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Microbial Characteristics and Potential Mechanisms of Souring Control for a Hypersaline Oil Reservoir

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Abstract

The production of hydrogen sulfide (H_2S) had caused huge economic losses and security risks to oil fields, which had attracted the attention of many researchers. Studies focused on hypersaline oil reservoirs were scarce. Here, we simulated H_2S inhibition by adding NO_3^- and NO_2^- in anaerobic bottles and up-flow sand-packed bioreactor in the laboratory using water samples from injection wells in the Jianghan reservoir. The microbial characteristics, pH, salinity, sulfate, sulfide, NO_3^- and NO_2^- were measured at every stage. The content of H_2S and elemental sulfur (S^0) gradually reduced to 0 mg/L with injected 400 mg/L of NO_3^- and 300 mg/L of NO_2^-. Halophilic nitrate-reducing bacteria (NRBs) related to *Halomonas* and *Arcobacter* could suppress the activity of sulfate-reducing bacteria (SRBs) through the accumulation of nitrite toxicity and the competition of organic substrates with SRBs. When SRBs activity could not be suppressed, the sulfur-oxidizing bacteria related to *Sulfurimonas*, *Thiomicrospira* and *Acrobacter* were accumulated for souring control through microbial sulfur cycle of H_2S production and oxidation. Salinity could be a key parameter affecting microbial development and interactions. This study provides a fundamental data to souring control in the hypersaline oil reservoir by reinjection produced water amended with NO_3^- and NO_2^-.

Key words Microbial characteristics · Souring control · Sulfate-reducing bacteria (SRBs) · Nitrate-reducing bacteria (NRBs) · Salinity

Nomenclature list

Symbols of abbreviations	Explanation
H_2S	Hydrogen sulfide
S^0	Elemental sulfur
NO_3^-	Nitrate ion
NO_2^-	Nitrite ion
NRBs	Nitrate-reducing bacteria
SRBs	Sulfate-reducing bacteria
MIC	Microbiologically influenced corrosion
so-NRBs	Sulfide oxidizing-nitrate reducing bacteria
SO_4^{2-}	Sulfate ion
COD	Chemical oxygen demand

1. Introduction

Microbial production of H_2S in oil reservoirs is an undesirable process that enhances corrosion of metal facilities and block of pores (Almahamedh et al. 2011). The annual global cost of corrosion is about $2.5 trillion, more than 3.4% of total GDP, not including the cost of safety and environmental consequences, and corrosion losses caused by microorganisms account for about 20% (Koch et al. 2016, Hou et al. 2017). SRBs were considered to be main culprits of microbiologically influenced corrosion (MIC). In fact, SRBs could produce hydrogen sulfide which caused souring of reservoir and corrosion equipment leading to serious economic damages in the petroleum industry (Voordouw 2011, Anandkumar et al. 2016). The production of H_2S by SRBs

has been as their major metabolic processes, especially in secondary oil recovery because sulfate in injected water stimulated the SRB activity recognized in reservoirs or surface facilities (Hubert and Voordouw 2007). Biogenic corrosion control is facilitated either by the physical removal of sulfate from the injection waters or by mitigation of the activity of sulfate reducers (Gieg et al. 2011).

Despite successful attempts for H_2S inhibition by nitrate injection in oil reservoirs, many recent studies have reported novel insights associated with nitrate and sulfate reduction, especially for hypersaline environments. Halophilic bacteria were reported to possess the ability to reduce nitrate to nitrite, and nitrite accumulation inhibited the activity of SRBs in high-salinity Bakken shale oil reservoirs, indicating the mechanism of nitrite toxicity to SRBs for souring control in high-salinity oil reservoirs (An et al. 2017). In industrial wastewaters containing organic nitrogen and sulfur-based pollutants, microorganisms such as *Arcobacter* and *Pseudomonas* played key roles in denitrification and desulfurization systems which could remove simultaneously organic compounds (estimated by COD), nitrogen and sulfide, as well as S^0 recovery (Liu et al. 2020, Chen et al. 2020). Deng et al. (2022) reported that the production of H_2S in the M71 oilfield was successfully inhibited by injecting 400mg/L NO_3^- and 300mg/L NO_3^-. Souring control in oil reservoirs was associated with complex microbial and chemical energetically processes. Though many studies had reported that microorganisms were used to reduce nitrate and sulfate, very few studies focused on the high-salinity environments, especially the mechanism of souring control in high-reservoir oil reservoirs. Removing H_2S through stimulating NRBs and sulfur-oxidizing bacteria with

NO_3^- and NO_2^- injection, this work provided a low cost and environmentally friendly method for souring control in hypersaline oil reservoirs.

Unravelling the microbial ecology in biological treatment systems is always the first and the most crucial step for understanding *in situ* bioprocesses (Fuhrman 2009). Collected samples of enriched cultures amended with NO_3^- and NO_2^- in 30 mL anaerobic bottles with low-salinity medium. Then established an up-flow were injected sand-packed bioreactor injected NO_3^- and NO_2^- with high-salinity cultures which handled with six stages and operated for 150 days to souring control of simulated oil reservoir conditions. The variation of microbial community was revealed by 16S rRNA gene sequencing, and the changes of pH, salinity, sulfate, sulfide, NO_3^- and NO_2^- were detected by chemical analysis in anaerobic bottles and bioreactor. The results illustrated that reinjected produced water with NO_3^- and NO_2^- removed H_2S in the hypersaline oil reservoir.

2. Materials and Methods

2.1 Sampling and media

Water samples were collected from the wellhead of an injection well (Xin 138#) in the year of 2016 from Xingou oil field (Jianghan Basin, China). The oil reservoir was a typical hypersaline reservoir with an average total salinity of 265,851.2 mg/L and was rich in divalent anion SO_4^{2-} of 4,211.5 mg/L (Table 1). The concentration of H_2S increased to 36.9 mg/L on the average, and pitting corrosion was severe for metal

facilities. A total of 200 L of water samples were transferred into bottles (10 L), transported to the lab and stored in a refrigerator.

E0 medium was prepared as follows: the collected water samples (Xin 138#) were passed through a 30–50 μm filter (Beimu, China), supplied with 3,600 mg/L lactate, then put them into 1 L anaerobic bottles with butyl rubber stoppers. The head space was filled with N_2 (99.99%), sterilized at 121 °C for 15 min and marked E0.

An anaerobic Coleville synthetic brine medium K (CSBK) (An et al. 2017) was supplied with 3,976 mg/L Na_2SO_4 and 3,280 mg/L sodium lactate and divided into 30-mL anaerobic bottles with an iron nail. The head space was filled with N_2 (99.99%) and sterilised at 121 °C for 15 min and marked ESRB for SRB enrichment. An anaerobic CSBK medium supplied with 3,976 mg/L Na_2SO_4, 3,535 mg/L KNO_3 and 3,280 mg/L sodium lactate was divided into 30 mL anaerobic bottles with an iron nail, sterilized at 121 °C for 15 min and marked ENRB for nitrate souring control.

2.2 Up-flow sand-packed bioreactor setup and operating conditions

ESRB and ENRB enrichment media were inoculated with 5% of the water (Xin 138#) collected from the oil field and incubated at 37 °C for 2 weeks.

An anaerobic up-flow bioreactor with a working Perspex column (10 cm inner diameter and 200 cm total height) was packed with quartz sand. The bottom layer with 30 cm height was filled with coarse sand (D=1–2 mm), however the upper layer was filled with fine sand (D=125–250 μm) (Figure 1). The final simulated column pore space was approximately 2.56 L. The constant flow rate was 0.7 L/d; thus, it took a

volume of 0.7 L medium for approximately 4 days from the bottom (influent) to the top (effluent).

The bioreactor was operated for 150 days and processed in six stages (stages I–VI). Stage I: day 0–10, in the injection tank, E0 medium was inoculated with 5% vol/vol Xin 138# injection water, flushed with N_2 (99.9%) and pumped to the bioreactor from the bottom to the top. Stage II: day 11–30, E0 medium supplied with 400 mg/L NO_3^- and 300 mg/L NO_2^- was pumped into the bioreactor from the bottom to the top; stage III: day 31–60, E0 medium was supplied with 200 mg/L NO_3^- and 150 mg/L NO_2^-; stage IV: day 61–90, the pump was stopped, and the bioreactor was maintained in the stationary phase; stage V: day 91–120, E0 medium was supplied with 200 mg/L NO_3^- and 150 mg/L NO_2^- and stage VI: day 121–150, E0 medium was supplied with 400 mg/L NO_3^- and 300 mg/L NO_2^-. During the whole experiment, a total of 70 samples of influent and effluent samples (50 mL each) were collected into sterilized bottles sealed with butyl rubber stoppers, and the head space was filled with nitrogen for the further chemical and sequencing analyses.

2.3 Chemical analysis

A total of 70 collected samples were used for chemical analysis of pH, salinity, sulfate, sulfide, NO_3^- and NO_2^-. The methylene blue method (DO-HX-10) could be used to determine the concentration of aqueous sulfide (Leggett et al. 1981). The pH value was detected using a pH meter (PHSJ-4F). The salinity was measured using a salinity meter (ZWC-2001, Jiangsu New High-tech Analytical Instrument, China). The ion chromatography (ICS-3000, Thermo Scientific™ Dionex™, USA) was used to detect

the concentration of SO_4^{2-}, NO_3^- and NO_2^-. The sulfur content in the solution was detected using a Microcoulometric Titrator (KY-200, Jiangsu Keyuan Electronic Instrument Co., Ltd., China).

2.4 High-throughput 16S rRNA gene sequencing and data analysis

Samples of SRBs and NRBs enrichment cultures (ESRB and ENRB) and bioreactor samples were collected at the end of each stage: stage I (day 10), stage II (day 30), stage III (day 60), stage IV (day 90), stage V (day 120) and stage VI (day 150) were used for DNA extraction. Approximately 30 mL of samples were centrifuged to collect bacteria at 10,000×g for 10 min. According to the manufacturer's instruction, genomic DNA was obtained by a Fast DNA SPIN kit for soil (Mp Biomedicals, USA). The V1–V3 regions of bacterial 16S rRNA gene were performed to amplify the DNA template with the primer pair 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 533R (5'-TTACCGCGGCTGCTGGCAC-3') to 454 pyrosequencing.

The raw multiplexed sequence data were analysed using QIIME (www.qiime.org) and R package that calculated the number of operational taxonomic units and the number of reads in each library (Bokulich et al. 2018). Removing the singleton statistically analysed the feature table, and the composition of microbial community of samples was visualized as violin at the at the six classification levels of phylum, class, order, family, genus and species. The obtained raw data using 16S rDNA sequencing had been to submit to NCBI database (PRJNA: 683706).

Using the Mothur software, the Spearman rank correlation coefficient between the dominant genus with the top 50 abundance was calculated. For the related advantage of

rho > 0.6 and P value < 0.01, the associated network was constructed and imported into Cytoscape (http://www. Cytoscape.org/) (Shannon et al. 2003) software for visualisation. The nodes represented the dominant genus. The network inferences were used to examine the interactions between members of different communities and to find the interaction patterns of co-occurrence or co-exclusion in different habitats through association analysis.

3. Results and Discussion

3.1 Souring control in low-salinity conditions

After 14 days of incubation in the CSBK medium, ESRB and ENRB enrichments showed an obvious difference. ESRB enrichments became black with numerous black solid components suspended, while ENRB cultures became turbid and yellow-green in colour. The iron nails can be clearly seen in the NRB bottles (Figure 2). In the cultures of ESRB, sulfate concentration decreased from 874±9 mg/L to 67±11 mg/L, while a low concentration of 35±6 mg/L sulfide was detected. This result might be due to the formation of black solid suspensions (FeS) that used the sulfide produced by SRBs. In the ENRB cultures, the sulfide concentration was also decreased to below the detection level o and the sulfide concentration was 12±3 mg/L, however S^0 concentration was higher (436±7 mg/L). The nitrate in the ENRB cultures was also decreased from 653 mg/L to 39±7 mg/L, with an increase in nitrite from 0 mg/L to 69±4 mg/L (Table 1). These experiments demonstrated high activity of sulfate reduction and possibility to control of sulfide production through addition of nitrate.

The composition of bacterial communities in ESRB and ENRB enrichment cultures was analysed. Figure 3 showed that at the genus level, the microbial communities of the two enrichment cultures were slightly different. In ESRB, the dominant bacteria were affiliated with *Desulfovibrio* (39.6%), *Luteibacter* (17.9%), *Geotoga* (17.9%), *Marinobacter* (13.1%) and *Marinobacterium* (2.8%). In ENRB, the dominant bacteria were *Desulfovibrio* (50.8%), *Luteibacter* (7.7%), *Marinobacter* (30.1%) and *Marinobacterium* (2.3%) (Table 3). In both samples, sulfidogenic bacteria were dominant comprising approximately 70% and 58%, respectively.

Based on the concentration of sulfide and the clear images of iron nails in the ENRB enrichment cultures, souring control seemed to be successful (Figure 2). However, the results of microbial community compositions were inconsistent with the macroscopic phenomena. Although the bacteria related to the *Geotoga* genus were absent, members of *Desulfovibrio* genus were still dominant in ENRB, indicating that sulfate reduction was active. However, numerous studies shown that nitrate and sulfate reducers were active concurrently for both denitrification and desulphurisation with H_2S production (Achtnich et al. 1995, Zhou et al. 2014 a and b). Another evidence was the detection of 436±7 mg/L of S^0, suggesting that SRBs were not suppressed. Sulfide could be firstly produced by the dominant SRBs and then was oxidised to S^0 in the ENRB cultures. Sulfide oxidation included chemical and biological pathways (Pokorna et al. 2015). Chemical oxidation seemed to be prominent in the ENRB cultures as few sulfur-oxidising bacteria (SOBs) or so-NRBs were stimulated. In the ENRB cultures, the excess nitrate with 2828 mg/L KNO_3 added in the enrichments probably produced

S^0. The S^0 production was reported to cause a more severe corrosion (Lahme et al. 2019). Therefore, the souring control in the enrichment cultures was unsuccessful although H_2S was not detected in the low-salinity cultures.

3.2 Dynamics of microbial community in the bioreactor

During the whole operation of the bioreactor, the microbial diversity slightly changed, and the dominant microbial groups were sulfate-reducing, nitrate-reducing, sulfur-oxidizing, and fermentative (H_2 producing bacteria) (Table 3), which indicated the potential microbial resources for souring control. The microbial structure showed considerable changes. With the first round of NO_3^- and NO_2^- injection (stages II–IV), the typical SRBs *Desulfobacter*, *Desulfovibrio* and *Desulfomicrobium* were suppressed, and the NRBs *Halomonas* and *Arcobacter* were activated. Halophilic, heterotrophic and denitrifying representatives of *Halomonas* genus (Miao et al. 2020, Zhang et al. 2020), can synthesise compatible solutes and accumulate them in the cell to resist high osmotic pressure environment (Fu et al. 2019). Members of *Arcobacter* genus were reported as anaerobic denitrifiers that could reduce nitrate to nitrite or an autotrophic sulfide-based denitrifiers (Chen et al. 2020, Xia et al. 2019). It was proposed that nitrate reduction was the dominant microbial process in stages I–IV.

During the second round of NO_3^- and NO_2^- injection (stages V–VI), the activity of SRBs was not suppressed with the increase of *Desulfomicrobium* and *Desulfobacter*. The SOBs of *Sulfurimonas* and *Thiomicrospira* and so-NRBs of *Acrobacter* also increased. *Sulfurimonas* and *Thiomicrospira* were genera dominant as sulfur-based autotrophic denitrifiers and played an important role in the biodesulfurization of high

concentrations of H_2S in alkaline environments (Hansen et al. 2015, Wu et al. 2020, Song et al. 2020). A microbial sulfur cycle of H_2S production and oxidation medium may have occurred in the bioreactor.

3.3 Response of bioreactor performance to souring control

During stage I (day 1–10), SRBs were accumulated in the bioreactor to produce H_2S. A high concentration of sulfide (315 mg/L) was detected in the effluent at the sixth day, and was stable at 400–410 mg/L (Figure 4a), showing the presence of SRB community. In stage II (day 11–30), a high concentration of 400 mg/L NO_3^- and 300 mg/L NO_2^- was added to E0 for souring control. The concentration of H_2S in the effluent decreased to 49 mg/L on the second day of Stage II and decreased to 0 mg/L at the third day. The S^0 production was decreased from 79 mg/L to 0 mg/L. The nitrate in the effluent decreased to approximately 40 mg/L (Figure 4b) with the nitrite increased to approximately 650 mg/L. In stage III (days 31–60), low concentrations of NO_3^- (200 mg/L) and NO_2^- (150 mg/L) were added to E0. The H_2S concentration remained 0 mg/L during the stage with a slight change in sulfate concentration (maintained at approximately 4000 mg/L). The nitrate reduction rate remained approximately 90% in comparison with stage II, and nitrite was accumulated to 302±5 mg/L at the last day of the stage.

During stages II–IV in the bioreactor, the nitrate treatment was rapidly effective in the decrease of H_2S content to undetectable levels. Based on the relative abundances of SRBs, sulfate reduction activity was obviously suppressed (Table 2). Aside from the osmotic stress caused by high salinity, the specificity of nitrite toxicity to sulfate

reduction was proposed to be a major stress condition in stages II–IV in the bioreactor as previously suggested (He et al. 2010, Voordouw et al. 2017). The main cause of nitrite production in the bioreactor was from the influent medium E0 that was supplied with 300 mg/L NO_2^- (stage II) and 150 mg/L NO_2^- (stage III). Another cause of nitrite production was microbial nitrate reduction by *Halomonas* and *Arcobacter*. The concentration of nitrite in the effluent of stage II was as high as 653±9 mg/L, indicating nitrite accumulation. The nitrite accumulation through microbial denitrification has been reported to be highly dependent on differences in salinity, nitrate concentrations and pH (Albina et al. 2019). In the present study salinity determining the microbial composition could be the most important factor for nitrite accumulation, which is consistent with the findings of Voordouw et al. (Voordouw et al. 2017).

In stage IV (day 61– 90), the pump was stopped, and the bioreactor was kept still to evaluate the sustained effect of souring control. One effluent sample at the last day (day 90) was obtained during this stage. The concentrations of sulfide, NO_3^- and NO_2^- decreased below the detection level. The concentration of sulfate was unchanged and was 3989±16 mg/L (Table 2). The bio-competitive exclusion effect in stage IV was noticeable with the lowest relative abundance of SRBs (Table 3). With the consumption of organic nutrients in injected water (E0), the substrate competition became stronger within bacteria. NRBs became predominant due to their higher ability of competing electron donors of nitrate reduction than sulfate reduction (Dofing and Hubert 2017).

In the stages V and VI, the SRBs *Desulfomicrobium* and *Desulfobacter* unexpectedly increased, and sulfide was detected. The low amount of nitrite in stage V

could not reach the threshold of toxicity effect to SRBs, resulting in the development of SRBs. A few studies carried out the thermodynamic conditions and optimal rations of SO_4^{2-}/NO_3^- and COD/SO_4^{2-} to sulfate and nitrate reduction (Zhou et al. 2014 a and b, Dofing and Hubert, 2017). However, few studies could give an universal formula to any environment as the different microbial compositions, diverse microbial metabolic pathways, and the applied aims for different environments. According to the H_2S and S^0 concentrations in the effluents, the existed SRBs did not cause serve souring in our bioreactor. Effective souring control can be attributed to *Acrobacter*, *Sulfurimonas* and *Thiomicrospira*. The oxidation of sulfide by so-NRB depend on the molar ratio of nitrate to sulfide (N/S) (Lahme et al. 2019). In stage V, the detection of H_2S and S^0 indicated incomplete oxidation, implying that the amounts of NO_3^- (200 mg/L) and NO_2^- (150 mg/L) were below the expected optimal concentrations with 400 mg/L NO_3^- and 300 mg/L NO_2^-. With the increase in NO_3^- (400 mg/L) and NO_2^- (300 mg/L) in stage VI, the souring was completely controlled, and H_2S and S^0 were not detected.

Our results showed that the optimal amount of NO_3^- and/or NO_2^- for souring control can be calculated through chemical reaction equations (Pokorna et al. 2015). In natural environments, microbial parameters could be the most crucial for controlling the whole process according to our results. In contrast to chemicals, bacteria are present and can be stimulated or suppressed with the changes in environmental conditions.

3.4 Effect of the salinity on souring control

The stimulated bacteria in ESRB, ENRB and the bioreactor showed a clear distinction despite the same sample (Xin 138#) used in the study (Figure 3). Bacteria

accumulated in the ESRB enrichment were *Desulfovibrio*, *Luteibacter* and *Geotoga*; however, the dominant bacteria stimulated in the bioreactor (Stage I) were *Desulfobacter*, *Desulfovibrio*, *Desulfomicrobium* and *Dethiosulfovibrio* which are capable of sulfate-, sulfur- or/and thiosulfate-reduction. The other detected dominant bacteria in stage I were halophilic *Halomonas*, *Halanaerobium*, *Marinobacter* and *Arcobacter* obtained using. Spearman association network analysis indicated that the dominant species were divided into two dominant camps. One consisted of bacteria detected in cultures (right camp), and the other consisted of bacteria detected in the bioreactor (left camp). Moreover, microbial interactions (lines between nodes) in the bioreactor were more rich/diverse than those in the cultures (Figure 5). According to Spearman association network analysis, *Desulfovibrio* represented negative interaction with *Thiomicrospira*, *Sulfurimonas*, *Halanaerobium*, *Dethiosulfatibacter*, *Tangfeifania*, *Fusibacter*, *Arcobacter*, *Sphaerochaeta*, *Sulfurospirllum* and *Desulfobacter*. *Halomoas* shown strong negative interactions with microbes in the low salinity enrichment bottles. Microbes with the same function of sulfate reduction would present different environmental durations, SRBs of *Desulfovibrio* shown negative interaction (co-extinction) with most of NRBs, while SRBs of *Desulfomicrobium* and *Desulfobacter* shown positive interactions (co-occurrence) with NRBs. The co-extinction and co-occurrence shaped the microbial interactions in our study. Moreover, many microbes could survive mutations (Wu et al. 2020). Salinity in the media was proposed to be the most important environmental factor in forming the two apparent camps. ESRB and ENRB were obtained using traditional laboratory enrichment media

with 5 g/L of NaCl in the study, making the salinity in ESRB to be 10,367±13 mg/L. Collected water samples was used to prepare E0 causing its salinity to reach 267,356±17 mg/L.

An et al. studied microbial communities in high- and low-salinity bioreactors and enrichments for souring control; they found that *Desulfovibrio* and *Marinobacter* were dominant in low-salinity conditions, whereas *Holomonas* and *Halanaerobium* were dominant in high-salinity conditions (An et al. 2017). Therefore, two assumptions could be drawn from the results: firstly, the most probable number used commonly to count microbial numbers in samples does not reflect the actual number of bacteria in samples from oil industries (An et al. 2017). Secondly, the unsuccessful souring control in the ENRB cultures in our study could be due to the non-adaptation of potential halophilic NRBs and/or so-NRBs in the low-salinity ENRB medium. Based on our results, ongoing injection of low-salinity water by mixing clean water with production water could result in more serious souring in high-salinity oil reservoirs.

An et al. studied microbial composition and diversity of cultured samples from Bakken shale oil reservoirs at different salinities, found that SRBs could be suppressed in the presence of nitrate in cultures at 2.5 M NaCl, but still lacked the specific nitrate concentration for souring control and the applied salinity was not enough high in this article (An et al. 2017). Wu et al. also reported that microorganisms (such as *Sulfurimonas* related to sulfur-oxidizing bacteria) played the most key role in bio-desulfurization process and the pH values from 7.5 to 8.5 could enhance the ability of removing H_2S in slightly alkaline biotricking filter (Wu et al. 2020). Very few studies

focused on souring control for specific concentration of nitrate injection in hypersaline oil reservoirs. Our work explored the effect of souring control between the low-salinity cultures and high-salinity cultures by injected 400 mg/L of NO_3^- and 300 mg/L of NO_2^-. The results of this study showed not performing well in the low-salinity cultures, whereas successful inhibited the production of H_2S in the high-salinity cultures for souring control. This work provides feasibility studies and basic data for lower cost souring control in a hypersaline oil reservoir.

Conclusion

In this work, we explored microbial souring control under high salinity conditions through NO_3^- and NO_2^- injection in anaerobic bottles and simulated up-flow sand-packed bioreactor in the lab. Underlying mechanisms were revealed by examining chemical characteristic and microbial community composition at different stages. The results indicated that souring control were feasible. Halophilic nitrate-reducing, sulfur-oxidizing bacteria were the stimulated target groups. The results indicated that the activity of SRBs was inhibited by bacteria related to *Halomonas* and *Arcobacter*, and *Sulfurimonas*, *Thiomicrospira* and *Acrobacter* could control the production of H_2S when SRBs could not be suppressed. The salinity was an important factor for the efficiency of souring control in the oil reservoir studied. Therefore, the reinjection of high salinity production water for the studied petroleum reservoir could be recommended.

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Authors' Contributions

YHS and FZ conceived and designed the research. CC, HX and SS Su conducted the experiments. IMB contributed new reagents or analytical tools. SS Sun and HFM investigated. WB and DSY wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Figure legends

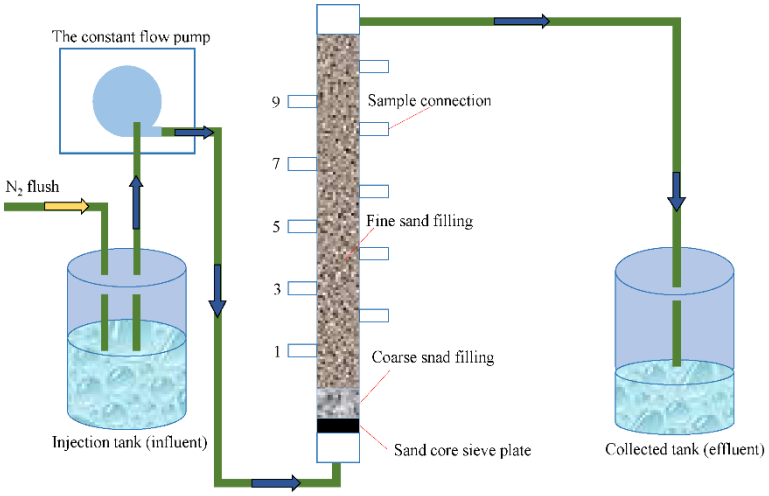

Fig. 1. Schematic of up-flow sand-packed bioreactor modeling a souring oil reservoir treated with NO_3^- and NO_2^- injection.

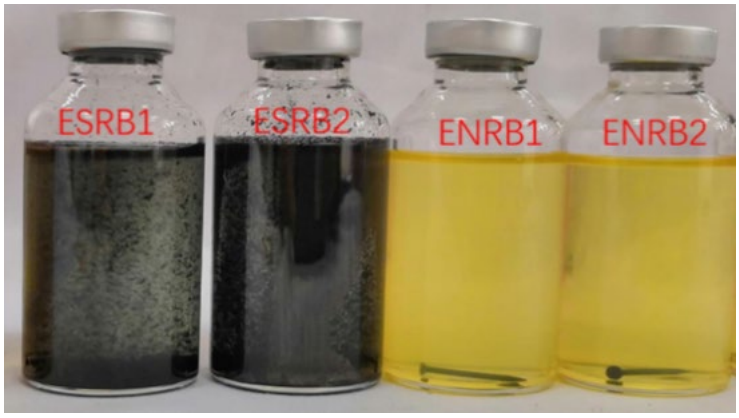

Fig. 2. Enrichment cultures for SRBs (ESRB1 and ESRB2) and nitrate souring control of SRBs (ENRB1 and ENRB2) in bottles with duplication.

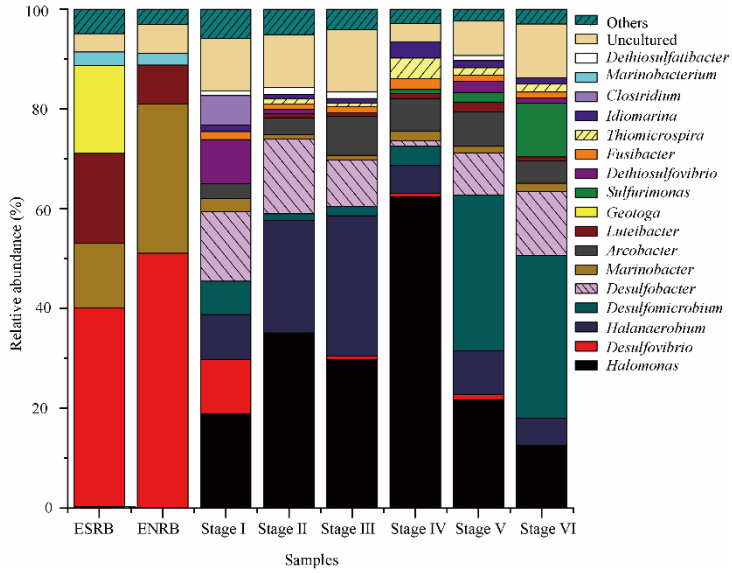

Fig. 3. Histograms showing the dominant bacteria at the genus level in the samples from enrichments (ESRB and ENRB) and from the bioreactor (Stage I- Stage VI).

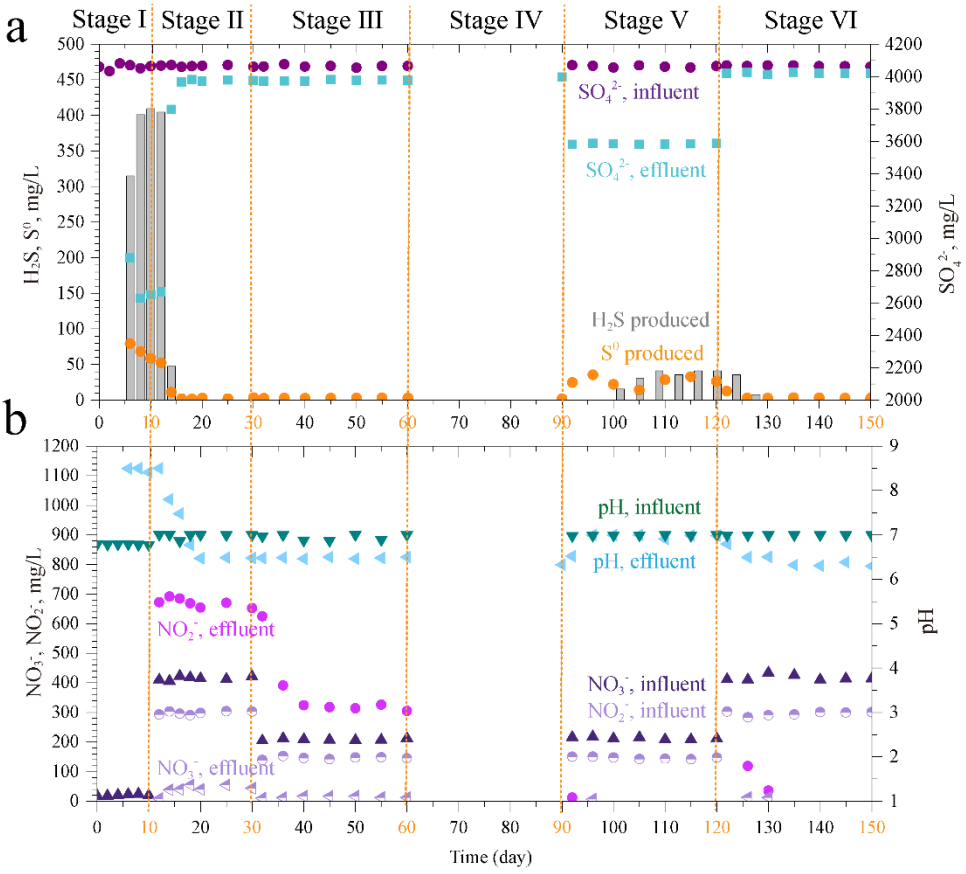

Fig. 4. Profiles of influent and effluent concentrations for SO_4^{2-}, H_2S and S^0 (Fig.4a), NO_3^-, NO_2^- and pH (Fig.4b) in the souring control performance (150 days) in the

bioreactor.

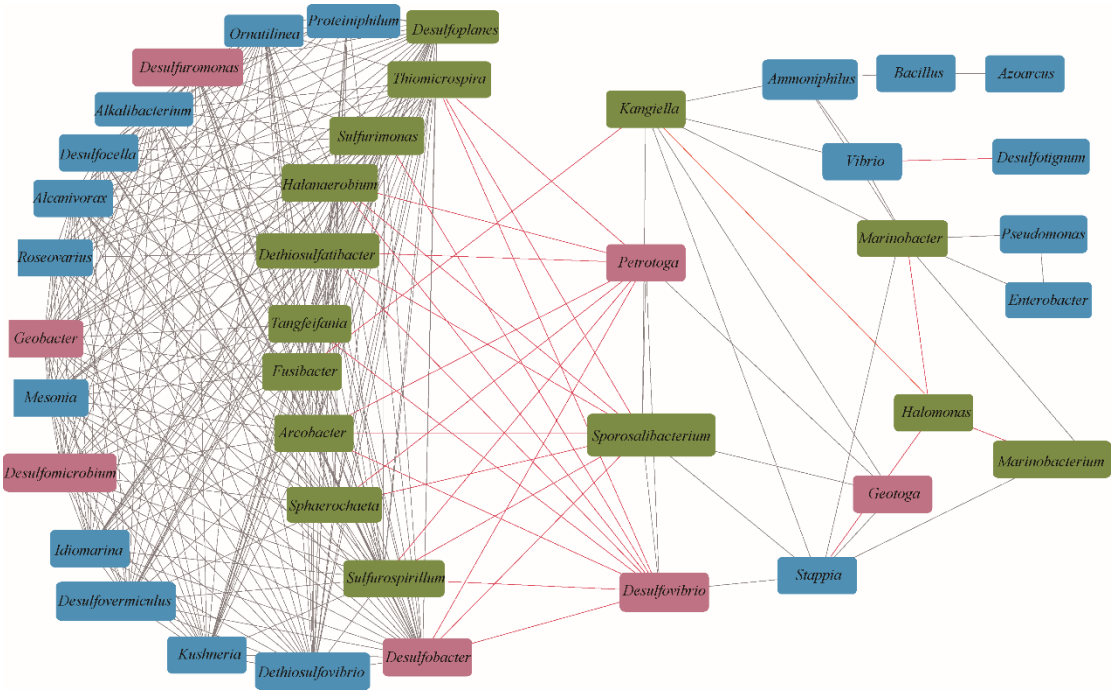

Fig. 5. The network inferences of dominant bacteria in the ESRB and ENRB enrichments and in the bioreactor. The nodes represent the dominant genus, and the connections between the nodes indicate a correlation between the two genera, the red line indicates a positive correlation, and the grey line indicates a negative correlation. The more connections through a node, the more associations the genus has with other members of microbial community.