IMPACT OF BIO-COMPETITIVE EXCLUSION ON NITRATE-REDUCING BACTERIA IN OIL RESERVOIR WATER TREATMENT

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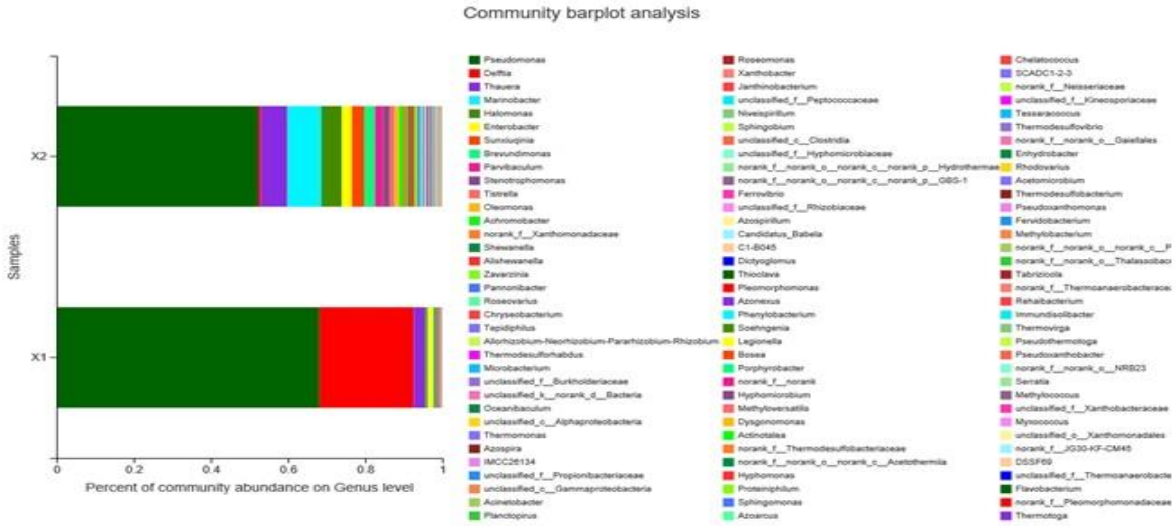
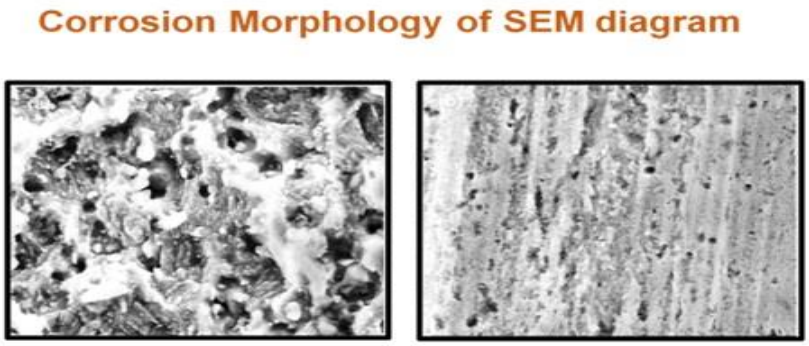
Graphical Abstract





Abstract:

In the presence of nitrogen oxides, nitrate-reducing bacteria (NRB) may use nitrate as an electron acceptor, reduce nitrate to nitrite, and finally convert nitrite to nitrogen or ammonium (NO and N_2O). Studies have shown that BCX (Bio-Competitive Exclusion) is a viable , ecologically benign, and low-cost technique for regulating NRB in its first

phases of study and application. More precisely, Pseudomonas sp. to isolate nitrate-reducing bacteria from oil-field wastewater using 16S rDNA sequencing and selection (NRB). Including NO_3 and NO_2 as bio-activators has helped researchers get a deeper understanding of the processes that govern NRB activation. The potential for molybdate and NRB to inhibit Sulfate-Reducing Bacteria (SRB) was also investigated. The results showed that NO_2 was more effective than NO_3. The effect was greatest at a 1:4 ratio of NO_3 to NO_2. NRB activation synergized with low molybdic acid solution concentrations (5%). Static corrosion model experiments demonstrated the importance of SRB in the corroding process. Biological inhibitors were shown to have the lowest corrosion rates, proving their effectiveness. Scanning electron microscopy was used to examine the corroded steel sheet's surface morphology. In addition, there was almost no surface corrosion or pitting on the steel sheet.

Keywords: Nitrate-reducing bacteria (NRB), Bio-Competitive Exclusion (BCX), Sulfate-Reducing Bacteria (SRB), static corrosion simulations, biological inhibitors

1. Introduction

Water flooding reservoirs have been shown to include a wide variety of microorganisms, including aerobic bacteria, sulfate-reducing bacteria (SRB), nitrate-reducing bacteria (NRB), iron-reducing bacteria, fermentative bacteria, and methanogens. On the basis of their metabolic mechanisms, NRB may be divided into three broad categories: heterotrophic nitrate-reducing bacteria (hNRB), sulfide-oxidized nitrate-reducing bacteria (SO-NRB), and anaerobic ammonia-oxidizing bacteria (aaoRB) (Anammox). Hydrocarbons and other organic compounds may provide energy for HNRB [1]. Carbon and energy for so-called sulfate-reducing bacteria (SO-NRB) come only from CO_2 and inorganic molecules (like sulphide). concerns about industrial waste and pollution. That's why reducing SRB activity in the oil field system is such an important scientific goal. Because of its potential to protect the environment and limit expansion over the long term, bio-competitive exclusion (BCX) has drawn a lot of interest from experts throughout the world [2]. Oil field workers often use nitrate or nitrite inhibitors to put a stop to the spread of SRBs. Due to the rivalry between hNRB and SRB for electron donor materials like organic acids, this strategy may promote the development of NRB while inhibiting the development of SRB. . However, SO-NRB has the potential to oxidise H_2S, eliminate it, and prevent SRB from producing sulphide. Nitrite, formed after nitrate reduction by NRB, may be utilised to directly block SRB [3].

Due to the high concentration of SO_4^{2-} in seawater, SRB growth and metabolism are greatly stimulated, resulting in the production of H_2S, which in turn causes acid corrosion in the reservoir and a number of other serious environmental problems. This occurs in most offshore oil fields at some point during production. It has been anticipated from the commencement of this study into microbiological energy-enhanced recoveries that certain Sulfate-Reducing Bacteria (SRB) break down crude oil. As SRB populations expand inside reservoirs, they begin to emit increasing quantities of the very toxic gas hydrogen sulphide. The oil industry has long been concerned about H2S from biological sources due to its corrosive qualities. Strata damage, decreased wellbore life, and inefficient production processes [4] are all possible outcomes of the rising sulphur content in oil and gas, which may also cause blockage of reservoirs and corrosion of oil pipelines. There has been a recent proliferation of innovative

architectures that use SRB's biological suppression. When a functional excitation adviser or endogenous DNB is introduced to the environment, the natural beneficial bacteria are activated, and they begin competing with SRB for dwelling space and nutritional sorbents, thereby eliminating the need for the traditional technique of SRB eradication. One technical strategy for dealing with the increased competition for dietary substrates caused by DNB is the biological suppression of SRB. This deprives SRB of the fuel it requires, slowing or even stopping its activity and so rendering sulphide formation impossible. As a result of the threat they provide, SRB have been the primary focus of research on bacteria in oil field water. In general, corrosion at the top facilities was caused by mesophilic SRB, whereas souring in the in-situ reservoirs was caused by thermophilic SRB [5]. The bulk of mesophilic SRBs identified from oil field water belonged to the genus Desulfovibrio. Microbiologists frequently use 16S ribosomal RNA sequencing to identify diversity in prokaryotic organisms and other organisms and subsequently investigate the phylogenetic relationships between them. Ribosomal RNA and ribosomes are present in all cells, which makes their use in molecular techniques advantageous. Based on the 16S rRNA gene sequence analysis, 21 bacteria were isolated from 15 samples of generated water during a comprehensive investigation of SRB in oil fields [6]. In addition to being regularly used for mycobacteria identification, 16S rRNA gene sequence alignment can help identify poorly described, infrequently isolated, or phenotypically abnormal strains and help identify novel microbes and noncultured microbes. Therefore, thermophilic Desulfotomaculum sp. has been extensively studied since the 1990s. existence is possible in oil field environments. Biocides like glutaraldehyde have been recommended for injection as a method of preventing H_2S generation [7]. Because SRB grew in the covered microorganisms and the biocide was inactivated in the area around the film and the elements, this treatment was costly and had little to no impact. Biocides that fail to degrade over time also contribute to environmental devastation. The SRB substrates may be recoverable after the biocides have degraded. Pipeline corrosion and other unfavourable effects might be avoided with the help of sulphide treatment, which was necessary for safety reasons [8]. At each stage of a well's lifecycle, SRB degradation poses a hazard that must be identified and counteracted [9]. According to the United States Geological Survey (USGS), one oil barrel requires 1,850 water gallons to retrieve and refine over the course of its lifetime. This is especially true of freshwater hydraulic pipes and oil tubes used in oil-pumped systems. By providing genus identification in >90% of cases and species recognition in 65-83percentage points of these, 16S rDNA sequence analysis is especially beneficial in determining unexpected microbes that are challenging to define by normal techniques. By using NRB's biological competitor exemption technique [10], scientists and engineers are able to keep sulfate-reducing bacteria under control with no additional pollution issues and far less environmental damage compared with the use of conventional chemical fungicides. To lessen the risk of sulphate corrosion in the complex storage tank, the optimum ratio of inoculating NRB to nitrate, nitrite, and molybdate was identified over a range of conditions. By using this measure, we were able to prevent sulphate-related damage to the reservoir [11].

Sulfate-reducing bacteria (SRB) in the injection fluids induce "souring," the buildup of sulphides in oil reservoirs. Nitrate is often added to injection fluids in oil fields as a means of mitigating the adverse effects of waste sulphide disposal [12]. In a nutshell, nitrate is useful for both sulfide-oxidizing bacteria and heterotrophic nitrate-reducing bacteria (soNRB and hNRB). Heterotrophic nitrate-reducing bacteria (hNRB) inhibit the development of sulfur-oxidizing nitrate-reducing bacteria (SoNRB) by more efficiently using oil organics. In order to decrease nitrate

levels, hNRB suggests using low molecular weight hydrocarbons such as alkylbenzenes and oil organics such as volatile fatty acids (VFA, a combination of acetate, butyrate, and propionate). Incomplete conversion of nitrate to nitrite allows for this method of avoiding sourness [13]. When oil reservoir temperatures are over 50 to 70 degrees Celsius, nitrite tends to accumulate more rapidly. With the abundance of electron donors in oil fields, nitrite may be transformed to dinitrogen (N_2) at temperatures below 50 °C.

Chronic nitrate injection into the chilly (30 °C) Medicine Hat Glauconitic C (MHGC) field led to nitrate breakthrough in certain producing wells. There was a low concentration of toluene and other alkylbenzenes in the oil that was extracted from these wells, suggesting that they were not suitable substrates for hNRB. Alkylbenzene-oxidizing bacteria, such as Thauera and Azoarcus, have been shown to form part of the bacterial community in MHGCs [14]. Nitrate reduction seems to be impeded by the presence of any alkylbenzenes, however minute. It's probable that the concentration of alkylbenzenes is greater in lighter oils. In lab microcosms, the use of light oil resulted in a 51% reduction in nitrate whereas the use of heavy oil resulted in a 15% reduction. The lingering oils had negligible amounts of toluene. When oil field hNRB was incubated with 4 mM toluene, all of the nitrate was reduced (probably to N_2), but when incubated with 4 mM m-xylene, only a tiny amount was changed to nitrite [15]. Nitrate was not degraded by incubation with ethylbenzene, propylbenzene, m-ethyltoluene, o-ethyltoluene, or p-ethyltoluene. Isolates of varying purity have been discovered to use alkylbenzenes for nitrate reduction. More research has been done on toluene consumption by hNRB isolates than any other substance. Betaproteobacteria, namely Azoarcus and Thauera, make up the vast bulk of these samples. Nitrate-reducing bacterium ToN1 was discovered in toluene, a petroleum waste, and has been demonstrated to rely on crude oil as a carbon source. In-depth research revealed that toluene is the sole oil component consumed by this particular isolate. Toluene was also used by two other strains, mXyN1 and EbN1, which were isolated from m-xylene and ethylbenzene, respectively. The PbN1 strain found in crude oil did not show any ability to decrease nitrate levels in laboratory tests. These findings show that the sort and quantity of alkylbenzenes present in oil significantly affect the amount of nitrate reduction done by hNRB that employ oil as a substrate. Nitrate reduction seems to need the use of toluene as an electron source. But because hNRB needs oil components to complete the nitrate-to-N_2 conversion, so NRB can only be used for sulphide oxidation. Since nitrate reduction is essential for using nitrate effectively in limiting sulphide development in oil reservoirs, that is the primary focus of the present contribution. The rRNA gene 16S conveniently contains of the both bounded and changeable areas. While genetic analysis the sequence variants enables differentiation between varying microorganisms like bacteria, archaea, and microbial eukarya, the conserved region enables universal amplification.

Studying methods to limit SRB variability was a primary focus of [16]. Denitrifying bacteria (DNB), methanogenic bacteria (MGB), saprophytic bacteria (SPB), and zymophyte bacteria (ZPB) were among the functional microorganisms and environmental factors examined in relation to their impacts on SRB. Denitrifying bacteria, methanogenic bacteria, often known as MGB, and iron bacteria are only a few of these organisms (IB). These tests were performed to determine if the preliminary coating facilitates bacterial colonisation by altering the electrostatic charges and hydrophilicity of the metal substrate [17]. Biofilm is a major problem in oil and gas facilities because

the corrosive environment speeds up the dissolving rates of steel and other metals, and it increases the difficulty of maintenance and operation. Because of the organisms found in biofilms' greater resistance to antibacterials and their prospects to afflict clients who have indwelling pharmaceutical products, biofilms pose a serious threat to the community and economy. Cleanup is much more difficult when biofilm is present. It's possible that in the long run, these problems may reduce heat transmission and flow. rates; scaling, corrosion, and fouling are further causes for worry. As a solution to the issue of polypropylene test solutions becoming less viscous when combined with untreated wastewater in the Shengli Oilfield, researchers [18, 37] provided an overview of the data and the implementation of the biocompetitive exemption methodology to remove sulphides produced in sewage and prevent SRB from coming up with new sulphides. The capacity of SRB to manufacture new sulphides was inhibited as a consequence of the deletion of sewage-produced sulphides using this method. This strategy was used to eliminate the preexisting sulphides in wastewater and to stop the formation of SRB. We were able to remove the sulphides already in the effluent and stop the SRB from adding any more using this procedure. Sulfides that had accumulated in the wastewater and those created by SRB were removed using this technique. The recommended approaches use biomineralization, a method for filling fissures that is somewhat slow. Due of this, they are at risk of the rebar corroding as the fracture heals. By incorporating corrosion inhibition produced by microorganisms into MSCC, we are able to greatly increase the amount of protection offered to rebar in this context. Because of this, rebar is shielded from injury to a greater degree. In this study, NO_3^- reducing bacterial granules were shown to reduce NO3 levels, therefore producing NO_2, and so reduce rebar corrosion. It takes the granules just 28 days to heal a fracture 300 micrometres wide [19]. Here are the most recent findings [20, 38] from controlled laboratory tests of SRBs for removing heavy metals. An in-depth breakdown of the findings is provided below. The first stage in this procedure is to specify how SRB cleans the air of metals. In the next part, we will go into further depth on the parameters that influence microbial activity and the efficiency of metal removal. This research is helpful for treating oil reserves with nitrate injection because it expands our knowledge of the corrosion mechanisms at work in hypersaline conditions characterised by the presence of NRP or SRP [21, 39]. The microorganism populations that make their homes in crude oils, tar sands, and biogas reservoirs are investigated in great detail in this study [22, 40]. It is crucial to study the diversity of various key groups in order to demonstrate the dynamic and variable nature of the resident microorganisms. These groups include sulphate and nitrate-reducing bacteria, naturally occurring anaerobic species, and fermentative bacteria.

This overview [23] educates readers on the vast range of organisms that may cause biocorrosion, the mechanisms of action associated with these organisms, and the variables that might affect the pace at which corrosion may develop. There has been a lot of study on biofilm and the function it plays in corrosion. Moreover, research has been conducted to explore the many methods that may be employed to counteract biocorrosion. Additional studies using surface morphology had confirmed B. Possible acceleration of pitting corrosion in X80 steel due to the presence of cereus [24]. This review [25] concludes that a management technique called as "control from farm to folk" is necessary to eliminate aflatoxin contamination and stop the spread of toxigenic fungus. This approach includes a variety of actions, such as monitoring for the presence of toxigenic fungi and aflatoxin levels on-site, halting the

growth of fungal development and aflatoxin-generating fungi to prevent pollution, and disposing of any affected materials.

Contributions to the study

- Using 16S rDNA sequencing, researchers in this work isolated a nitrate-reducing bacterium (NRB) from oil-production water and determined that it was a heterotrophic Pseudomonas sp.
- NRB and molybdate's additive inhibitory effect on SRB was also investigated.
- Scanning electron microscopy was used to examine the corroded steel sheet's topographical features. Moreover, there was little to no pitting or other signs of severe corrosion on the steel sheet's surface.

2. Materials and methods

2.1 Samples

NRB medium: "0.02 g/L $CaCl_2$, 5 g/L NaCl, 0.3 g/L NH_4Cl, 1.8 g/L $MgCl_2$, 0.2 g/L K_2HPO_4, 3 g/L KNO_3, 0.5 g/L KCl, 6 mL/L sodium lactate (50%), pH 7.0–7.4, 1 g/L yeast powder". We obtained the quaternary ammonium salt ZH-B-01 and the imidazoline RX-402 from two petrochemical companies in Beijing and Shanxi respectively. All of the chemical reagents that were used were of analytical quality, with the exception of those that came with a particular notice. Generally speaking, the hydraulic oil's water content shouldn't be higher than 0.12%. The critical range is already 0.10 to 0.12%. At the very least, oil-care steps must be taken here. However, because the oil contains quite so much moisture beyond a composition of 0.12%, it is imperative to replace the oil.

2.1.1 Fisher's exact test

The test helps determine the significance of a connection (contingency) between both the types of classification for categorical variables that come from the classification of objects in a number of ways. Therefore, in Fisher's initial statement, one categorization requirement might be whether milk or tea has been placed in the bowl initially, while another might be whether Bristol claims to believe that the milk or tea has been put in the first.

2.1.2 Segment cluster analysis

In genetics and related disciplines, centralised clusters in code each time are frequently revealed using cluster histogram. When creating a histogram, a rectangle grid is drawn that corresponds to the columns and the rows of the data matrix, and the cells are coloured according to the value systems of the data set.

2.2 Analysis procedures

2.2.1 Determination of S^{2-}

In this investigation, spectrophotometric analysis was performed using a dye solution. You may learn more about the sulfide-methylene spectrometer's water performance specifications by searching for "GBT-16489-1996 water performances of the sulfide-methylene" on the internet.

2.2.2 Determination of NO^{-3}

The following are the parameters utilised by thymol spectrophotometry to arrive at a diagnosis. To prepare the sample, we put 1 millilitre of it and 100 millilitres of ammonium solution into a 50 millilitre colorimetric tube. After letting the solution sit undisturbed for five minutes, 2 mL of the H_2SO_4-A_gSO_4 mixture was added and the mixture was agitated. Then, to get the right consistency, 8 ml of sterile distilled water and 9 mL of ammonia water were added, and the mixture was mixed and diluted. Finally, 25 mL of filtered water was added to complete the mixture. We used a UV spectrometer to find that 420 nm provided the clearest visibility of the solution's absorption.

2.2.3 Determination of NO^{-2}

-Naphthylamine spectrophotometry with hydrochloric acid was utilised. In order to arrive at a precise figure, we used these procedures. One millilitre of a solution comprising was added to a 50-milliliter colorimetric tube holding a predetermined quantity of sample. "p-aminobenzene sulfonic acid" and one millilitre of beta-naphthylamine were added. After 10 minutes of standing and vigorous shaking, the absorbance of the combination was found to be 520.

2.2.4 Quantification of NRB

While three parallel tubes of extinction dilution utilizing the color development with Griess reagent were used to quantify NRB.

2.3 Separation of NRB

A 90 mL NRB medium container was inoculated with 10 mL of generated water from the 72649 oil well using an anaerobic medium. Using nitrogen gas at a pressure of 15 megapascals and a purity of 99.99 percent, oxygen levels in the anaerobic container and the cultured organisms were restored to their former levels, and the mixture was allowed to rest in an incubator at 350 degrees Celsius for four days. Before "a ten percent inoculation of the culture was moved to a new NRB medium," there was no media blackout. History may be broken down into three distinct epochs, each of which experienced unprecedented levels of cultural advancement and global trade. To separate the NRB from the enriched NRB medium, three rounds of enrichment were performed using dilute coatings and sandwich growth on stacked plates. This was only possible after three iterations of enrichment.

2.4 NRB performance in reducing $NO^{-}{}_3$

Strains of interest were plated onto NRB liquid medium and then cultured for four days at 350℃ before being sampled for analysis. A 0.2 mL volume of culture medium was placed in each of the designated tubes, with one additional tube serving as a control. If the media turned red, orange, or brown almost immediately, suggesting that NO_3 was converted into NO_2, this was a positive indicator that "Griess Solvent A" The worst possible consequence would be for the media to turn blue, indicating that no reduction of NO3 was taking place. In addition to preventing the medium from becoming blue, success required converting the NO3 and produced NO2 to non-toxic molecules like N_2 and NH_3.

3. Result and discussion

3.1 Identification and screening of NRB

Due to advances in media identification, NRBs may be successfully countered in mass by a large number of NRBs. Therefore, before being utilised to cover the plates, the culture media had to be isolated from the remainder of the culture medium. A streak plate is used to obtain single colonies of bacteria by applying the dilution principle to a single plate. A streak culture was also performed, with four representative colonies obtained until no viable mixed bacteria were found. Each of these settlements was given a number between 72649-1 and -72649-4. In contrast to an oxygen atmosphere, which is abundant in oxygen, an anaerobic environment is characterised by the absence of free oxygen (O_2). This kind of environment is devoid of oxygen, but it may contain atomic oxygen bound in sulfites, nitrite, and nitrates. An anaerobic environment was maintained and cultivated with four different bacteria. At the end of day four of cultivation, results from a NO_3 reduction performance test are tallied. Table 1 represents the results of nitrate reduction tests.

Table 1: Results of nitrate reduction tests following a four-day culture

Adding liquid A and liquid B	Sample	Adding diphenylamine reagent
Colorless	Control	Blue
Red	72649-1	-
Colorless	72649-3	Colorless
Red	72649-2	-
colorless	72649-4	colorless

After 4 days in culture, the NO_2 concentration of the medium had reduced and the liquid A and B enhanced media were significantly lighter in colour. After adding three reagents to the medium for 72649-3 and 72649-4, the NO_3 and NO_2 were colourless. Therefore, 72649-3 and 72649-4 were superior than NO3 in terms of efficiency and rate of decrease. Both strains, 72649-1 and 72649-3, were shown to be facultative anaerobes by the findings. As a consequence, 72649-3 became the focal point of the subsequent study. E. coli, Salmonella, and Pseudomonas spp. The results of the 16S rDNA analysis positively confirmed that strain 72649-3 was in fact the target organism. Due to the 16S rDNA sequences' low probability of HGT, comparatively slow growth rate, and propensity for concerted evolution among the 16S operons within a single genetic code, they have come to be used as the gold standard for identification of bacteria and phylogeny restructuring.

3.2 NRB activation system optimization

Most of the NRBs in the reservoir were inactive since there weren't enough electronic donors to keep them alive. Pumping NO_3 or NO_2 into the reserve activates the native NRB, which helps battle the growth of SRB and the

subsequent biological deterioration due to the creation of H_2S. These two issues combined to force us to take this measure. The conditions in the culture sample introduced into the enrichment medium were optimal for the development of SRB. The dilution theory underlies the operation of a streak plate. A loopful of microbes is smeared on an agar medium so that with each streak, it becomes fragmented and forms single colonies. Research looked at how introducing NO_3 and NO_2 to an SRB altered its capacity to reduce sulphate. On day eight, the "inhibitory effect of 1.2 g/L NO_2" was at its maximum, when a lot of S_2 figure 2 was made. Combining NO_3 and NO_2 increases their inhibitory effect on SRB. This research looked at how changing the NO_3/NO_2 ratio affected S_2 production and SRB inhibition. When the ambient SRB level was high, the results showed that injecting an NRB solution was more effective at preventing SRB and H_2S formation than activating the reservoir's internal NRB using NO3 and NO2. Strain 72649-3 was used because it is an efficient NO3 reducer and SRB blocker. As can be shown in Fig. 4, when just the active NRB solution was used, its inhibitory effect on S2 was quite weak. When NO_3 and NO_2 were mixed together, the full potential of SRB regulation was realised, and both the engaged NRB and S_2 contents of the state were kept under tight control. As can be observed in Figure 3, the addition of NO3 and NO2 reduces the number of NRBs. The effect of NO_3 and NO_2 additions on S_2 is seen in Figure 5.

When molybdate was used in conjunction with exogenous NRB, the inhibitory effect on SRB was enhanced. The NRB activation system includes nitrate, nitrite, molybdate, and exogenous NRB, all of which work together to prevent sulfate-reducing bacteria (SRB), H2S production by SRB, and biological corrosion. Oral bacteria that break down nitrate, including Rothia, Neisseria,Actinomyces, Veillonella, Haemophilus, Kingella and Corynebacterium nitrate is reduced to nitrite. That molybdate can block SRB's activity is quite exciting. The following elevation in NO-3 content in the responding vessel is seen in Figure 1. As can be observed in Table 2, the response rates are affected by the kind of inhibitor used.

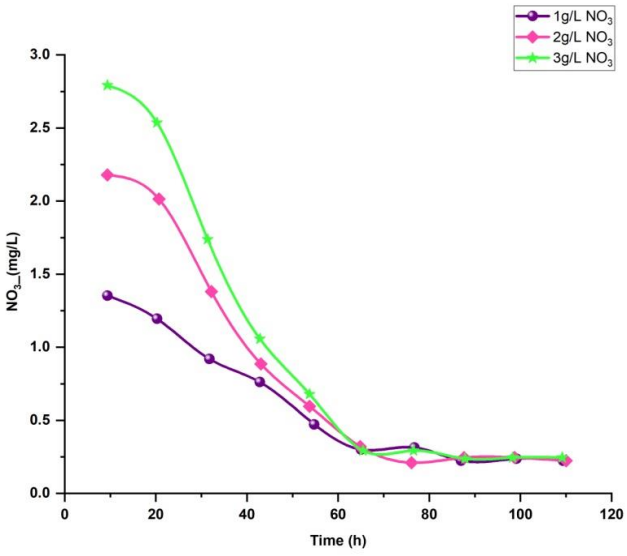


Figure 1: Rise in NO^-_3 content in the reacting container after NO^-_3 addition

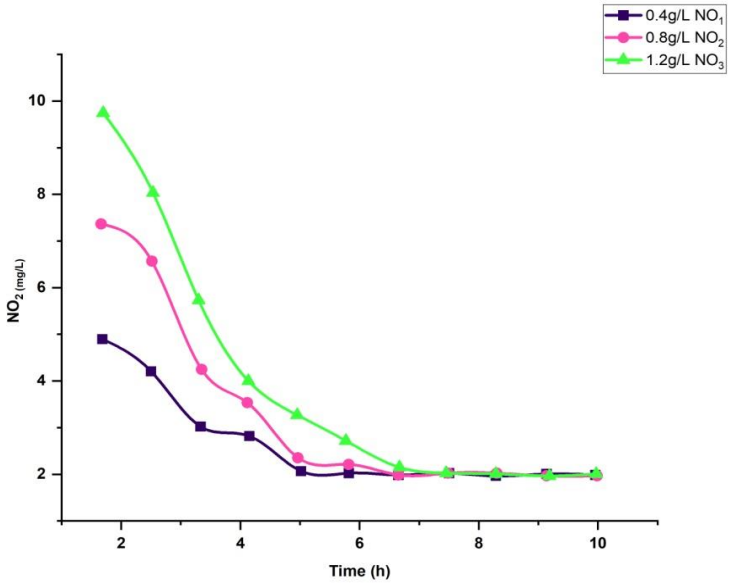


Figure 2: Rise in NO^{-}_{2} concentrations in reacting vials with NO^{-}_{2} supplied

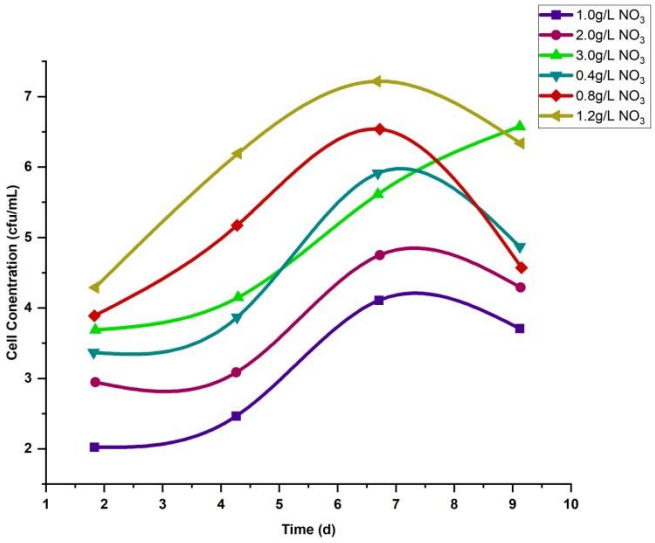


Figure 3: Impact of NO^{-}_{3} and NO^{-}_{2} addition on the abundance of NRB

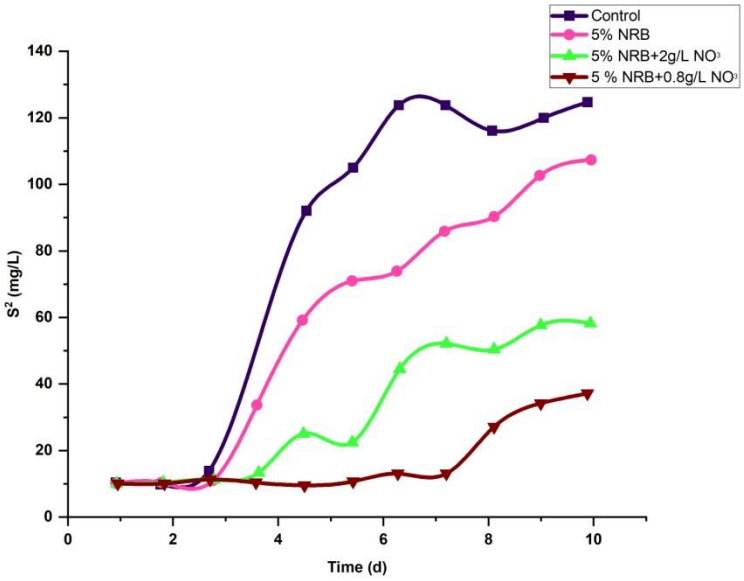


Figure 4: Efficacy of NRB addition upon s^{2-} inhibitory

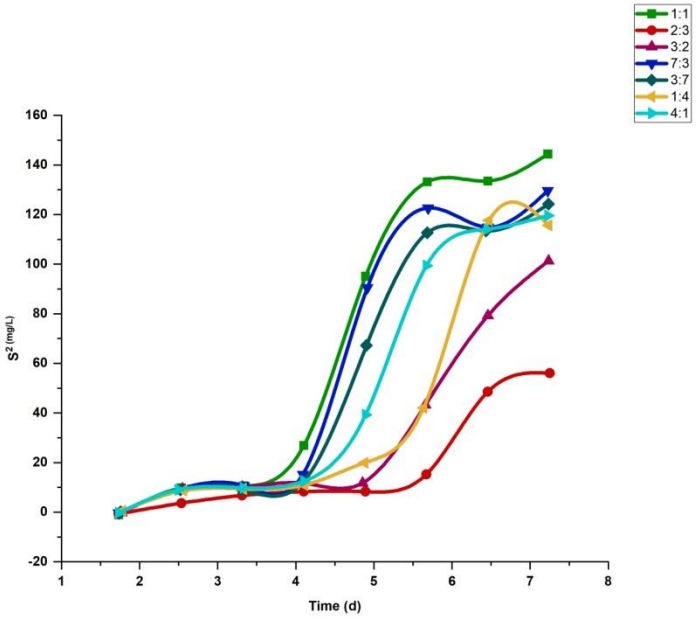


Figure 5: Result of introducing various levels of NO^{-}_{3}, NO^{-}_{2} on s^{2-}

Table 2: Reaction rate variation for various inhibitors

Inhibitor dosage	Corrosion rate (mm/y)
"5%NRB+5 mg/L Na+20mg/LNa"	0.0178
"5%NRB+2.5 mg/L Na+10mg/LNa	0.0281
"5%NRB+10mg/L Na+40mg/LNa"	0.0316
"5%NRB+7.5 mg/L Na+30mg/LNa"	0.0156

As a result, the following conditions "were necessary for the ideal inhibitory system: five percent NRB, a NO_3/NO_2 ratio of 1, and a molybdate" content that was 1/5 that of NO_3 were all present.

3.3 Corroded steel sheet SEM image

A representation of "the surface morphology of the corroded steel sheet" is shown in Figure 6. SEM is an exterior imaging technique that employs occurrence electron beam scanning to produce secondary and backscattered electrons that are then used to reconstruct an image of the specimen. The steel sheet illustrated in Figure 6(a) was subjected to static erosion modelling tests using the first 72649 water sample collected from the generated waters from the oil field. The biologically inhibited steel sheet used in the static corrosion modelling of sample water 72649 is shown in Figure 6. (b). Corrosion pits were seen in huge numbers only on the surface of the metal after a corrosion simulation experiment was run with 72649 steel sheets and the first water sample. Pitting corrosion was the most prevalent kind, and the corrosion pits were broad and dense, both of which are indicative of SRB corrosion. Throughout the pit, dislodged corrosion byproducts had spread, making SRB's presence all the more crucial (Figure 6(a)). Experiments simulating corrosion on Steel Sheet 6(b) in biologically inhibited water indicated much decreased surface corrosion and just a few minor places. As can be observed in Figure 6(a), the distribution was very even, there were no glaring signs of erosion, and the pit damage was minimal.

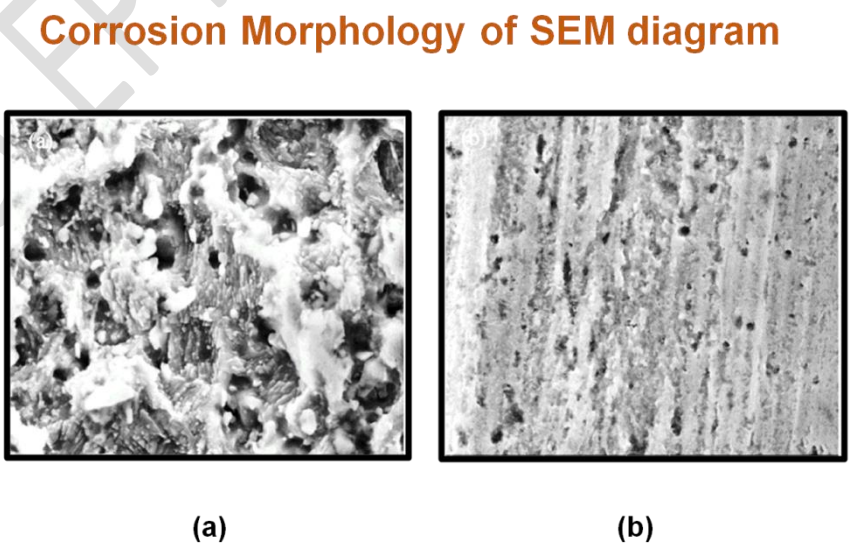


Figure 6: Morphology of corrosion in the SEM diagram (2000×) (a) the steel sheet following the initial 72649 water sample's static corrosion simulation test. (b) The steel sheet following the addition of a biological inhibitor during the static corrosion simulation test using a 72649 water sample.

3.4 Analysis of bacterial community composition

Selected typical OTU (operational taxonomic unit) sequences from bacterial 16S rRNA gene sequencing were compared with the NCBI database to get bacterial annotation information, which was then used to examine the bacterial composition and diversity of the samples. Calculations of relative bacterial abundances and distribution maps were performed using the aforementioned data (Figure 7). Figure 7 shows that NRB constituted 3.9% of X1, with 10 different types present. Figure 8 shows that when BCX was added to the hydrogen sulfide-treated well, the number of NRB species climbed to 26 and the percentage increased to 26.8 percent.

Among them, hNRB with up-regulated abundance included Rhizobiales, Xanthomonadales, Deferribacteraceae and Rhodobacteraceae. In Rhizobiales, Xanthobacter, Xanthobacter, Pleomorphomonas, Hyphomicrobium, Chelatococcus, Pseudoxanthobacter, Tepidamorphus, Kaistia and Bradyrhizobium only existed in X2. In Xanthomonadales, the abundance of Stenotrophomonas increased the most from 0.03% to 2%, and Thermomonas, Pseudoxanthomonas, Rehaibacterium also only existed in X2. The same went to Thioclava, Tabrizicola, Mesorhizobium and Azospirillum.

At genus level, Mesorhizobium, Thauera, Achromobacter, Azospirillum, Marinobacter and Tepidiphilus were up-regulated. The abundance of Thauera increased from 3% to 7%, Achromobacter from 0.03% to 1%, Marinobacter from 0.1% to 9% and Tepidiphilus from 0.004% to 0.5%. SO-NRB with up-regulated abundance consisted of Bosea, Halomonas, Rhizobium and Roseovarius, among which the abundance of Bosea increased from 0.004% to 0.03%, Halomonas from 0.5% to 5%, Rhizobium increased from 0.02% to 0.4% and Roseovarius from 0.01% to 0.7%.

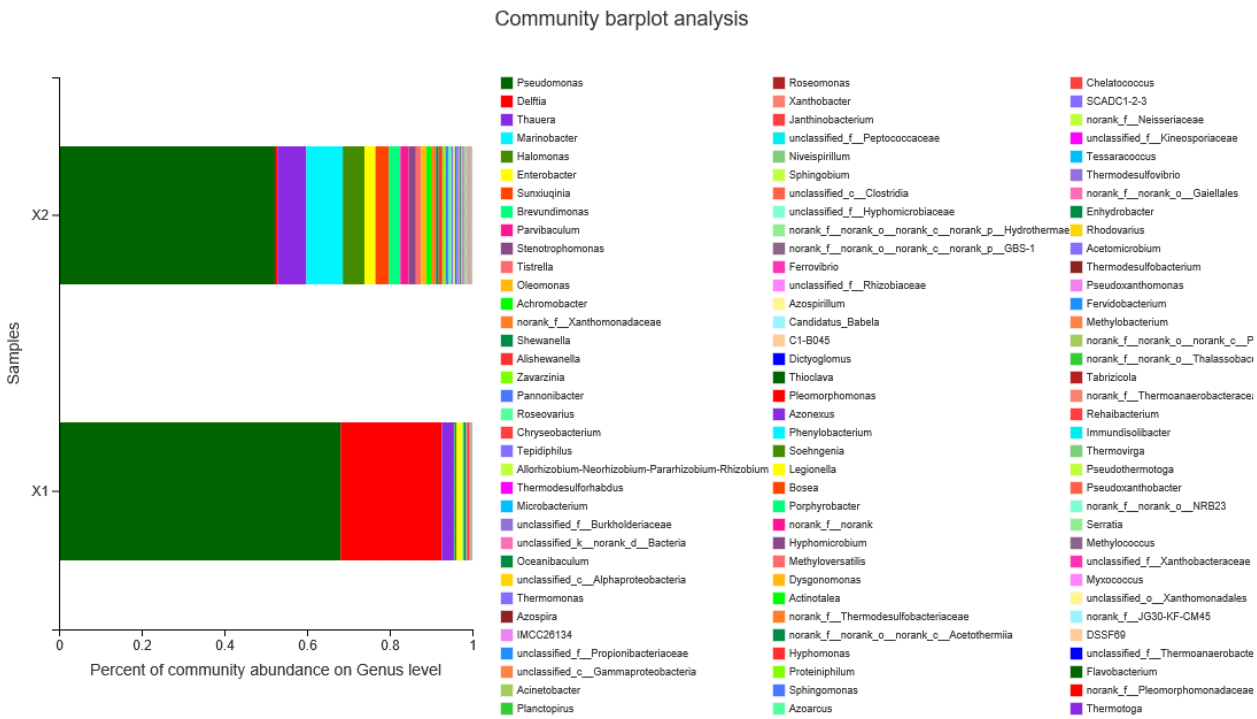


Figure 7 Relative distribution map of bacteria abundance

(The ordinate is the sample, X2 is the hydrogen sulfide-treated well, X1 is the non-treated well. The abscissa is the percent of community abundance on genus level.)

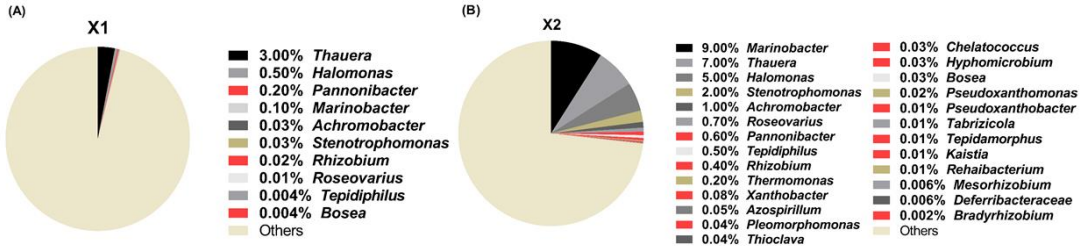


Figure 8 Comparison of NRB abundance in X1 and X2

(A: X1 is the non-treated well. B: X2 is the hydrogen sulfide-treated well.)

3.5 Fisher's exact test

Figure 9 displays the relative abundances of many NRB, including Thauera, Halomonas, Marinobacter, and Tepidiphilus, between X1 and X2. All four of these NRB were strongly elevated in X2 after being exposed to BCX, as evidenced by the red dots.

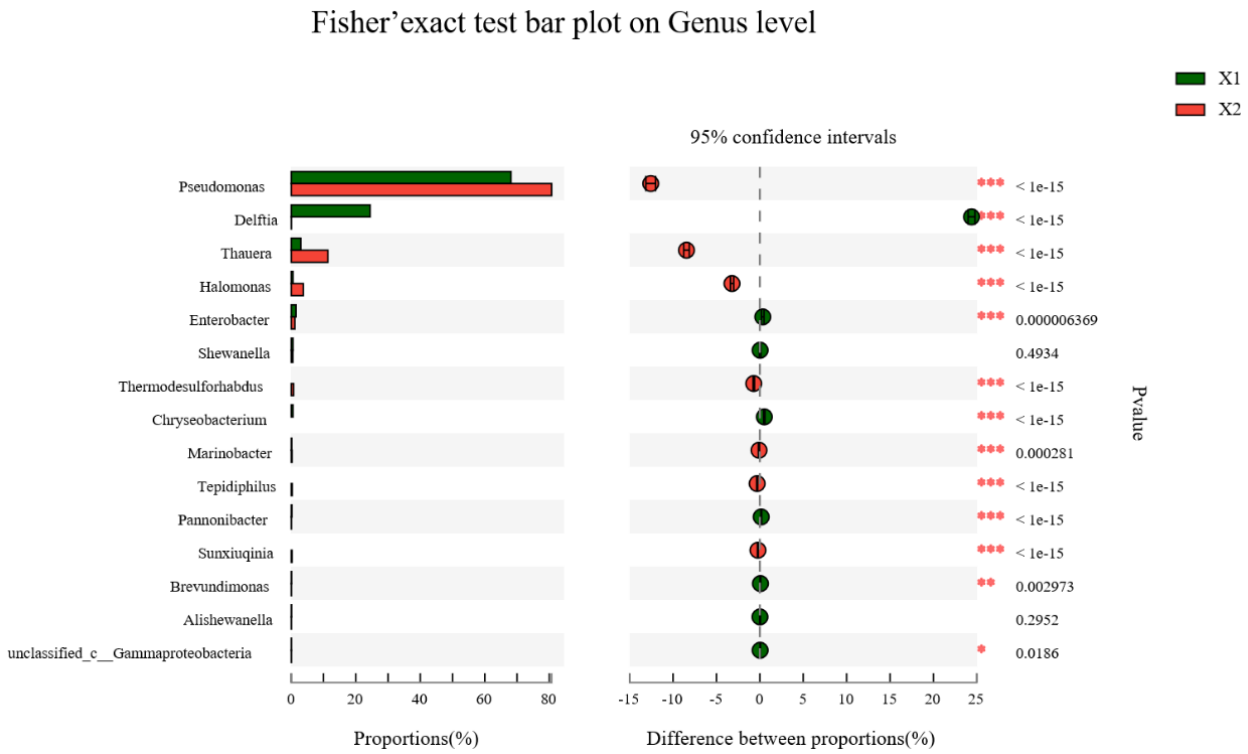


Figure. 9 The relative abundance of bacteria detected by Fisher's exact test

(The ordinate is the bacteria, and its corresponding column represents its abundance in the sample. The dot position indicates the abundance difference in the sample, and the dot color shows the sample color with higher bacteria abundance. X1 is the non-treated well, and X2 is the hydrogen sulfide-treated well. *, $0.01<P\leq0.05$; **, $0.001<P\leq0.01$; ***, $P\leq0.001$.)

3.6 Cluster analysis of Heat map

More red indicates a greater relative abundance of that bacterium in the sample in the heatmap of its relative abundance (Figure 10).

It could be seen from Figure 4 that there existed significant differences in bacterial composition between hydrogen sulfide-treated well X2 and non-treated well X1. In X2, NRB with increased abundance included Azospirillum, Thermomonas, Xanthobacter, Halomonas, Thauera, Marinobacter, Tepidiphilus, Rhizobium, Stenotrophomonas, Pannonibacter, Achromobacter and Roseovarius, which indicated that BCX promoted the proliferation of NRB in hydrogen sulfide-treated well.

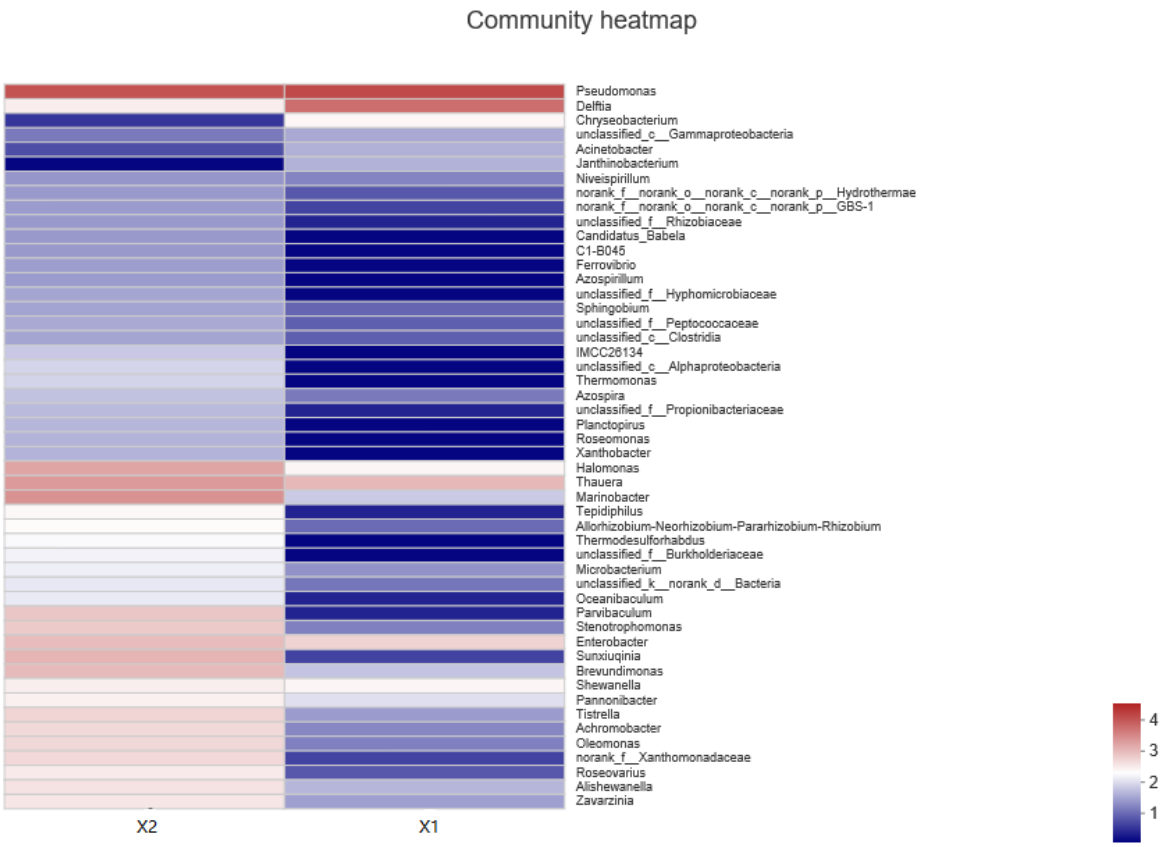


Figure 10 Cluster heatmap of bacterial abundance

(The abscissa represents the sample, while the ordinate represents the bacteria; the ordinate changes in colour to reflect the amount of the bacteria in the sample; a redder hue indicates a greater abundance of bacteria.)

Numerous studies have revealed that nitrate has a major influence on NRB variety and distribution in oil reservoir. Researchers Gittel et al. [26] found that hNRB (Deferribacter) was more prevalent in nitrin-injected oil fields than in those without by conducting molecular biological analysis of the produced fluid of high-temperature nitrin-injected oil fields in the Danish North Sea. The effects of nitrate injection in western Canadian medium-temperature oil fields were studied by Telang et al. [27], who found that SO-NRB (Thiomicrospira sp. CVO) was the most common NRB in both the production well and the injector. Figures 7 and 8 suggest that nitrate inhibitor may promote the growth of a wide variety of NRB. Bosea Thiooxidans, found in farmland soil, can oxidise reduced inorganic sulphur molecules to sulphate [28] and produce gas in a heterotrophic matrix including nitrate by denitrification. Sulfide oxidation during nitrate reduction may allow Halomonas to propagate across a rehabilitated oilfield's ecosystem [29]. Nitrate may be converted to nitrite or gas by Roseovarius and Rhizobium [30, 31]. Thauera may employ organic acids, aromatic compounds, and aliphatic chemicals to reduce nitrate, nitrite, and N_2O in the absence of oxygen [32]. Marinobacter is often found in contaminated marine environments and oilfields. Marine ecosystems adjacent to oil refineries commonly feature Marinobacter hydrocarbonoclasticus, a bacterium that can grow anaerobically on hydrocarbons and convert nitrate to nitrogen through denitrification [33, 34]. Most Rhizobiales can

denitrify nutrient-poor settings effectively due to their nitrate reduction abilities and strong environment adaption [35, 36].

4. CONCLUSION

The following findings are the end result of several studies and testing conducted to determine the level of corrosion resistance shown by SRB. The addition of, NO-3-. Inhibited S2 production to varied degrees. The growth conditions of a culture are improved upon when it is switched to SRB enrichment media. Different concentrations of, NO-3- were employed to test how the SRB's sulphate reduction activity was influenced. and, NO-2-⁻... On the other hand, NO-2-. Effect NO-3-. As well as being a crucial component of SRBs, NO-3- is also a potent stimulant of NRBs. Combine to have an impact on SRB that is larger than the sum of its parts. The effects of inhibiting SRB with, NO-3- were enhanced and, NO-2-⁻. were coupled because they could be supplied at precisely regulated levels of active NRB and S2. SRB functioned best in the presence of, NO-3-. and, NO-2-⁻. at a ratio of one to four, the two groups were merged into one. The addition of sodium molybdate at a concentration of 0.04 g/L had a synergistic inhibitory impact on SRB. Adding 5% NRB to SRB enrichment medium maximises the effectiveness of this synthetic biological inhibitor. However, the proportion seems substantially different when compared to, NO-3-. to ,NO-2-⁻. , NO-3-. Whereas the molybdate concentration was just 1:4. This technique was beneficial in lowering SRB activity and, by extension, SRB-induced corrosion.

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