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Review Article

Nitrate-Reducing Sulphide-Oxidising Bacteria (NR-SOB) in Corrosion Control: A Review

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ABSTRACT

Microbiologically influenced corrosion (MIC) and reservoir souring caused by sulphate-reducing bacteria (SRB) represent major challenges in the oil and gas industry, leading to infrastructure degradation, safety risks and substantial economic losses. In response to these challenges, nitrate injection has emerged as a promising biological control strategy that stimulates nitrate-reducing sulphide-oxidising bacteria (NR-SOB), which will oxidise hydrogen sulphide (H_2S) while competing with SRB for available resources. While previous reviews have broadly discussed nitrate injection as a biocide alternative, this paper uniquely traces the 27-year evolutionary trajectory of NR-SOB research by bridging early field-scale successes with recent molecular and thermodynamic discoveries. This review synthesises evidence from early studies published between 1999 and 2026, examining the presence, mechanisms, and effectiveness of NR-SOB in controlling corrosion rates following nitrate injection. Field studies reported reductions in corrosion rates of approximately 40-50% and sustained SRB suppression over 7-8 years, with NR-SOB genera such as *Sulfurimonas*, *Thiomicrospira*, and *Sulfurovum* identified as the key contributors. However, this review also reveals the complexities of the nitrate-treated system, including potential localised corrosion during sulphide oxidation, the formation of corrosive intermediate products such as elemental sulphur, the ability of certain SRB

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strains to counter nitrite inhibition through nitrite reductase activity, and the microbial response to nitrate treatment. Overall, this review synthesises the mechanisms and field applications of NR-SOB in nitrate-treated systems, drawing on studies from 1999 to 2026, and outlines future directions for optimising NR-SOB-mediated corrosion control.

Keywords: Corrosion, MIC, nitrate injection, NR-SOB, reservoir souring

INTRODUCTION

One of the earliest definitions of corrosion appeared in Uhlig's Corrosion Handbook in 1948, where corrosion was defined as the destruction of a metal through chemical or electrochemical reaction with its environment (Uhlig, 1948). Given that much of the world's critical infrastructure, including highways, buildings, water systems, and oil and gas facilities, relies heavily on metallic materials. These assets remain highly vulnerable to the destructive effects of corrosion (Alamri, 2020). Recent surveys have estimated that global economic losses caused by corrosion amount to approximately US$2.5 trillion, equivalent to 3.4% of the global Gross Domestic Product (GDP) (Procópio, 2022). Corrosion prevention costs an estimated US$875 billion annually, excluding indirect expenses such as specialised labour and productivity losses from maintenance-related shutdowns. Many of these metallic structures in the oil and gas industry are exposed to harsh operating conditions—including elevated pressures on submerged pipelines, extreme temperatures, salinity, pH levels, and naturally corrosive environments—which worsen as oil facilities age. Another major contributor to corrosion is the presence and activity of microorganisms on metal surfaces, also known as microbiologically influenced corrosion (MIC).

MIC can be defined as an electrochemical process involving microorganisms that initiate, facilitate and accelerate corrosion reactions through interactions among three main components: the metal, the surrounding solution and microorganisms (Reza, 2008). The initiation of MIC resembles that of a conventional corrosion occurrence, in which corrosion processes occur in the presence of an electron donor, an electron acceptor, an electrolyte, an energy source and also a carbon source that supports microbial activity (Khan et al., 2021). Since the microorganisms occur in a wide range of environments, MIC has been associated with more than 75% of corrosion cases in producing oil wells and more than 50% of pipeline failures (AlAbbas et al., 2012). Furthermore, MIC costs the oil and gas industry more than US$ 100 million annually and accounts for about 20-30% of internal pipeline corrosion costs (Khan et al., 2021).

The involvement of microorganisms adds a distinctive feature to the electrochemical nature of corrosion, namely the formation of biofilms. A biofilm is an undesirable microbial deposit on a solid surface in contact with water that may reduce operational capacity, shorten equipment lifespan, and cause substantial financial losses. The stages of biofilm

development are shown in Figure 1. As illustrated in Figure 1a, biofilm formation begins with the development of a conditioning film on a solid surface, while microorganisms are initially present in their planktonic state. A conditioning film is a surface coating formed through the adsorption of biomolecules from the surrounding environment (Bhagwat et al., 2021). Bacteria subsequently colonise the conditioning film, as depicted in Figure 1b, and secrete extracellular polymeric substances (EPS). The EPS functions as an adhesive matrix that anchors cells onto the surface before they become sessile (Javaherdashti & Raman, 2001).

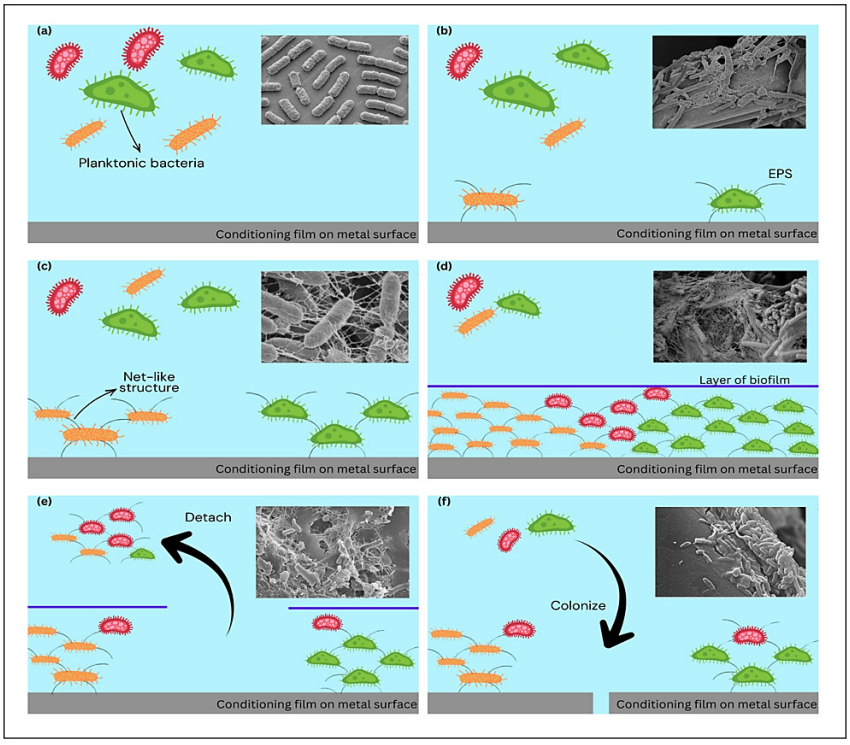

Figure 1. Schematic representation of the stages of biofilm formation: (a) bacteria at the planktonic stage; (b) bacteria forming colonies with secretion of EPS; (c) replication of bacteria; (d) formation of biofilm within microcolonies; (e) detachment of bacteria and (f) recolonisation of exposed or new surfaces. Each schematic illustration is accompanied by field-emission scanning electron microscopy (FESEM) analysis

Sessile bacteria subsequently continue to replicate on the metal surface, forming a net-like structure (Figure 1c) that traps additional particles and promotes biofilm development. The close association between microcolonies of different species, as in Figure 1d, increases layer thickness, eventually leading to the development of a mature biofilm. The presence of this biofilm, together with complex interactions among the bacterial species within it, alters the electrochemical conditions at the metal-biofilm interface compared with the surrounding

environment. This electrochemical alteration is known as ennoblement, a process that shifts the corrosion potential towards a more positive value and increases the likelihood of pitting corrosion. As shown in Figure 1e, portions of the biofilms may detach or disperse into the environment, colonising new surfaces. Meanwhile, in Figure 1f, the exposed areas may then be recolonised by other planktonic bacteria from the surrounding medium.

The offshore industry often experiences corrosion of infrastructure due to the use of seawater in secondary recovery methods (Nicoletti et al., 2022). This method involves injecting outer fluids, such as seawater, into the oil reservoir to displace oil and increase recovery rates (Mohammadi et al., 2024), thereby initiating reservoir souring. Under marginally sour conditions, corrosion behaviour shifts between uniform and localised attack depending on environmental and microbial activity, underscoring the instability of sulphate-reducing bacterium (SRB)- dominated systems (Azhar et al., 2026). Although the seawater is treated before injection, this process is intended to remove debris that could clog the oil pipeline, while leaving the chemical properties unchanged. As a result, studies on marine environments have shown that SRB activity promotes severe localised corrosion and pitting on carbon steel through biofilm formation and hydrogen sulphide (H_2S) production (Zulkafli et al., 2023).

Additionally, higher H_2S concentrations have been reported to increase both uniform and localised corrosion rates, showing the aggressiveness of SRB action in sour environments (Azhar et al., 2025). Over time, the oil and gas industry has developed a method called nitrate injection to counter the effects of reservoir souring by influencing the growth of heterotrophic nitrate-reducing bacteria (hNRB), including types that can oxidise sulphide at the expense of nitrate, known as nitrate-reducing sulphide-oxidising bacteria (NR-SOB), to suppress SRB (Nemati & Voordouw, 2000). While several recent reviews have comprehensively covered the general microbiology of reservoir souring and the broad mechanisms of MIC, they often overlook the specific dual role of NR-SOB in operational environments. Existing literature lacks a focused synthesis that connects the historical field successes of NR-SOB with the modern understanding of its operational risks, such as incomplete sulphide oxidation and nitrite-induced localised corrosion. To fill this gap, this review uniquely focuses on the 27-year trajectory (1999 to 2026) of NR-SOB-mitigated corrosion control. It systematically evaluates not only the biological mechanisms and field performances of NR-SOB but also critically assesses the emerging thermodynamic and molecular complexities that challenge its universal efficacy.

Nitrate Injection in Controlling Reservoir Souring

MIC in the oil and gas industry is primarily associated with the metabolic activities of SRB, which utilise sulphate as a terminal electron acceptor under anaerobic conditions and produce H_2S as a metabolic byproduct (Dall'Agnol & Moura, 2014; Rizk, 2016).

The presence of SRB indicates simultaneous corrosion and reservoir souring in the petroleum system, along with H_2S. This process can cause detrimental effects, including corrosion of infrastructure, equipment failure, higher oil-refining costs due to sulphur content, and safety hazards for crew members (Gao & Fan, 2023). This issue is particularly severe in offshore water-injection systems and secondary oil-recovery methods, where sulphate-containing seawater is injected into oil reservoirs (Bødtker et al., 2008). Although reservoir souring is often associated with seawater flooding, conventional chemical injection struggles to control it because the biologically active zones within the reservoir are vast (Callbeck et al., 2011).

Nitrate injection has therefore been widely applied in oilfields as a method to mitigate reservoir souring, often referred to as Bio-Competitive Exclusion (BCE) technology (Al-Tamimi & Mehdi, 2017; Kiss et al., 2010). The introduction of nitrate shifts the terminal electron-accepting process from sulphate reduction to nitrate reduction, which is thermodynamically more favourable and yields more energy for the participating microbial communities (Davidova et al., 2001; Xue & Voordouw, 2015). This shift involves two main functional groups of bacteria, namely hNRB and NR-SOB, each of which plays distinct but complementary roles in controlling the sulphur cycle (Gittel, 2010; Grigoryan & Voordouw, 2008). The presence of hNRB is vital for long-term suppression of SRB activity. Their primary mechanism of action is bio-competitive exclusion, in which they outcompete SRB for limiting electron donors—volatile fatty acids (VFAs), including acetate, propionate, and butyrate (Al-Tamimi & Mehdi, 2017; Hubert & Voordouw, 2007; Suri et al., 2017).

Because nitrate provides more Gibbs free energy than sulphate reduction, hNRB can achieve higher biomass yields and growth rates than SRB when utilising the same organic substrates (Gittel, 2010; Xue & Voordouw, 2015). This competition deprives SRB of the necessary electron donor required for sulphate reduction, thereby decreasing sulphide production (Kumaraswamy et al., 2011; Voordouw, 2008). The reduction of nitrate by hNRB can proceed via denitrification to nitrogen gas. The following equations represent the stoichiometry of nitrate reduction using acetate as an electron acceptor, with the actual products varying with reservoir conditions, pH, and specific microbial species. Equation 1 shows the general nitrate-to-nitrite reduction, and Equation 2 represents complete denitrification.

$$CH_3COO^- + 4NO_3^- \rightarrow 2HCO_3^- + 4NO_2^- + H_2O \quad [1]$$

$$5CH_3COO^- + 8NO_3^- + 3H^+ \rightarrow 10CO_2 + 4N_2 + 7H_2O + 8OH^- \quad [2]$$

While hNRB focus on preventing new H_2S production, NR-SOB, such as *Sulfurimonas* and *Arcobacter* are responsible for the removal of existing sulphide (Bødtker et al., 2008;

Lahme et al., 2019; B. Wang et al., 2024). These bacteria act as chemolithotrophs, utilising nitrate or nitrite as electron acceptors to oxidise H_2S (Davidova et al., 2001; Jahanbani Veshareh & Nick, 2019). NR-SOB facilitate the conversion of sulphide into less harmful or reusable sulphur species. The primary pathways include partial oxidation, where sulphide is oxidised to elemental sulphur, which may appear as an intermediate product and complete oxidation, where sulphide is fully oxidised to sulphate, effectively recycling the sulphur back into the reservoir system (Deng et al., 2022; Xue & Voordouw, 2015). In general, NR-SOB carry out a process critical to removing existing H_2S from the reservoir. The following equations represent the representative stoichiometry for sulphide oxidation by NR-SOB with a complete oxidation pathway, Equation 3:

$$5HS^- + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O \quad [3]$$

The presence of nitrite after reaction carried out by hNRB and NR-SOB is arguably the most potent inhibitor of biogenic sulphide production. It functions through three primary mechanisms: metabolic inhibition, abiotic reaction, and toxicity. Nitrite specifically inhibits the dissimilatory sulphite reductase (Dsr) enzyme in SRB. By binding to the Dsr, nitrite prevents the reduction of sulphate to sulphide, effectively halting H_2S production without necessarily killing the SRB cells (Al-Tamimi & Mehdi, 2017; Gittel et al., 2009; Jahanbani Veshareh & Nick, 2019). In the second mechanism, nitrite can react chemically with dissolved sulphide to produce elemental sulphur and nitrogen gas, leading to direct removal of H_2S (Davidova et al., 2001). Lastly, if nitrite is present at high concentrations, it can exert direct toxicity on SRB and other anaerobic microorganisms, contributing to a rapid reduction in their activity (Grigoryan & Voordouw, 2008; B. Wang et al., 2024). Moreover, nitrite, generated as a metabolic intermediate, specifically inhibits the SRB dissimilatory sulphite reductase (Dsr), providing multifaceted suppression of biogenic souring. Multiple bacterial genera capable of nitrate reduction and sulphide oxidation have been identified in oil field systems following nitrate injection. The most frequently documented genera include *Sulfurimonas*, *Thiomicrospira*, *Sulfurovum*, *Sulfuricurvum*, *Thiobacillus denitrificans* and *Arcobacter* sp., as summarised in Table 1.

Table 1
Genera of NR-SOB discovered and used in studies

NR-SOB Genera	Location	Biocorrosion Control Mechanism	Ref.
Sulfurimonas sp.	Veslefrikk injection wellhead	Thrive in oligotrophic conditions	Bødtker et al. (2008)
Thiomicrospira sp.	Laboratory	Dramatically shift the environmental redox potential, creating conditions unfavourable for SRB activity	Greene et al. (2003)

Table 1 (*continued*)

NR-SOB Genera	Location	Biocorrosion Control Mechanism	Ref.
Sulfurovum sp.	Bioreactor	Responsive to nitrate treatment in sulfide-rich environments	Yang et al. (2021)
Sulfuricurvum sp.	Petroleum reservoir	Responsive to nitrate treatment	Tian et al. (2024)
Thiobacillus denitrificans & *Arcobacter* sp.	Sediments in Balongan Bay, Indramayu	Reduce sulfide production	Ihsan et al. (2022)

Figure 2 shows the reaction pathway involving SRB, hNRB, and NR-SOB in reservoir souring conditions. Figure 2a depicts the role of NR-SOB in oxidising hydrogen sulphide to sulphate or intermediate sulphur species. In addition to NR-SOB controlling the souring, its by-product, which is nitrite, acts as an inhibitor of the dissimilatory sulphite reductase (Dsr) enzyme of SRB, hindering its action of reducing sulphate to sulphite (Greene et al., 2003; Veshareh et al., 2021; Zuo et al., 2024) as shown in Figure 2b. Figure 2c illustrates the reaction pathway in which hNRB presence causes SRB to compete for organic carbon, such as lactate. Both SRB and hNRB use lactate as their organic electron donor, which is then oxidised incompletely to acetate and CO_2 (Hubert & Voordouw, 2007). This type of souring control by hNRB is known as competitive exclusion, whereby SRB gain reduced access to lactate, leading to slower metabolic activity and decreased H_2S production (Gao & Fan, 2023).

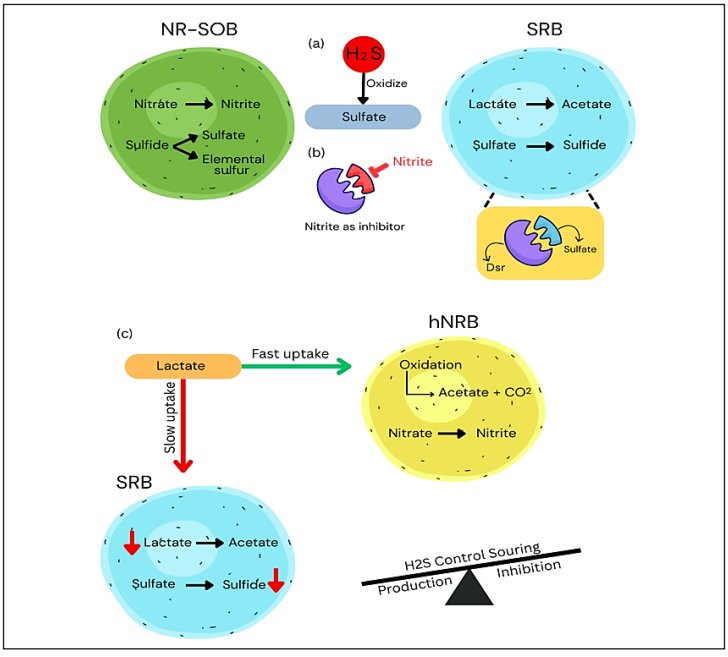

Figure 2. The reaction pathway for (a) NR-SOB, (b) SRB and (c) hNRB in reservoir souring

The sulphur cycle in oil reservoirs involves complex interactions between various microbial functional groups. SRB reduces sulphate in the reservoir to sulphide, while sulphur-oxidising bacteria (SOB) oxidise previously reduced sulphur compounds back to sulphate or intermediate oxidation states (Gao & Fan, 2023; Ihsan et al., 2022; Tian et al., 2024). Without counteracting activity from other microbial groups, SRB activity dominates under anaerobic conditions, leading to sulphide accumulation and associated corrosion in the system (Streets & Eden, 2024). The biogenic production of H_2S not only causes reservoir souring but also drives an electrochemical reaction that initiates, accelerates, and sustains the degradation of metallic infrastructure. Furthermore, the establishment of SRBs within complex biofilms creates a localised environment that shifts the corrosion potential positively, significantly increasing the risk of severe pitting and equipment failure.

As shown in Figure 3, nitrate injection alters this sulphur cycle by introducing an alternative electron acceptor that is thermodynamically more favourable than sulphate (Dolfing & Hubert, 2017; Veshareh et al., 2021). This method stimulates two main microbial groups: hNRB, which compete with SRB for organic electron donors, and NR-SOB, which oxidise sulphide using nitrate or nitrite as electron acceptors (Grigoryan & Voordouw, 2008). This results in a shift in microbial community structure and metabolic activity, thereby controlling sulphide production and accumulation (Deng et al., 2022; Deng et al., 2025).

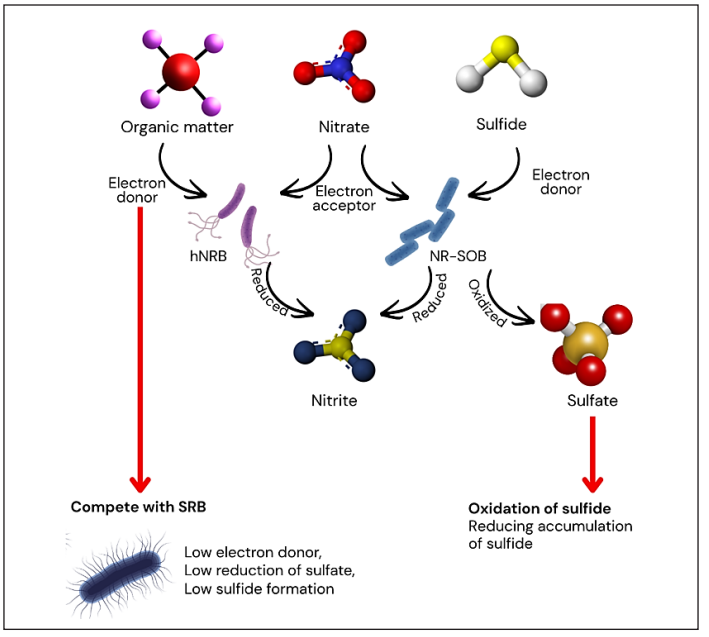

Figure 3. Microbial pathways involved in nitrate-reducing and sulphide-oxidising processes

Compared with conventional biocides like glutaraldehyde, nitrate injection offers a sustainable, cost-effective, and environmentally friendly alternative (Bødtker et al., 2008; Eckford & Fedorak, 2004; Sunde et al., 2004; Xue & Voordouw, 2015; Yuk et al., 2020). Field and laboratory evidence consistently demonstrates that nitrate can equal or surpass the performance of glutaraldehyde in suppressing souring. In sandstone column experiments, nitrite was found to be more effective than glutaraldehyde, with SRB recovery being delayed significantly longer after nitrite treatment (Reinsel et al., 1996). While biocides like glutaraldehyde may lead to rapid regrowth as residual biofilms enhance transport and sorption, nitrate provides a more stable long-term inhibition. Recent research highlights that nitrate can be highly effective at lower dosages when used synergistically. Sodium nitroprusside (SNP), for instance, has been shown to be highly synergistic with nitrate, where 1 ppm of SNP combined with 85 ppm of sodium nitrate was sufficient to inhibit souring (Fida et al., 2018). This synergistic approach allows for a substantially lower overall chemical loading than traditional biocide shock dosages.

Beyond reducing chemical loading, nitrate injection is a highly effective way to structurally alter the microbial ecosystem. Proven effective in North Sea oil field applications, this strategy stimulates beneficial microbial communities that mitigate souring through three primary pathways: (1) direct oxidation of sulphide by NR-SOB, (2) competitive exclusion by hNRB for electron donors, and (3) production of nitrite that hinders SRB activity (W. Liu et al., 2025; Zhou et al., 2025). However, the method's efficacy depends on maintaining precise nitrate concentrations. Deviations can alter the localised electrochemical environment within biofilms, potentially inducing incomplete oxidation cycles that inadvertently facilitate pitting corrosion. These incomplete oxidation cycles refer to metabolic pathways where a substrate is not fully oxidised to its highest possible oxidation state, resulting in the accumulation of reactive intermediates. In biofilm-mediated corrosion, two incomplete cycles are critical: incomplete nitrate reduction and incomplete sulphide oxidation.

Nitrate reduction typically proceeds via denitrification or dissimilatory nitrate reduction to ammonium (DNRA) (Fu et al., 2022; Herath, 2019). In many biofilm environments, nitrate reduction is incomplete, leading to transient or sustained nitrite accumulation. Accumulating nitrite acts as a strong oxidising agent and cathodic reactant that significantly alters the electrochemical balance at the metal-biofilm interface. Nitrite accumulation in the biofilm provides a potent cathodic reactant. In studies using *Shewanella oneidensis* MR-1, nitrite was found to cathodically protect specific areas of the metal surface while accelerating the corrosion of nearby unprotected (anodic) areas (Miller et al., 2018). This creates a large cathode-to-small anode ratio, the classic prerequisite for aggressive pitting (Nicoletti et al., 2022; Street et al., 2015).

In a system where nitrate is present, NR-SOB will utilise nitrate to oxidise biogenic sulphide (Jia et al., 2017; Salgar-Chaparro et al., 2022) , where sulphide is oxidised back to sulphate. However, incomplete oxidation can lead to the accumulation of elemental sulphur, thiosulfate, and polysulfide (Lahme et al., 2019). The accumulation of these species depends on the nitrate-to-sulfide stoichiometry within the biofilm (Nicoletti et al., 2022). Elemental sulphur is highly corrosive to carbon steel. When NR-SOB incompletely oxidise sulphide, the resulting elemental sulphur can deposit on the metal surface, promoting localised acid generation and providing a localised cathodic site that drives pit growth.

The implementation of nitrate injection represents a significant paradigm shift in managing MIC and reservoir souring, moving from broad-spectrum chemical suppression to targeted ecological modulation. This strategy capitalises on nitrate's thermodynamic advantage as an electron acceptor, enabling hNRB to marginalise SRB through competitive exclusion for essential organic carbon sources such as lactate. Beyond simple nutrient competition, the established microbial community provides a multi-layered defence; NR-SOB, such as *Sulfurimonas* and *Thiomicrospira*, actively remediate existing souring by oxidising biogenic H_2S into less harmful compounds or sulfate (Gao & Fan, 2023; Nemati et al., 2001). Furthermore, nitrite accumulation—a metabolic intermediate—acts as a potent biochemical inhibitor that specifically disrupts the Dsr enzyme, thereby halting the SRB's primary mechanism for sulphide production (Greene et al., 2003; Veshareh et al., 2021).

Biological Mechanisms of Reservoir Souring Control

NR-SOB, such as *Sulfurimonas spp.*, oxidise sulphide while reducing nitrate to nitrite or other nitrogen species. Their metabolic activities produce intermediates such as nitrite and elemental sulphur, both of which can accelerate corrosion. NR-SOB thrive in nitrate-rich, low-oxygen environments typical of nitrate-injected systems. The biofilms produce a steep chemical gradient that exacerbates localised corrosion. Although the effect of NR-SOB is significant, knowledge gaps remain regarding long-term behaviour, temperature response, and nitrate availability.

NR-SOB use sulphide as an electron donor while reducing nitrate or nitrite as the electron acceptor, removing H_2S from the system (Lahme et al., 2019; Rizk, 2016). Sulfide oxidation can proceed via multiple pathways, with endpoints varying by environmental conditions and the microbial group involved (Dolfing & Hubert, 2017). Complete oxidation of sulphide to sulphate displays the most thermodynamically favourable pathway under some conditions. However, incomplete oxidation of sulphide to intermediate sulphur compounds, such as elemental sulphur, is also common (Buckingham & Eckert, 2021; Dolfing & Hubert, 2017). Laboratory studies have reported that NR-SOB strains such as *Thiomicrospira sp.* strain CVO are able to oxidise up to 15 mM sulphide while increasing the redox potential of the environment from -400 mV to +100 mV (Greene et al., 2003).

This significant shift in redox potential creates an environment less favourable to SRB activity.

Thermodynamic modelling has significantly enhanced the understanding of which oxidation pathways are favoured in nitrate-treated systems. The coexistence of both NRB and NR-SOB in a nitrate-treated system highlights two distinct metabolic routes: nitrate-driven oxidation of sulphide and nitrate-driven oxidation of organic compounds. To determine the more thermodynamically favourable route, (Dolfing & Hubert, 2017) demonstrated that nitrate-driven oxidation of acetate was more exergonic than nitrate-driven oxidation of sulphide. The corresponding reactions are shown in Equations 4 and 5. The change in Gibbs free energy ($\Delta G°'$), normalised to 2 mol of electrons transferred, was −200.4 kJ per 2 mol e^- for acetate oxidation and −186.1 kJ per 2 mol e^- for sulphide oxidation. Thus, acetate oxidation yielded approximately 14.3 kJ more energy per 2 mol e^- transferred than sulphide oxidation under the specified standard conditions.

$$5acetate + 13H^+ + 8NO_3^- \rightarrow 4N_2 + 10CO_2 + 14H_2O\ ,\ \Delta G^{o'} = -200.4\ kJ/2e^- \quad [4]$$

$$5HS^- + 3H^+ + 8NO_3^- \rightarrow 4N_2 + 5SO_4^{2-} + 4H_2O\ ,\ \Delta G^{o'} = 186.1\ kJ/2e^- \quad [5]$$

Researchers have calculated Gibbs free energies for various sulphide oxidation reactions, showing that sulphide oxidation by NR-SOB under nitrate conditions can lead to either complete oxidation to sulphate or incomplete oxidation to elemental sulphur, depending on the availability of nitrate and other environmental factors (Dolfing & Hubert, 2017; Valdés et al., 2023). This finding has vital implications for corrosion control, as elemental sulphur can be corrosive under certain conditions. Another major mechanism by which nitrate injection controls SRB activity is the competitive exclusion of hNRB for organic electron donors (Gieg et al., 2011; Grigoryan & Voordouw, 2008; Hagar et al., 2022; Londry & Suflita, 1999). The reduction of nitrate is thermodynamically and energetically more favourable than the reduction of sulphate, thereby providing hNRB with higher growth yields and rates when competing for the same substrates (Xie et al., 2025; Youssef et al., 2009). Thermodynamics allows hNRB to outcompete SRB for VFAs, lactate, and other organic compounds originally used to fuel sulphate reduction (Grigoryan et al., 2008).

Laboratory studies using oil field samples from Argentina showed that competitive oxidation of volatile fatty acids by SRB and NRB, in conjunction with nitrate-reducers, has kinetic advantages (Grigoryan et al., 2008). Substrate availability, along with fermentative and oil-degrading bacteria that generate electron donors, influences the dynamics of oxidation competition and sustains both sulphate and nitrate reduction (Gao & Fan, 2023). The formation of nitrite as an intermediate product during nitrate reduction strongly and specifically inhibits SRB, serving as a biological mechanism controlling reservoir souring

(Grigoryan & Voordouw, 2008; Grigoryan et al., 2008; B. Liu et al., 2021). Both hNRB and NR-SOB produce nitrite through partial reduction of nitrate, and this nitrite accumulation plays a critical role in suppressing SRB (Al-Humam et al., 2022; Tao et al., 2025; Zhou et al., 2025). This inhibitory effect of nitrite on SRB activity has been reported in various laboratory and field studies (Mand et al., 2017).

However, the relationship between nitrite production and SRB inhibition is more complex than initially discovered. While nitrate is frequently introduced to suppress reservoir souring, certain SRB possess the ability to reduce nitrite, which is the intermediate produced during nitrate reduction, to ammonium through DNRA (Bourceau et al., 2023; Greene et al., 2003). The nitrite reductase enzyme (Nrf) mediates this process, and the presence of the nrfA gene confers resistance. By further reducing inhibitory nitrite to ammonia, SRB with Nrf activity prevent nitrite accumulation, which would otherwise disrupt their metabolic activity.

Consequently, these specific SRB populations can persist and continue to produce sulphide even in the presence of high nitrate concentrations (Mohanakrishnan et al., 2011). Treatment failure occurs primarily when the microbial community is dominated by or contains significant populations of SRB capable of Nrf-mediated nitrite ammonification. For example, *Desulfovibrio vulgaris* Hildenborough utilises Nrf to respire nitrite, allowing it to bypass the inhibitory effects typically associated with nitrite accumulation (Korte et al., 2015). For other SRB strains that lack Nrf activity, they will have to face a long-lasting inhibition of nitrite. This discovery is important for understanding that the effectiveness of nitrate treatment can vary across oil field environments with different SRB populations.

Treatment efficacy depends heavily on the applied concentration and dosing strategy. High concentrations of nitrite can suppress all microbial activity, while gradual application of nitrite at low concentrations has a less pronounced effect on corrosion (Youssef et al., 2009). This study suggests that periodic and continuous nitrate injection allows moderate nitrite production, which can be more effective than high-dose, irregular injection. Nitrate injection stimulates a shift in microbial community composition, with marine bacterial taxa and autotrophic groups responding rapidly to nitrate availability (Gao & Fan, 2023; P. Liu et al., 2025). Molecular analyses using 16S rRNA gene sequencing, DGGE (denaturing gradient gel electrophoresis), and functional gene quantification have revealed the complexity of microbial community dynamics (Bødtker et al., 2008; Tian et al., 2024).

At the Veslefrikk oil field, bacteria associated with NR-SOB, such as *Sulfurimonas sp.,* formed a major population at the injection wellhead throughout the 7 to 8-year treatment period. However, their presence was less pronounced downstream of the deaerator and finally disappeared after 40 months of nitrate treatment at some locations (Bødtker et al., 2008). This spatial and temporal variation in NR-SOB populations shows the importance of environmental conditions in regulating community structure. Such heterogeneity suggests that localised factors, including oxygen availability, nutrient concentrations, and flow rates,

play a critical role in determining the persistence and distribution of these beneficial consortia. Furthermore, the eventual disappearance of NR-SOB at specific downstream points may indicate a shift in metabolic niches or the completion of sulphide oxidation, highlighting the dynamic nature of microbial succession within nitrate-treated injection systems.

Recent research has shown that nitrate and oxygen significantly alter the abundance and structure of SRB and SOB in petroleum reservoir water (Tian et al., 2024). The highest SRB inhibitory effect and the lowest gene-encoding ratio for Dsr and sulphur oxidation (soxB) were obtained under high concentrations of nitrate and oxygen, suggesting synergistic effects. Commonly detected SRB genera include *Desulfovibrio*, *Desulfobacca*, *Desulfobulbus*, *Sulfuricurvum,* and *Dechloromonas*. During nitrate treatment, the biofilm community tends to remain highly diverse and stable for long periods (Abd Rahman et al., 2022; Alkan et al., 2024; Bødtker et al., 2008). The stability of the biofilm community may contribute to the prolonged effectiveness of nitrate treatment, as multiple microbial groups can be more resilient to environmental disruption. However, the persistence of certain microorganisms across different treatment phases suggests either metabolic flexibility or spatial zonation based on nutrient availability (Gao & Fan, 2023). This ecological robustness is further exemplified by the successful replacement of deleterious SRB populations with beneficial nitrate-reducing consortia, marking a fundamental shift toward a balanced microbial system. Consequently, the established biofilm acts as a stable biological barrier, in which the continuous presence of functional groups such as NR-SOB ensures the long-term suppression of sulphate reduction and sustained mitigation of localised corrosion.

Field Performances and Optimisation of Nitrate Injection

In 1999, among the earliest studies, the Veslefrikk and Gullfaks fields replaced glutaraldehyde biocide treatment with continuous low-concentration nitrate injection, allowing extended monitoring and analysis of treatment outcomes (Bødtker et al., 2008). At the Gullfaks field, inhibition of H_2S by nitrate injection on platforms B (GFB) and C (GFC) was compared with the use of biocide. During nitrate treatment, SRB activity remained low, averaging 0.86 ± 0.32 μg H_2S/cm^2/day (±SE, n = 76) at GFB and 0.84 ± 0.28 μg H_2S/cm^2/day (±SE, n = 59) at GFC. In contrast, SRB activity was substantially higher during biocide treatment, with averages of 4.98 ± 0.51 μg H_2S/cm^2/day (±SE, n = 72) at GFB and 11.73 ± 1.40 μg H_2S/cm^2/day (±SE, n = 60) at GFC. The study outcomes showed stable, long-term SRB inhibition in seawater injection systems after nitrate treatment. Community analysis of the 16S rRNA gene by Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) showed that bacteria belonging to NR-SOB, such as *Sulfurimonas sp.* formed a major population at the injection wellhead throughout the treatment period at Veslefrikk. This provides direct evidence of the establishment and persistence of NR-SOB communities following nitrate injection.

Following nitrate injection, the SRB population was replaced by an equally large NRB population in the biofilm, showing a complete shift in the dominant microbial functional group (Halim et al., 2011; Sunde et al., 2004; Voordouw, 2008). The shift in microbial groups was accompanied by a significant decrease in H_2S production upon the treated water reaching the production wells, confirming the effectiveness of the biological control strategy at the field scale. Figure 4 shows a significant reduction in corrosion rates following nitrate treatment, with corrosion levels in the water injection system decreasing by more than 50%.

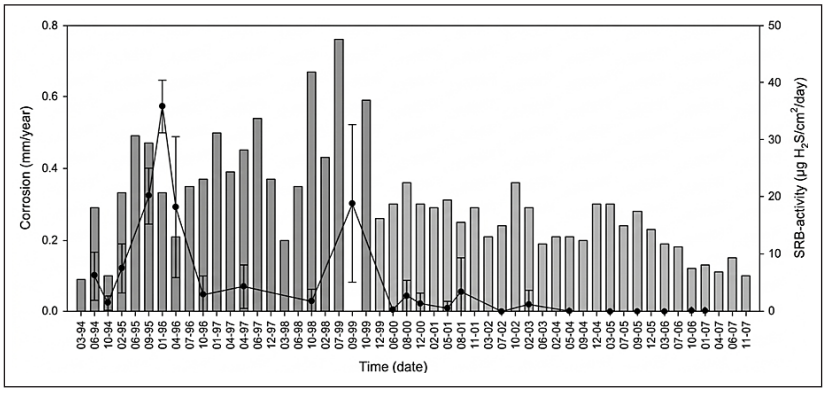

Figure 4. Corrosion rates at GFC during biocide treatment (dark greys bars) and nitrate treatment (light grey bars) (Bødtker et al., 2008)

Quantitative corrosion rate data from field studies showed the significance of these reductions. Another field study also reported a decrement of corrosion rates from 0.7 mm/year to 0.2 mm/year after 4 months of nitrate injection (Eckford & Fedorak, 2004). This result represents a 3.5-fold reduction in corrosion rate, extending infrastructure lifespan while lowering maintenance costs. The sustained nature of these corrosion reductions is particularly notable: SRB activity remained low at ≤0.3 and ≤0.9 µg H_2S/cm^2/day at Veslefrikk and Gullfaks, respectively, over a 7 to 8 year monitoring period (Bødtker et al., 2008).

On a laboratory scale, the addition of *Thiomicrospira* sp. as the NR-SOB into a fully grown culture of SRB consortium at 120-160 hours was reported to show a significant increase in the population towards the end of the experiment (Nemati et al., 2001). The biofilm community during nitrate treatment was highly diverse and stable over time, indicating the successful establishment of a balanced microbial system. This replacement or shift in the microbial community reflects not only reduced total microbial biomass, but also the substitution of one functional group with another that performs beneficial activities rather than damaging ones (Sunde et al., 2004). Both laboratory and bioreactor studies have provided controlled environments for examining NR-SOB mechanisms and optimising nitrate treatment methods. These studies have carried out various experimental designs,

including sand-packed columns, sandstone cores, up-flow packed-bed bioreactors, and oilfield-simulating bioreactors (Xue & Voordouw, 2015; Youssef et al., 2009).

A study by Xue and Voordouw (2015) conducted bioreactor research to simulate real oilfield conditions and investigate the control of microbial sulphide production with nitrate and biocide. This study showed that NR-SOB oxidise sulphide and suppresses SRB activities while linking nitrate dosing to corrosion mitigation. Yang et al. (2021) carried out four lab-scale bioreactors to imitate force mains over 8 months and evaluate the effect of ferric iron and nitrate on hydrogen sulphide control. This study found that the co-existence of sulphide and nitrate stimulated the development of NR-SOB, particularly *Sulfurovum sp.*, which was responsible for sulphide removal in the presence of nitrate. This shows that NR-SOB can be enriched under conditions of simultaneous availability of sulphide and nitrate.

Few laboratory studies have interpreted the specific mechanism by which NR-SOB controls corrosion. Early research has investigated bacterial interactions in detail, testing various NR-SOB strains for sulphide oxidation capacity while measuring changes in environmental redox potential (Greene et al., 2003; Janssen et al., 1998; Ter Heijne et al., 2018). *Thiomicrospira sp.* strain CVO can oxidise up to 15 mM sulfide, while escalating redox potential from -400 to +100 mV and producing 0.6 moles of nitrite for every mole of nitrate reduced.

Few other studies have examined corrosion-rate dynamics associated with nitrite or nitrate-mediated control of souring under biological conditions that simulate an oil reservoir (Lahme et al., 2019; Rempel et al., 2006). Through microbial cultures and electrochemical techniques such as potentiodynamic scans and linear polarisation, the study found that adding nitrate and NR-SOB had similar effects on sulphide oxidation and reduction. However, nitrate and NR-SOB alterations also caused pitting corrosion, with maximum corrosion rates of 0.72 mm/year. This study highlighted a potential complication: nitrate treatment reduces overall corrosion but may also promote localised corrosion during the sulphide oxidation phase. Recent lab-based research has examined the optimum nitrate treatment strategies by investigating the effects of different nitrate concentrations while co-treating with other agents and environmental factors. Tian et al. (2024) studied how nitrate and oxygen levels affect the abundance and structure of sulphate-reducing and sulphur-oxidising bacteria in petroleum reservoir water. The study found the highest SRB inhibitory effect and the lowest dsrB/soxB ratio under high concentrations of both nitrate and oxygen, suggesting that combined treatments may be more effective than nitrate alone.

Recognising that corrosion can occur solely through ionic or electrochemical reactions in seawater or other media, previous studies have established several analytical techniques to distinguish MIC from abiotic corrosion. To isolate the contribution of microbial activity, researchers employ comparative experimental designs that contrast biotic environments with abiotic, sterile controls (Lei et al., 2009; Machuca et al., 2011; Makama et al., 2016).

These controls typically involve filter-sterilised seawater or abiotic artificial seawater to establish a baseline for electrochemical and material degradation. Establishing the contribution of microbial activity requires a multi-faceted approach, as relying on a single diagnostic method can be misleading. Strong evidence for MIC is typically validated by integrating surface analysis and molecular profiling (Ayodele et al., 2025).

The first approach to establishing the contribution of microbial activity is mineralogical analysis. Corrosion products on biofilm surfaces often show mineral compositions that differ significantly from sterile controls; for instance, biofilm carbon steel may show high concentrations of $CaCO_3$ (aragonite) compared with the goethite (α-FeOOH) that dominates sterile surfaces (Makama et al., 2016). Second, molecular profiling can confirm microbial activity. 16S rRNA gene amplification and sequencing enable researchers to identify specific bacterial taxa, such as sulfate- and iron-reducing bacteria, associated with MIC (L. Jones et al., 2024; L. M. Jones et al., 2024; Machuca et al., 2011). Lastly, previous studies have used comparative untargeted metabolomics to reveal disparities between the metabolic products of biofilm-associated cells and planktonic populations, providing insight into the specific biochemical pathways driving corrosion. This is because planktonic cells do not accurately represent the bacterial population within a biofilm. Therefore, assessing MIC risk by sampling the surrounding seawater is insufficient for an accurate diagnosis.

Corrosion Risks Associated with Nitrate Treatment

Generally, nitrate treatment reduces overall corrosion rates, but several studies have reported the potential for localised corrosion and pitting during sulphide oxidation. Rempel et al. (2006) found that amendments of nitrate and NR-SOB led to localised corrosion in the form of pitting, with a maximum corrosion rate of 0.72 mm/year. The corrosion rate accelerates during sulphide oxidation and removal, suggesting that sulphide oxidation may create conditions conducive to corrosion. This condition highlights a significant challenge for field applications, as localised corrosion can cause fatal failures even when average corrosion rates are reduced. The mechanisms that lead to localised corrosion are still not fully understood, but may involve galvanic cells, localised pH changes or the accumulation of corrosive intermediates such as sulphur compounds (Rempel et al., 2006). Field reports show increased corrosion rates in some applications, but each case should be examined, as conditions can vary (Bolaji et al., 2019). The variability in the outcomes shows the need for thorough monitoring and optimisation of nitrate treatment based on site conditions.

Thermodynamic modelling by Dolfing and Hubert (2017) revealed that the oxidation of sulphide by NR-SOB can be incomplete, producing elemental sulphur as an intermediate product. Elemental sulphur in the system can be highly corrosive to oil field infrastructure. This is because elemental sulphur is highly reactive yet has low water solubility, thereby facilitating the formation of corrosive layers, such as iron sulphide (Fe_3S_4), on carbon steel

surfaces (Drønen et al., 2014). Sulphide oxidation may be a problem in topside oil/water separation due to potential NR-SOB-mediated corrosion when nitrate is limited, which favours incomplete oxidation of sulphide to elemental sulphur (Dolfing & Hubert, 2017; Wang et al., 2016; Xu et al., 2025). The formation of a cryptic sulphur cycle, in which sulfide produced by SRB is oxidised back by NR-SOB, can generate corrosive intermediates, posing a critical challenge (Dolfing & Hubert, 2017). Although this cycle reduces H_2S accumulation in the reservoir, the intermediate products might contribute to corrosion under certain conditions. In previous years, both operators in produced water reinjection (PWRI) facilities and researchers have similarly reported an increase in corrosion rate due to mixed microbial communities and the addition of nitrate (MacDonald et al., 1978; Nemati et al., 2001; Rempel et al., 2006)

During the oxidation of sulphide, elemental sulphur, which is the intermediate NR-SOB, has been speculated to be responsible for corrosion under the nitrate treatment (Nemati et al., 2001; Vik et al., 2007) Due to its characteristics as a strong oxidant of iron (MacDonald et al., 1978; Schmitt, 1991). In one corrosion study, metal coupons used after the corrosion test were covered with a layer of black precipitates, identified by X-ray analysis as iron sulphide, iron oxide, iron hydroxide, and elemental sulfur. The thickness of the precipitation was increasing towards the upper region of the bioreactor, where high corrosion rates occurred (Hubert et al., 2005), which correlates with the finding that the penetration of oxygen near the top of the bioreactor may have led to the oxidation of sulphide to corrosive elemental sulphur (Lee et al., 1993). Elemental sulphur in a pipeline system may significantly increase corrosion rates by forming sulfuric acid and H_2S, which inhibit repassivation and promote both crevice corrosion and stress corrosion cracking (Schmitt, 1991).

In addition to elemental sulphur, nitrite formation also causes MIC under nitrate-reducing conditions (Beeder et al., 2007; Drønen et al., 2014; Vik et al., 2007). Nitrite is a key intermediate in nitrate-dependent corrosion; its presence promotes chemical oxidation of iron and can accelerate corrosion rates (Iino et al., 2021). The effect of nitrite accumulation on X80 steel has been found to induce both uniform and pitting corrosion, where the corrosion severity is highly dependent on the nitrite concentration gradient (B. Liu et al., 2021).

In a recent study, the researchers conducted a detailed study of NR-SOB-mediated corrosion by observing the initial nitrate-to-sulphide (N/S) ratios relating to oil field operations, and reported that corrosion rates are up to 0.27 millimetres per year (mm y^{-1}) and up to 60-µm-deep pitting corrosion for only 9 days (Lahme et al., 2019). Various N/S ratios directly affect sulphide oxidation and lead to the accumulation of byproducts, as shown in Figure 5. Figure 5a shows the complete oxidation of sulphide to sulphate, which is happening at N/S ratios of ≥1.6 (Equation 6). As for Figure 5b, it depicts the accumulation of corrosive S0 due to incomplete oxidation at N/S ratios <1.6, especially when nitrate is the limiting factor (Equation 7). However, Figure 5c shows the presence of excess nitrate when the N/S ratio >1.6, leading to the accumulation of nitrite (Equation 8).

Therefore, nitrate availability and the N/S ratio play a crucial role in corrosion rate because they directly affect the accumulation of different NR-SOB metabolites (Lahme et al., 2019). Understanding and managing these intermediate sulphur compounds and nitrite buildup are critical to optimising the effectiveness of nitrate treatment.

$$HS^- + 1.6\ NO_3^- + 0.6\ H^+ \rightarrow SO_4^{2-} + 0.8\ N_2 + 0.8\ H_2O,\ \Delta G^0 = -784.3\ \text{kJ mol}^{-1} \quad [6]$$

$$HS^- + 0.4\ NO_3^- + 1.4\ H^+ \rightarrow S^0 + 0.2\ N_2 + 1.2\ H_2O,\ \Delta G^0 = -197.2\ \text{kJ mol}^{-1} \quad [7]$$

$$HS^- + 4\ NO_3^- \rightarrow SO_4^{2-} + 4\ NO_2^- + H^+,\ \Delta G^0 = -510.4\ \text{kJ mol}^{-1} \quad [8]$$

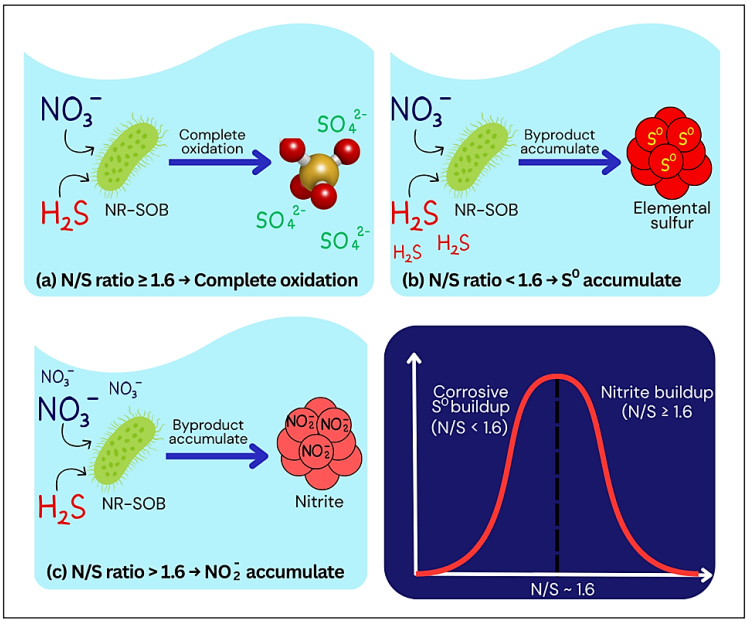

Figure 5. Effect of N/S ratios with (a) ≥ 1.6; (b) < 1.6 and (c) > 1.6 on sulphide oxidation and byproduct accumulation

However, the discovery of some SRB strains with Nrf activity shows a clear limitation to the universal effectiveness of nitrate treatment (Greene et al., 2003). Related SRB strains such as *Desulfovibrio vulgaris* and *Desulfobulbus propionicus* have the ability to reduce nitrite to ammonia, thereby eliminating the inhibitory effect of nitrite produced by both hNRB and NR-SOB (Greene et al., 2003). This ability of certain SRB strains translates into resistance mechanisms against nitrite, indicating that the effectiveness of nitrate treatment will vary with the composition of the indigenous SRB population in the system. In a system

dominated by Nrf-positive SRB strains, nitrate injection may be less effective at suppressing sulphate reduction, and H_2S can continue to accumulate, worsening reservoir souring. In this study, it is critical to identify and characterise indigenous microbial communities before initiating nitrate injection.

Although this review primarily focuses on NR-SOB, studies involving other NRB remain highly relevant as they provide important insights into the broader microbial and corrosion responses associated with nitrate treatment. A recent study investigating mixed cultures of *Desulfovibrio vulgaris* and *Pseudomonas aeruginosa* showed that nitrate addition can unexpectedly enhance corrosion despite suppressing SRB activity (Sun et al., 2022). In the experiment, nitrate addition stimulated NRB growth, particularly *Pseudomonas aeruginosa*, increasing corrosion risk through enhanced biofilm activity and microbially mediated electron transfer. Although nitrate treatment effectively reduced sulphide production by SRB, some findings indicate that it does not guarantee corrosion mitigation, as it may stimulate highly corrosive NRB populations, further increasing the corrosion risk.

Advances in nr-sob Research for Souring Control

Various environmental and operational factors directly affect nitrate treatment and the establishment of NR-SOB in the system. Environmental factors, such as temperature, are vital for optimising nitrate treatment strategies. Nitrate treatment is utilised in oil reservoirs at temperatures ranging from low to approximately 80 °C (Magot, 2005). Temperature dictates the mechanism by which nitrate treatment operates, directly affecting its efficacy in controlling reservoir souring or enhancing oil recovery. At lower temperatures (up to 45 °C), nitrate is reduced to N_2, whereas at 45–65 °C, it is reduced to nitrite (Okpala et al., 2017). Since nitrite is a potent inhibitor of thermophilic SRB, maintaining a nitrate-reducing zone at 45–65 °C can effectively suppress souring in hotter reservoir zones (65-80 °C).

Although research exists on high-temperature reservoirs, thermophilic NR-SOB populations and their functionality at rising temperatures remain less well characterised than mesophilic populations. In addition, concentration and application strategy are critical to treatment success. Continuous low-dose nitrate injection between 0.25 mM and 0.33 mM has proven effective in field applications (Bødtker et al., 2008), but the optimal concentration may vary with sulphate levels, organic carbon availability, temperature, and site-specific factors. In short, nitrate overdose can be wasteful and could promote undesirable microbial activities, while underdose might even fail to establish NR-SOB populations and the effectiveness of this treatment.

Comparing early studies from 1999 to 2008 with recent findings from 2017 to 2024 revealed continuity and evolution in knowledge of NR-SOB-mediated corrosion control, as shown in Figure 6. Figure 6a shows earlier studies between 1999 and 2008 that used nitrate to control sulphide production and SOB, which were first reported in 1999 (Jenneman &

Gevertz, 1999; Londry & Suflita, 1999). Field studies in 2004 and 2008 attained the first long-term evidence for NR-SOB establishment, alongside sustained corrosion control in operational oil fields (Bødtker et al., 2008; Sunde et al., 2004). Early studies reported a significant reduction in SRB populations, and both H_2S generation and corrosion rates decreased, confirming nitrate injection as an alternative to biocide treatment. In 2003, one study identified an SRB resistance mechanism through Nrf activity, raising awareness of the effectiveness of nitrate treatment, which is not universal and depends on indigenous microbial community composition (Greene et al., 2003). Another study in 2008 revealed competitive oxidation of VFAs by SRB and NRB, giving mechanistic insights into the dynamics of the competition (Grigoryan et al., 2008).

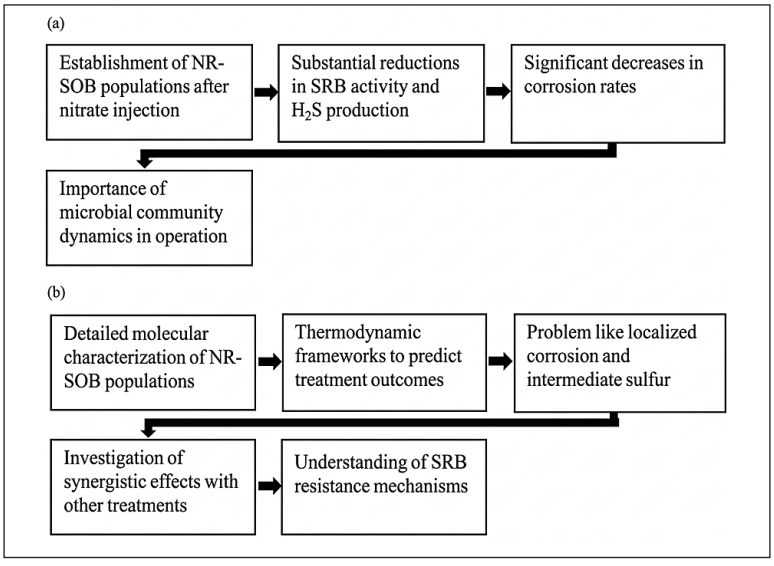

Figure 6. Advancement of findings linked to NR-SOB control corrosion in (a) early studies (1999-2008) and (b) recent studies (2017-2026)

In contrast, Figure 6b depicts recent research conducted on these foundations, using more elaborate molecular, thermodynamic, and systemic approaches. In 2017, thermodynamic modelling was applied to predict the outcomes of nitrate-based interventions, revealing the potential for incomplete sulphide oxidation and production of corrosive intermediate products. This marked a shift from purely observational approaches to predictive modelling based on thermodynamic principles (Dolfing & Hubert, 2017). Tian et al. (2024) used advanced molecular techniques, including 16S rRNA gene sequencing and functional gene quantification, to show that nitrate and oxygen significantly altered the abundance of SRB and SOB rather than their structure. This finding suggests that nitrate treatment works by shifting the abundances of existing functional groups rather than introducing an

entirely new community structure. Current studies also examined the synergistic effects of combined treatments. Yang et al. (2021) compared ferric iron and nitrate effects, while Tian et al. (2024) observed the combined effect of nitrate and oxygen treatments on SRB inhibition. Synergistic effects are still being studied in recent years; for example, one study uses nitrate/nitrite coupling with NRB to suppress SRB while reducing corrosion rates in high-temperature oil reservoirs (Guo et al., 2026; Zhou et al., 2025).

Summary of nr-sob Research in Souring Control (1999-2026)

During the initial phase between 1999 and 2008, research focused on establishing the fundamental efficacy of nitrate injection. These early studies successfully demonstrated that the introduction of nitrate facilitates the establishment of NR-SOB populations, which in turn leads to substantial reductions in SRB activity and biogenic H_2S production. Ultimately, this period provided the first long-term evidence that these microbial shifts translate into significant decreases in overall corrosion rates within operational oil fields, underscoring the vital role of microbial community dynamics in industrial operations. In contrast, the more recent research era spanning 2017 to 2024 reflects a move towards detailed molecular and thermodynamic characterisation of these systems.

Current studies utilise advanced molecular tools to characterise NR-SOB populations and employ thermodynamic frameworks to predict treatment outcomes more accurately. Researchers use high-throughput sequencing and metagenomic analysis to characterise the functional potential and microbial composition of NR-SOB (Cui et al., 2024; Jian et al., 2023; F. Liu et al., 2021; S. Liu et al., 2021; T. Wang et al., 2024). Metagenomics is used to assess the abundance of specific functional genes—such as narG and napA—that are often associated with autotrophic sulphur-oxidising denitrifiers. These tools enable the mapping of metabolic pathways, including complete denitrification, sulphur disproportionation, and DNRA. In addition, modelling efforts have shifted from conventional bulk biomass models towards trait-based process modelling (Sampara et al., 2022). Traditional models often fail to predict ecosystem dynamics in functionally diverse microbiomes because they rely on aggregated concentrations rather than on specific microbial physiological traits. As for current research, trait-based models calibrated at the genus level demonstrate superior accuracy in predicting nitrite accumulation and nitrate removal outcomes.

However, this increased resolution has also revealed new complexities, such as localised corrosion and the corrosive potential of intermediate sulphur species, such as zero-valent sulphur. Modern investigations are now prioritising the understanding of SRB resistance mechanisms, such as Nrf activity, and exploring the synergistic effects of combining nitrate with other treatments to optimise corrosion control in challenging environments. Table 2 presents a summary of methodologies extracted from various studies spanning 1999 to 2026. The most common analytical method is measuring sulphide production, alongside

Table 2
Summary of nitrate injection in controlling souring corrosion extracted from studies between 1999 and 2026

Bacterial Strain	Analytical Method	Corrosion Assessment	Microbial Analysis	Key Parameters	Year	Ref.
SOB isolated from oil field	Sulfide measurement	Field-based monitoring	Culture-based identification and characterisation	Sulfide concentrations, bacterial activity	1999	Jenneman and Gevertz (1999)
SRB from oily waste	Sulfide quantification	Indirect assessment through sulfide control	SRB activity monitoring	Sulfide production rates, nitrate consumption	1999	Londry and Suflita (1999)
Mixture of SRB and NRB	H_2S measurement, nitrate/nitrite quantification, corrosion rate determination	Weight loss and corrosion rate calculation	Most probable number (MPN) enumeration of SRB and NRB	Sulfide concentration, nitrate/nitrite levels, corrosion rates, bacterial populations	2001	Nemati et al. (2001)
Pure cultures of SRB and NR-SOB	Enzyme assays for nitrite reductase, sulfide measurements	Not primary focus	Growth curves, enzyme activity measurements, molecular techniques	Nitrite reductase activity, sulfide production, bacterial growth	2003	Greene et al. (2003)
Indigenous microbe from Gullfaks oil field	Field monitoring of H2S, nitrate, sulfate	Corrosion coupon monitoring, pipeline inspection	Biofilm sampling and analysis	H_2S levels, nitrate concentration, corrosion rates, water chemistry	2004	Sunde et al. (2004)
SRB and NR-SOB	H_2S measurement for SRB activity, 16S rRNA gene-based community analysis by PCR-DGGE	Field-based monitoring	16S rRNA gene-based community analysis by PCR-DGGE	SRB activity (μg H_2S/cm^2/day), corrosion rate, bacterial community composition	2008	Bødtker et al. (2008)
Nitrate-utilising consortium of SRB from Algerian oil field	Weight loss measurements, surface analysis, sulfide measurements	Corrosion rate calculations, SEM analysis of corroded surfaces	16S rRNA gene sequencing, culture-based identification	Corrosion rates, sulfide production, bacterial growth, nitrate consumption	2012	Kebbouche-Gana and Gana (2012)
Thermodynamic modeling of SRB and nitrate-reducing bacteria	Thermodynamic calculations, Gibbs free energy analysis	Indirect through souring control prediction	Modeling of microbial metabolism	Thermodynamic parameters, substrate availability, product formation	2017	Dolfing and Hubert (2017)
Mixed microbial communities from wastewater	H_2S measurements, ion chromatography, molecular analysis	Not primary focus	16S rRNA gene sequencing	H_2S, nitrate, ferric iron, sulfate, microbial community	2021	Yang et al. (2021)

Table 2 (*continued*)

Bacterial Strain	Analytical Method	Corrosion Assessment	Microbial Analysis	Key Parameters	Year	Ref.
Mixed microbial communities for simultaneous sulfide and nitrate removal	Sulfide and nitrate measurements, high-throughput sequencing	Not primary focus	16S rRNA gene sequencing, functional prediction	Sulfide removal, nitrate removal, microbial community structure	2024	Guo et al. (2024)
Co-culture of SRB and nitrate-reducing bacteria	Electrochemical measurements, surface analysis, metabolite analysis	Weight loss, electrochemical impedance spectroscopy, SEM, XRD	qPCR, biofilm characterisation	Corrosion rates, bacterial populations, metabolites, deposit characteristics	2024	Sun et al. (2024)
Sulfate-reducing bacteria (SRB) in seawater	Microbial fuel cell voltage monitoring; cyclic	Corrosion-rate comparison, mass loss, surface morphology, and sulfur/iron-sulfide deposits	Antibiotic susceptibility test (ABST) for SRB inhibition	ZnO nanoparticles; SRB increased corrosion rate by 21.3% versus control; ZnO reduced corrosion rate by 21.7% relative to control	2025	Ambepitiya et al. (2025)
Sulfate-reducing bacteria (SRB) with denitrifying bacteria (DNB)	Electrochemical impedance spectroscopy (EIS), linear polarisation resistance (LPR), weight loss, SEM, and maximum pit depth	Average corrosion rate, maximum pit depth, EIS capacitive arc, and polarisation resistance	16S rDNA sequencing	Combined glutaraldehyde and DNB treatment; corrosion rate reduced 44.2% to 0.01608 mm/a; maximum pit depth reduced 84.3% to 1.53 μm; SRB abundance decreased from 62.7% to 11.9% over 14 days	2026	Guo et al. (2026)

quantifying nitrate and nitrite over the experimental period.

Discussions and Future Directions

This review synthesises evidence from early field applications to evaluate the presence, biological mechanisms, and overall effectiveness of NR-SOB in mitigating corrosion following nitrate injection. Although nitrate injection has consistently reduced corrosion rates in both laboratory and field studies, reports of localised pitting during sulphide oxidation highlight a knowledge gap in mechanistic understanding (Lahme et al., 2019; Rempel et al., 2006). The formation of elemental sulphur from the incomplete oxidation of sulphide poses a technical challenge in the operation. Figure 7 summarises the current research gap and the areas for future research, including quantifying the production of corrosive intermediates and accounting for corrosivity as a function of temperature, flow rate, and nitrate concentration. Strategies to promote complete oxidation to sulphate should also be developed to fully implement corrosion control through this nitrate treatment in the oil field system.

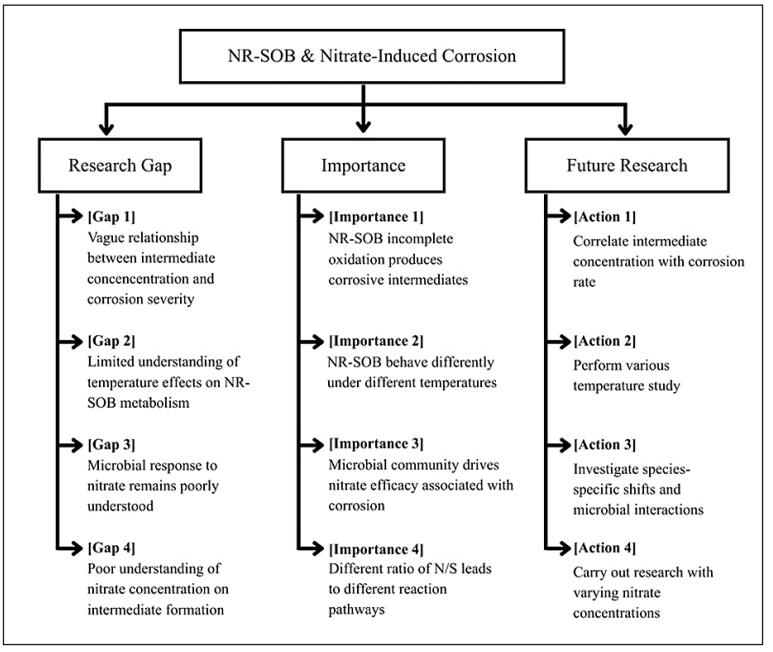

Figure 7. Overview of current knowledge gaps and future research directions in understanding NR-SOB mediated corrosion

Related studies demonstrate that corrosion outcomes under nitrate injection are strongly condition-dependent, rather than driven solely by NR-SOB activity. Negative effects are always linked to incomplete sulphide oxidation and the accumulation of

corrosive intermediate products. The effectiveness of nitrate treatment is also influenced by the composition of the indigenous microbial community, specifically the presence of Nrf-positive SRB that can reduce nitrite, thereby mitigating nitrite accumulation. These interactions highlight that nitrate treatment does not operate independently; instead, it is influenced by complex microbial dynamics. Therefore, rapid and cost-effective molecular diagnostics for pre-treatment analysis will aid in predicting system response and further reducing the risk of nitrate treatment failure. Integrating field datasets with thermodynamic models can help optimise treatment strategies, reduce analysis costs, and provide early warning systems before applying them in the oilfield. Further research on biofilm-focused strategies might help NR-SOB establish populations at key interfaces, enhancing its ability to control souring and limit corrosion rates.

CONCLUSION

This review article synthesises evidence from highly relevant studies spanning from 1999 to 2026, documenting the presence, mechanisms, and effectiveness of NR-SOB in controlling corrosion rates after nitrate injection in oil and gas systems. Findings from the articles indicate that nitrate injection stimulates the growth and establishment of NR-SOB populations, including genera such as *Sulfurimonas, Thiomicrospira, Sulfurovum, Sulfuricurvum, Thiobacillus denitrificans,* and *Arcobacter sp.*, which play important roles in sulphide oxidation and corrosion control. However, the literature indicates that the corrosion outcomes of nitrate injection depend heavily on the conditions and specific operational risks. The documented challenges include incomplete sulphide oxidation, nitrite accumulation, localised corrosion, SRB ability to counter nitrite inhibition, and variability in microbial community responses, which can substantially alter treatment outcomes. Ultimately, while nitrate-stimulated NR-SOB populations provide a viable biological control for reservoir souring, optimising this treatment requires site-specific evaluations of microbial composition, environmental conditions, and thermodynamic factors to ensure complete sulphide oxidation and prevent localised corrosion.

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