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ORIGINAL PAPER

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Effects of nitrate treatment on a mixed species, oil field microbial biofilm

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Abstract Biofilms of bacteria, indigenous to oil field produced water, were grown in square section, glass capillary flow cells at 45 °C. Initially, in situ image analysis microscopy revealed predominantly coccoid bacteria (length-to-width ratio measurements (l_c:w_c) of bacterial cells gave a mean value of 1.1), while chemical measurements confirmed sulphate reduction and sulphide production. After nitrate ion addition at 100 and 80 mg/l, in the two repeat experiments respectively, the dominance of rod-shaped bacteria (mean l_c:w_c = 2.8) was observed. This coincided with the occurrence of nitrate reduction in the treated flow cells. Beneficially, no significant increase in biofilm cover was observed after the addition of nitrate. The dominant culturable nitrate-reducing bacterium was *Marinobacter aquaeolei*. The l_c:w_c ratio measured here concurs with previously reported cell dimensions for this organism. Several *Marinobacter* strains were also isolated from different oil fields in the North Sea where nitrate treatment has been applied to successfully treat reservoir souring, implying that this genus may play an important role in nitrate treatment.

Keywords Biofilm · Nitrate · Sulphate-reducing bacteria · Nitrate-reducing bacteria · *Marinobacter* · Oil field microbiology

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Introduction

Sulphate-reducing bacteria (SRB) are a group of anaerobic bacteria that reduce SO_4^{2-} to S^{2-} during dissimilatory anaerobic respiration. SRB are problematic in the oil industry where they cause crude oil souring by H_2S (which is also toxic) [22] and the pitting corrosion of iron and steel in pipelines and tanks [17]. A number of SRB have been found to form biofilms readily in laboratory reactors [23] and it is now known that such biofilms metabolise in subsurface reservoir environments to sour the crude oil, gas and associated produced water [11].

As an alternative to toxic biocides, nitrate (calcium or sodium) has been shown to prevent H_2S production by SRB in various laboratory trials [4, 15, 18, 19] and, more recently, in the field [16, 30]. Competition between nitrate-reducing bacteria (NRB) and SRB, inhibition of SRB by nitrite, or changes in environmental redox potential have all been proposed as possible mechanisms [28]. In addition, biological oxidation of sulphide by nitrate-reducing, sulphide-oxidising bacteria (NRSOB) can occur [10] and some true SRB are able to use nitrate as an electron acceptor, even in the presence of sulphate [6]. However, to our knowledge no studies document the direct in situ observation of a mixed species biofilm composed of oilfield species being treated with nitrate. Additionally, in the rare cases where the important competing organisms have been identified, autotrophic NRSOB have been found [31], which are significantly ecologically distinct from the heterotrophic bacteria discussed here.

SRB biofilm cannot be monitored directly in subsurface oil reservoirs and so laboratory experiments are essential to determine the mechanisms that prevent souring in a given situation and to help optimise nitrate dosing. In this paper two similar experiments are described, using methods previously used to investigate 'live' SRB biofilms [5], to determine the changes occurring in an SRB biofilm upon application of nitrate.

Important NRB species, which became dominant in the laboratory, were also found in various field locations.

Materials and methods

Experimental procedure

This study was divided into four parts. Preliminary bottle tests were carried out in order to ensure that the produced water received from the field supported bacterial growth and that no inhibitory chemicals were present. Two similar laboratory flow cell experiments were then conducted which used two different inoculum sources and two different concentrations of nitrate. Finally, the dominant cultivable NRB, isolated in the laboratory and directly from a variety of field samples, were identified.

For the preliminary bottle test, acetate at 1,000 mg/l and propionate at 100 mg/l were added to 90 ml of produced water with 10 ml of artificial seawater (Tropic Marin® Dr. Biener, Wartenberg, Germany). This was placed in a 100 ml serum vials and incubated at 45 °C, without added inoculum. The bottle was then tested regularly for sulphide.

In experiment 1, two parallel flow cells were each inoculated with a mixed SRB culture and a mixed NRB culture (1 ml of each at 10^9 cells/ml), previously enriched from a North Sea oilfield produced water (see Culture and medium for more details). After 9 days, one of the flow cells was dosed with nitrate ion at 100 mg/l as calcium nitrate.

For experiment 2, in order to avoid an inoculum culture step in synthetic growth medium, a 90 ml subsample of freshly collected produced water was again incubated at 45 °C with acetate at 1,000 mg/l and propionate at 100 mg/l together with 10 ml of artificial seawater (Tropic Marin®). Sulphide was measured over 14 days and 2 ml of this water was subsequently used as inoculum for two parallel flow cells. After 8 days growth under flowing conditions, one flow cell (termed batch) was dosed with nitrate at 80 mg/l nitrate ion continuously for 24 h. The other flow cell was dosed continuously with 80 mg/l nitrate ion for the remainder of the experiment. A diagram of the relative timing of nitrate dosing in the two experiments is shown in Fig. 1.

Finally, samples were taken in the field using the SRB and NRB most probable number (MPN) serial dilution extinction technique (see Culture and medium). The most dominant bacteria cultured from several oil field sites were then sequenced and compared to bacteria identified during the flow cell experiments.

Flow cell once-through system

Two pieces of square section glass tubing (3 mm × 3 mm × 20 cm length) [S-103 Camlab Ltd, Cambridge, UK] were incorporated in parallel into a once-through flow loop made with neoprene tubing (Fig. 2). Nutrients (acetate at 1,000 mg/l and propionate at 100 mg/l) were added to filter-sterilised produced water and 5% artificial seawater (Tropic Marin®) contained in two medium reservoirs. Nutrients were added to simulate the high concentrations found in some formation waters. These vessels were sparged with oxygen-free argon at intervals during the test and a constant argon head was maintained throughout. A peristaltic pump controlled nutrient medium flow through both flow cells at 10 ml/h.

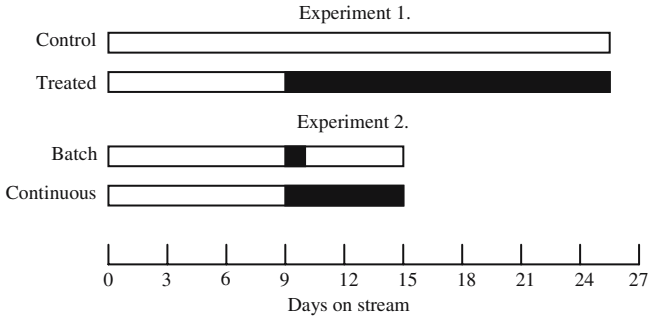

Fig. 1 Relative timing of nitrate dosing in Experiment 1 (100 mg/l) and Experiment 2 (80 mg/l)

Initially, each flow cell was pre-wetted, as sterile medium was allowed to flow through the cell, and the temperature was increased to 45 °C using the heated stage to establish a steady state. The flow was then stopped and the flow cells were inoculated directly through septa by sterile syringe (see Culture and medium). After allowing attachment of bacterial cells for 24 h (the flow cell was rotated through 180° after 15 h to ensure colonisation on both the upper and lower surfaces), the flow was re-started and biofilm growth was monitored in situ by image analysis.

Culture and medium

Sterilisation of the entire flow cell apparatus was carried out by autoclaving it at 121 °C for 30 min. Produced water was collected from the BP Shiehallion field in a sterile 20 l plastic container and transferred within 7 days to the laboratory. This field is located in the North Sea, approximately 200 miles north of Aberdeen. The treatment of injection water with calcium nitrate to control reservoir souring was initiated in 2001. The supplied produced water was diluted with 5% artificial seawater (Tropic Marin®), to give a sulphate concentration of 900 mg/l, before it was filter-sterilised using 0.22 μm filters directly into two sterilised medium reservoirs. Nutrients (acetate at 1,000 mg/l and propionate at 100 mg/l) were added to the two medium reservoirs which were sparged with argon. Before and after dosing with nitrate, MPN tests were carried out for SRB and DNB, which were incubated anaerobically, for samples from both flow cells and medium reservoirs. Ingredients for SRB

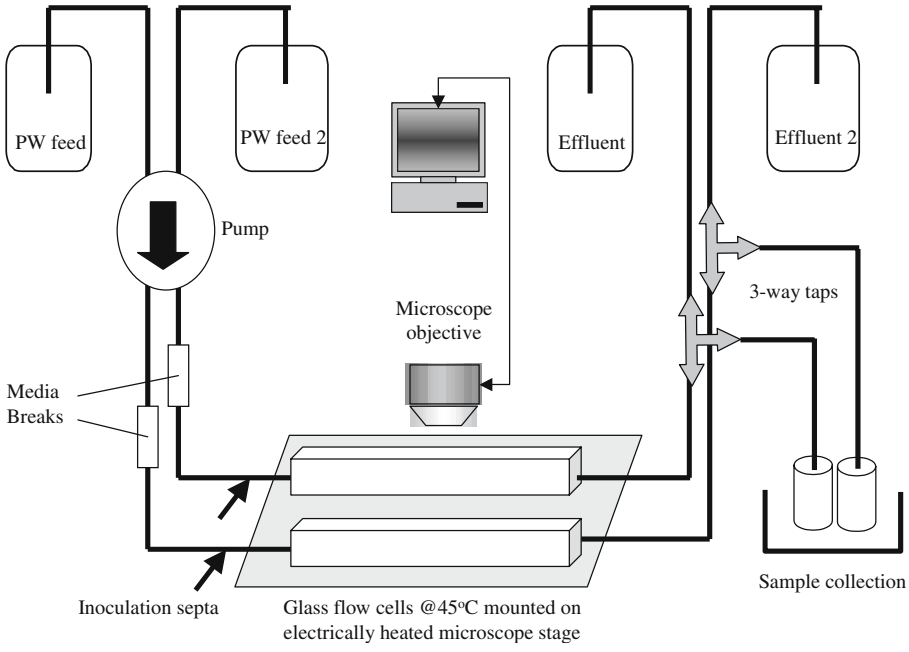

Fig. 2 Schematic diagram of flow cell apparatus

MPN medium were (g/l) KH_2PO_4 (0.05), $MgSO_4{\cdot}7H_20$ (0.2), sodium lactate (4 ml of 50% solution), Yeast extract (1), NaCl (10), ammonium ferrous sulphate (0.1), sodium thioglycollate (0.1), ascorbic acid (0.1), made up in 750 ml filtered aged seawater: 250 ml distilled water. Ingredients for NRB medium were (g/l) lab-lemco (3.0), peptone (5.0) potassium nitrate (1.0).

Image analysis

Image analysis was carried out using a Cohu closed-circuit television camera (CCTV) linked to a Leica IBM type PC equipped with Leica QWin software and a digital frame grabber (Leica Microsystems, Milton Keynes, UK). Ten images along the length of the flow cell were captured periodically using a ×5 objective on a Leitz Orthoplan microscope. The percentage biofilm surface area, covered by cell clusters, in each field was measured by setting a threshold so that the cell clusters were black and the surrounding voids white [29]. In addition, 20× and 40× long working distance objectives were used to look more closely at the individual cells and images were recorded so that cell morphology could later be quantified using the QWin software. This process involved using 'image detect' to select the greyscale that represented the cells on the surface. Then 'binary amend' allowed delineation of the individual cells before the software was ordered to automatically measure feature roundness (length-to-width ratio, $l_c{:}w_c$).

The thickness of biofilm, on the inner top and bottom surfaces of the flow cell, could also be measured by focusing from the top to the bottom of a biofilm stack and then calibrating the focus graticule [1].

Chemical measurement

The flow cell loops were specially modified to allow fluid samples to be taken directly from the flow lines. These samples were run off, either directly into fixative, or into cooled sterile vessels for chemical analysis. Sulphide was measured from zinc acetate-fixed samples using the methylene blue method [3]. Sulphate was determined from cooled samples by ion chromatography. Nitrate and nitrite were measured using a Palintest^{TM} (Professional Test Systems, Powys, UK) and by Nitratest sticks (Camlab Ltd).

16S rRNA gene sequencing and phylogenetic analysis

Strains were characterised phylogenetically by 16S rRNA gene sequencing. 16S rRNA gene fragments were generated by PCR [14]. An almost complete fragment of the 16S rRNA gene was amplified from DNA by PCR using universal primers pA (positions 8–28, AGAGTTTGATCCTGGCTCAG *Escherichia coli* numbering) and pH* (positions 1542–1522, AAGGAGGTGATCCAGCCGCA). The PCR products were directly sequenced using a *Taq* dye-Deoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an automatic DNA sequencer (model 373A, Applied Biosystems). Large fragments (approximately 1,450 base pairs) of the 16S rRNA genes were amplified by PCR and directly sequenced using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolates were determined by performing database searches using the program FASTA [26]. These sequences and those of other known

related strains were retrieved from GenBank and aligned with the newly determined sequences using the program DNATools [27]. The resulting multiple sequence alignment was corrected manually using the program GeneDoc [21]. A phylogenetic tree was constructed according to the neighbour-joining method with the programs DNATools and TREEVIEW [25] and the stability of the groupings was estimated by bootstrap analysis (1,000 replications) using the same programs. In addition, maximum parsimony analysis was performed [8].

Three laboratory samples were sequenced to identify the most numerous, culturable, NRB. These were from: NRB medium, inoculated from the Schiehallion produced water as received; NRB medium, inoculated with flow cell effluent before nitrate dosing; and NRB medium, inoculated with flow cell effluent after nitrate dosing. Further field samples were also sequenced (see Field testing).

Field testing

Two planktonic MPN samples were taken directly from the BP Schiehallion water injection and produced water streams, and two samples were taken from the same streams on the Foinaven platform. Continuous treatment of injected water with calcium nitrate had been carried out for 32 months on Foinaven and 34 months on Schiehallion. Two sessile samples were also taken from surface-associated solids from another North Sea field (water injection pipeline and producing wellhead. Continuous treatment with sodium nitrate had been carried out for 29 months, when the samples were taken.

Results

Preliminary bottle test

In the static bottle test, conducted prior to the flow cell experiments, SRB growth was indicated by a black precipitate, presumably iron sulphide, and 72 mg/l sulphide was measured after 14 days of incubation (Fig. 3).

Experiment 1

Effect of nitrate on biofilm cover

In both flow cells, bacteria, which had attached during the inoculation period, began to divide and form biofilm cell clusters (dense aggregates of cells). Prior to any nitrate addition, percentage surface area of biofilm cover increased from 0% to peaks of 28 and 27% in the Control- and Treated-flow cells, respectively (Fig. 4). On day 9 continuous addition of 100 mg/l of nitrate ion to the Treated-flow cell caused no significant change in percentage biofilm cover. After 15 days of nitrate treatment in the Treated-flow cell, biofilm cover in both flow cells was at approximately 22%. A two-sample *t*-test, assuming equal variances, showed that there was no significant difference in percentage biofilm cover in the two flow cells, over the entire experiment ($P < 0.05$). In addition, biofilm thickness measurements, before and after nitrate addition, showed biofilm clusters had a maximum thickness of 80–90 μm. However, most biofilm was much thinner, often only a single layer of cells on the surface.

Effect of nitrate on cell morphology

Prior to nitrate treatment, bacterial cells in both flow cells were predominantly cocci, approximately 2 μm in diameter. In addition, a very few rod-shaped cells were observed. Image analysis was used to calculate mean cell l_c:w_c ratio in the Control- (l_c:$w_c = 1.14$) and Treated-flow cells (l_c:$w_c = 1.20$) on day 8, before nitrate treatment. Many of these cells were motile and could be seen twitching or moving, often against the direction of fluid flow. After nitrate treatment of the Treated-flow cell, on day 9, a distinct difference in the cell morphology could be seen compared to the Control-flow cell (Fig. 5). Many more rod-shaped cells were observed, which became dominant with few cocci remaining on day 23 (l_c:$w_c = 2.80$, indicating cells that were longer than they

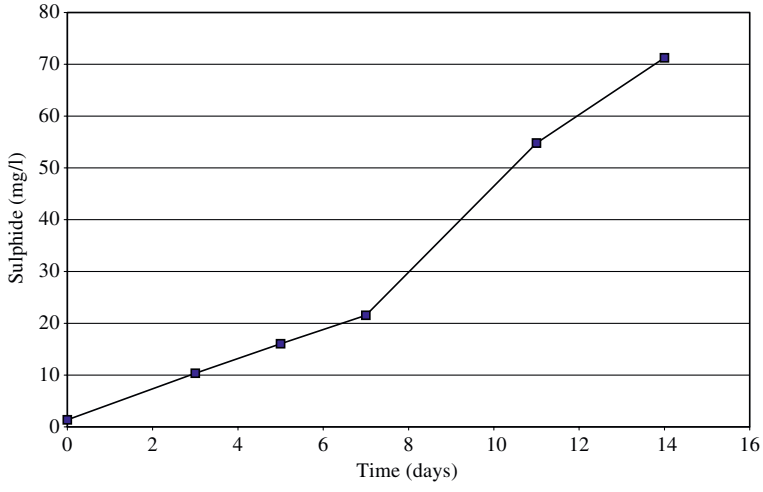

Fig. 3 Preliminary bottle test. Sulphide production in artificial seawater (10 ml) mixed with produced water (90 ml) amended with acetate (1,000 mg/l) and propionate (100 mg/l) in a serum bottle incubated at 45 °C. Bacteria were not added

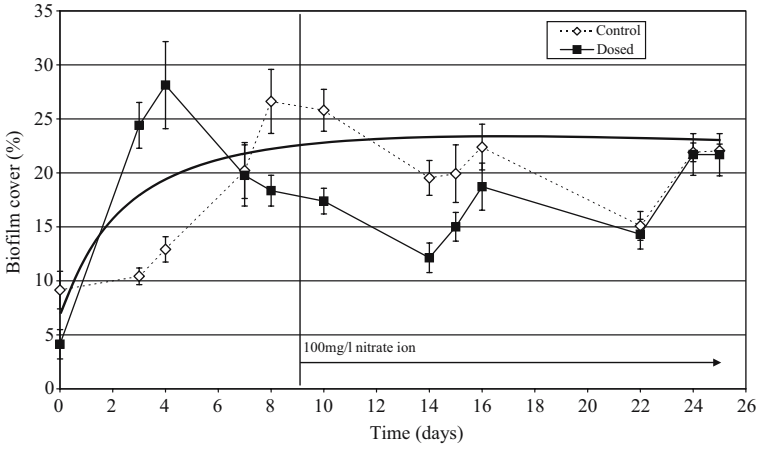

Fig. 4 Experiment 1. Biofilm cover on inner upper surface of flow cell before and after continuous addition of nitrate (100 mg/l) to dosed cell relative to control

were wide). In contrast, cell morphology in the Control-flow cell was relatively unchanged (l_c:w_c day 23 = 1.24).

Effect of nitrate on bacterial metabolism

After nitrate dosing, 5% of the available nitrate was reduced in the Treated-flow cell (Table 1). In addition, nitrite was measured at approximately 5 mg/l in the effluent. In contrast, no nitrate or nitrite was measured in the Control-flow cell effluent.

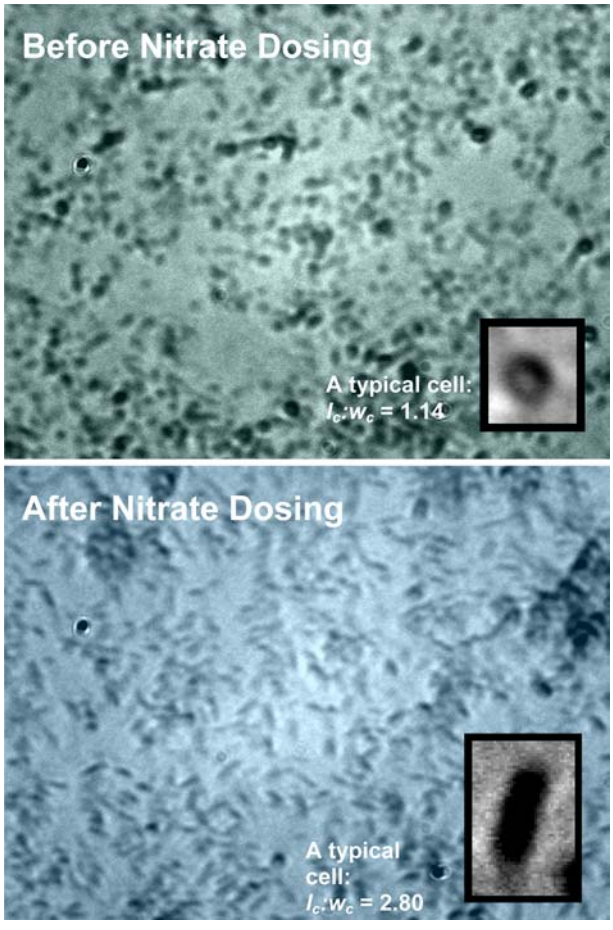

Fig. 5 Experiment 1. Cell morphology within biofilm 1 day before (**a**) and 14 days after (**b**) continuous addition of nitrate (100 mg/l) to flow cell

Bacterial population

Monitoring of the bacterial population showed approximately 140 SRB/ml and >140,000 NRB/ml in the effluent from both flow cells, prior to nitrate dosing (day 8). After nitrate dosing to the Treated-flow cell (day 17), 25 SRB/ml and >140,000 NRB/ml were again measured in both flow cells. The dominant bacterium isolated in the NRB medium was identified as *Marinobacter aquaeolei* [13] at a value of greater than 99% sequence similarity, from all three samples tested. This species was isolated as the dominant culturable NRB present in the produced water as received, the flow cell prior to nitrate treatment and the flow cell after nitrate treatment.

Experiment 2

Effect of nitrate on biofilm cover

Again, bacteria that had attached to the glass in both flow cells during the inoculation period began to divide and form biofilm cell clusters. The percentage surface area of biofilm cover increased from 0% to a peak of 15% in the Batch-flow cell and to 19% in the Continuous-flow cell by day 8, before any nitrate dosing (Fig. 6). On day 9 continuous addition of 80 mg/l of nitrate ion to both flow cells was begun. This was discontinued in the Batch-flow cell after 24 h and allowed to continue for the remainder of the experiment in the Continuous-flow cell. After nitrate dosing, biofilm cover reached a maximum of 22.5% and 14% in the Batch and Continuous flow cells, respectively. Over the 15 day experiment, a two-sample *t*-test, assuming equal variances, showed that there was no significant difference in percentage biofilm cover in the two flow cells ($P < 0.05$).

Table 1 Changes in chemical composition of effluent from flow cells during experiments 1 and 2

Experiment 1	Sulphate reduction		Sulphide produced		Nitrate reduction		Nitrite produced	
	Control (%)	Treated (%)	Control (mg/l)	Treated (mg/l)	Control (%)	Treated (%)	Control (mg/l)	Treated (mg/l)
Before nitrate dosing (day 8)	1	0	0	0	0	0	0	0
After nitrate dosing (day 23)	3	0	0	0	0	5	0	5

Experiment 2	Sulphate reduction		Sulphide produced		Nitrate reduction		Nitrite produced	
	Batch (%)	Continuous (%)	Batch (mg/l)	Continuous (mg/l)	Batch (%)	Continuous (%)	Batch (mg/l)	Continuous (mg/l)
Before nitrate dosing (day 8)	28	12	0.13	0.03	0	0	0	0
After nitrate dosing (day 9/10)	11	3	0	0	5	5	1	1

Sulphate reduction and nitrate reduction were calculated as the respective percentage loss of sulphate or nitrate measured across the flow cells. Nitrate was added on day 9 in both experiments

Effect of nitrate on cell morphology

Again, prior to nitrate treatment, bacterial cells in both flow cells were predominantly cocci with very few rod-shaped cells observed (Fig. 7). Mean cell l_c:w_c ratio were calculated for the Continuous-(l_c:w_c = 1.10) and Batch-flow cells (l_c:w_c = 1.12) on day 8. After nitrate treatment some rod-shaped cells became evident in both flow cells within 24 h. However, on day 13, rods dominated the Continuous-flow cell (l_c:w_c = 3.11), while the cocci ultimately remained predominant in the Batch-flow cell (l_c:w_c = 1.14).

Effect of nitrate on bacterial metabolism

Initially, sulphate reduction occurred in both flow cells (28 and 12% in the Batch-flow cell and the Continuous-flow cell, respectively, on day 8, Table 1). In addition, despite the poor suitability of the equipment for sulphide measurement, a trace of sulphide was detected in the effluent from both flow cells (up to 0.13 mg/l). After nitrate dosing (day 9), nitrate reduction at 5% occurred in both flow cells and nitrite was detected in the effluent from both flow cells (1 mg/l). Approximately 5% nitrate reduction continued in the Continuous-flow cell throughout the experiment and ceased immediately on cessation of nitrate in the Batch-flow cell on day 9.

Bacterial population

SRB (2 and 25 ml^{-1}) were detected in the effluent from the Batch and Continuous-flow cells, respectively, prior to nitrate dosing (day 8). This decreased to 0 and 2.5 SRB/ml on day 9. NRB (2,500 ml^{-1}) were recovered from the effluent from both flow cells, prior to nitrate dosing (day 8). After nitrate dosing to the Treated-flow cell (day 9), 2,500 NRB/ml and > 140,000 NRB/ml were measured in the Batch- and Continuous-flow cells, respectively.

Field testing

Two strains, OP117 and OP118, from samples taken from the surface solids from inside the water injection pipeline, proved to be *Marinobacter litoralis* [32]. This was identified as the dominant culturable NRB in the pipeline (> 140,000 cells/ml). A further sample was taken from a producing wellhead. This strain, OP129, was identified as *Marinobacter hydrocarbonoclasticus* [9] (> 15,000 cells/ml).

Another *Marinobacter* was identified from the BP Schiehallion water injection system (450 cells/ml). Strain OPL201 is closely related to *M. aquaeolei*; however, the degree of homology between DNA extracted from strain OPL201 and that of *M. aquaeolei* is sufficiently great that further work is required to elucidate

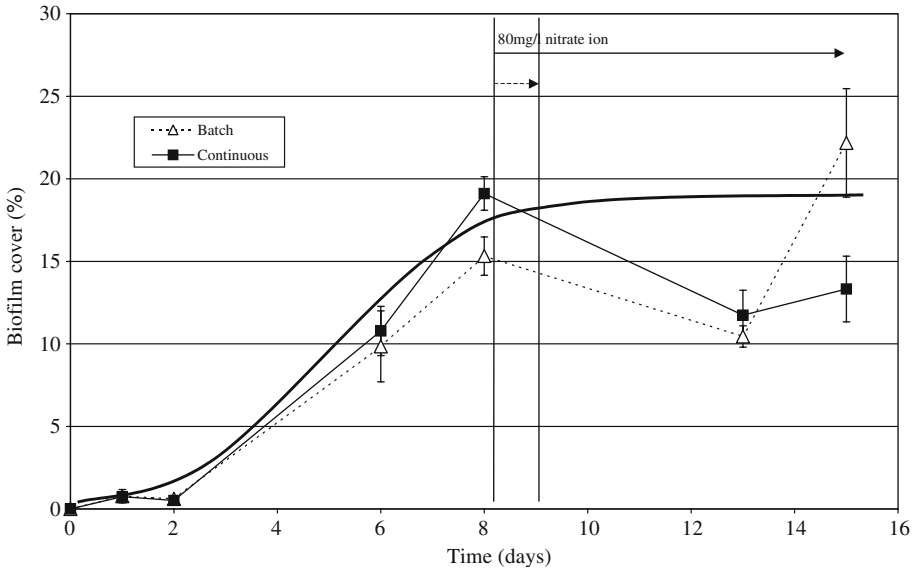

Fig. 6 Experiment 2. Biofilm cover on inner upper surface of flow cell before and after continuous addition of nitrate (80 mg/l) relative to batch-treated cell, to which nitrate was added for only 1 day.

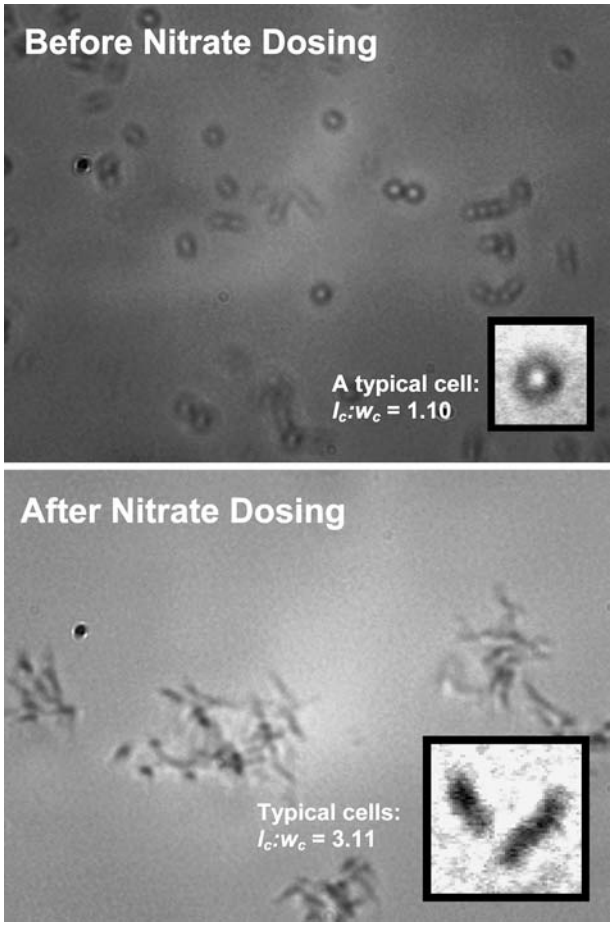

Fig. 7 Experiment 2. Cell morphology in biofilm 1 day before (**a**) and 4 days after (**b**) continuous addition of nitrate (80 mg/l) to flow cell relative to batch-treated cell, to which nitrate was added for only 1 day

the precise taxonomic position of OPL201. Finally, a further NRB species, isolated in low numbers (2.5 cells/ml) from BP Foinaven produced water, strain OPL202, has been identified as *Halomonas venusta*. This species was first isolated from a marine environment and was described as a facultative nitrate reducer [2].

Discussion

Initial tests showed that the microflora indigenous to the produced water sample used in these experiments was capable of producing sulphide and, furthermore, the nutrients supplied for these experiments, which are common constituents of produced waters, were sufficient to support SRB. SRB biofilms were then established using a method that has previously been successful [5]. We were interested to determine in situ, the effects of nitrate on such an SRB biofilm, and to identify the important competing NRB both in the laboratory and in the field.

Effect of nitrate on bacterial population

Continuous nitrate addition, at 80 and 100 mg/l, caused sulphate reduction rates to decrease and nitrate reduction to occur in these produced water tests. While nitrate has been shown to prevent sulphide generation in several once-through flowing laboratory experiments [18, 19], they have either been with simulated seawater injection [19] or synthetic medium [18], rather than simulated produced water reinjection. These laboratory experiments suggest that high doses of nitrate may not be necessary to control souring, even with reinjection of produced water (which contains more available carbon). They also suggest that nitrate has a residual effect on the bacterial population and further work could be conducted into optimisation of dosing. Presently, field use has been based on continuous dosing [16, 30], which can be prohibitively expensive for offshore fields where nitrate solution must be shipped to site.

In these experiments, direct microscopic observation showed that when nitrate was applied, rod-shaped cells replaced the bacterial population assemblage of mainly coccoid cells. *M. aquaeolei* has not previously been reported to grow as cocci or reduce sulphate, and length-to-width ratio measurements, made on cells in the flow cell biofilms after nitrate treatment, are consistent with previous descriptions of *M. aquaeolei* [13], which give length-to-width ratios of 2.8 to 4. We therefore conclude that the bacterial population changed from one dominated by coccoid SRB species to a population dominated by rod-shaped NRB. *M. aquaeolei* was suited to growth in this system as it is a moderately halophilic, mesophilic bacterium, and is a facultative nitrate-reducer, capable of using acetate, amongst other carbon sources. *M. aquaeolei* was present in the planktonic phase before and after nitrate addition and so must be capable of growth under the experimental conditions, even in the absence of added nitrate. It is clear that the rather rapid change in biofilm morphology was triggered by the introduction of nitrate and the onset of nitrate reduction. We cannot, however, discount the possibility that in the absence of nitrate, the morphology of *M. aquaeolei* is atypically coccoid, although we can find no reports of this.

SRB and NRB could be cultured from these mixed biofilms and evidence is presented which showed some increases in NRB and decreases in SRB numbers upon nitrate treatment. Although relatively few SRB were detected in the effluent from flow cells, even prior to nitrate dosing, the culture of cells from the planktonic phase may not directly reflect the composition of biofilm populations.

Effect of nitrate on biofilm cover

Nitrate did not cause any significant increase in the biomass on surfaces within the flow cells. It is likely that another nutrient and not the electron acceptor was the limiting factor for growth. This work suggests that the potential detrimental effects of nitrate, such as biomass build-up and reservoir plugging may be minimal. However, further work is needed in this little-studied area of research into nitrate treatments.

Field testing

Marinobacter aqueolei was isolated and identified from produced water samples which originated from an offshore oil-producing platform. This species had previously been isolated from an oil-producing wellhead in a Vietnamese oilfield [13]. It was then important to attempt to isolate NRB from diverse oil field locations where nitrate is being used to treat reservoir souring successfully. This sampling programme has revealed numerous species of *Marinobacter*. Planktonic and sessile samples have been taken from seawater and produced water systems. *Marinobacter* have even been found in two separate oilfields: one a chalk reservoir treated with sodium nitrate and the other a sandstone reservoir injected with calcium nitrate. *M. litoralis* is a moderately halophilic bacterium isolated previously from seawater from the East Sea in Korea [32]. *M. hydrocarbonoclasticus* is very closely related to *M. aqueolei*. It is an extremely halotolerant, hydrocarbon-degrading marine bacterium [9], previously isolated from seawater, near the effluent outlet of a petroleum refinery. The final *Marinobacter* strain was again closely related but may, in fact, be a novel species. All are well suited to growth in petroleum reservoirs, which are usually highly saline.

Much previous work has centred on the autotrophic NRSOB promoted by nitrate treatment of oilfield waters [12, 20, 31]. However, elsewhere, in 12 out of 15 oilfield water samples, heterotrophic NRB have been found to be in equal or greater numbers than NRSOB [7]. This implies that heterotrophic NRB may have more potential in the successful use of nitrate to control reservoir souring. Indeed, *Marinobacter* have also been isolated from waters associated with oil production during a comprehensive molecular survey [24]. We therefore propose that species of *Marinobacter* are likely to have an important role in out-competing SRB for nutrients or producing inhibitory nitrite [28] in oil field environments. Further work will show whether this genus may be used as an indicator for the successful application of nitrate, which could be usefully detected before any impact on field souring can be identified (sometimes this may be years owing to transit times between water injectors and producers)—or perhaps selected species could be inoculated into systems where nitrate treatment has not previously proved successful.

Conclusions

1. Nitrate treatment had a clear influence on the SRB biofilms developed in the flow cell.
2. The cells in the nitrate-treated biofilms were more rod-shaped ($l_c{:}w_c = 2.5$) than those cells growing under sulphate-reducing conditions ($l_c{:}w_c = 1.1$).
3. Nitrate ions (100 and 80 mg/l) caused the dominant metabolic process to switch from sulphate reduction to nitrate reduction in these produced water tests.
4. No significant increase in biofilm surface area cover or biofilm thickness was seen due to nitrate treatment in these tests.
5. The dominant culturable NRB genus in these laboratory tests was identified as *Marinobacter*. *Marinobacter* have also been found to be the dominant culturable species in various field samples and may be important for the success of nitrate as a treatment for preventing sulphide generation.

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