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ORIGINAL PAPER

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Dynamics of corrosion rates associated with nitrite or nitrate mediated control of souring under biological conditions simulating an oil reservoir

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Abstract Representative microbial cultures from an oil reservoir and electrochemical techniques including potentiodynamic scan and linear polarization were used to investigate the time dependent corrosion rate associated with control of biogenic sulphide production through addition of nitrite, nitrate and a combination of nitrate-reducing, sulphide-oxidizing bacteria (NR-SOB) and nitrate. The addition of nitrate alone did not prevent the biogenic production of sulphide but the produced sulphide was eventually oxidized and removed from the system. The addition of nitrate and NR-SOB had a similar effect on oxidation and removal of sulphide present in the system. However, as the addition of nitrate and NR-SOB was performed towards the end of sulphide production phase, the assessment of immediate impact was not possible. The addition of nitrite inhibited the biogenic production of sulphide immediately and led to removal of sulphide through nitrite mediated chemical oxidation of sulphide. The real time corrosion rate measurement revealed that in all three cases an acceleration in the corrosion rate occurred during the oxidation and removal of sulphide. Amendments of nitrate and NR-SOB or nitrate alone both gave rise to localized corrosion in the form of pits, with the maximum observed corrosion rates of 0.72 and 1.4 mm year^{-1}, respectively. The addition of nitrite also accelerated the corrosion rate but the maximum corrosion rate observed following nitrite addition was 0.3 mm year^{-1}. Furthermore, in the presence of nitrite the extent of pitting was not as high as those observed with other control methods.

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Introduction

Oil reservoirs harbour a diverse microbial community, including sulphate-reducing bacteria (SRB), methanogenic, fermentative and iron-reducing bacteria, as well as heterotrophic nitrate-reducing (NRB), and nitrate-reducing, sulphide-oxidizing bacteria (NR-SOB) [6, 20, 30]. The association of SRB with microbially influenced corrosion (MIC) of ferrous metals and their alloys has been known for many years [2, 3]. Furthermore, the activity of SRB in oil reservoirs contributes to the production of hydrogen sulphide which in turn reduces the quality of the produced oil and gas, increases the corrosion risk, and contributes to the plugging of reservoir formations [16, 34]. Based on tests in laboratory systems and oil fields various strategies for control of biogenic sulphide production have been proposed. These include the removal of sulphate from water prior to injection [1], addition of metabolic inhibitors such as molybdate to the injection water [16, 24], application of a variety of biocides [8, 31, 32] and exposure of water to microwave and ultrasonic irradiations [4]. Nitrate or nitrite mediated control of souring has emerged as an attractive alternative in recent years and a number of studies aiming to assess the effectiveness of this approach have been conducted in model laboratory systems [6, 7, 12, 16, 23–25, 27, 28] and in onshore and off shore oil fields [15, 18, 19]. Continuous amendment of water injected into the Coleville oil field with 500 ppm ammonium nitrate for 50 days led to complete removal of sulphide at one of the two injectors, and a 50–60% reduction in the sulphide level in the co-produced brine and a significant increase in population of NR-SOB *Thiomicrospira* sp. CVO [15, 30]. Using microbial cultures enriched from the Coleville produced water, Nemati et al. [25] demonstrated that the addition of nitrate and an NR-SOB

culture dominated by *Thiomicrospira* sp. CVO to a growing SRB consortium inhibited the production of sulphide by this consortium. The activity of NR-SOB eventually led to oxidation and removal of the sulphide. The addition of nitrate alone did not impose an inhibitory effect but stimulated the activity of NR-SOB leading to the removal of sulphide. Employing immobilized cells of SRB in continuous packed bioreactors, Hubert et al. [12] reported that the amount of nitrite or nitrate required to prevent the activity of SRB was dependent on the level of the available electron donor, Na-lactate and suggested the use of 0.7 mole nitrate or 0.8 mole nitrite per each mole of present Na-lactate. Myhr et al. [23] reported that the injection of 0.5 mM nitrate or 0.12 mM nitrite to model columns containing crude oil as carbon source and an SRB consortium from the produced water of the Statfjord oil field in the North Sea led to the complete elimination of sulphide from these systems. Tests conducted at the Halfdan and Skjold oil fields in the North Sea have confirmed the efficacy of nitrate additions in control of souring in offshore reservoirs [18, 19].

While containment of souring through the addition of nitrate and nitrite has been successful in model systems and in the field tests, the impact of these treatments on corrosion is poorly understood. Preliminary studies have indicated that the addition of nitrate or a combination of NR-SOB and nitrate to an SRB culture increases the extent of corrosion [13, 26]. These studies, however, have been based on gravimetric methods in which metallic coupons were exposed to conditions which varied during the course of the experiments and average corrosion rates were determined at the end of the experiments. No information regarding the instantaneous corrosion rate and its variation during the treatment process has been provided. The work described in here represent the first study in which electrochemical techniques such as potentiodynamic scan and linear polarization have been used to establish the time dependent corrosion rate profiles associated with control of biogenic sulphide production through addition of nitrite, nitrate and a combination of NR-SOB and nitrate.

Materials and methods

Microbial cultures and medium

The sulphide oxidizing bacterium used in this study was a pure culture of *Thiomicrospira* sp. CVO (NRRL-B−21472), provided kindly by Dr. G. Voordouw, University of Calgary, Canada. To simulate the biological conditions of an oil reservoir, a consortium of SRB, enriched from the produced water of a Canadian oil field, located in Coleville, Saskatchewan was used as an inoculum in the corrosion experiments. This SRB enrichment has been characterized previously and is dominated mainly by species of *Desulfovibrio* [25]. *Thiomicrospira* sp. CVO, referred to as CVO for the remaining part of the paper, was maintained in Colville Synthetic Brine, containing 2–3 mM sulphide and 10 mM Nitrate [9]. A modified form of Coleville Synthetic Brine (m-CSB), containing per l: 7 g NaCl, 0.027 g KH_2PO_4, 0.02 g NH_4Cl, 0.24 g $CaCl_2.2H_2O$, 0.975 g $MgSO_4.7H_2O$, 1.075 g $(NH_4)_2SO_4$, 1.9 g $NaHCO_3$, 5.5 g Na-lactate (60% w/w) and 0.5 ml trace element solution was used for the maintenance of the SRB culture and as a medium in the corrosion experiments. The trace element solution used in CSB and m-CSB contained per litre: 0.5 ml concentrated H_2SO_4, 2.28 g $MnSO_4.H_2O$, 0.5 g $ZnSO_4.7H_2O$, 0.5 g H_3BO_3, 0.025 g $CuSO_4.5H_2O$, 0.025 g $Na_2MoO_4.2H_2O$, 0.045 g $CoCl_2.6H_2O$ and 0.58 g $FeCl_3$.

Experimental set-up and electrodes

The main part of the experimental set-up used in the corrosion studies was an electrochemical cell, consisting of a glass vessel with a working volume of one litre, equipped with inlet and outlet tubes for the transfer and drainage of the liquid medium, a sampling port and inlet and outlet glass tubes for injection of nitrogen gas (Fig. 1). This configuration allows the operation of the system in either batch or continuous flow modes. The mixing in the electrochemical cell was achieved by a magnetic stirrer. The electrochemical cell was equipped with reference, working and counter electrodes. The electrodes were connected to a GAMRY Instruments PCI4/300 Potentiostat integrated into a computer that was running version 4.21 of GAMRY Instruments DC105 Corrosion Techniques software. Two identical electrochemical cells were used in this study. The working electrodes were cut from cold drawn SAE 1018 low carbon steel ¼″ rod (Russel Metals, Saskatoon, Canada) and machined to form the coupons. The coupons were approximately 1.2 cm long and 0.95 cm in diameter. Prior to each experiment, the working coupon was polished sequentially with 240, 320, 400 and 600 silicon carbide paper for approximately 60 s at each grit on a South Bend Precision lathe rotating at 665 RPM (Robert Morse Corporation Ltd., model A, CL 944 Z). The coupon was then cleaned ultrasonically in ethanol for 2 min in a 150 HT Aquasonic ultrasonic cleaner (VWR), rinsed with acetone and dried in air. To minimize the risk of leaks and the possibility of crevice corrosion, a coupon holder was designed and manufactured in our laboratories. The coupon holder consisted of a threaded 316 stainless steel rod and a Teflon tube. The reference electrode was a Beckman Coulter saturated calomel electrode (SCE) model BK511100. During the course of the experiment plugging of the SCE by bacteria and extracellular polysaccharide excreted from the bacteria was noticed and at times was severe enough to necessitate the replacement of the electrode. To remedy this problem a positive pressure (10 psi) was applied on the filling hole of the SCE, using a low flow rate of gaseous nitrogen.

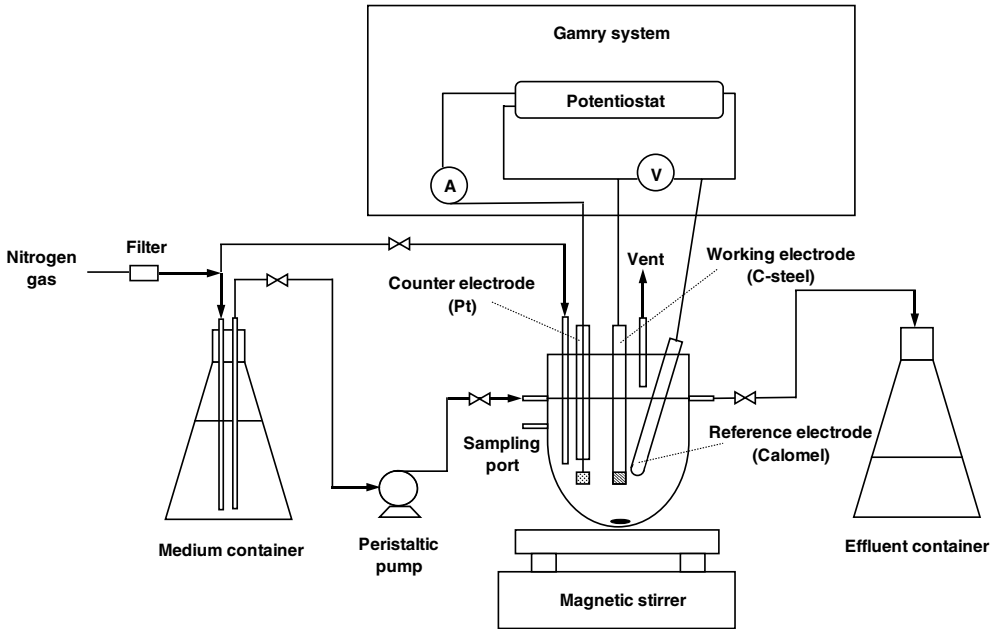

Fig. 1 Schematic diagram of the experimental set-up

The counter electrode used in this system was designed and constructed using 1 cm^2 piece of 0.25 mm-thick platinum foil tack welded to a 5 cm long 18 gauge platinum wire. The wire was extended out of the top of a 640 mm diameter glass tube by a 14 gauge copper wire silver soldered to the platinum wire inside the glass tube. The electrode end was sealed using cobalt glass.

Experimental procedures

Modified Coleville Synthetic Brine was used as a medium for the growth of sulphate reducing bacteria and to stimulate the biogenic production of sulphide in the electrochemical cell. The medium was prepared in 1 l aliquot by dissolving all the medium components in reverse osmosis water. Following the adjustment of pH to 7.0 with 2 M HCl, the medium was sterilized for 30 min at 121°C. Prior to assembly, the electrochemical cell and all components were rinsed with a 75% solution of ethanol in water, followed by a rinse with sterilized reverse osmosis water. The electrochemical cell was charged with 900 ml of m-CSB at room temperature (22°C). To establish the anaerobic condition, the liquid content of the electrochemical cell was purged with filter sterilized gaseous nitrogen for 3–4 h. The working electrode was inserted into the vessel, prior to inoculation while the cell was still under the nitrogen pressure. This was followed by inoculation of medium with an SRB consortium (approximately 10% v/v). Sulphate and sulphide concentrations were determined prior and immediately after inoculation and then on a daily basis until completion of the experiment. The corrosion rate was also determined on a daily basis. The experiment was performed at room temperature and atmospheric pressure.

To investigate the dynamics of the corrosion rate associated with control of souring through addition of nitrate and sulphide-oxidizing bacteria, nitrite, or nitrate alone, three additional experimental runs were conducted. In each case the electrochemical cell was charged with 900 ml of m-CSB and inoculated with the SRB consortium (10% v/v). In one set of experiment, prior to the complete reduction of sulphate (produced sulphide concentration: 10 mM), 50 ml of a fresh CVO culture and a concentrated solution of nitrate were added into the liquid (final nitrate concentration 10 mM). The effects of nitrite and nitrate amendments were studied by addition of concentrated solutions of nitrite (final concentration 5 mM) or nitrate (final concentration 10 mM) to a growing culture of SRB (mid exponential phase; sulphide concentration 5–6 mM). The experiments were performed at room temperature and atmospheric pressure. The concentrations of sulphide, sulphate and nitrite, as well as corrosion rate were monitored on a daily basis. During the critical parts of the experiments (i.e. following the addition of nitrate and CVO, nitrite or nitrate) the corrosion rate was determined every 12 h. The exposed corrosion coupons were examined by scanning electron microscope at the end of each experimental run. To establish a base line for the corrosion rate, a control experiment was conducted in the electrochemical cell, using sterile m-CSB without addition of inoculum.

Measurement of the corrosion rate

The electrochemical tests used to determine instantaneous corrosion rate were performed every 24 h and included a potentiodynamic scan and a linear polarization measurement. During some experiments an addi-

tional linear polarization measurement was conducted 12 h after the first test. Both electrochemical tests were performed using a Gamry PC4 Potentiostat with a version 4.21 of the proprietary software. The following equations were used to calculate the corrosion rate:

$$\mathrm{CR} = \frac{i_{\mathrm{corr}} \mathrm{AW}}{\mathrm{ZF}\rho} \quad (1)$$

$$i_{\mathrm{corr}} = \frac{1}{2.3}\left(\frac{\beta_{\mathrm{a}}\beta_{\mathrm{c}}}{\beta_{\mathrm{a}}+\beta_{\mathrm{c}}}\right)\frac{1}{\mathrm{R_p}} \quad \text{Stearn} - \text{Gearyexpression}, \quad (2)$$

where,

CR corrosion rate (mm s^{-1})
i_{Corr} current density (A cm^{-2})
AW atomic weight of coupon (g mol^{-1})
ρ density of the coupon (g cm^{-3})
β_a anodic Tafel constant (V $decade^{-1}$)
β_c cathodic Tafel constant (V $decade^{-1}$)
R_p polarization resistance (ohm).

The value of the cathodic and anodic Tafel slopes were determined by conducting a potentiodynamic scan over the range of ±150 mV from E_{OC} (open circuit potential) and at a scan rate of 0.5 mV s^{-1}. The slopes of the linear portion of anodic and cathodic curves were used as Tafel slopes (β_a, β_c). Preliminary tests were conducted to determine the appropriate potential scan range and scan rate required for the electrochemical tests. In these tests freshly polished coupons were placed in m-CSB and various ranges of potential, ±50, ±100 and ±150 mV from E_{OC} were applied. The analysis of the resulting data indicated that a scan range of ±150 mV provided the most consistent values for the Tafel slope without damaging the coupon. Further experiments with this scan range were conducted with various scan rates of 0.1, 0.3 and 0.5 mV s^{-1}. Analysis of the results indicated that a scan rate of 0.5 mV s^{-1} would give the suitable details in the Tafel region. Standard linear polarization scans (scan range: ±20 mV and scan rate: 0.167 mV s^{-1}) were conducted to determine the polarization resistance.

Chemical analysis and scanning electron microscopy

Concentrations of sulphate, sulphide and nitrite were determined, using spectrophotometric methods described elsewhere [25]. The exposed coupons were visually examined using a Joel Scanning Electron Microscope (JSM−840A) at an accelerating voltage of 15 kV. Prior to examination, the exposed coupons were inserted into 1 M HCl for several seconds to remove the precipitated compounds. This was followed by a thorough washing with reverse osmosis water, rinsing in acetone and drying in air. In the case of the nitrate amended SRB culture, the corrosion coupon was first used to examine the biofilm formed on the coupon. The coupon was then cleaned according the procedure given above and examined again for the corrosion characteristics.

Results

Control experiment and biogenic production of sulphide by SRB consortium

In the control experiment in which the corrosion coupon was exposed to m-CSB for a period of 30 days low values of corrosion rate fluctuating in the range 0.005–0.025 mm $year^{-1}$ were observed. The concentration of sulphate remained constant at a value close to the sulphate content of m-CSB, while no sulphide was detected in the liquid phase.

Figure 2 shows the profiles of sulphate reduction and sulphide production and the associated corrosion rate in the m-CSB inoculated with SRB culture. As can be seen, after a lag period of 4 days, bacterial activity initiated and the sulphide concentration increased steadily and reached to a maximum value of 12.5 mM after 10 days. The increase in sulphide concentration was accompanied by a continuous decrease in sulphate concentration that eventually attained a negligible level. The concentration of sulphide remained constant at a high level for 3–4 days and then slowly decreased continuously. The decrease in sulphide concentration could be attributed to transfer of sulphide into the head space gas or the slow oxidation of sulphide. Prior to inoculation the measured corrosion rate was around 0.01 mm $year^{-1}$. The addition of inoculum and reaction of sulphide present in the inoculum with the carbon steel coupon, led to formation of a black precipitate (possibly iron sulphide) and a slight decrease in corrosion rate. During the exponential phase of bacterial growth the corrosion rate fluctuated in the range 0.003–0.01 mm $year^{-1}$. The highest corrosion rates of 0.01–0.02 mm $year^{-1}$ were observed toward the end of the experimental run and during the period when sulphide concentration decreased. The average corrosion rate calculated over the entire period

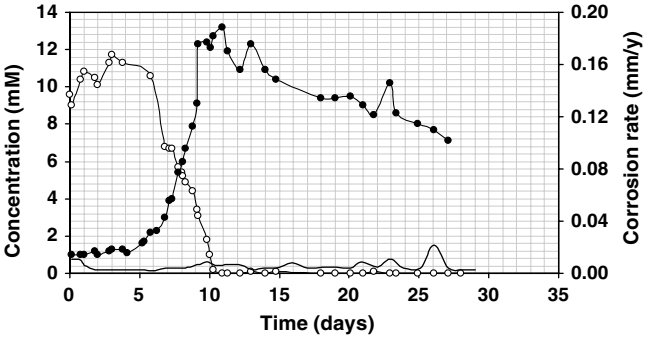

Fig. 2 Profiles of sulphate (*open circle*) and sulphide (*filled circle*) concentrations and associated corrosion rate (*solid line*) in SRB consortium

of experiment was around 0.006 mm year^{-1}. Visual and scanning electron microscopic examinations of the exposed coupon indicated that the corrosion occurred uniformly over the entire surface of coupon and no evidence of localized corrosion or pitting was seen (Fig. 6b).

Control of biogenic sulphide production by nitrite injection

Profiles of sulphate, sulphide and nitrite concentrations, and the associated corrosion rate for a growing culture of SRB amended with 5 mM nitrite in the middle of exponential growth phase (6 mM sulphide) are summarized in Fig. 3. In this experiment the lag phase in the SRB activity was around 10 days. Following the lag phase the concentration of sulphide increased linearly and reached 6 mM by day 17. This corresponded to the middle of the exponential phase of bacterial activity, as was evident from the residual concentration of sulphate. At this stage a concentrated solution of nitrite was added to the culture to obtain a nitrite concentration of 5 mM. Following the addition of nitrite, the trend of increasing sulphide concentration was reversed and a continuous decrease in sulphide concentration was observed until it stabilized at a value in the range 1.3–1.5. The concentration of nitrite decreased continuously from an initial value of 4.6 mM to a negligible level at day 40. The production of sulphide resumed as soon as nitrite concentration dropped to a low level. Consistent with the previous run, the corrosion rate measured in the SRB culture prior to nitrite injection was less than 0.01 mm year^{-1}. The injection of nitrite led to increases in corrosion rate in two distinct phases. In the first phase, which coincided with a fast decrease in concentration of sulphide (from 6 to 2.5 mM), the corrosion rate increased to a maximum value of 0.09 mm year^{-1}. In the second phase, coinciding with a slower decrease in sulphide concentration (from 2.5 to 1.3 mM), the corrosion rate passed through a maximum value of 0.3 mm year^{-1} and then decreased to 0.03 mm year^{-1}.

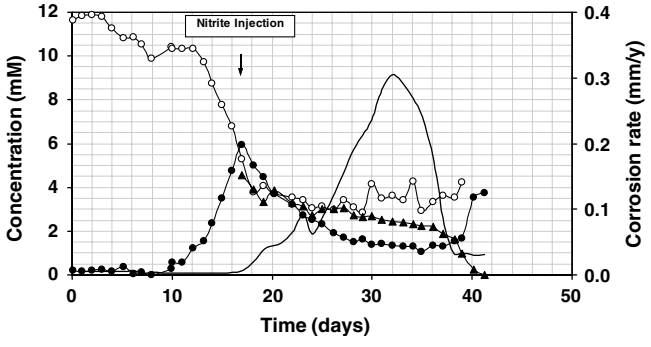

Fig. 3 Profiles of sulphate (*open circle*), sulphide (*filled circle*) and nitrite (*filled triangle*) concentrations and associated corrosion rate (*solid line*) in SRB consortium amended with 5 mM nitrite

The average corrosion rates calculated over the entire period of experiment and for the period after the injection of nitrite were 0.08 and 0.13 mm year^{-1}, respectively. Contrary to what was observed in the untreated SRB culture, the corrosion occurred locally and evidence of pitting was seen near the edges and on the bottom part of the coupon (Fig. 6c).

Control of biogenic sulphide production by injection of sulphide-oxidizing bacteria and nitrate

Figure 4 summarizes the profiles of sulphate, sulphide and nitrite concentrations, and the associated corrosion rate for a growing SRB consortium amended by 10 mM nitrate and a fresh culture of CVO (10% v/v) toward the late exponential phase of SRB growth. The profiles of sulphate and sulphide concentrations prior to injection of nitrate and CVO were similar to those observed in the previous cases. The addition of nitrate and CVO did not seem to affect the production sulphide. However, since this addition was done toward the end of sulphide production phase (residual sulphate concentration: 0.7 mM), the immediate effect on sulphide production cannot be clearly identified and compared with that of nitrite. Following the complete reduction of sulphate, the concentration of sulphide decreased from 10.3 to 9.2 mM over a period of seven days (an indication of the lag phase in activity of CVO) and then decreased sharply from 9.2 mM to a negligible level in less than 3 days (exponential phase of CVO growth). The oxidation of sulphide by CVO could result in formation of sulphur or sulphate with concomitant reduction of nitrate to either nitrite or nitrogen [9], and according the following reactions:

$$HS^- + NO_3^- + H^+ \rightarrow S^\circ + NO_2^- + H_2O \quad (3)$$

$$HS^- + 0.4NO_3^- + 1.4H^+ \rightarrow S^\circ + 0.2N_2 + 1.2H_2O \quad (4)$$

$$HS^- + 4NO_3^- + 5H^+ \rightarrow SO_4^{2-} + 4NO_2^- + 6H^+ \quad (5)$$

$$HS^- + 1.6NO_3^- + 0.6H^+ \rightarrow SO_4^{2-} + 0.8N_2 + 0.8H_2O. \quad (6)$$

During the oxidation of sulphide both turbidity and sulphate concentration increased in the culture. However, the level of produced sulphate (3.6 mM) was significantly lower than that expected from the stoichiometry of reactions 3 and 4, an indication that sulphide was partially oxidized to elemental sulphur and sulphate. The level of nitrite detected in the culture during the oxidation of sulphide was insignificant. The highest corrosion rate observed prior to injection of CVO and nitrate was around 0.012 mm year^{-1}. The slow decrease in concentration of sulphide during the lag phase in activity of CVO coincided with a relatively slow increase in corrosion rate from 0.012 to 0.13 mm year^{-1}. The shift in CVO activity from the lag phase to exponential phase caused a sharp decrease in concentration

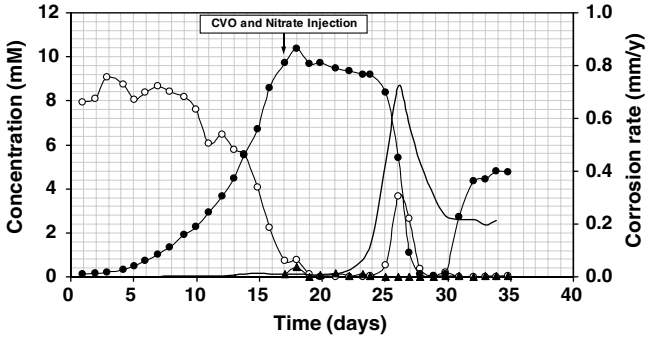

Fig. 4 Profiles of sulphate (*open circle*), sulphide (*filled circle*) and nitrite (*filled triangle*) concentrations and associated corrosion rate (*solid line*) in SRB consortium amended with 10 mM nitrate and NR-SOB CVO

of sulphide and a significant increase in corrosion rate to a maximum value of 0.72 mm year^{-1}. The corresponding concentration of sulphide at this point was around 5.5 mM. The observed corrosion rate when concentration of sulphide eventually reached to a negligible level was around 0.4 mm year^{-1}. Following this the activity of SRB resumed and an increase in concentration of sulphide to a final value of 4.8 mM was observed. The corrosion rate also decreased slightly and eventually stabilized at 0.21 mm year^{-1}. The average corrosion rates calculated over the entire period of experiment and that observed following the injection of CVO and nitrate were 0.12 and 0.22 mm year^{-1}, respectively. Visual and microscopic examination of the exposed coupon indicated that in this case the corrosion occurred locally and evidence of extensive pitting was seen in the various parts of the coupon (Fig. 6d).

Control of biogenic sulphide production by injection of nitrate

Profiles of sulphate, sulphide and nitrite concentrations, and the associated corrosion rate for an SRB consortium amended by 10 mM nitrate in the mid-exponential phase of growth are summarized in Fig. 5. In this experiment, following a short lag phase bacterial activity initiated and sulphide concentration reached to a value of 4.5 mM in 10 days. At this point nitrate at a final concentration of 10 mM was injected into the system. The addition of nitrate did not have any effect on the activity of SRB and production of sulphide continued at a rate similar to that observed before addition of nitrate. The addition of nitrate, however, led to a sharp increase in corrosion rate from an initial value of 0.05–0.65 mm year^{-1}over a period of 9 days, during which sulphide concentration reached to a maximum value of 10.4 mM (complete reduction of sulphate). Following this period, sulphide concentration decreased continuously in two distinct phases. In the first phase (days 19–23) sulphide was removed at a fast rate with concomitant formation of nitrite, with the highest nitrite concentration being around 4.6 mM. In the second phase (days 23–37) sulphide removal proceeded with a much slower rate and accompanied by consumption of nitrite. The SRB consortium enriched from Coleville produced water has been shown to contain a small population of NR-SOB, mainly CVO and addition of nitrate to this consortium stimulated the growth and activity of this population [25]. The decrease in concentration of sulphide following the addition of nitrate that was accompanied by production and subsequent consumption of nitrite can be considered as a strong evidence of the NR-SOB activity. During the first phase of sulphide oxidation concentration of sulphate increased, while in the second phase sulphate concentration initially decreased and then increased. The symbiotic interaction of SRB and NR-SOB in anaerobic environments has been documented in the literature [10, 17]. According to the proposed mechanism, SRB utilize the present organic carbon source as the electron donor for reduction of sulphate, while the NR-SOB use nitrate as the electron acceptor to reoxidize the produced sulphide. The reduction of nitrate by NR-SOB could result in formation of nitrite that, in the absence of sufficient nitrate, is used as an electron acceptor for oxidation of sulphide by NR-SOB. From the data presented in Fig. 5, it appears that during the first phase of sulphide removal (day 19–23), due to the absence of sulphate the activity of SRB was insignificant and biooxidation of sulphide by NR-SOB was the main process occurred in the system. However, during the second phase (day 23–37) both SRB and NR-SOB were active and as a result removal of sulphide by oxidation and reduction of sulphate to sulphide occurred simultaneously though at different rates resulting in a slower removal rate of sulphide as compared with the first phase. Following the consumption of nitrite (day 38), due to the lack of an electron acceptor, oxidation of sulphide stopped but sulphate reduction continued and as a result an increase in sulphide concentration was observed. During the removal of sulphide and for a period of 20 days the corrosion rate fluctuated in the range 0.6–1.4 mm year^{-1} and finally stabilized at a value around 0.7 mm year^{-1}. The average corrosion

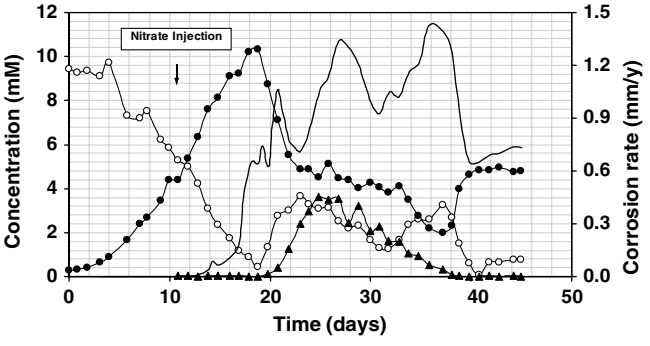

Fig. 5 Profiles of sulphate (*open circle*), sulphide (*filled circle*) and nitrite (*filled triangle*) concentrations and associated corrosion rate (*solid line*) in SRB consortium amended with 10 mM nitrate

rates calculated over the entire period of experiment and that observed following the injection of nitrate were 0.65 and 0.74 mm year^{-1}, respectively. Evidence of extensive pitting was observed on various parts of the coupon upon visual and microscopic examinations (Fig. 6e).

Discussion

Sulphate-reducing bacteria have been implicated in pitting corrosion of ferrous metals and their alloys since 1934 [2]. The hydrogenase activities in SRB promoting the cathodic depolarization, formation of iron sulphide, and secretion of extra cellular polysaccharides are regarded as key factors in SRB induced corrosion [2, 14, 33]. In the present work, the quantity of Na-lactate was in excess of that required for reduction of sulphate, thus consumption of hydrogen by SRB and cathodic depolarization was unlikely to be the dominant mechanism of corrosion. The corrosion rates of 0.003–0.02 mm year^{-1}, observed with SRB alone, are in agreement with the values reported in the literature. Measuring the weight loss of the carbon steel coupons, Nemati et al. [26] reported average corrosion rates of 0.01 and 0.06 mm year^{-1} for pure culture of *Desulfovibrio* sp. Lac6 and an SRB consortium, respectively. Hubert et al. [13] examined carbon steel coupons in continuously operated up-flow packed-bed bioreactors inoculated with an SRB mixed culture and reported the average corrosion rates of 0.01 and 0.4 mm year^{-1}, for the regions near the bioreactor inlet and outlet, respectively. Applying electrochemical techniques, Hernández Gayosso et al. [11] measured a maximum corrosion rate of 0.5 mm year^{-1} in the presence of a bacterial consortium enriched from the samples collected from a gas pipeline in the Marine Region of Pemex, Mexico. The, observed corrosion was attributed to activity of *D. vietnamensis*, the dominant SRB species on the metallic surface. Pitonzo et al. [29] investigated the corrosion induced by various bacterial species isolated from the deep subsurface at Yucca mountain. The maximum corrosion rates observed with iron-oxidizing, sulphate-reducing and exopolysaccharide (EPS)-producing bacteria were 0.06, 0.08 and 0.07 mm year^{-1}, respectively. Various microbial combinations showed higher corrosion rates in the range 0.08–0.12 mm year^{-1}.

In the present work amendment of 5 mM nitrite stopped the production of sulphide immediately. This was followed by a continuous decrease in concentrations of present sulphide and nitrite at a relatively slow rate, possibly as a result of chemical reaction between nitrite and sulphide as suggested by other researchers [16, 23]. In contrast addition of nitrate alone did not have an immediate effect on the activity of SRB. However, the

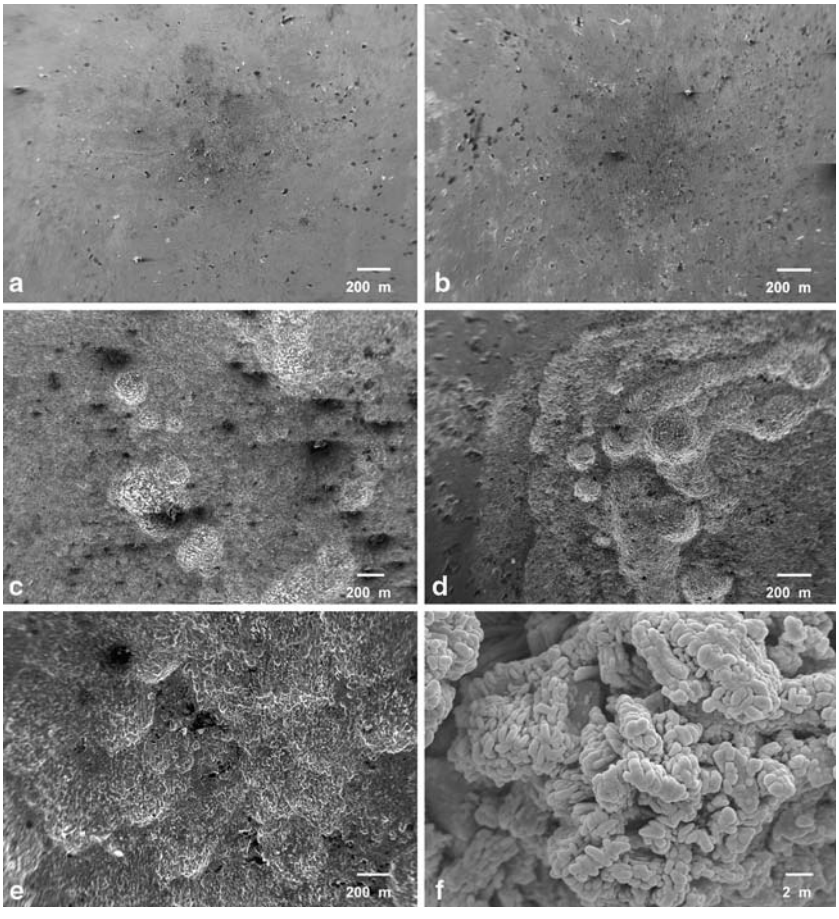

Fig. 6 Scanning electron micrographs of a fresh carbon steel coupon (**a**); carbon steel coupons exposed to: a growing culture of SRB (**b**); a growing culture of SRB, amended with 5mM nitrite (**c**); a growing culture of SRB, amended with 10 mM nitrate and NR-SOB CVO (**d**); a growing culture of SRB, amended with 10 mM nitrate (**e**); biofilm and corrosion products on the surface of a carbon steel coupon exposed to a growing culture of SRB, amended with 10 mM nitrate (**f**)

produced sulphide was removed from the environment eventually. The addition of nitrate and NR-SOB also led to the oxidation and removal of sulphide but the decrease in concentration of sulphide in the presence of NR-SOB and nitrate was significantly faster than that observed with nitrate alone. Due to addition of nitrate and NR-SOB toward the end of sulphide production phase, the assessment of immediate effect on biogenic sulphide production was not possible. In all three cases the impact of treatment was temporary and toward the end of experiments activity of SRB and sulphide production resumed.

The mechanisms proposed for the control of biogenic sulphide production through addition of nitrite or nitrate, or a combination of NR-SOB and nitrate include: (1) exclusion of SRB as a result of competition with heterotrophic nitrate- or nitrite-reducing bacteria (NRB) [6, 7, 13], (2) the preferential use of nitrate or nitrite instead of sulphate by some species of SRB [5, 13, 22], (3) simultaneous oxidation of present sulphide and reduction of nitrate by NR-SOB, which either added or already present in the system [10, 21, 25, 30], and (4) inhibition of SRB activities by nitrite and removal of sulphide as a result of chemical oxidation mediated by nitrite [8, 16, 28]. In the present work the immediate cessation of sulphide production after addition of nitrite appears to be a result of SRB inhibition, since stimulation of NRB activity or shift in SRB metabolism for preferential use of nitrite are unlikely to occur immediately. The inhibition of SRB activity in a continuous system could lead to a decrease in sulphide concentration. However, in a batch system other mechanisms such as chemical oxidation of sulphide mediated by nitrite must be responsible for the decrease in sulphide concentration. The decreases in sulphide concentration observed after addition of NR-SOB and nitrate or nitrate alone is a direct result of NR-SOB activity and biooxidation of sulphide as reported previously [25].

All three scenarios used to control the souring led to accelerated corrosion rates and gave rise to localized corrosion, though at various intensities. In all cases following the amendment of the controlling agent and during the oxidation of sulphide a sharp increase in corrosion rate was observed which passed through a maximum and eventually decreased. This transient behaviour is likely to be the result of formation of aggressive intermediates such as elemental sulphur and thiosulphate which are known to increase the extent of corrosion and give rise to pitting. In case of nitrite, the highest corrosion rate observed during this transient phase was 0.3 mm year^{-1}. Hubert et al. [13] reported that continuous addition of 20 mM nitrite completely eliminated the corrosion of carbon steel coupons placed in an up-flow packed-bed bioreactor with active SRB biofilms. Stepwise increases in concentration of nitrite from 0 to 20 mM, however, led to localized corrosion with the average rate being in the range 0.05–0.1 mm year^{-1}. In the present work the maximum corrosion rates measured after addition of NR-SOB CVO and nitrate (0.72 mm year^{-1}) or nitrate alone (1.4 mm year^{-1}) were significantly higher than that observed with nitrite. Nemati et al. [26] reported average corrosion rates of 0.1 and 0.14 mm year^{-1} for carbon steel coupons exposed to SRB cultures which treated by addition of nitrate and NR-SOB, or nitrate alone, respectively. The average corrosion rate determined in the present work for the containment of souring by NR-SOB and nitrate (0.12 mm year^{-1}) is comparable with that reported by Nemati et al. [26], while in the case of nitrate amendment a higher average corrosion rate (0.65 mm year^{-1}) was observed in the present work.

In conclusion the results of the present study indicated that the addition of nitrate and NR-SOB, nitrate or nitrite to an SRB culture would lead to oxidation and removal of sulphide from the system. The real time monitoring of the system revealed that in all three cases a transient increase in the corrosion rate occurred during the removal of sulphide. Amendments of nitrate and NR-SOB or nitrate alone both accelerated the corrosion rate and gave rise to localized corrosion in the form of pits. In the case of nitrite the acceleration of corrosion rate and the extent of pitting were not as significant as those observed with other control methods.

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