a simulated formation water container till they were saturated with water. Next, and the mass of the two models was weighed. This step could calculate the porosity.

The permeability was measured: Darcy's formula was adopted to measure the permeability of the two models, respectively. After the calculation, the high-permeability model K=1500 md, and the low permeability model K=328 md.

The simulated crude oil was configured: the crude oil was configured from an oil field in eastern China and the aviation kerosene at a ratio of 1:5 to produce the simulated crude oil with 5.5 mPa•s viscosity. The mentioned was conducted to obtain a crude oil exhibiting better flow ability at the ambient temperature, as well as to facilitate the experimental manipulation;

The polymer was configured: the simulated formation water (2000 $mg.L^{-1}$ NaCl solution) and the polymer (Polyacrylamide, molecular weight 18 million) commonly used in oilfields were configured into a 2000 $mg.L^{-1}$ polymer solution.

The entire system was connected, which covered the storage container, the peristaltic pump, the pressure gauge, the parallel heterogeneous physical model. The process and form on the plane are illustrated in **Figure. 1**.

Saturated oil: the peristaltic pump was driven to saturate the simulated oil configured in step 4 at an injection flow rate of 1 $mL.min^{-1}$. Such a saturating process was sampled per 20 min, and the saturation was stopped when the oil content in three consecutive samples exceeded 98 %. After oil the saturation, all the preparations for the oil displacement experiment were completed.

3.4. Oil Repelling Experiment Flow

The water injection development reservoir was simulated: the water injection development reservoir was simulated at a speed of 1 $mL.min^{-1}$. The samples were taken per 15 min with a scaled test tube. Next, the oil displacement efficiency and water cut of the two models were recorded, respectively. The experiment was stopped till the water content in the liquid at the outlet end exceeded 98% in three consecutive samples

Polymer injection: the water injection was changed to the injection of polyacrylamide solution to perform the displacement experiment. 0.2 PV was injected to make the solution freely distributed, and then the water injection was initiated;

Water injection: step 1 was repeated till the water content of the high permeability reservoir exceeded 98% in three consecutive samplings.

Microbial-induced calcium carbonate precipitation to plug the reservoir: 500mL (OD_{600} value 1.12) was pre-cultured for 24 h, 2 $moL.L^{-1}$ urea and $CaCl_2$ were mixed at 1:1 as a cementitious solution. Then, the solution was mixed with the bacterial solution at 10:1 and then poured into the sandbox model. The cumulative injection volume reached 0.2 PV, which could be allocated freely, and then the injection was stopped. By referencing conclusion 4.1, the sandbox model was incubated in an incubator at 35 °C for 48 h. The mentioned process aimed to completely metabolize *Bacillus subtilis* in the sandbox and form the calcium carbonate precipitation to block the percolation channel.

Water injection after plugging: Repeat step 1 to increase the water level in three consecutive samples to more than 98 % and stop all experiments.

3.5. Comparison of the Oil Displacement Effects Exhibited by Microbial Induced Calcium Carbonate Precipitation and Microbial Growth/Biomass Production

In this group, the incubation and experimental steps were basically identical to step 3.3 and 3.4, except that Ca^{2+} was not added to the solution of bacteria to be oil repelled. The permeability of the experimental model simulated in this group was 1680 md and 355 md for the high permeability and the low permeability, respectively.

4. Results

4.1. Glucose Metabolism in Bacillus Subtilis

Since the precipitation by binding Ca^{2+} by the bacterium requires an alkaline environment formed by decomposing urea, the addition of glucose is of critical significance, which can prevent the precipitation close to the bottom of the injection well and increase the depth of depth of blockage. The results are illustrated in the **Figure 2**, the higher the glucose content in the medium, the longer it would take for the pH value of the bacterial solution to reach 7 and the longer the end of the logarithmic phase of growth would be.

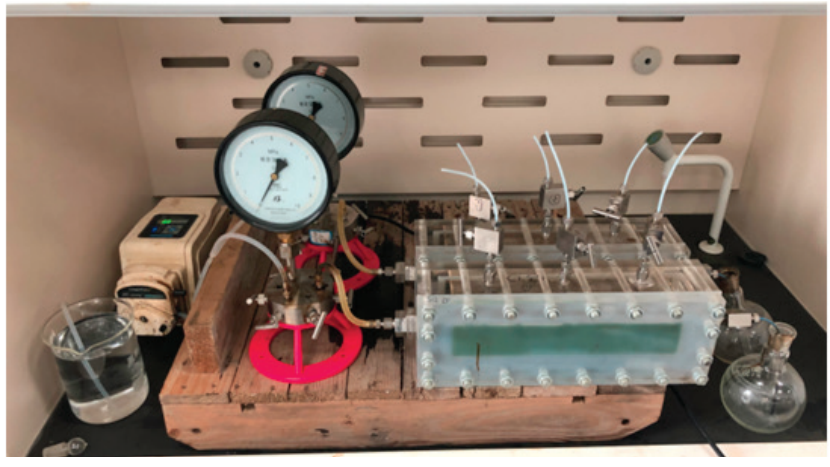

Figure 1. Flow chart of oil repelling experiment

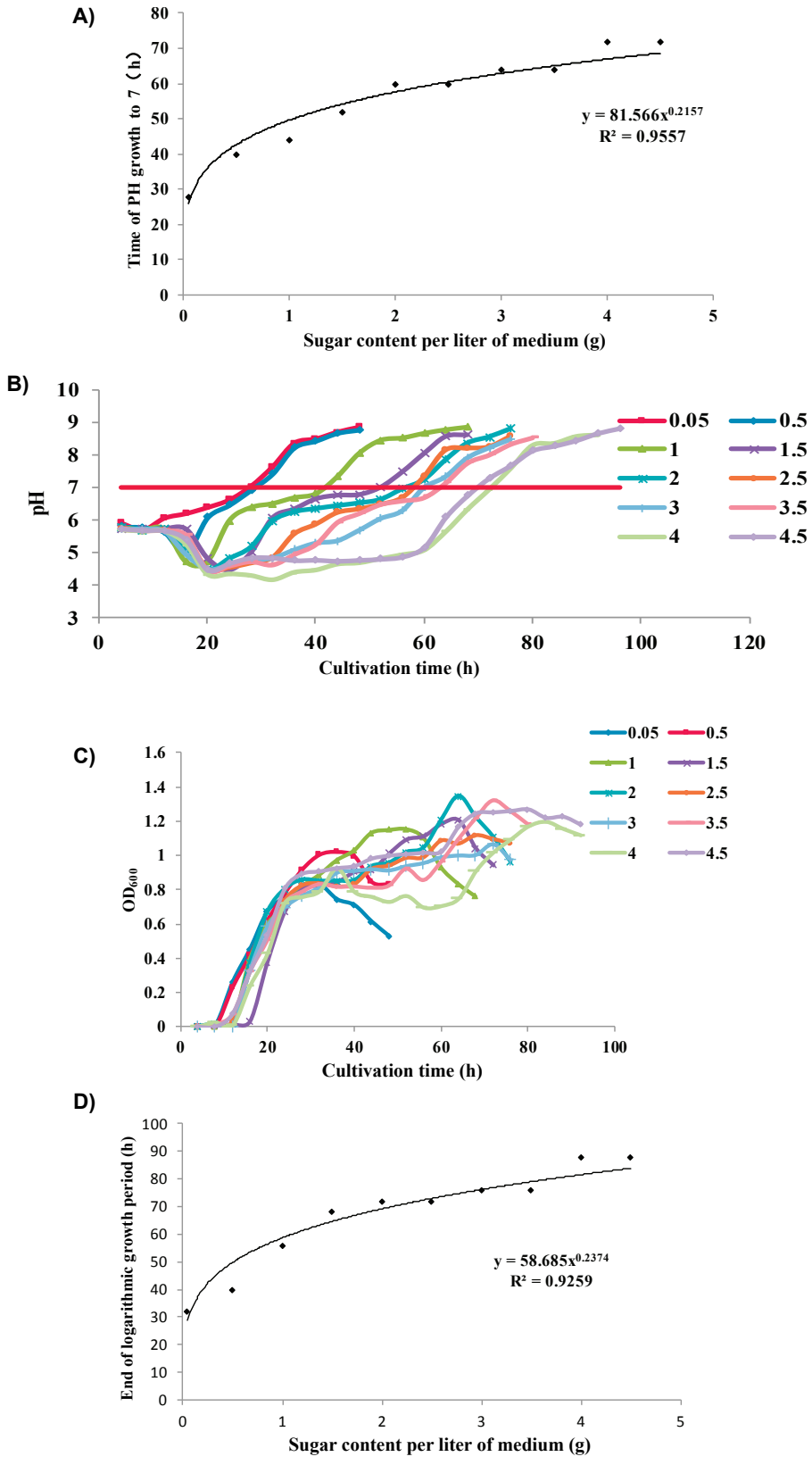

Figure 2. Growth and metabolism of Bacillus subtilis at different glucose contents. **A)** shows the change of pH value at different glucose contents of the medium; **B)** shows the time of reaching pH 7 at different glucose contents of the medium; **C)** shows the change of absorbance at different glucose contents; **D)** shows the time of ending the logarithmic phase of growth of the bacterium at different glucose contents

In another group of the control test, different glucose content media were used simultaneously to take equal amounts of the bacterial solution for the precipitation test. a 15mL bacterial solution was taken, 2 moL.L^{-1} $CaCl_2$ was add to the solution, and then the volume was fix to 20 mL. The OD_{600} value of the bacterial solution and the supernatant was monitored before and after the precipitation, and ($OD_{600}1$-$OD_{600}2$)/$OD_{600}1$ acted as the consumption of bacteria, in which $OD_{600}1$ and $OD_{600}2$ were the concentrations of bacterial solution before and after the precipitation, and the precipitation was weighed after the filtration with phosphate buffer till the mass remained unchanged (**Fig. 3**). As revealed from the results, the glucose content in the 48h medium displayed a significant correlation with the consumption of bacteria and the quality of precipitation.

4.2. Oil Displacement Experiment

The experimental results are shown in **Table 1**. As

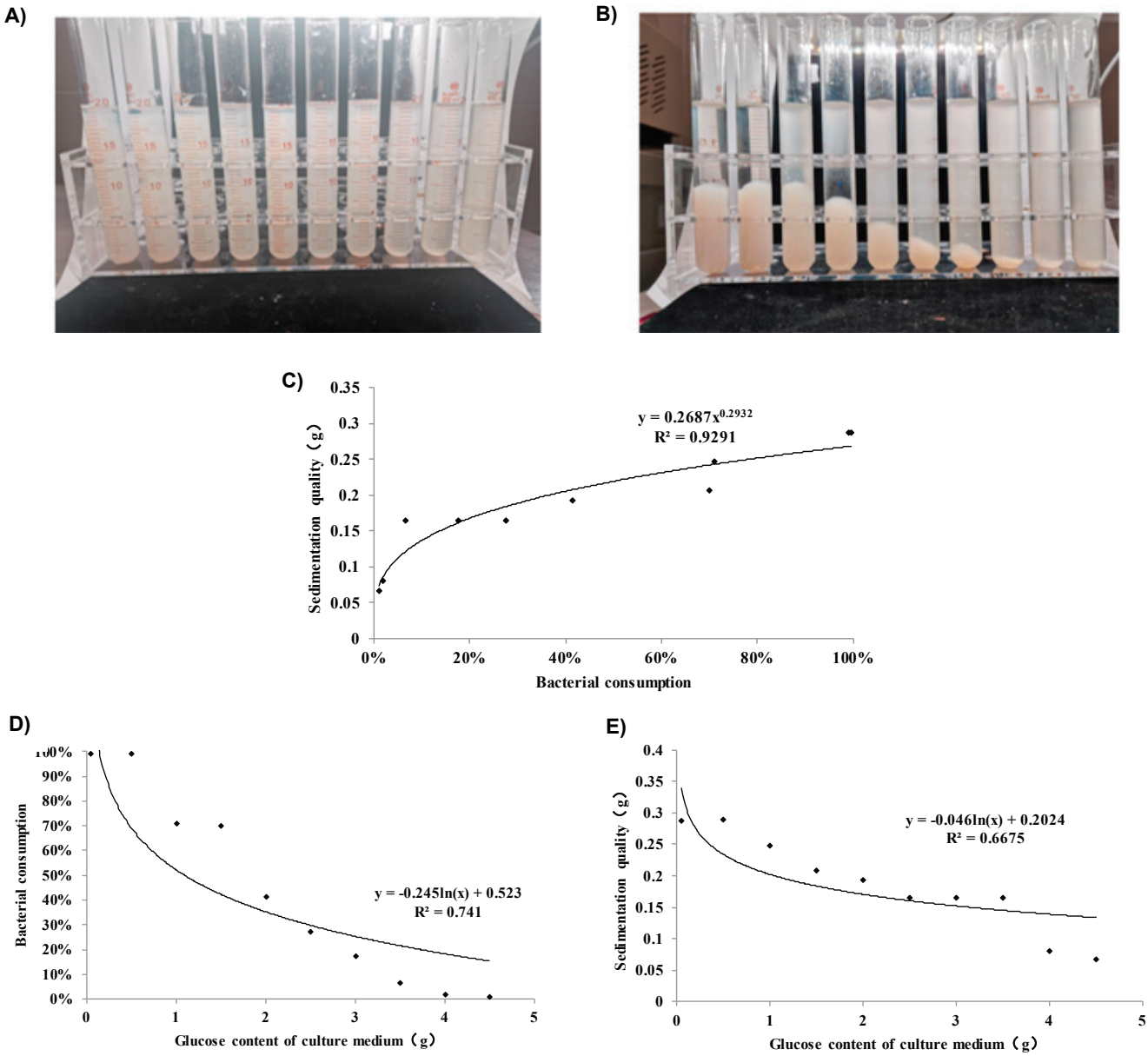

Figure 3. Precipitation of bacterial solution with different glucose content at 48 hours. **A)** is the image before precipitation; **B)** is the image after precipitation; **C)** is the relationship between bacterial consumption and precipitation; **D)** is the relationship between glucose content and bacterial consumption per liter of medium; **E)** is the relationship between glucose content and precipitation per liter of medium

Table 1. Experimental result table

Simulated reservoir	Stage	Control group A		Experimental group B	
		Effective permeability (md)	Recovery ratio (%)	Effective permeability (md)	Recovery ratio (%)
High permeability reservoir	Water flooding	1680	64.2	1500	46.4
	Polymer flooding	/	72.9	/	74.8
	Microbial flooding	1556	72.9	136	74.8
Low permeability reservoir	Water flooding	355	20.9	328	32.4
	Polymer flooding	/	56.1	/	52.1
	Microbial flooding	324	56.7	306	60

indicated from the data of the oil discharge experiment in the control group A, the increase in the microbial colonization and the biomass did not lead to an improvement of the oil recovery. The viscosities of water, polyacrylamide and the bacterial solution were measured after the incubation by viscometer for 48h (American BROOKFIELD Co., DVN type), and their viscosities were examined as 1.7 mPa·s, 169 mPa·s and 2.4 mpP·s, respectively.

The experimental group is presented in **Figure 4B**. The permeability of the high-permeability model was reduced from 1500md to 136md with a reduction of 88.6 %, and the permeability of the low permeability model was down-regulated by 22 md with an increase in the recovery of 7.9 %. For this reason, the recovery was significantly improved.

5. Discussion

5.1. Discussion on Glucose Metabolism in Bacillus Subtilis

Predecessors have conducted extensive investigations to make the bio mineralization more uniform. *Tobler* (24) found that bacteria were easier to fix in a homogeneous sandstone matrix, and that the longer the seepage distance, the smaller the number of the bacteria would be received at the outlet. According to *Mountassir* (25), the bacterial fluid would form a preferential seepage path in the seepage process. The solution they proposed is to reduce the flow rate or inject multiple times, since the bacteria will expand its spread as impacted by the deposited calcium carbonate and form more sediments, whereas it is inconsistent with the possibility of clogging close to the well end. Some researchers proposed lowering the temperature of the bacterial solution to inhibit the urease activity (26) or adding H^+ into the initial solution to lower the pH to reduce the urease activity for delaying the precipitation time (27). Nevertheless, the bacteria have already metabolized the urea, so the extracellular polymer contains considerable negative charge. In practical engineering applications, the Bacteria based on this method is easy to be diluted by the injected liquid and precipitate directly.

As shown in **Figure 2**, in the presence of glucose and urea simultaneously, *Bacillus subtilis* first ferments glucose and metabolizes organic acid, so the pH decreases. When glucose is sufficient, the pH declines to nearly 4.5. In addition, when the energy supply of glucose cannot meet the energy required for the growth and development of the bacterium, and the microorganism starts to decompose urea. Moreover, the pH will increase rapidly to 8. Subsequently, it slowly increases to 9, and it is basically kept at this value. Our explanation for the mentioned phenomenon, complies with that in the literature (28). Under the pH of the bacterial solution of 4.5-8, the urease within the bacteria starts to catalyze the urea to be hydrolyzed. This process can fall into two steps below. First, as catalyzed by the urease, 1 molecule

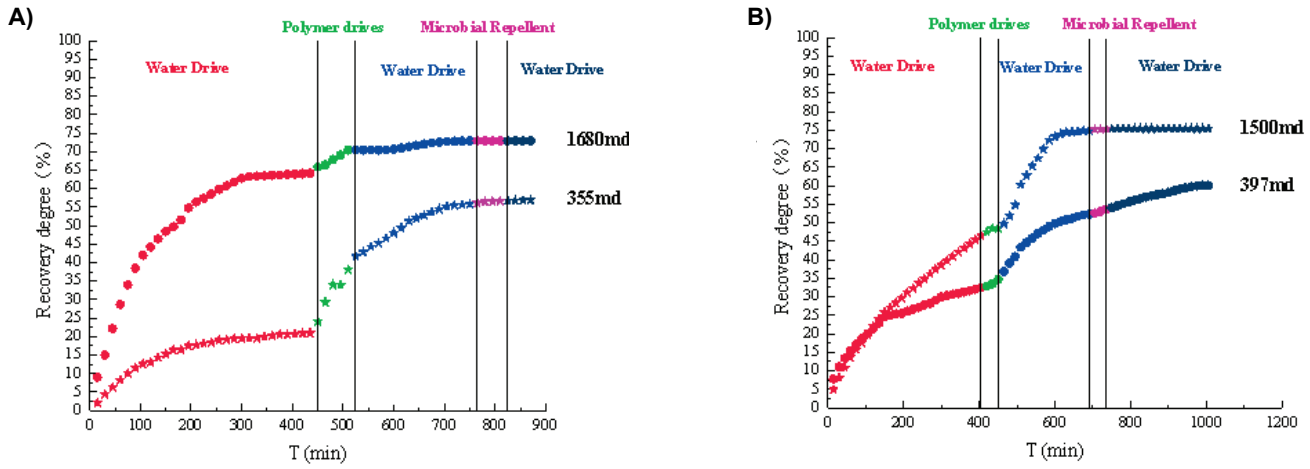

Figure 4. Results of two groups of oil displacement experiments. In the control group **A),** because there is no Ca^{2+}, no plugging occurs, and the ability to improve oil recovery is limited. In the Group **B)**, sealing with MICP after polymer drive can further mobilize the residual polymer and residual oil in the low permeability reservoir to achieve enhanced recovery.

of urea is hydrolyzed into 1 molecule of carbamate and 1 molecule of ammonia. Second, 1 molecule of carbamate is spontaneously hydrolyzed to synthesize 1 molecule of carbonic acid and a 2 molecules of ammonia. Accordingly, 1 molecule of urea hydrolysate consists of 2 molecules of ammonia and 1 molecule of carbonic acid. Under the physiological pH, carbonic acid protons are dissociated, and ammonia molecules are protonated under the action of water molecules, thereby resulting in a net increase in the pH (29). This process constitutes Positive feedback adjustment, and it is suggested to exceed the suitable reaction of the urease inside *Bacillus subtilis* when the environmental pH is 8. The urease activity decreases, and the pH increases slowly. The mentioned process is transformed into negative feedback adjustment till the pH is close to 9, and the urea decomposition reaction is terminated. Accordingly, the glucose content in the medium in the oil repelling experiment was set to 2 $g.L^{-1}$. On the one hand, the bacterium reached the logarithmic stage of growth with high activity in the 24-h incubation. On the other hand, the pH of the bacterium ranged from 5 to 6 at this time, the microorganism did not decompose considerable urea in the medium, and the bacterium failed to be combined with cations (e.g., Ca^{2+} and Mg^{2+}) required for the mineralization to produce precipitation in the subsurface. Thus, the microorganisms exhibited a long transport distance in the subsurface environment, thereby increasing the depth and efficiency of the drive, instead of gathering around the injection well. As a consequence, wear and tear of production equipment and damage to the reservoir were caused (30-31). However, it is noteworthy that we only discussed the metabolism of the medium containing glucose in this study. It was inferred that in the engineering application of using *Bacillus subtilis* for microbial mineralization, using common industrial fermentation carbon sources (e.g., molasses or corn syrup) may exert the same effect, whereas further research should be conducted to determine the amount of addition and its effect on the engineering effect.

5.2. Discussion of Oil Repelling Experiment

In the oilfield development, the heterogeneity of the reservoir significantly impacts the development effect. The “water finger” phenomenon is suggested to artificially supplement energy at the respective stage of the development of the oilfield, considerable unused crude oil remains in the pores (33), and selective plugging of high-permeability layers is a common practice in oilfields (33-35). For the plugging of substances (e.g., extracellular polysaccharides) secreted by biomass and microorganisms, the reservoir should have matching pore throats and appropriate supplementary carbon sources to maintain the growth and metabolism of microorganisms (33), and popular chemical plugging agents aim to achieve long-term effects. Such an aim imposes a higher burden on the water treatment

equipment and the stratum environment on site (34-37). Existing studies elucidated the mechanism of various mineralized microorganisms to plug porous media (38-39) and proposed their potential applications of the microorganisms in facilitating oil recovery. Our experiments revealed that microbial mineralization still has promising applications after chemical flooding (**Fig. 4**). In the control A, with the growth of bacteria and the increase in the biomass, the oil recovery was not significantly improved. The analysis of this study was based on the higher overall reservoir permeability. The bacteria and the biomass would be carried out of the reservoir with the water flow and could not be blocked in the pores and throats of the reservoir. Moreover, the viscosity data after 48 h revealed that the bacteria failed to proliferate extracellular polymers in the reservoir to cause the viscosity to drastically change (40). Given the mentioned two reasons, a conclusion was drawn that the blockage of calcium carbonate precipitation induced by *Bacillus subtilis* in the high permeability reservoir could be the cause of the increase in oil production of Group B.

The oil drive experiment group B achieved the better plugging effect of the high permeability model, with the subsequent oil production of 0 and unchanged degree of recovery. Besides, the fluid production of the low permeability model fluctuated and then increased, and lastly it was stabilized. As a result, residual polymer was produced in the water collected. The reason for this result is presented below. The high-permeability reservoir was breached by water introduced, and then the water primarily flowed in the high-permeability reservoir. Subsequently, the crude oil could not be displaced by polymer in the low-permeability reservoir, thereby causing the polymer to be partially residual in the low-permeability model after the high permeability reservoir was plugged, the subsequent water entered the low permeability model and moved the participating polymers, which to a certain extent caused the fluid production to fluctuate. Furthermore, our experiment verified the conclusion of Kirkland *et al*. (32) that under the same oil and water, microorganisms can still metabolize the urea to complete the mineralization process.

6. Conclusion

By analyzing the utilization of glucose and urea by *Bacillus subtilis*, we found that the addition of appropriate amount of glucose in the medium can delay the fermentation glucose metabolism by the bacteria first to produce bio acid, and using this metabolism can delay the microorganism to enter the decaying period and the time of precipitation thus preventing the blockage near the well end.

Bacillus subtilis can induce mineralization under the condition of simultaneous presence of oil and water, and the effect can be seen after one treatment, and the reservoir permeability decrease rate reaches 88.6%. Recovery rate increased again by 7.9%.

The effect of adjusting the water absorption profile can be achieved by injecting 0.2 PV bacterial solution and cementing solution into the high permeability layer after polymer drive, and at the same time, the polymer retained in the low permeability model can be used to increase the polymer utilization rate.

Acknowledgment

Supported by Open Fund of Key Laboratory of Exploration Technologies for Oil and Gas Resources (Yangtze University), Ministry of Education, NO PI2021-06.

Educational Commission of Hubei Province of China, D20201302

Supported by the National Natural Science Foundation of china, Grant No.52174019

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