Supplementary file

Fracture permeability reduction and sealing mechanisms of microbial cementation in underground fractured media: Application to low-permeability reservoirs

Yanlong He[1,2,3], Shizi An[1,4], Tayfun Babadagli[1,5], Keyi Liu[1,3], Lu Bai[1,3], Hai Huang[1,2,3*]

[1] *School of Petroleum Engineering, Xi'an Shiyou University, Xi'an 710065, P. R. China*

[2] *Shaanxi Key Laboratory of Carbon Dioxide Sequestration and Enhanced Oil Recovery, Xi'an 710065, P. R. China*

[3] *Engineering Research Center of Development and Management for Low to Ultra-Low Permeability Oil & Gas Reservoirs in West China, Ministry of Education, Xi'an 710065, P. R. China*

[4] *College of Petroleum Engineering, China University of Petroleum, Beijing 102249, P. R. China*

[5] *Department of Civil and Environmental Engineering, Faculty of Engineering, University of Alberta, Edmonton T6G 1H9, Canada*

E-mail address: stpnet@126.com (Y. He); anshizi1234@163.com (S. An); tayfunbabadagli@hotmail.com (T. Babadagli); 24212010182@stumail.xsyu.edu.cn (K. Liu); 13299197451@163.com (L. Bai); huanghai@xsyu.edu.cn (H. Huang).

[*]Corresponding author (ORCID: 0000-0002-8741-2897 (H. Huang))

He, Y., An, S., Babadagli, T., Liu, K., Bai, L., Huang, H. Fracture permeability reduction and sealing mechanisms by microbial cementation in underground fractured media: Application to low-permeability reservoirs. Advances in Geo-Energy Research, 2026, 20(1): 85-97.

The link to this file is: https://doi.org/10.46690/ager.2026.04.07

Appendix A: Method. S0 Preparation of urea culture medium

The first medium was a urea liquid medium containing 5 g/L NaCl, 20 g/L urea, 2 g/L glucose, and 2 g/L peptone. First, a liquid culture medium without urea and glucose was prepared, and 0.1% resazurin solution and phenol red reagent were added. The medium was heated to boiling in an electric furnace and maintained at this temperature for 30 min. During this process, high-purity nitrogen was injected into the medium. The deoxygenated medium was packed into 30 mL serum bottles with rubber stoppers. After sterilization, high-purity nitrogen was used to replace residual oxygen in the sample using a vacuum pump and a sterile needle (Dong et al., 2022). Second, urea and glucose solutions were injected into the above deoxygenated and sterilized culture medium in an anaerobic glove box using a syringe and a 0.22 μm filter. The urease screening medium was similar to the urea liquid medium in terms of preparation. The only difference was the addition of 20 g/L agar to the urease screening medium. The above urea liquid medium and urease screening medium were used as anaerobic mediums.

Appendix B: Method. S1 Enrichment, screening, and cultivation of microorganisms

The rock cuttings were gathered at the drilling site, placed in sterile bottles, and stored at 4 °C. The rock cuttings were tested immediately upon arrival at the laboratory. Briefly, 5 g of rock cuttings were added to 50 mL of sterile normal saline, shaken, and allowed to rest. Then, 2 mL of supernatant was injected into the urea liquid medium using a syringe, which was then cultured in an anaerobic incubator. The medium was observed to see if it turned red. The red culture medium samples were selected, and the red urea liquid medium was pipetted into a centrifuge tube. The cell concentration was diluted with sterile water to the target gradient of 10^{-4} and 10^{-5}. In addition, the diluted culture was coated on a urease screening medium in an anaerobic glove box. Finally, the strains that caused the medium to turn red were selected from the urease screening medium and transferred to a urea liquid medium in an anaerobic glove box. The urea liquid medium was placed in the anaerobic glove box at 47 ℃ for 24 h. The isolated single-bacteria medium was continually coated and streaked in a urease screening medium. The above operations were repeated until only one type of microorganism existed in the urease screening medium. The single isolated strain was preserved in glycerol solution at -4 °C.

Appendix C: Method. S2 Microbial concentration measurement

One milliliter of stimulated strain solution was inoculated into the above urea liquid medium and cultured at 47 °C and 180 r/min. A spectrophotometer (*Shanghai Jinghua Technology Instrument Co., Ltd.*) was used to measure the OD_{600} value of the culture medium, which indicated the strain concentration in the vial. Samples were taken every 3 h for 72 h. The OD_{600} value of the medium inoculated with only the strain was used to calibrate the spectrophotometer's zero point. The urease activity was measured by the conductivity method. The pH of the culture medium was measured by a pH meter.

Appendix D: Method. S3 Urease activity measurement

The degree of urea hydrolysis is proportional to the solution conductivity. The urease activity of the strain was determined by measuring the change in solution conductivity during urea hydrolysis over time (Maleki et al., 2022). The urease activity was determined with the following two-step process (Wang and Bai, 2018): (1) The strain solution was diluted by ten times. Two milliliters of diluted bacterial solution were combined with 18 mL of 1.5 mol/L urea solution. The solution conductivity was measured with a conductivity meter (*DDS-307, Shanghai INESA Scientific Instrument Co., Ltd.*) at 28 °C for 5 min. (2) The urea hydrolysis degree per unit of time was calculated according to Eq. (1), and the urea hydrolysis degree per unit of time reflected the urease activity.

$$\text{Urea decomposed (mmol/(L·min))} = \text{Conductivity (mS/(cm·min))} \times 10.54 \times 10 \quad \text{(S1)}$$

Appendix E: Fracture model

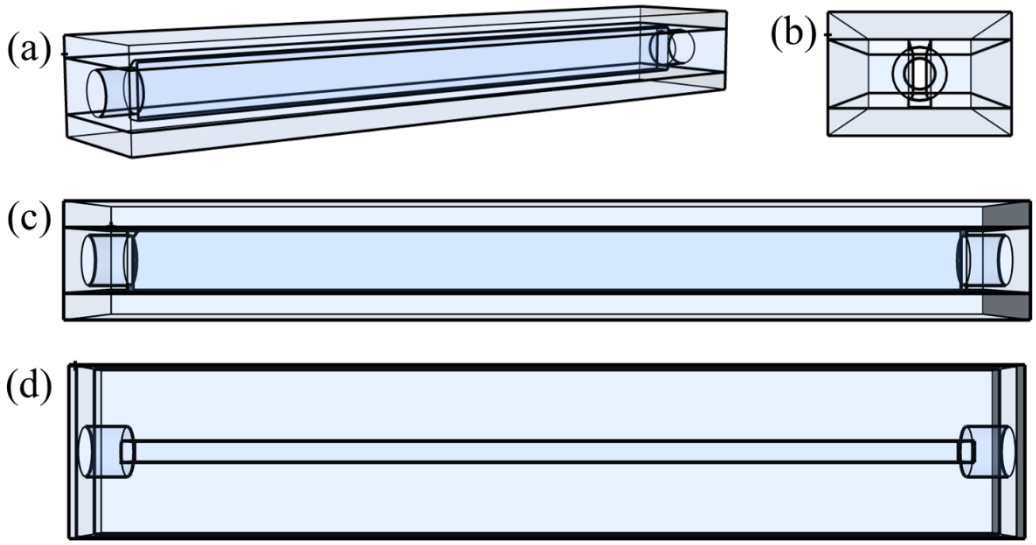

Fig. S1. Fracture model: (a) 3D model; (b) front view; (c) left view; (d) vertical view.

Appendix F: Original and enrichment strain

The culture medium turns red. An indigenous urease-producing anaerobic microorganism was found in the reservoir of the Yanchang Formation by comparing the original culture medium with the final culture medium.

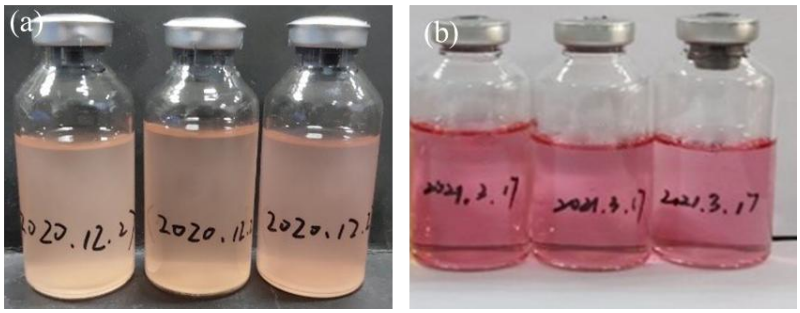

Fig. S2. Original culture medium of strain (a) and enrichment medium of strain (b).

Appendix G: Sequencing results of strains HN-01

The microorganism was identified as Bacillus megaterium based on 16S rDNA evidence.

AAGTCGAGCGAACTGATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACA
CGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGG
ATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGC
GGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAG
AGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAG
GGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTT
CGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACAAGAGTAACTGCTTGTACCTTGAC
GGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGC
AAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGA
AAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGA
AAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGA
AGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATT
AGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTT
AGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTC
AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCG
AAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGG
GGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT
CCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGAC
TGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTG
GGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAAT
CCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGC
TAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGAGTAACCGTAAGGA

Fig. S3. Sequencing results of strains HN-01.

Appendix H: Single factor optimization experiment results

Based on single-factor experimental results, sucrose was identified as the best carbon source and peptone as the best nitrogen source.

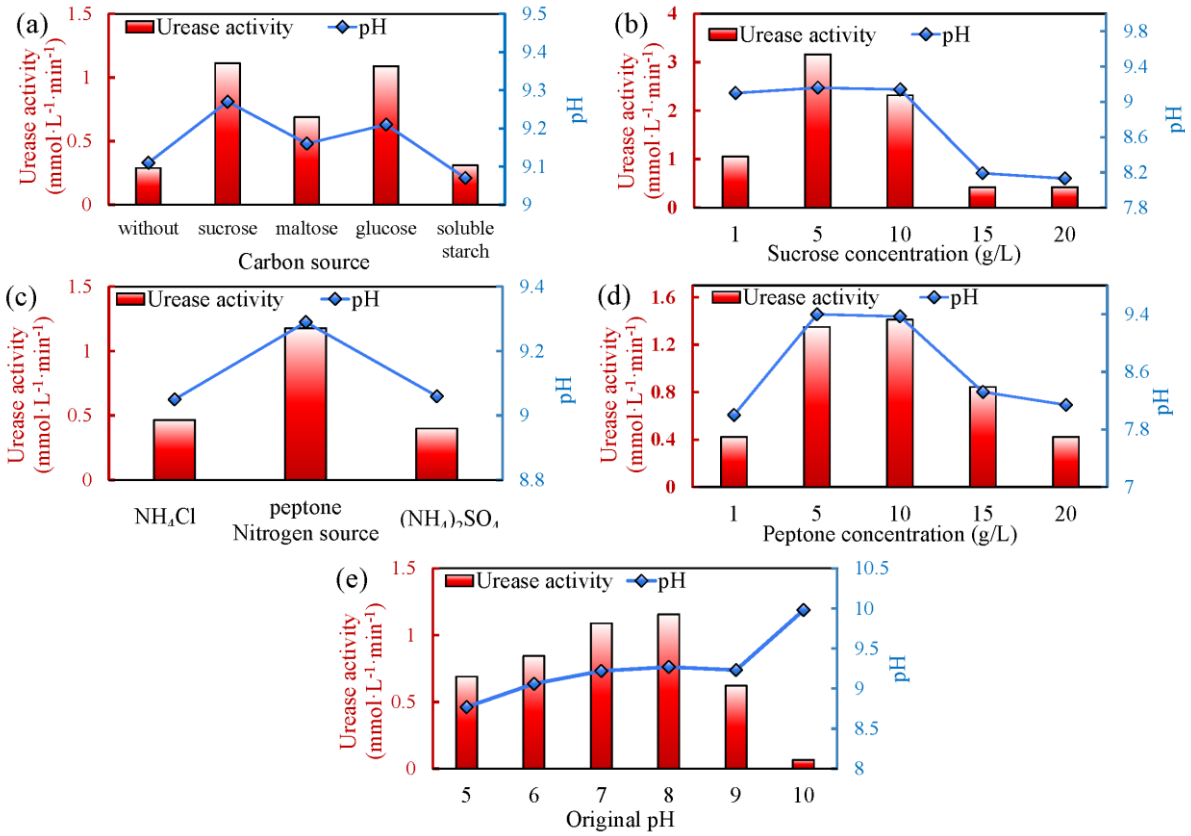

Fig. S4. Single factor optimization experiment results: (a)-(e) Optimization of Carbon source, sucrose concentration, nitrogen source, peptone concentration, initial urea concentration.

Appendix I: Static growth process of microbial cement

On the third day, large amounts of extracellular polymers (EPS) were produced in the anaerobic bottle. Microbial cement was not produced, and the medium pH gradually increased.

All the extracellular polymers were dissolved on the seventh day, and microbial cement crystals were produced at the bottom of the anaerobic bottle. According to classical nucleation theory, heterogeneous nucleation (nucleation on surfaces) is energetically more favorable than homogeneous nucleation (nucleation within a uniform phase).

A large amount of microbial cement was produced at the bottom and on the walls of the anaerobic bottle by the 20th day of culture.

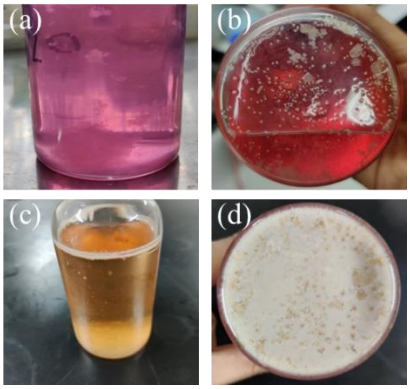

Fig. S5. Static growth process of microbial cement: (a) 3rd day of cultivation of microorganisms; (b) 7th day of cultivation of microorganisms; (c), (d) 20th day of cultivation of microorganisms.

Appendix J: Concentration of injected bacteria

The concentration of the injected microorganisms is OD600=2.3-2.6

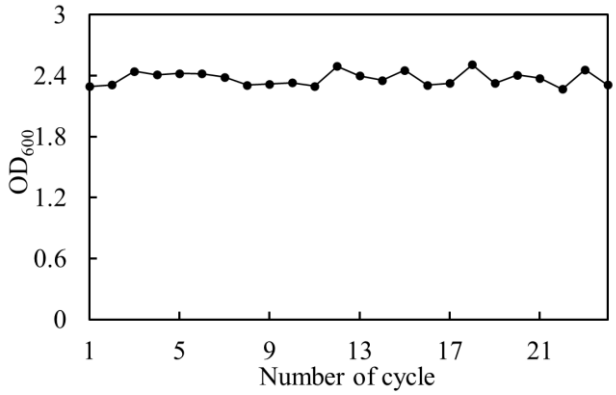

Fig. S6. Concentration of injected bacteria.

Appendix K: Repair effect of fractures with different aperture

Distribution of microbial cement in fractures of 0.5, 0.3, and 0.05 mm.

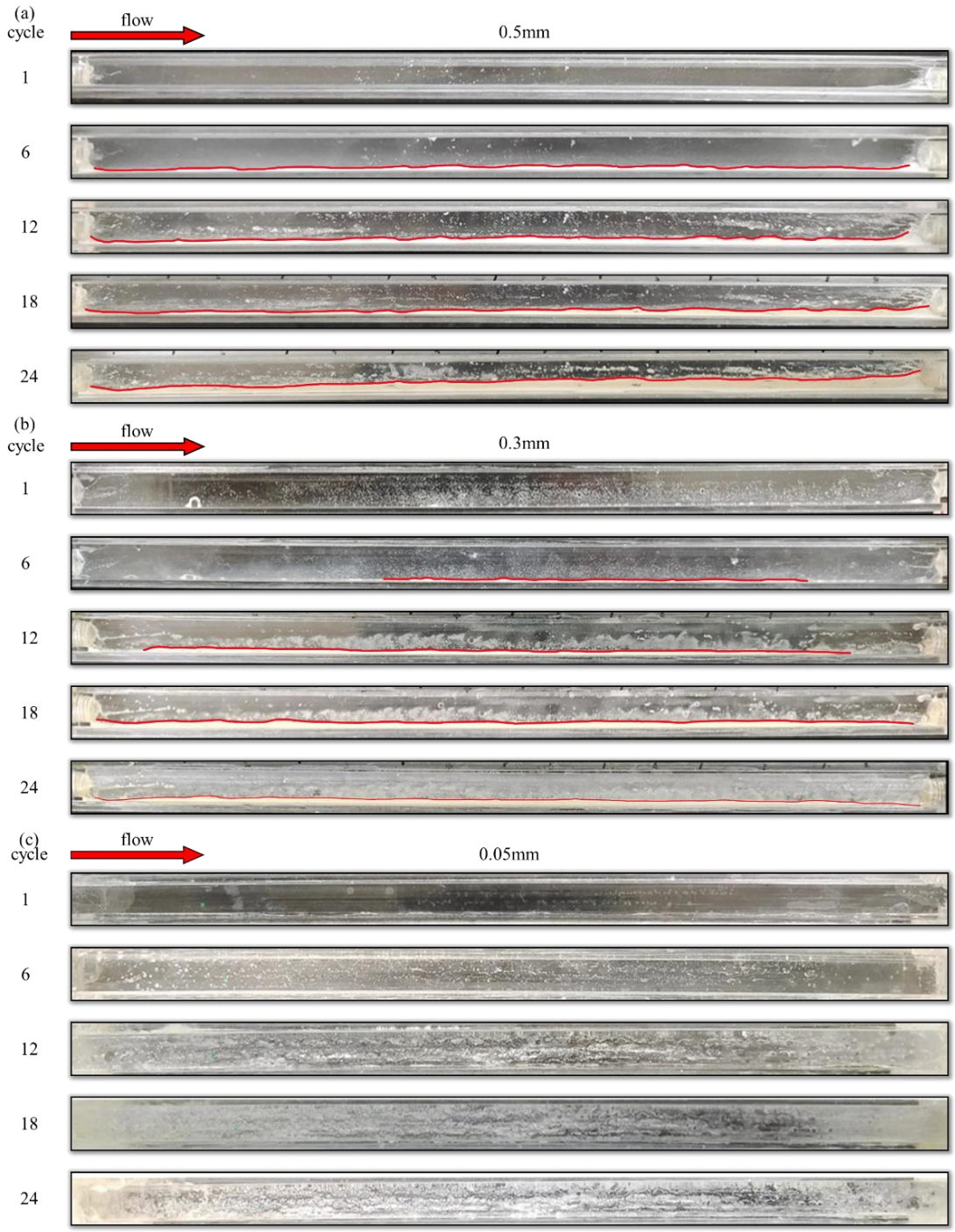

Fig. S7. The repair effect of fractures with different aperture: (a-c) the fracture aperture is 0.5, 0.3, and 0.05 mm.

Appendix L: Repair effect of core with 0.3mm fracture aperture

In the 0.3 mm, fracture permeability decreased from 3.305 to 0.219 μm^2, with a reduction of 93.38%. After the 8th injection cycle, the decline rate of fracture permeability decreased. The permeability data were fitted, and the slope of the fitting curve decreased from 0.3486 to 0.0596.

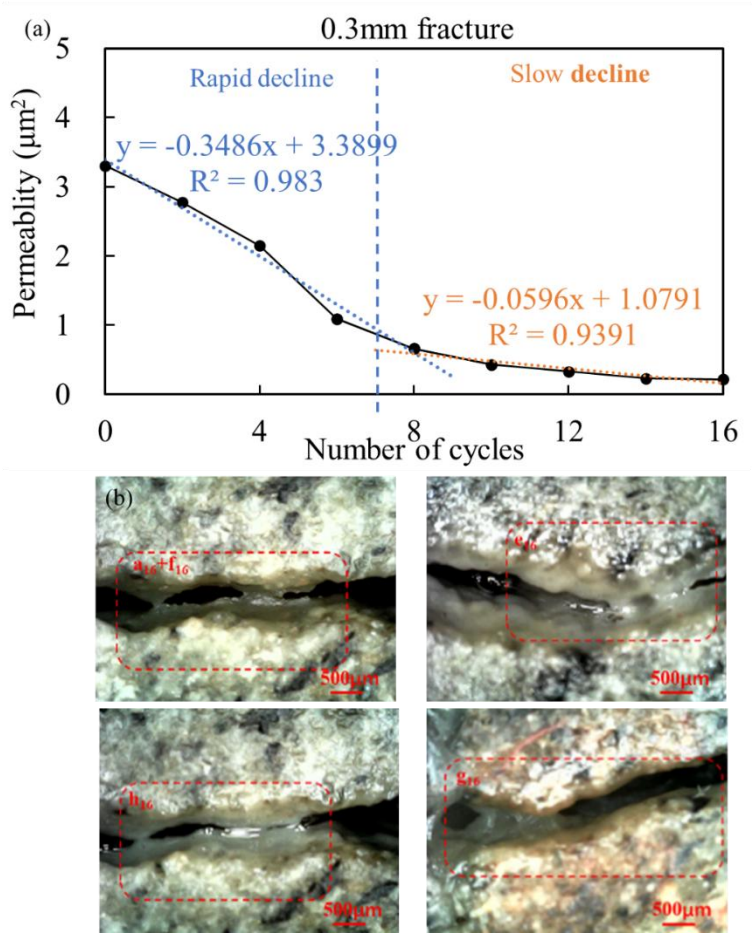

Fig. S8. The repair effect of core with 0.3mm fracture aperture: (a) Permeability change and (b) distribution of microbial cement on the end face of fractured core.

Appendix M: Variance Analysis

The results of the variance analysis were obtained from the quadratic model. The influencing factor was significant when the p-value was less than 0.05. Table S1 shows that the two factors with the greatest influence on urease activity were peptone concentration and urea concentration.

Table S1. Analysis of variance (ANOVA) for response surface Quadratic model obtained from experimental design.

Source	Urease activity			pH		
	Sum of Squares	DF	*p*-value	Sum of Squares	DF	*p*-value
Model	24.34	9	<0.0001	20.48	9	<0.0001
X_1	0.1557	1	0.1661	0.9180	1	0.0033
X_2	0.6535	1	0.0158	1.70	1	0.0006
X_3	1.57	1	0.0017	7.07	1	<0.0001
X_1X_2	0.0000	1	0.9837	0.3364	1	0.0330
X_1X_3	1.42	1	0.0023	0.1406	1	0.1305
X_2X_3	0.0687	1	0.3387	0.0056	1	0.7420
X_1^2	2.81	1	0.0003	0.9104	1	0.0033
X_2^2	11.49	1	<0.0001	0.0009	1	0.8922
X_3^2	4.25	1	<0.0001	9.01	1	<0.0001
Residual	0.4564	7		0.3357	7	
Lack of Fit	0.0833	3		0.0787	3	
Pure Error	0.3731	4		0.2570	4	
Cor Total	24.80	16		20.82	16	

Reference

Dong, H., Zhang, F., Xu, T. et al. Culture-dependent and culture-independent methods reveal microbe-clay mineral interactions by dissimilatory iron-reducing bacteria in an integral oilfield. Science of the Total Environment, 2022, 840: 156577.

Maleki-Kakelar, M., Aghaeinejad-Meybodi, A., Sanjideh, S. et al. Cost-effective optimization of bacterial urease activity using a hybrid method based on response surface methodology and artificial neural networks. Environmental Processes, 2022, 9(1): 7.

Wang, Z., Bai, B. Preformed-particle-gel placement and plugging performance in fractures with tips. SPE Journal, 2018, 23(6): 2316-2326.