MATERIALS SCIENCE

Charge-reversal surfactant antibiotic material for reducing microbial corrosion in petroleum exploitation and transportation

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The corrosions caused by sulfate-reducing bacteria (SRB) are serious problems in petroleum exploitation and transportation, which can lead to safety problems, environmental pollutions, and economic losses. Here, a charge-reversal surfactant antibiotic material *N*-dodecyl-1-carboxylic acid-1-cyclohexenyl-2-carboxamide (C12N-DCA) is designed and synthesized. C12N-DCA is a negatively charged surfactant, which cannot be adsorbed by soil and rock in a large amount. Therefore, it can reach the "lesion location", with enough concentration. After being hydrolyzed and charge reversed under the acceleration of H_2S produced by SRB, C12N-DCA becomes a positively charged surfactant dodecane ammonium salt to kill SRB. Through a simulating experiment, it is found that C12N-DCA can reach the SRB inhibition ratio of almost 100%, and it can reduce iron corrosion by 88%. Such an antibiotic material or its homologs may be added to the chemical flooding fluids, killing SRB during petroleum exploitation and reducing the SRB-induced corrosion in the petroleum exploitation and transportation.

INTRODUCTION

Corrosion is a serious problem in the petroleum industry (*1*–*3*). On the one hand, corrosion causes device damage or pipeline perforation, resulting in short circuit, leakage, or other serious problems of safety and environmental pollution (*4*, *5*). On the other hand, the corrosion of steel also gives rise to great economic losses (*2*, *3*). Among all the corrosions in the petroleum industry, microbial corrosion accounts for a large proportion (*6*), mainly in the following three aspects. First, the polymer corrosion of chemical flooding fluids in the oil field comes from the decomposition of polymers in chemical flooding fluids by anaerobic microorganisms (*7*), which leads to the decrease in efficiency of displacement of reservoir oil. Second, corrosion of steel in exploitation and pipeline transportation basically arises from the metabolic processes and metabolites, which results in device damage, pipeline blockage, or perforation (*3*, *8*). Last, corrosion of steel in water stations and recycled water pipelines (*9*) is same as that in pipeline transportation, which causes frequent maintenance and replacement of devices and pipelines. In general, microbial corrosion is mainly caused by the metabolic process and metabolites of microorganisms (*10*), while microorganisms in the soil extracted during petroleum exploitation are the source of the above microorganisms.

Of all the microorganisms that cause corrosion, sulfate-reducing bacteria (SRB) are generally considered to be the most harmful ones (*10*–*13*). This kind of bacteria can reduce sulfate to sulfur ions, being in the form of hydrogen sulfide or ferrous sulfide, thus causing serious corrosion to steel (*14*, *15*). In addition, SRB, as an anaerobic microorganism, may cause decomposition of the components in the chemical flooding fluids and reduce the efficiency of displacement of reservoir oil (*16*). There are many SRB antibiotics used in petroleum exploitation, including quaternary ammonium surfactants, chlorine dioxide, etc. However, they are not satisfactory enough. For example, quaternary ammonium surfactants lose efficacy after being mixed with anionic surfactants (*17*–*19*), which are usually used as chemical flooding agents. In addition, they are easily adsorbed by negatively charged soil, thereby not being able to reach the "lesion location" and interact with SRB. They even cause the overall retention of the chemical flooding fluids due to the adsorption, thus increasing the cost of displacement of reservoir oil. Chlorine dioxide, as another example, has poor stability and persistence. Consequently, these antibiotics are only applicable to water stations and recycled water pipelines. Other anti-SRB methods such as biological competition with denitrifying microorganisms are not mature enough (*20*). Pipeline protection by painting lacquer is too expensive to achieve 100% coverage (*21*). Therefore, it is important to develop new kinds of antibiotic materials in petroleum exploitation.

We wondered whether there was an antibiotic material that could kill SRB during petroleum exploitation and reduce the corrosion of SRB accordingly. To this end, the antibiotic material requires to be cheap and of good compatibility with the chemical flooding fluids, which means it will not have much negative impact on the performance of chemical flooding agents. It is necessary for the antibiotic material to avoid being completely adsorbed by soil or extracted by petroleum, for it to be able to reach the lesion location to interact with SRB. Considering that the negatively charged species could meet the requirements of compatibility and nonadsorbability while positively charged surfactants were widely used in antibiosis (*22*–*27*), we designed and synthesized a charge-reversal surfactant antibiotic material, *N*-dodecyl-1-carboxylic acid-1-cyclohexenyl-2-carboxamide (C12N-DCA), as an SRB antibiotic material. Charge-reversal system refers to a kind of system that can realize the positive and negative inversion of charge under certain external conditions (*28*–*32*). As shown in Fig. 1 and movie S1, C12N-DCA is a negatively charged surfactant initially, which might have good compatibility with the chemical flooding fluids. It would not be adsorbed by soil and rock in a large amount. Therefore, it could reach the lesion location with enough concentration. Because H_2S, the metabolite of SRB (*11*), increases the acidity of the microenvironment, the hydrolyzation and charge reversal of C12N-DCA would be accelerated. As a result,

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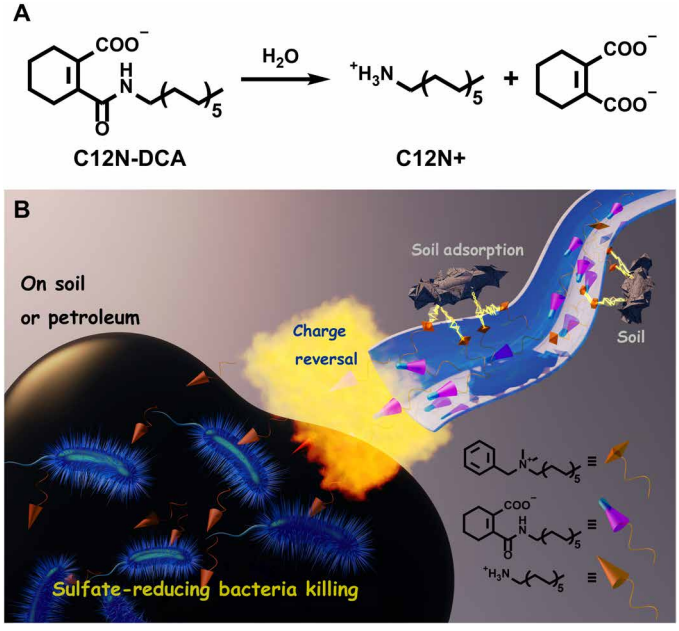

Fig. 1. Charge-reversal and antibacterial function of C12N-DCA. (**A**) Charge-reversal reaction of C12N-DCA. (**B**) Schematic representation of the antibacterial process of charge-reversal antibacterial agent during petroleum extraction.

C12N-DCA would become a positively charged surfactant dodecane ammonium salt (C12N+) to kill SRB. Besides, as a surfactant, C12N-DCA could be partially distributed in the water phase, so it might take effect even in the presence of petroleum. In addition, the raw materials to synthesize C12N-DCA are cheap and the synthetic process is simple (fig. S1). It is anticipated that such an antibiotic material or its homologs can be added to the chemical flooding fluids, killing SRB during petroleum exploitation and reducing the SRB-induced corrosion in petroleum exploitation and transportation accordingly.

RESULTS

Establishment of measuring method of relative content of *Desulfovibrio desulfuricans*

To evaluate the antibacterial activity of C12N-DCA to SRB, we established a new measuring method of relative content of SRB, taking *D. desulfuricans* (DSM 642) as a representative of SRB. Traditional measuring methods include extinction dilution method (*33*), APS (adenosine 5'-phosphosulfonate) reductase assay (*34*), ATP (adenosine 5′-triphosphate) bioluminescence assay (*35*), polymerase chain reaction assay (*36*), etc. These methods are complex in operation, or not suitable for high-throughput detection, or too expensive. So, it is highly demanded if a simple, efficient, and cheap method for SRB content measurement can be established. Cell Counting Kit-8 (CCK-8) assay is a method to detect cell viability based on electron transfer medium (*37*, *38*). Its advantages consist of simple operation, high-throughput detection, and low cost. However, no report has been published yet on measuring relative content of SRB by CCK-8 assay. Therefore, we explored the possibility of measuring relative content of SRB using CCK-8 assay. As shown in Fig. 2A, in the range of relative content of *D. desulfuricans* from 0 to 1 (about 10^3 per milliliter; fig. S2), the absorbance at 450 nm and relative content of *D. desulfuricans*

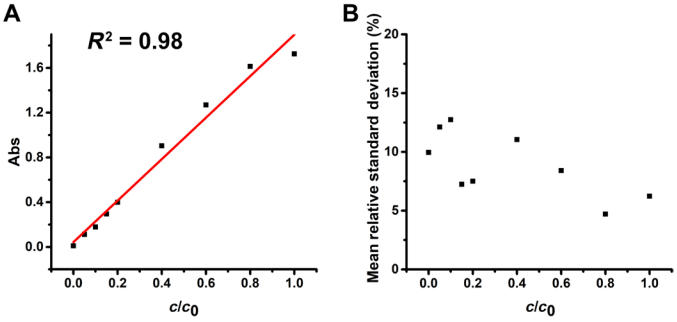

Fig. 2. The evaluation of CCK-8 assay. (**A**) Correlation between average absorbance at 450 nm and relative content of *D. desulfuricans* after incubation at 37°C for 30 min with CCK-8. (**B**) Mean relative SD of different relative content of *D. desulfuricans*. The concentration of cultured *D. desulfuricans* was about 10^3 per milliliter as indicated by QuickChek SRB detecting result (fig. S2). The adding amount of CCK-8 was 1:10 (v:v).

showed a linear relationship (R^2 = 0.98). This indicates that the relative content of SRB can be calculated by *D* value ratio of absorbance directly, which facilitates the measurement of the relative content of SRB and the evaluation of the antibacterial activity of C12N-DCA to SRB. Furthermore, the mean relative SDs under different relative contents were analyzed (Fig. 2B). The maximum mean relative SD was no more than 13%, which is precise enough to meet the requirements of the analysis of a biological sample. Besides, the detection limit could be reduced by extending the culture time or increasing the amount of CCK-8. Therefore, the CCK-8 assay is suitable for this work, in which the concentration of cultured SRB (without antibiotics) was about 10^3 per milliliter. Considering that SRB is also an important engineering strain for sewage treatment (*39*–*40*), this method is not only suitable for this study but may also be promoted as a conventional method for SRB detection.

Antibacterial activities and mechanism of C12N-DCA and C*n*N-DCA

We first studied the antibacterial activity of C12N-DCA with the measuring method described above. C12N+, the product of charge reversal, was used as the positive control. *N*-dodecyl-1-cyclohexanecarboxylic acid-2-carboxamide (C12N-CCA), which is similar in structure but hardly hydrolyzed under the experimental conditions, was used as the negative control. The antibacterial activities of C12N-DCA, C12N+, and C12N-CCA were tested, respectively. The experimental results are shown in Fig. 3A. The MIC_{50} (minimum inhibitory concentration required to inhibit the growth of 50% of organisms) of C12N-DCA was about 118 μM. C12N+ exhibited higher antibacterial activity than C12N-DCA, of which the MIC_{50} was about 25 μM. The negative control C12N-CCA did not exhibit clear antibacterial activity under 200 μM. Since the two control groups and C12N-DCA have the same hydrophobic alkyl chain structure, the difference in antibacterial ability should arise from the difference in the head group: C12N+ is the pure product of charge reversal, while C12N-CCA will hardly undergo charge reversal under experimental conditions. After added C12N+, as a positively charged surfactant, it could interact with SRB immediately, leading to the death of bacteria. While C12N-DCA needed time to undergo hydrolysis and charge reversal. It took about 11 hours for C12N-DCA to be hydrolyzed completely at pH 6 at 37°C as shown in fig. S3. C12N-DCA could interact with SRB only after

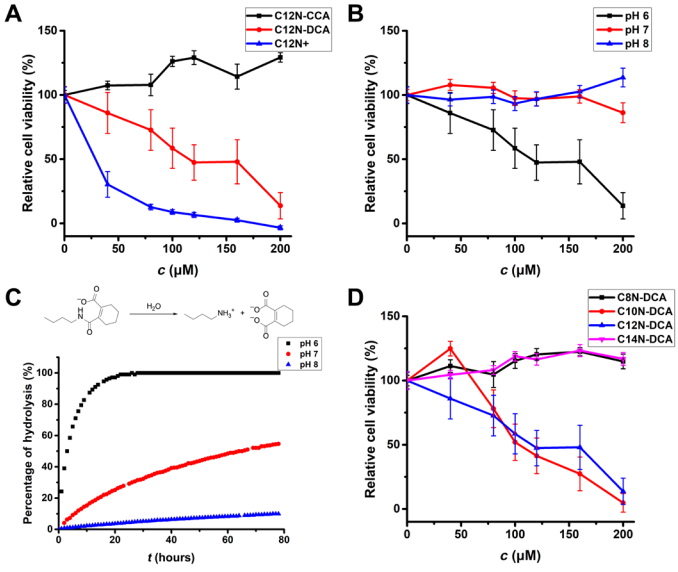

Fig. 3. The antibacterial activities and mechanism of C*n*N-DCA. (**A**) Relative content of *D. desulfuricans* after being anaerobically incubated at 37°C for 3 days with C12N-DCA, C12N+ (positive control), and C12N-CCA (negative control). Incipient pH 6, $n = 12$. (**B**) Relative content of *D. desulfuricans* after being anaerobically incubated at 37°C for 3 days with C12N-DCA at incipient pH 6, 7, and 8. $n = 12$. (**C**) Hydrolysis reaction and hydrolysis kinetics of C4N-DCA at pH 6, 7, and 8 (pH buffer) at 20°C. (**D**) Relative content of *D. desulfuricans* after being anaerobically incubated at 37°C for 3 days with C8N-DCA, C10N-DCA, C12N-DCA, and C14N-DCA. Incipient pH 6, $n = 12$. All relative cell viabilities were measured by CCK-8 assay on the basis of the method established above. Data represent means ± SE.

transforming into positively charged surfactant. Thus, the antibacterial activity of C12N-DCA was lower than the antibacterial activity of the positive control C12N+. As for C12N-CCA, it could hardly be hydrolyzed, and the charge could not be reversed to be a positively charged surfactant, thus exhibiting no antibacterial activity. The antibacterial activity of C12N-DCA is between that of C12N+ and C12N-CCA, which is high enough to inhibit the growth of SRB.

The antibacterial mechanism of charge reversal was supported by the antibacterial activities of C12N-DCA at different pH values. As shown in Fig. 3B, at pH 6, C12N-DCA exhibited antibacterial activity. However, when pH rose to 7 and 8, the antibacterial activities of C12N-DCA decreased significantly. This result accorded with the hydrolysis kinetics of the model molecule *N*-butyl-1-carboxylic acid-1-cyclohexenyl-2-carboxamide (C4N-DCA) (Fig. 3C). C4N-DCA was completely hydrolyzed in about 30 hours at pH 6, but it was hydrolyzed only in half in 78 hours at pH 7 and hardly hydrolyzed at pH 8. The consistency of antibacterial activities and hydrolysis rates under different pH values indicates that the antibacterial activity of C12N-DCA originates from the charge reversal and the transformation into positively charged surfactant consequently. The antibacterial activities of C12N-DCA at other pH and temperature also supported the mechanism. As shown in figs. S4 and S5, C12N-DCA exhibited better antibacterial activity at lower pH under acidic conditions. Besides, it also exhibited antibacterial activity at pH 11 at 37°C because of the hydrolysis of amide bond under a relatively strong basicity. The antibacterial activities of C12N-DCA at 37°C were higher than those at 25°C owing to the higher solubility and hydrolysis rate. This result suggests that it may work better at relatively high temperatures of oil reservoir.

To have a better understanding on the relationship between the antibacterial activities and the chemical structure of charge-reversal surfactant antibiotic materials, the antibacterial activities of C*n*N-DCA with different alkyl lengths ($n = 8$, 10, 12, and 14) were investigated. As shown in Fig. 3D, *N*-octyl-1-carboxylic acid-1-cyclohexenyl-2-carboxamide (C8N-DCA) and *N*-tetradecandioic-1-carboxylic acid-1-cyclohexenyl-2-carboxamide (C14N-DCA) did not exhibit antibacterial activities under experimental conditions, while *N*-decyl-1-carboxylic acid-1-cyclohexenyl-2-carboxamide (C10N-DCA) and C12N-DCA exhibited similar antibacterial activities. A possible reason could be that the alkyl chain of C8N-DCA was short, and the depth of membrane rupture was not enough after charge reversal, which was responsible for its low antibacterial activity. For C14N-DCA, its low solubility and effective concentration under the experimental conditions resulted in the low antibacterial activity as well. With balanced alkyl lengths and solubility, both C10N-DCA and C12N-DCA display good antibacterial activity. To confirm this hypothesis, the antibacterial activities of C8N+ and C14N+ were tested. As shown in fig. S6, C8N+ did not exhibit antibacterial activity under experimental conditions, indicating that C8N-DCA does not kill SRB even if it is hydrolyzed. Therefore, the short alkyl length is the reason why C8N-DCA does not kill SRB. In addition, C14N+ displayed lower antibacterial activity than that of C12N+, indicating the low effective concentration of C14N+. And, the low effective concentration of C14N+ leads to the low antibacterial activity of C14N-DCA.

Influence of petroleum and soil on antibacterial activity of C12N-DCA

As petroleum and soil may exist around SRB in the real environment, the influence of the presence of electric desalted petroleum and montmorillonite on the antibacterial activity of C12N-DCA was studied. The growth state of *D. desulfuricans* was observed directly as shown in Fig. 4A. The formation of black iron sulfide precipitate (in 0 and 100 μM groups) indicated the growth of *D. desulfuricans*. Further quantitative analysis showed that C12N-DCA could still kill *D. desulfuricans* (to detection limit) with the concentration above 200 μM in the presence of electric desalted petroleum and montmorillonite (Fig. 4B). The antibacterial activity of C12N-DCA was basically the same as that without adding electric desalted petroleum and montmorillonite. This result indicated that C12N-DCA was not adsorbed by soil immediately after charge reversal. The charge-reversed form of C12N-DCA (C12N+) had enough time to interact with SRB. Similarly, C12N-DCA and the charge-reversed form of C12N-DCA (C12N+) were not extracted to the oil phase completely by petroleum. Therefore, C12N-DCA can still exhibit good antibacterial activity on SRB in the presence of petroleum and soil.

Antibacterial activity and inhibiting effect on SRB-induced corrosion of C12N-DCA in a simulating system

The antibacterial activity of C12N-DCA on SRB during petroleum exploitation and the inhibiting effect of C12N-DCA on SRB-induced corrosion after exploitation were evaluated through a simulating system. The antibiotic material was first shaken with montmorillonite to simulate the adsorption of soil in the water injection process and then used in antibacterial and anticorrosive experiments. Benzalkonium chloride (BZK), as a representative of commercial quaternary ammonium surfactant antibiotics, was also studied in these experiments as a comparison. As shown in Fig. 5 (A and B),

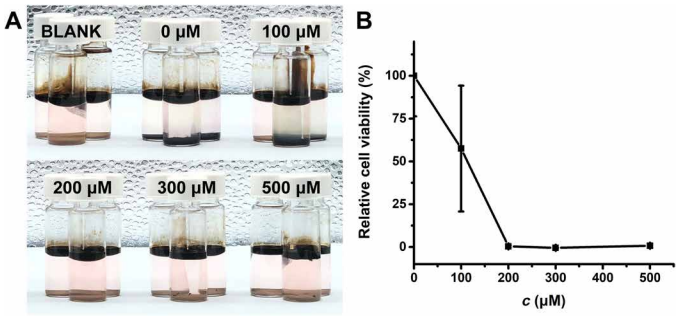

Fig. 4. The antibacterial activity of C12N-DCA in the presence of petroleum and montmorillonite. (**A**) Photos of culture systems after being anaerobically incubated at 37°C for 3 days with C12N-DCA in the presence of petroleum and montmorillonite. The upper layer in each vial was electric desalted petroleum. There was neither C12N-DCA nor *D. desulfuricans* in the BLANK group. The concentration referred to the concentration of C12N-DCA. Incipient pH 6. (**B**) Relative content of *D. desulfuricans* after being anaerobically incubated at 37°C for 3 days with C12N-DCA in the presence of petroleum and montmorillonite. Incipient pH 6, $n = 3$. All relative cell viabilities were measured by CCK-8 assay on the basis of the method established above. Data represent means ± SE. Photo credit: L.Z., Tsinghua University.

the relative *D. desulfuricans* viability in the C12N-DCA group was under detection limit after the first culture cycle. C12N-DCA exhibited good antibacterial activity after shaking with montmorillonite. While the inhibition ratio of *D. desulfuricans* in the BZK group was 20%, BZK almost lost its efficacy after shaking with montmorillonite. As the skeleton structure of montmorillonite is a silicate layer with negative charge on the surface, which has a strong cation exchange and adsorption property, the quaternary ammonium surfactant antibiotics are adsorbed by montmorillonite and become ineffective. While C12N-DCA is an anionic surfactant before charge reversal, its adsorption by montmorillonite was not serious. C12N-DCA could still be of enough concentration after interacting with montmorillonite. The situation is similar with the petroleum exploitation process. It is negatively charged on the surface of soil and rock, which leads to adsorption of the quaternary ammonium surfactant antibiotics. As a result, the quaternary ammonium surfactant antibiotics can only reach the lesion location of SRB in a very low concentration, exhibiting low antibacterial activity. In addition, the strong adsorption may even cause the overall retention of the chemical flooding fluids. Therefore, the commercial quaternary ammonium surfactant antibiotics are not suitable for petroleum exploitation. On the contrary, the charge-reversal surfactant antibiotic material C12N-DCA can avoid being adsorbed by rock and soil in a large amount in the water injection process. Therefore, it is expected that C12N-DCA can be distributed over soil and rock and then be hydrolyzed to the charge-reversed form to kill SRB.

The inhibiting effect on SRB-induced corrosion was evaluated after the second culture cycle. The second culture cycle simulated the situation after the petroleum was extracted, in which the water or the petroleum might contact with steel. As shown in Fig. 5(C and D), the *D. desulfuricans* grew both in the C12N-DCA group and the BZK group. However, the C12N-DCA group still had an inhibition ratio of 45%, different from the BZK group where *D. desulfuricans* grew abundantly. Since there was no remedication between the first culture cycle and the second culture cycle, 45% inhibition ratio is acceptable, especially considering that the normal culture cycle of SRB is half a month to 1 month. The inhibiting effect of C12N-DCA on SRB-induced corrosion was shown in Fig. 5 (E and F). The mean corrosion rate of iron foils in the C12N-DCA group was a little bit higher than the background corrosion rate (the mean corrosion rate in the BLANK group), and the net mean corrosion rate in the C12N-DCA group decreased by 88% compared with the net mean corrosion rate in the BACTERIA group. Such a great inhibiting effect on corrosion comes from the inhibition to SRB growth. As for BZK, to our astonishment, it aggravated corrosion markedly and the reason remained unclear. Combined with the results after the first culture cycle, the charge-reversal surfactant antibiotic material C12N-DCA can reach the lesion location with enough concentration and kill SRB after charge reversal other than being adsorbed by rock and soil in a large amount in the water injection process. After the petroleum is extracted, inhibition of C12N-DCA to SRB growth still exists (the second culture cycle), so the corrosion caused by SRB can be reduced significantly.

Compatibility of C12N-DCA and the chemical flooding fluids

To confirm whether C12N-DCA had good compatibility with the chemical flooding fluids, the influences of C12N-DCA on interfacial tensions, surface tensions, and viscosities to four typical small molecular and polymer flooding fluids were studied. As shown in table S1, for sodium dodecylbenzenesulfonate, anionic polyacrylamide, and xanthan gum, both interfacial tensions and surface tensions decreased. While for sodium 1-hexadecanesulfonate, the interfacial tension and surface tension increased a little. The viscosity did not change much for the four flooding fluids. The addition of C12N-DCA has positive effects on the performance of these typical small molecular and polymer flooding fluids, except for saturated sodium 1-hexadecanesulfonate, of which the influence is acceptable. Therefore, C12N-DCA has good compatibility with the chemical flooding fluids and can be added into the flooding system. Furthermore, as an additive, the critical micelle concentration (CMC) and partition coefficient of C12N-DCA were also measured. The CMC of C12N-DCA was about 415 μM (fig. S7), and the partition coefficient between oil and water was 0.29 at 300 μM (fig. S8).

DISCUSSION

The success of C12N-DCA in killing SRB provides a new perspective on reducing microbial corrosion: using charge-reversal surfactant antibiotic materials. Different from conventional quaternary ammonium surfactants, charge-reversal surfactant antibiotic materials are able to avoid being adsorbed by rock and soil in a large amount; thus, it can inhibit growth of bacteria. The charge-reversal functional group could be tailored according to the pH, temperature, mining time, and other conditions of the oil wells, such as different β-carboxylic acid-carboxamide or imine groups. This strategy could also be extended to antibiotic polymers.

In summary, we have successfully designed and constructed a charge-reversal surfactant antibiotic C12N-DCA. C12N-DCA can reach the lesion location with enough concentration and kill SRB after charge reversal other than being adsorbed by rock and soil in a large amount in the water injection process, and consequently reduce the corrosion caused by SRB significantly. As cationic surfactant is generally considered as a broad-spectrum antibiotic material, the inhibition on other microorganisms and relevant corrosions is highly expected. It is anticipated that such an antibiotic material or its homologs can be added to the chemical flooding fluids, killing

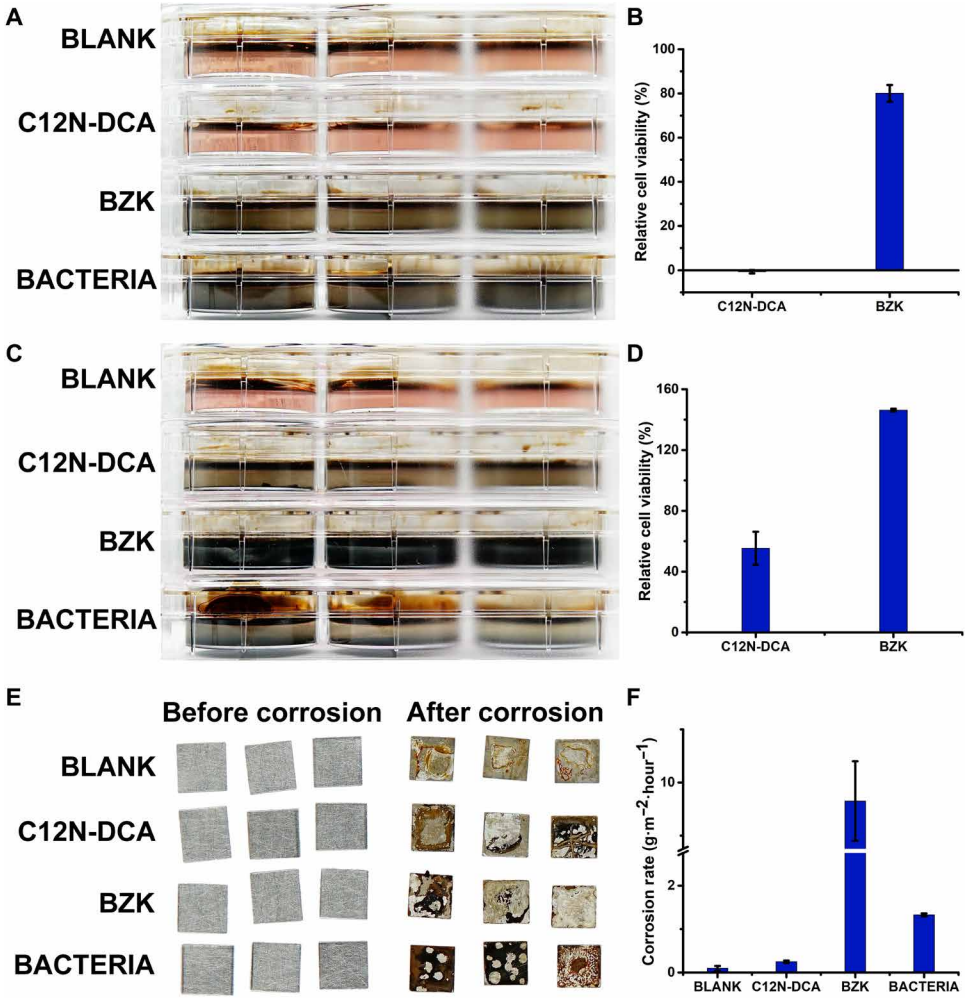

Fig. 5. The antibacterial activity and inhibiting effect on SRB corrosion of C12N-DCA after being adsorbed by montmorillonite. (**A**) Photos of culture systems after being anaerobically incubated at 37°C for 3 days (the first culture cycle). Incipient pH 6. The C12N-DCA and BZK experienced the adsorption of montmorillonite before being added, of which the concentrations were 300 μM if the antibiotics were not adsorbed by montmorillonite at all. There were neither antibiotics nor *D. desulfuricans* in the BLANK group. There were no antibiotics in the BACTERIA group. The blur of the photo was due to the antislip strip on the six-well plate. (**B**) Relative content of *D. desulfuricans* after being anaerobically incubated at 37°C for the first culture cycle. (**C**) Photos of culture systems after being anaerobically incubated at 37°C for another 3 days (the second culture cycle). Pieces of iron foils were put in each well before the second culture cycle. (**D**) Relative content of *D. desulfuricans* after being anaerobically incubated at 37°C for the second culture cycle. (**E**) Photos of iron foils before and after corrosion. The backgrounds were removed. (**F**) Mean corrosion rates of iron foils. All relative cell viabilities were measured by CCK-8 on the basis of the method established above. Data represent means ± SE, $n = 3$. Photo credit: L.Z., Tsinghua University.

microorganisms during petroleum exploitation and reducing the microbial corrosion in the petroleum industry.

MATERIALS AND METHODS

Materials

The charge-reversal surfactant antibiotics C12N-DCA and its homolog C4N-DCA, C8N-DCA, C10N-DCA, C14N-DCA, and C12N-CCA were synthesized according to the literature as shown in fig. S1 [their ^{1}H nuclear magnetic resonance (NMR) spectra are shown in fig. S9] (*31*). Octane ammonium salt (C8N+), dodecane ammonium salt (C12N+), and tetradecane ammonium salt (C14N+) were synthesized by adding corresponding amines to excess hydrochloric acid, followed by freeze drying. The electric desalted petroleum sample was offered by H. Chen in School of Chemistry and Chemical Engineering, Shandong University. Other chemicals were obtained from commercial suppliers. *D. desulfuricans* (DSM 642, revived) was bought from Beijing Cio-Med Technology Development. CCK-8 was bought from Beyotime Biotechnology. QuickChek SRB detection system was bought from Modernwater. The anaerobic culture of *D. desulfuricans* was performed with MGC Anaeropack Series.

Culture of *D. desulfuricans*

An appropriate amount of suspension of revived *D. desulfuricans* was added to disinfected SRB culture medium and then incubated at 37°C in an anaerobic bag in a constant-temperature incubator for 3 to 4 days. If there was black iron sulfide precipitate in the suspension after incubation, which meant successful reproduction, then the *D. desulfuricans* would be passaged in the same way. After that, 20% glycerin was added and the suspension was stored in the refrigerator at −80°C with 1.5 ml per tube (marked as 0 generation). Before the antibacterial test, one tube of *D. desulfuricans* was added

into an appropriate amount of SRB culture medium containing 5‰ dimethyl sulfoxide (volume fraction) and then incubated at 37°C in an anaerobic bag for 3 days (marked as first generation). *D. desulfuricans* would be passaged again in SRB culture medium containing 5‰ dimethyl sulfoxide (marked as second generation). The second and third generations could be used in bacterial experiments. The cultured *D. desulfuricans* could be stored in an anaerobic bag at 4°C for 1 month, and one tube of *D. desulfuricans* was used only once after it was opened.

Establishment of measuring method of relative content of *D. desulfuricans* by CCK-8 assay

Five, 10, 15, 20, 100, 0, 40, 60, 80, and 0 μl* of suspensions of cultured *D. desulfuricans* were added to 96-well plates horizontally. Then, 95, 90, 85, 80, 0, 100, 60, 40, 20, and 100 μl* of SRB culture mediums were added in turn, and 10 μl of CCK-8 was injected to each well except doses marked with *, which was set as baseline. Second, the plates were incubated at 37°C for 30 min. After that, the plates were centrifugalized, and the absorbance of 100 μl of supernatant at 450 nm was measured with a microplate reader. The experiment was repeated for three times, with six wells in the longitudinal direction. The linear correlation between the average absorbance and relative content was analyzed. And, the precision of the method was evaluated with the mean relative SD of three measurements. The concentration of cultured *D. desulfuricans* was about 10^3 per milliliter as indicated by QuickChek SRB detecting result (fig. S2).

D. desulfuricans viability assay of C12N-DCA and its positive and negative control, as well as C*n*N-DCA and C*n*N+ with different alkyl lengths

Part of the sterilized SRB culture medium was taken in advance, and the formula that made its pH value 6 (by adding HCl) after adding 5‰ dimethyl sulfoxide was recorded. The cultured *D. desulfuricans* and SRB culture medium were planted to 96-well plates by a volume ratio of 1:10. Then, C12N-DCA, C12N+ (positive control), C12N-CCA (negative control), C8N-DCA, C10N-DCA, C14N-DCA, C8N+, and C14N+ were dissolved by dimethyl sulfoxide and added at certain concentrations, respectively. And, dimethyl sulfoxide was replenished to 4.5‰ (i.e., 5‰ ignoring the volume of *D. desulfuricans* added). The pH was regulated to 6 according to the formula obtained. After being incubated at 37°C in an anaerobic bag for 3 days, the relative content of *D. desulfuricans* was measured by CCK-8 on the basis of the method established above.

Monitoring of hydrolysis kinetics of C12N-DCA by ^{1}H NMR

A pH 6 phosphate buffer with a concentration of 200 mM was prepared with D_2O. Then, C12N-DCA dissolved in the solution as much as possible. After that, a continuous monitoring program at 37°C was set up, and the ^{1}H NMR spectra were recorded. The hydrolysis ratio was calculated on the basis of the equation

$$\text{Hydrolysis ratio} = \frac{\text{integral}_{\sigma=3.20}}{(\text{integral}_{\sigma=3.20} + \text{integral}_{\sigma=3.01})} \times 100\% \quad (1)$$

D. desulfuricans viability assay of C12N-DCA at different pH values and temperatures

Part of the sterilized SRB culture medium was taken in advance, and the formulas that made its pH value 3, 4, 5, 6, 7, 8, 9, 10, or 11 (by adding HCl or NaOH) after adding 5‰ dimethyl sulfoxide were recorded. And, the *D. desulfuricans* viability assay of C12N-DCA at different pH was performed, as described in the "*D. desulfuricans* viability assay of C12N-DCA and its positive and negative control, as well as C*n*N-DCA and C*n*N+ with different alkyl lengths" section. After being incubated at 37° or 25°C in an anaerobic bag for 3 days, the relative content of *D. desulfuricans* was measured by CCK-8 on the basis of the method established above.

Monitoring of hydrolysis kinetics of C4N-DCA by ^{1}H NMR

Phosphate buffers with a concentration of 200 mM were prepared with D_2O, of which the pH would be 6, 7, and 8 after addition of 10 mM C4N-DCA. Then, a cycle monitoring program was set up ahead of the measurement, and the C4N-DCA solutions with different pH were prepared about 1 min before each sample was put to the sample case autosampler. The room temperature was 20°C during the measurement. The hydrolysis ratio was calculated on the basis of the Eq. 1.

Influence of the presence of petroleum and montmorillonite on the antibacterial activity of C12N-DCA

Part of the sterilized SRB culture medium was taken in advance, and the formula that made its pH value 6 (by adding HCl) after adding 5‰ dimethyl sulfoxide was recorded. Fifty milliliters of SRB culture medium was added to 200 mg of sterilized montmorillonite solid (240 m^2/g). After regulating the pH, the suspension was shaken well and divided into 2 ml each glass vial. Second, C12N-DCA was dissolved by dimethyl sulfoxide and added at certain concentrations, and dimethyl sulfoxide was replenished to 5‰. Then, 200 μl of suspension of *D. desulfuricans* and 200 μl of electric desalted petroleum were added. For the BLANK group, only 200 μl of electric desalted petroleum was added. After being incubated at 37°C in an anaerobic bag for 3 days, the relative content of *D. desulfuricans* was measured by CCK-8 on the basis of the method established above.

Influence of adsorption by montmorillonite on the antibacterial activity of C12N-DCA and BZK as well as the inhibiting effect of the antibiotics on corrosion

A 1.67 times concentrated SRB culture medium was prepared and sterilized. Part of it was taken in advance, to which 1 mM NaOH solution was added to make the concentration normal. Second, the formula that made its pH value 6 (by adding HCl) after adding 5‰ dimethyl sulfoxide was recorded. C12N-DCA/BZK was dissolved by dimethyl sulfoxide and added to sterilized montmorillonite suspension (10 mg/ml), in which there was 1 mM NaOH to avoid the precipitation of C12N-DCA/BZK. The concentration of C12N-DCA/BZK was 825 μM in the suspension (which would be 300 μM when incubated with *D. desulfuricans* if the antibiotics were not adsorbed by montmorillonite at all), and the volume fraction of dimethyl sulfoxide was 1.25%. For the BLANK and BACTERIA group, only dimethyl sulfoxide was added with the volume fraction of 1.25%. After shaking in the constant-temperature shaker (220 rpm, 37°C) for 30 min, the suspension was centrifugalized, and 2.4 ml of supernatant was added to 3.6 ml of concentrated SRB culture medium in six-well plates. Then, HCl was injected according to the formula recorded. Six hundred microliters of suspension of *D. desulfuricans* was planted for the C12N-DCA, BZK, and BACTERIA group, while 600 μl of normal SRB culture medium was added for the BLANK group. Besides, 600 μl of electric desalted petroleum was added.

After being incubated at 37°C in an anaerobic bag for 3 days, 450 μl of aqueous suspension was sucked out carefully and divided into three parts of 150 μl to measure the relative content of *D. desulfuricans* by CCK-8 based on the method established above. In addition, pieces of preweighed iron foils [about 5 × 5 mm^2, 0.127 mm (thick), 99.5% (metals basis)] were washed successively by 75% alcohol and SRB culture medium and then put in each well. The surface area of each piece of iron foil was computed as a cuboid using mass, density, thickness, and ignoring side area. After being incubated at 37°C in an anaerobic bag for another 3 days, the relative content of *D. desulfuricans* was measured by CCK-8 again. In addition, the iron foils were taken out, ultrasonic cleaned with water and ethanol, and weighed. Last, the corrosion rates were calculated.

Measurement of interfacial tensions, surface tensions, and viscosities

A solution of sodium dodecylbenzenesulfonate (2 mg/ml), a suspension of sodium 1-hexadecanesulfonate (2 mg/ml), a solution of anionic polyacrylamide [1 mg/ml; MW (weight-average molecular weight), 8×10^6], and a solution of xanthan gum (1 mg/ml), as typical chemical flooding fluids, were prepared with the ultrasonic method. C12N-DCA was dissolved by 0.04 M NaOH solution to a concentration of 0.02 M. Then, a small amount C12N-DCA solution was added to the four chemical flooding fluids and water, respectively, until the concentration of C12N-DCA becomes 300 μM.

The interfacial tensions of the four chemical flooding fluids and five C12N-DCA solutions were measured by the spinning drop method at room temperature (25° ± 1°C), taking dodecylbenzene as oil phase. The surface tensions of the nine liquid samples were recorded using the Wilhelmy method at room temperature (25° ± 1°C). And, the viscosities were obtained using a Ubbelohde viscometer with water bath at 25°C.

Measurement of CMC

C12N-DCA was dissolved by 0.1 M 1:4 Na_2CO_3/$NaHCO_3$ buffer. Then, the surface tensions of the C12N-DCA solution at different concentrations were recorded using the Wilhelmy method at room temperature (25° ± 1°C).

Measurement of partition coefficients

C12N-DCA was dissolved by 0.1 M 1:4 Na_2CO_3/$NaHCO_3$ buffer to different concentrations. And, the ultraviolet-visible (UV-vis) spectra were measured (Abs-before). Then, the solutions were extracted by petroleum ether. After centrifugation, the oil phase and the emulsion layer were removed. Then, the UV-vis spectra of the aqueous phase were measured (Abs-after). The experiment was carried out at room temperature (25° ± 1°C). The partition coefficients were calculated on the basis of the equation

$$\text{Partition coefficients}_{O/W} = \frac{(\text{Abs} - \text{before}_{\lambda=240\ nm} - \text{Abs} - \text{after}_{\lambda=240\ nm})}{\text{Abs} - \text{after}_{\lambda=240\ nm}} \quad (2)$$

Instruments

Interfacial tension was measured using a KINO Industry Co. TX500 spinning drop interfacial tensiometer. Surface tension measurements were carried out on a Dataphysics DCAT21 high-sensitive microeletromechanical balance system with a Wilhelmy plate. Viscosities were obtained using an Ubbelohde viscometer. NMR spectra were recorded on BRUKER 600M AVANCE III HD with a cryoprobe, BRUKER 600M AVANCE III HD with a cryoprobe, and JEOL ECS-400. The absorbance data of 96-well plates were collected by a PerkinElmer EnVision multimode plate reader. The UV-vis spectra were measured using a HITACHI U-3010 spectrophotometer.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/25/eaba7524/DC1

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