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# Comparison of microbial communities involved in souring and corrosion in offshore and onshore oil production facilities in Nigeria

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**Abstract** Samples were obtained from the Obigbo field, located onshore in the Niger delta, Nigeria, from which oil is produced by injection of low-sulfate groundwater, as well as from the offshore Bonga field from which oil is produced by injection of high-sulfate (2,200 ppm) seawater, amended with 45 ppm of calcium nitrate to limit reservoir souring. Despite low concentrations of sulfate (0–7 ppm) and nitrate (0 ppm), sulfate-reducing bacteria (SRB) and heterotrophic nitrate-reducing bacteria (NRB) were present in samples from the Obigbo field. Biologically active deposits (BADs), scraped from corrosion-failed sections of a water- and of an oil-transporting pipeline (both Obigbo), had high counts of SRB and high sulfate and ferrous iron concentrations. Analysis of microbial community composition by pyrosequencing indicated anaerobic, methanogenic hydrocarbon degradation to be a dominant process in all samples from the Obigbo field, including the BADs. Samples from the Bonga field also had significant activity of SRB, as well as of heterotrophic and of sulfide-oxidizing NRB. Microbial community analysis indicated high proportions of potentially thermophilic NRB and near-absence of microbes active in methanogenic hydrocarbon degradation. Anaerobic incubation of Bonga samples with steel coupons gave moderate general corrosion rates of 0.045–0.049 mm/year, whereas near-zero general corrosion rates (0.001–0.002 mm/year) were observed with Obigbo water samples. Hence, methanogens may contribute to corrosion at Obigbo, but the low general corrosion rates cannot explain the reasons for pipeline failures in the Niger delta. A focus of future work should be on understanding the role of BADs in enhancing under-deposit pitting corrosion.

**Keywords** Oil field · Sulfide · Souring · Corrosion · Methanogen · Sulfate-reducing bacteria

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## Introduction

With a maximum daily production capacity of 2.5 million barrels per day, Nigeria is Africa's largest producer of oil and is among the top ten oil producers worldwide (http://www.indexmundi.com). Oil production often involves injection of freshwater or of seawater in onshore and in offshore fields, respectively. Water injection can boost the activity of oil-associated, sulfate-reducing prokaryotes (SRP), which couple the oxidation of oil organics to the

reduction of sulfate to sulfide (souring). Souring is catalyzed by sulfate-reducing bacteria (SRB) at low temperature or by sulfate-reducing archaea (SRA) at higher temperature [6, 32, 36]. Souring can be especially severe when seawater with a high concentration of sulfate is injected. Oil organics include alkanes and aromatics, as well as volatile fatty acids (VFA) and other organic acids derived from these [2]. Lack of nutrients (e.g., phosphate), high salinity, or high reservoir temperature are also factors that may limit growth of SRB and SRA [17, 32]. Ideal conditions for microbial growth may occur in the near-injection wellbore region (NIWR), where seawater sulfate and oil organics mix and where temperatures can be cooler than in the bulk of the reservoir. Souring increases the concentration of sulfide in produced oil, water, and gas, which in turn increases corrosion risk [3, 5, 32]. Increased pipeline corrosion and resulting oil spills have caused environmental degradation in the Niger delta, providing strong incentives for a detailed study of microbes present in Nigerian oil fields and in production operations.

In this work we have analyzed samples from the Obigbo North field, located onshore in the Niger delta, which is injected with low-sulfate groundwater. The estimated reservoir temperature is 40 °C. Produced oil, water, and gas are transported through a delivery flow-line to high pressure (HP) and low pressure (LP) separators, in which gas is separated from liquid by pressure reduction. The liquids from the separators are then transported to the terminal for crude oil dehydration and processing. Solids from two pipelines, transporting mostly water or mostly oil from the Obigbo field, were also examined. Results for these samples are compared with those obtained for samples from the Bonga field, which is located 120 km offshore, southwest of Warri, Nigeria, where water depths range from 950 to 1,200 m and which is injected with about 300,000 barrels of seawater per day for production of 180,000–200,000 barrels of crude oil per day [13, 14]. The reservoir temperature at Bonga is 63 °C. Because the high concentration of sulfate in seawater (2,200 ppm at Bonga) increases the potential for souring, injection water at Bonga is treated with chlorine, subjected to regular biocide treatment, and amended with 45 ppm of calcium nitrate to limit SRB activity. At Obigbo fresh groundwater, which is essentially free of sulfate and has few microbes, is injected. Hence, one expects less SRB-mediated souring and associated corrosion risk at Obigbo. However, flowline/pipeline failures in onshore fields in Nigeria occur raising the question whether these relate to microbiologically influenced corrosion (MIC) and, if so, which microbial groups are responsible. In addition to SRB, microbial communities in oil fields include fermenting bacteria, as well as methanogenic archaea, which have been implicated in corrosion [4, 20, 30]. Because it is often difficult to cultivate these anaerobic microbes, oil field microbial communities are most easily characterized by culture-independent methods, such as the sequencing of 16S rRNA genes [8, 16, 21, 31].

## Materials and methods

### Site and process description

Samples 2N1 to 2N6 and samples 3N1 to 3N4 (Table 1) were collected from the Obigbo and Bonga fields, respectively, in sterile 500-ml Nalgene sample bottles, filled to the brim to exclude air and shipped to the University of Calgary, Alberta, Canada for further analysis within 1 week of collection. Sampling sites have been ordered in Table 1 to reflect fluid flow (from 2N3 to 2N4 and from 3N3 to 3N1). Cleaned, produced water from Obigbo and other producing onshore assets is piped offshore for disposal through a bulk discharge header pipeline at the terminal. Solids scraped from a water- and from an oil-transporting pipeline (Table 1, PPL1 and PPL2) were also obtained and shipped in closed 500-ml Nalgene sample bottles. These solids were removed 2 months after the lines had been excavated to repair a failure. Hence these samples experienced significant air-exposure. All samples were transferred to a Coy anaerobic hood with an atmosphere of 90 % (v/v) $N_2$ and 10 % (v/v) $CO_2$ upon arrival. Sample analysis was started within 3 weeks of collection, except for PPL1 and PPL2, for which analysis was started within 11 weeks of collection.

### Chemical analyses

Water samples were analyzed without further treatment. For analysis of pipeline solids, 10 g of sample (wet weight) was combined with 10 ml of deionized water. Following vigorous shaking the solids were allowed to settle and the supernatants, PPL1-S and PPL2-S, were used for analysis. The pH of all samples (water samples as well as supernatants of solid samples) was measured using an Orion pH meter. Aqueous sulfide was analyzed using the diamine method [29] and $NH_4^+$ with the indophenol method [1]. Sulfate, nitrate, nitrite, and the VFA acetate, propionate, and butyrate were analyzed by high-performance liquid chromatography (HPLC), as described elsewhere [10]. For analysis of inorganic anions 100 μl of sample was combined with 400 μl HPLC anion buffer, whereas for analysis of VFA 300 μl of the sample was combined with 20 μl 1 M phosphoric acid. PPL1-S and PPL2-S (100 μl) were combined with 0.5 ml of 0.5 N HCl and incubated for 15 min; 100-μl aliquots of the extracts were then used for the determination of ferrous iron by the FerroZine assay [20].

**Table 1** Samples obtained from Nigerian oil fields and their water chemistry

| Sample | Field | Description | pH | Site *T* (°C) | Sulfate (mM) | $NH_4^+$ (mM) | Ace[a] HPLC | Pro[a] HPLC | But[a] HPLC |
|---|---|---|---|---|---|---|---|---|---|
| 2N3/CR6 | Obigbo North | Low-sulfate groundwater (injection water) | 5.5 | 25 | 0.03 | 0.06 | 0.5 | 1.3 | 1.8 |
| 2N1 | Obigbo North | Produced water and oil from delivery line | 7.1 | 30–40 | 0.02 | 0.17 | 29.6 | 2.4 | 0.4 |
| 2N2 | Obigbo North | Produced water and oil from HP separator | 7.1 | 30–40 | 0.07 | 0.2 | 60.0 | 5.8 | 1.2 |
| 2N6 | Obigbo North | Produced water from inlet T1401 | 7.2 | 30–40 | 0.05 | 0.18 | 1.9 | 0.3 | 1.3 |
| 2N5/CR4 | Obigbo North | Produced water from bulk discharge header | 7.1 | 30–40 | 0 | 0.16 | 2 | 0.1 | 5.7 |
| 2N4 | Obigbo North | Produced water from sludge tank 1201B (Bobi Tank Farm) | 7.1 | 30–40 | 0 | 0.17 | 0.7 | 0.6 | 2.4 |
| PPL1 | Obigbo North | Pipeline solids from 10-in. mainly water-transporting pipeline[b] | 6.8 | 30–40 | 28.6 | 0.53 | ND[c] | ND | ND |
| PPL2 | Obigbo North | Pipeline solids from 20 in. mainly oil-transporting pipeline[b] | 6.4 | 30–40 | 13.6 | 2.43 | ND | ND | ND |
| 3N3/CR1 | Bonga | Biocide-treated Injection water | 6.3 | 25 | 21.6 | 0.31 | 0.063 | 0 | 0 |
| 3N4 | Bonga | Partially treated produced water B (65 ppm oil) | 7.1 | 28–32 | 11.8 | 0.38 | 2.05 | 0.63 | 0.85 |
| 3N2 | Bonga | Partially treated produced water A (56 ppm oil) | 7.3 | 28–32 | 11.7 | 0.62 | 2.16 | 0.25 | 0 |
| 3N1/CR2 | Bonga | Treated produced water (<25 ppm oil) | 7.2 | 28–32 | 10.4 | 0.39 | 3.20 | 1.25 | 0 |

Samples labeled CR were used for corrosion rate measurements (Fig. 3). Additional water chemistry data for injection and produced waters are provided in Table S1

[a] Concentrations (mM) of the volatile fatty acids acetate (Ace), propionate (Pro), and butyrate (But) determined by HPLC

[b] Solids (10 g) were suspended in 10 ml of deionized water. The suspension was vortexed and the supernatant was used for analysis. The results are for the supernatant

[c] Not determined

## Measurement of microbial counts and activities

The activities of SRB, as well as of heterotrophic and of sulfide-oxidizing nitrate-reducing bacteria (hNRB and soNRB) were measured. Coleville synthetic brine (CSB) medium [19], containing 7 g of NaCl/l was used throughout. Medium was anaerobically dispensed in 70-ml aliquots into 125-ml serum bottles with a gas phase of 90 % $N_2$ and 10 % $CO_2$ and closed with sterile butyl rubber stoppers. Medium was amended with 40 mM lactate and 20 mM sulfate or with 3 mM VFA (3 mM each of acetate, propionate, and butyrate) and 20 mM sulfate for measurement of SRB activity. Medium was amended with 3 mM VFA and 10 mM nitrate for measurement of hNRB activity and with 5 mM sulfide and 10 mM nitrate for measurement of soNRB activity. The medium bottles were inoculated by injection of 3.5 ml of sample and were incubated at 37 °C with shaking. This temperature is in the range of values at sites from which samples were collected (Table 1). A sterile syringe needle was used to periodically remove 1 ml of medium and to determine the sulfide, sulfate, nitrate, and nitrite concentrations. Microbial activities were calculated as $100/t_{1/2}$ units/day, where $t_{1/2}$ is the time (days) needed to reduce half of the sulfate (SRB activity) or nitrate concentration (hNRB and soNRB activities), or oxidize half of the sulfide concentration (soNRB), as described by Voordouw et al. [35]. The count of lactate-utilizing SRB was determined using vials with 9 ml of anaerobic API RP-38 broth with an NaCl concentration of 5 g/l. These were inoculated with 1 ml of sample and with tenfold dilutions derived thereof and were incubated at 37 °C for 30 days. Formation of a black iron sulfide precipitate was used to score the presence of SRB.

## Corrosion testing

Coupons (2 × 1 × 0.1 cm) were cut from ASTM A366 carbon steel with 0.015 % (w/w) carbon (ASTM international

designation A 1008/A) and cleaned according to a standard protocol (NACE RP0775-2005) in which the coupons were polished with 400-grit sandpaper and then placed in a dibutylthiourea–HCl solution for 2 min. The coupons were then neutralized in a saturated bicarbonate solution for 2 min, rinsed with deionized $H_2O$ and then with acetone, and finally dried in a stream of air. The coupons were weighed three times and the average weight was recorded as the starting weight. Duplicate coupons were placed in 160-ml serum bottles containing 70 ml of sample under anaerobic conditions (headspace of 90 % v/v $N_2$ and 10 % $CO_2$; $N_2$–$CO_2$). One set contained unfiltered sample, whereas another set contained sample filtered through 0.2-μm Millipore Acrodisc syringe filters. Serum bottles with 70 ml of filtered Milli-Q purified water were used as a control. The samples were incubated at 32 °C while being shaken at 60 rpm. After a 4-week incubation period, the coupons were cleaned and dried according to the NACE standard protocol and weighed again. The corrosion rate (CR) was determined from the metal weight loss ($\Delta W$ in grams) as

$$\mathrm{CR} = 87,600 \times \Delta W(A \times D \times T)\ \mathrm{mm/year}$$

$A$, $D$, and $T$ represent the coupon area (4.6 $cm^2$), the density of the steel (7.85 $g/cm^3$), and the incubation time in hours, respectively. An experimentally determined value of 0.0021 g was subtracted from the measured weight loss to correct for iron removal by the second cleaning procedure.

### DNA extraction, amplification, sequencing, and bioinformatic analysis

Genomic DNA was extracted from 40 ml of samples 2N1–2N6 and 3N1–3N4 using the MP Biomedical FastDNA kit. For PPL1 and PPL2, 500 mg of sample was added to 978 μl of sodium phosphate buffer and 122 μl of MT buffer in a lysing matrix tube and homogenized in a FastPrep instrument for 40 s at a speed setting of 6; this was followed by centrifugation at 14,000 rpm for 15 min in a microcentrifuge to pellet the debris. The supernatant was transferred to a clean microfuge tube and 250 μl of protein precipitate solution was added and mixed by shaking the tube by hand 10 times; this was followed by centrifugation at 14,000 rpm for 5 min to pellet the precipitate and the supernatant was transferred to a clean 15 ml tube. Extracted DNA (2 ng $\mu l^{-1}$) was then amplified through 25 PCR cycles using primers 926f and 1392r. The PCR products were purified and subjected to a second round of 10 PCR cycles with pyrosequencing primers 454T-RA-X (which is barcoded) and 454T-FB, which have 926f and 1392r as their 3′ ends. PCR product quality was verified on an 0.7 % agarose gel and PCR products were purified with a QIAquick PCR Purification Kit (Qiagen) following which their concentrations were determined on a Qubit fluorimeter (Invitrogen), using a Quant-iT dsDNA HS Assay Kit (Invitrogen). Detailed procedures have been described elsewhere [20]. PCR products (typically 20 μl of 5 ng $\mu l^{-1}$) were sent to the Genome Quebec and McGill University Innovation Centre for pyrosequencing with an FLX Instrument, using a GS FLX Titanium Series Kit XLR70 (Roche Diagnostics Corporation). Data analysis was conducted with Phoenix 2, a 16S rRNA data analysis pipeline, developed in-house [27]. High-quality sequences, which remained following quality control and chimeric sequence removal, were clustered into operational taxonomic units (OTUs) at 5 % distance by using the average linkage algorithm [25]. A taxonomic consensus of all representative sequences from each of these was derived from the recurring species within 5 % of the best bitscore from a BLAST search against the SILVA102 SSU reference data set [22]. Amplicon libraries were clustered into a Newick-formatted tree using the UPGMA algorithm with the distance between libraries calculated with the thetaYC coefficient [37] as a measurement of their similarity in the Mothur software package [26]. The Newick format of the sample relation tree was visualized using Dendroscope [11]. The entire set of raw reads is available from the Sequence Read Archive at the National Center for Biotechnology Information (NCBI) under accession numbers SRR619059 to SRR619063, SRR619766 to SRR619769, SRR621568, and SRR621623.

## Results

### Chemical characterization of samples

Results for chemical analysis of samples are indicated in Table 1. The water samples from Obigbo North had a low sulfate concentration (0–0.07 mM), whereas injection water from Bonga had 21.6 mM sulfate, a typical value for seawater. Produced waters from Bonga had 10.4–11.8 mM sulfate. Ammonium concentrations were 0.06–0.18 and 0.31—0.62 mM for Obigbo and Bonga waters, respectively. Nitrate concentrations were below detection limits in all water samples (results not shown). Obigbo produced waters 2N1 and 2N2 had very high acetate concentrations (Table 1, 29.6 and 60.0 mM), which ranged from 0.1–3.2 mM in all other samples. The conductivity of Bonga produced waters was 56.9 mS/cm, corresponding to that of an NaCl concentration of 0.554 M, whereas that of Obigbo produced waters was 19.7 mS/cm, corresponding to that of an NaCl concentration of 0.193 M. Actual ion compositions of Bonga and Obigbo injection and produced waters are given in Table S1 in the Supplementary Material.

The pipeline solid extracts PPL1-S and PPL2-S had high sulfate concentrations of 28.6 and 13.6 mM (Table 1) and significant nitrate concentrations of 0.38 and 2.46 mM, respectively. These samples also had significant

ferrous iron concentrations of 1.84 and 3.68 mM, respectively. Pipeline solids PPL-1 and PPL-2 had a water content of 15 and 22 % (w/w), a carbonate concentration of 463 and 36 ppm, and a bicarbonate concentration of 3,608 and 426 ppm, respectively, as determined by on-site measurements. The presence of the last two was evident as bubble formation, when these solids were treated with HCl.

## Microbial activities and counts

At Obigbo the groundwater used as the injection water had a low count of lactate-utilizing SRB (Table 2, 10/ml) and lacked activities of lactate-utilizing and VFA-utilizing SRB, VFA-utilizing hNRB, and soNRB. All other water samples had significant activity of lactate-utilizing SRB and VFA-utilizing hNRB (Table 2). VFA-utilizing SRB and soNRB were less active. The pipeline solid samples PPL1 and PPL2 had the highest counts of lactate-utilizing SRB of $10^8$–$10^9$/g (Table 2), whereas water samples from the same field had $10^1$–$10^5$/ml. The SRB count of water samples did not correlate with their sulfate concentration (Tables 1, 2).

At Bonga produced water 3N1 had the highest count of lactate-utilizing SRB ($5 \times 10^5$/ml), whereas those in produced waters 3N2 and 3N4 and in injection water 3N3 were $10^2$/ml. SRB activities with lactate or with VFA were also highest in produced water 3N1 (Table 2). All Bonga produced waters and the injection water had significant hNRB and soNRB activity (Table 2), possibly because this field is injected with 45 ppm of calcium nitrate to prevent souring [13, 14].

**Table 2** Counts of lactate-utilizing SRB and activities of lactate-utilizing SRB, VFA-utilizing SRB, VFA-utilizing hNRB, and soNRB for samples listed in Table 1

| Sample code | Description | SRB/ml lactate | Activity[a] SRB, lactate | Activity[a] SRB, VFA | Activity[a] hNRB, VFA | Activity[a] soNRB |
|---|---|---|---|---|---|---|
| Obigbo | | | | | | |
| 2N3/CR6 | Low-sulfate groundwater (injection water) | $10^1$ | 0 | 0 | 0 | 0 |
| 2N1 | Produced water and oil from delivery line | $10^5$ | 34 | 3 | 66 | 0–10[b] |
| 2N2 | Produced water and oil from HP separator | $10^1$ | 25 | 0 | 14 | 0 |
| 2N6 | Produced water from inlet T1401 | $10^4$ | 25 | 3 | 66 | 0–10[b] |
| 2N5/CR4 | Produced water from bulk discharge header | $10^5$ | 40 | 4 | 66 | 0–10[b] |
| 2N4 | Produced water from sludge tank 1201B (Bobi Tank Farm) | $10^5$ | 17 | 2.5 | 66 | 0 |
| PPL1 | Pipeline solids from 10-in. water-transporting pipeline[c] | $10^8$ | ND[d] | ND | ND | ND |
| PPL2 | Pipeline solids from 20 in. oil-transporting pipeline[c] | $10^9$ | ND | ND | ND | ND |
| Bonga | | | | | | |
| 3N3/CR1 | Biocide-treated injection water | $10^2$ | 0–10[b] | 0 | 23 | 3.7 |
| 3N2 | Partially treated produced water B (65 ppm oil) | $10^2$ | 7.1 | 2.2 | 43 | 15 |
| 3N1/CR2 | Partially treated produced water A (56 ppm oil) | $5 \times 10^5$ | 48 | 5.6 | 25 | 22 |
| 3N4 | Treated produced water (<25 ppm oil) | $10^2$ | 5.8 | 1.6 | 26 | 40 |

The data from which these activities are derived are shown in Figures S1 to S8 in the Supplementary Material

[a] Calculated as $(100/t_{1/2})$, where $t_{1/2}$ is the time (days) needed to reduce half of the electron acceptor (sulfate or nitrate)

[b] Estimate because of incomplete reduction of electron acceptor

[c] SRB count is per gram of solids

[d] Not determined

### Microbial communities in the Obigbo and Bonga fields

DNA was successfully extracted and purified from all samples, except from 2N3 Obigbo injection water (groundwater). Failure to isolate DNA from this sample is in agreement with the low SRB count and zero microbial activities (Table 2). Following PCR amplification and purification by agarose gel electrophoresis, the 16S rRNA amplicons for the remaining 11 samples were subjected to pyrosequencing. After removal of poor-quality or chimeric sequences 3,074–15,992 good sequences remained for the seven Obigbo samples and 12,974–30,149 good sequences remained for the four Bonga samples (Table 3). These sequences clustered into 83–448 OTUs (groups of sequences with 95 % sequence identity) for Obigbo and into 33–339 OTUs for Bonga samples (Table 3). The relationship between the number of identified OTUs and the number of sequences is shown in rarefaction curves (Fig. 1). These indicate that microbial diversity was on average higher at Obigbo than at Bonga. This is also suggested by higher values for the normalized Shannon index for Obigbo, as compared to Bonga samples (Table 3). An exception was Obigbo produced water sample 2N2, which had the lowest number of estimated OTUs and the lowest Shannon index of all Obigbo samples (Table 3). Sequence analysis indicated the presence of 33–152 taxa in microbial communities in these fields (Table 3). Compositions for samples from Obigbo and Bonga were very different, forming two distinct clades in the relational tree shown in Fig. 2. These compositions are summarized in Table 3, which indicates fractions (%) of taxa for all samples. The sums of these ranged from 87.7 to 99 %, the balance being fractions of rarer taxa not indicated in Table 3.

A major difference between communities at Obigo and Bonga was that those at Obigbo were dominated by methanogenic archaea (Table 3, entries 2 to 9), which were mostly absent from Bonga. Anaerobic, fermenting *Porphyromonadaceae*, nitrate-reducing, potentially thermophilic, *Deferribacteraceae* [6, 7], *Rhodobacteraceae*, and *Marinobacterium* were present among the top 14 taxa in both fields (Table 3: entries 13, 21, 36, and 56).

At Obigbo samples 2N1 and 2N2 (produced waters from an oil delivery line and an HP separator) formed a distinct subgroup (Fig. 2). Microbial communities at these two sites were dominated by the methylotrophic methanogen *Methanolobus* (Table 3, entry 8), but lacked the acetotrophic methanogen *Methanosaeta* (Table 3, entry 7), which may explain the high acetate concentrations in these samples (Table 1, 29.6 and 60.0 mM, respectively). In the other Obigbo water samples, the presence of *Methanosaeta* (Table 3, 22–33 %), which converts acetate to methane and $CO_2$, may be responsible for the lower acetate concentrations observed (Table 1, 0.7–2.0 mM). Anaerobic, potentially oil-degrading *Firmicutes* (Table 3, entries 22–31) were found in all Obigbo samples. Potentially oil-degrading syntrophs (Table 3, entries 49–51) were found in all Obigbo samples, except 2N1 and 2N2. The microbial communities in pipeline solids samples PPL1 and PPL2 clearly treed with those from Obigbo water samples (Fig. 2). Pipeline solids sample PPL1 contained significant fractions of taxa capable of aerobic hydrocarbon degradation (Table 3, entries 15 and 40). These may have been increased by exposure to air during storage and during shipment. Low fractions of SRB were observed in Obigbo samples (Table 3, entries 44–48). Overall, the microbial community at Obigbo has potential for anaerobic, methanogenic hydrocarbon degradation through the action of firmicutes, syntrophs, and methanogens [9, 38].

At the Bonga field the microbial community of the 3N3 injection water (seawater) differed significantly from those in the produced waters with high fractions of the marine bacteria *Parvibaculum* and *Thalassobaculum* (Table 3, entries 35 and 38). *Parvibaculum* is a marine bacterium capable of aerobic alkane degradation [23]. Its high fraction (49 %) in the injection water (seawater) indicates the presence of hydrocarbons. Interestingly, the injection water also had high fractions of anaerobic *Clostridiales* (Table 3, entries 24 and 29), indicating potential anaerobic hydrocarbon degradation in the injection water following oxygen removal. No significant fractions of *Parvibaculum* and *Thalassobaculum* were found in produced waters 3N1, 3N2, and 3N4, indicating that these marine bacteria did not survive passage through the reservoir. Produced waters 3N2 and 3N4 had high fractions of *Rhodobacteraceae*, *Rhodospirillaceae*, *Marinobacter*, and *Marinobacterium* (Table 3, entries 36, 39, 53, and 56). These taxa may not represent the community in the reservoir, as they likely emerged by growth in the cooler produced-water handling units (Table 1, 28–32 °C). Produced water 3N1 was distinct from 3N2 and 3N4 with a high fraction of *Chlorobiales*_BSV26 and *Petrobacter*, a potentially thermophilic hNRB [24]; (Table 3, entries 20 and 41). This sample had a more anaerobic signature than 3N2 and 3N4 through the presence of the methanogen *Methanolobus*, the nitrate-reducing *Deferribacteraceae*, and the *Deltaproteobacteria* (the class that includes the SRB) *Desulfovibrio, Desulfuromonas*, and *Geoalkalibacter* (Table 3, entries 8, 21, 46, 47, and 48, respectively). The significant presence of anaerobes (including SRB) in this sample, as determined by pyrosequencing, is in agreement with the fact that 3N1 had the highest count and activities of SRB in the Bonga samples (Table 2).

### Corrosion rates of coupons exposed to Obigbo and Bonga samples

The corrosivity of samples 2N3, 2N5, 3N3, and 3N1 from the Obigbo and Bonga fields was examined as part of a

**Table 3** Phylogenetic classification of pyrosequencing reads for samples from the freshwater injected at Obigbo North field and from the seawater injected at Bonga field

| Sample name | | PPL1 | PPL2 | 2N1 | 2N2 | 2N4 | 2N5 | 2N6 | 3N1 | 3N2 | 3N3 | 3N4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Type of sample | | Pipe solids | Pipe solids | PW | PW | PW | PW | PW | PW | PW | IW | PW |
| Sequence code | | V17_677 | V17_678 | V11_395 | V11_396 | V11_397 | V11_398 | V11_399 | V14_538 | V14_539 | V14_540 | V14_541 |
| Total reads | | 4,483 | 15,992 | 5,844 | 3,074 | 6,386 | 2,963 | 6,225 | 23,623 | 30,149 | 12,974 | 22,463 |
| Number of OTUs | | 239 | 398 | 241 | 83 | 448 | 265 | 414 | 339 | 160 | 315 | 133 |
| Number of taxa | | 109 | 149 | 80 | 33 | 156 | 108 | 151 | 151 | 75 | 152 | 71 |
| Estimated OTUs (Chao) | | 469 | 574 | 372 | 122 | 864 | 515 | 913 | 678 | 427 | 835 | 263 |
| Normalized Shannon index | | 3.20 | 3.22 | 3.04 | 2.01 | 3.73 | 3.89 | 3.68 | 2.63 | 1.52 | 2.02 | 1.90 |
| Entry | Taxon (phylum; class; order; family; genus) | | | | | | | | | | | |
| 1 | *Euryarchaeota; Archaeoglobi; Archaeoglobales; Archaeoglobaceae; Archaeoglobus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.031 | 0.004 |
| 2 | *Euryarchaeota; Methanobacteria; Methanobacteriales; Methanobacteriaceae; Methanobacterium* | 1.227 | **17.528** | 0.034 | 0.033 | **3.148** | 0.742 | 0.884 | 0.013 | 0.003 | 0 | 0 |
| 3 | *Euryarchaeota; Methanobacteria; Methanobacteriales; Methanobacteriaceae; Methanothermobacter* | **3.056** | **2.839** | 0 | 0 | 0.329 | 0 | 0.08 | 0 | 0 | 0 | 0 |
| 4 | *Euryarchaeota; Methanomicrobia;* ANME-1;ANME-1b | **1.093** | 0 | 0 | 0 | 0.016 | 0.034 | 0.064 | 0 | 0 | 0 | 0 |
| 5 | *Euryarchaeota; Methanomicrobia; Methanomicrobiales; Methanocalculus* | 0 | **1.301** | 0.257 | 0 | **10.523** | **6.75** | **10.41** | 0.004 | 0 | 0 | 0.004 |
| 6 | *Euryarchaeota; Methanomicrobia; Methanomicrobiales; Methanomicrobiaceae; Methanoculleus* | **8.164** | 0.269 | 0.086 | 0 | **5.371** | **2.936** | **4.418** | 0.013 | 0 | 0.031 | 0.013 |
| 7 | *Euryarchaeota; Methanomicrobia; Methanosarcinales; Methanosaetaceae; Methanosaeta* | **43.408** | **12.094** | 0.137 | 0 | **33.636** | **22.241** | **31.373** | 0.013 | 0.003 | 0.062 | 0.004 |
| 8 | *Euryarchaeota; Methanomicrobia; Methanosarcinales; Methanosarcinaceae; Methanolobus* | **2.677** | **3.508** | **26.489** | **39.753** | **2.177** | **2.835** | **2.345** | **5.465** | 0 | 0.008 | 0 |
| 9 | *Euryarchaeota; Methanomicrobia; Methanosarcinales; Methanosarcinaceae; Methanomethylovorans* | 0.089 | 0.006 | 0.017 | 0 | **1.08** | **6.649** | **1.976** | 0 | 0 | 0.015 | 0 |
| 10 | *Euryarchaeota; Thermoplasmata;* WCHA1-57 | 0 | 0.013 | 0.068 | 0 | **1.112** | 0.877 | 0.803 | 0 | 0 | 0 | 0 |
| 11 | *Actinobacteria; Actinobacteria; Coriobacteridae; Coriobacteriales; Coriobacterineae; Coriobacteriaceae* | 0.022 | 0.944 | 0.017 | 0 | 0.423 | 0 | 0.016 | 0 | 0 | 0 | 0 |
| 12 | *Bacteroidetes; Bacteroidia; Bacteroidales; Marinilabiaceae; Anaerophaga* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **1.029** | **1.201** | 0 | 0.508 |

**Table 3** continued

| Entry | Taxon (phylum; class; order; family; genus) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | *Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae* | **5.041** | **5.522** | 0.719 | **2.472** | 0.157 | 0.371 | 0.129 | 0.525 | **3.791** | 0 | **4.234** |
| 14 | *Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Paludibacter* | 0 | 0.006 | 0 | 0 | 0.094 | 1.856 | 0.305 | 0 | 0 | 0 | 0 |
| 15 | *Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae* | **1.338** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.031 | 0 |
| 16 | *Bacteroidetes; Sphingobacteria; Sphingobacteriales;* SB-1 | 0.959 | 0.063 | 0.65 | 0 | 0.11 | 0.742 | 0.289 | **1.888** | 0 | 0 | 0 |
| 17 | *Bacteroidetes;* VC2.1 | 0.87 | 0.331 | **4.586** | 0 | 0.157 | 0.101 | 0.145 | 0.423 | 0 | 0 | 0 |
| 18 | Candidate_division_OP9 | **4.104** | 0.331 | 0 | 0 | **2.49** | 0.304 | 0.627 | 0 | 0 | 0 | 0 |
| 19 | Candidate_division_WS6 | 0 | 0.663 | **4.141** | 0 | **3.101** | **4.185** | **4.562** | 0 | 0 | 0 | 0 |
| 20 | *Chlorobi; Chlorobia; Chlorobiales*; BSV26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **26.61** | 0 | 0 | 0.013 |
| 21 | *Deferribacteres; Deferribacteres; Deferribacterales; Deferribacteraceae* | **2.32** | **2.464** | **2.977** | **5.172** | 0.251 | **2.599** | **1.639** | **8.695** | **1.028** | 0.008 | **1.211** |
| 22 | *Firmicutes* | 0 | 0.081 | **11.174** | 0.911 | **1.378** | **6.075** | **6.12** | 0.004 | 0 | 0 | 0 |
| 23 | *Firmicutes; Clostridia; Clostridiales* | 0.067 | 0.213 | **2.31** | **3.578** | 0.423 | 0.337 | 0.675 | 0 | 0 | 0.008 | 0 |
| 24 | *Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium* | 0 | 0.031 | 0 | 0.033 | 0 | 0 | 0 | **5.334** | 0.716 | **16.479** | 0.539 |
| 25 | *Firmicutes; Clostridia; Clostridiales; Eubacteriaceae; Acetobacterium* | 0 | **10.093** | 0 | 0 | 0.094 | 0.337 | 0.53 | 0 | 0 | 0 | 0 |
| 26 | *Firmicutes; Clostridia; Clostridiales; Eubacteriaceae; Alkalibacter* | 0 | **1.476** | 0 | 0 | 0.031 | 0 | 0.064 | 0 | 0.003 | 0 | 0 |
| 27 | *Firmicutes; Clostridia; Clostridiales; Family_XI_Incertae_Sedis; Sedimentibacter* | 0.736 | **1.144** | 2.19 | 0 | 0.188 | 0.337 | 0.145 | 0 | 0 | 0 | 0 |
| 28 | *Firmicutes; Clostridia; Clostridiales; Family_XI_Incertae_Sedis; Tissierella* | 0 | 0.375 | **13.347** | **4.88** | 0.752 | **2.734** | **1.398** | 0.313 | 0 | 0 | 0 |
| 29 | *Firmicutes; Clostridia; Clostridiales; Lachnospiraceae;Incertae_Sedis* | 0.112 | 0.144 | 0 | 0.033 | 0.141 | 0.067 | 0 | **4.271** | 0.577 | **12.872** | 0.508 |
| 30 | *Firmicutes; Clostridia; Clostridiales; Syntrophomonadaceae; Syntrophomonas* | 0.022 | 0.131 | **1.626** | 0 | 0.235 | 0 | 0.225 | 0 | 0 | 0 | 0 |
| 31 | *Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Acholeplasma* | 0.022 | 0.025 | **14.87** | 0 | 0.031 | **1.89** | 0.305 | 0 | 0 | 0 | 0 |
| 32 | *Lentisphaerae; Lentisphaeria* | 0.201 | 0 | 0 | 0 | 0.063 | 0 | **1.655** | 0 | 0 | 0 | 0 |
| 33 | *Lentisphaerae; Lentisphaeria;* WCHB1-25 | 0 | 0 | 0 | 0 | 0.031 | **1.012** | 0.032 | 0 | 0 | 0 | 0 |
| 34 | *Proteobacteria; Alphaproteobacteria* | 0.446 | 0.069 | 0.017 | 0 | 0.016 | 0.337 | 0.08 | 0.063 | 0.1 | **4.147** | 0.085 |
| 35 | *Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhodobiaceae; Parvibaculum* | 0 | 0 | 0.017 | 0 | 0 | 0 | 0 | 0.381 | 0 | **49.337** | 0 |

**Table 3** continued

| Entry | Taxon (phylum; class; order; family; genus) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 36 | *Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae* | 0.803 | **13.182** | 0.53 | 0 | **2.756** | 0.405 | 0.193 | 0.483 | **10.644** | 0.755 | **28.126** |
| 37 | *Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae* | **1.472** | 0.488 | 0 | 0 | 0.016 | 0 | 0 | 0.017 | 0.02 | 0.84 | 0.018 |
| 38 | *Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Thalassobaculum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.042 | 0 | **5.503** | 0 |
| 39 | *Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; uncultured* | 0 | 0 | 0 | 0 | 0 | 0.034 | 0 | 0.186 | **10.531** | 0.008 | 8.57 |
| 40 | *Proteobacteria; Alphaproteobacteria; Sphingomonadales* | **5.331** | 0.006 | 0 | 0 | 0 | 0 | 0 | 0.004 | 0 | 0.008 | 0 |
| 41 | *Proteobacteria; Betaproteobacteria; Hydrogenophilales; Hydrogenophilaceae; Petrobacter* | 0 | 0.094 | 0.222 | 0 | 0.376 | 0.506 | 0.096 | **12.509** | 0 | 0.008 | 0 |
| 42 | *Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae* | 0.268 | 0.138 | 0.017 | 0 | **1.143** | 0.472 | 0.643 | 0.004 | 0 | 0.123 | 0 |
| 43 | *Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Thauera* | 0.089 | 0.757 | **1.728** | 0 | **1.957** | 0.27 | 0.514 | 0.004 | 0.003 | 0 | 0 |
| 44 | *Proteobacteria; Deltaproteobacteria; Desulfobacterales; Desulfobacteraceae; Desulfotignum* | 0 | 0.044 | 0.24 | 0 | 0.11 | 0.776 | **1.558** | 0 | 0 | 0 | 0 |
| 45 | *Proteobacteria; Deltaproteobacteria; Desulfobacterales; Desulfobulbaceae; Desulfobulbus* | 0 | 0.069 | 0.582 | 0.716 | 0.188 | 0.877 | 0.98 | 0 | 0 | 0.008 | 0 |
| 46 | *Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio* | 0 | 0.019 | 0.034 | 0.098 | 0 | 0 | 0.016 | **2.023** | 0 | 0.015 | 0 |
| 47 | *Proteobacteria; Deltaproteobacteria; Desulfuromonadales; Desulfuromonadaceae; Desulfuromonas* | 0.045 | 0.181 | 0.154 | 0 | 0.752 | 0.169 | 0.145 | **3.276** | 0 | 0 | 0 |
| 48 | *Proteobacteria; Deltaproteobacteria; Desulfuromonadales; Geobacteraceae; Geoalkalibacter* | 0.112 | **1.032** | 0 | 0 | 0.172 | 0.067 | 0 | **1.3** | 0.474 | 0.039 | 0.009 |
| 49 | *Proteobacteria; Deltaproteobacteria; Syntrophobacterales; Syntrophaceae; Smithella* | 0.022 | 0 | 0 | 0 | 0.016 | 0.337 | 0.418 | 0 | 0 | 0 | 0 |
| 50 | *Proteobacteria; Deltaproteobacteria; Syntrophobacterales; Syntrophaceae; Syntrophus* | 0.892 | 0.05 | 0 | 0 | 0.611 | 0.236 | 0.707 | 0 | 0 | 0 | 0 |

**Table 3** continued

| Entry | Taxon (phylum; class; order; family; genus) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 51 | *Proteobacteria; Deltaproteobacteria; Syntrophorhabdaceae; Syntrophorhabdus* | 0.022 | 0.019 | 0 | 0 | 0.345 | 0.169 | 0.594 | 0 | 0 | 0 | 0 |
| 52 | *Proteobacteria; Epsilonproteobacteria; Nautiliales; Nautiliaceae; Nitratifractor* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **1.384** | 0 | 0 | 0 |
| 53 | *Proteobacteria; Gammaproteobacteria; Alteromonadales; Alteromonadaceae; Marinobacter* | 0.112 | **1.588** | 0.051 | 0 | 0 | 0 | 0 | 2.388 | **9.274** | 0.301 | **12.875** |
| 54 | *Proteobacteria; Gammaproteobacteria; Chromatiales; Ectothiorhodospiraceae; uncultured* | 0.067 | **7.485** | 0 | 0.195 | 0 | 0 | 0 | 0 | 0.043 | 0.008 | 0 |
| 55 | *Proteobacteria; Gammaproteobacteria; Oceanospirillales; Halomonadaceae; Halomonas* | 0.022 | 0.006 | 0 | 0 | 0 | 0 | 0 | 0.114 | 0.743 | 0.008 | **7.483** |
| 56 | *Proteobacteria; Gammaproteobacteria; Oceanospirillales; Oceanospirillaceae; Marinobacterium* | **4.528** | **1.244** | **1.01** | 0.098 | **4.244** | 9.72 | 0.916 | **17.978** | **56.237** | 0.077 | **33.553** |
| 57 | *Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas* | **1.785** | **3.17** | 0.907 | **30.547** | 0.031 | 0 | 0.048 | 0.237 | **3.612** | 0.802 | 0.49 |
| 58 | *Spirochaetes; Spirochaetes* | 0.022 | 0.225 | 0.359 | **4.88** | 0.407 | **2.025** | **2.088** | 0 | 0 | 0 | 0 |
| 59 | *Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Spirochaeta* | 0.335 | 0.194 | **1.3** | **3.611** | 0.235 | 0.945 | 0.594 | 0.004 | 0 | 0 | 0 |
| 60 | *Synergistetes; Synergistia; Synergistales; Synergistaceae; Thermanaerovibrio* | 0.468 | 0.188 | 0.291 | 0.325 | **1.769** | **1.451** | **1.944** | 0.004 | 0 | 0 | 0 |
| 61 | *Thermotogae; Thermotogae; Thermotogales; Thermotogaceae; Kosmotoga* | 0.468 | 0.819 | 0.24 | 0 | **5.622** | **2.7** | **3.598** | 0 | 0 | 0 | 0 |
| | Sum | 92.8 | 92.7 | 93.4 | 97.3 | 88.3 | 87.5 | 86.3 | 97.0 | 99.0 | 91.5 | 98.2 |

The number of good sequence reads for each sample (Total reads), the number of OTUs (95 % sequence identity) and the number of taxa that these represent are indicated. The estimated maximum number of OTUs (Chao) and the Shannon index (normalized for the same number of reads) are also listed. The fractions of reads (%) are indicated for all taxa which have at least one fraction in excess of 1 % (indicated in bold). Some taxa not meeting this criterion (entries 1, 49–51) are discussed in the text

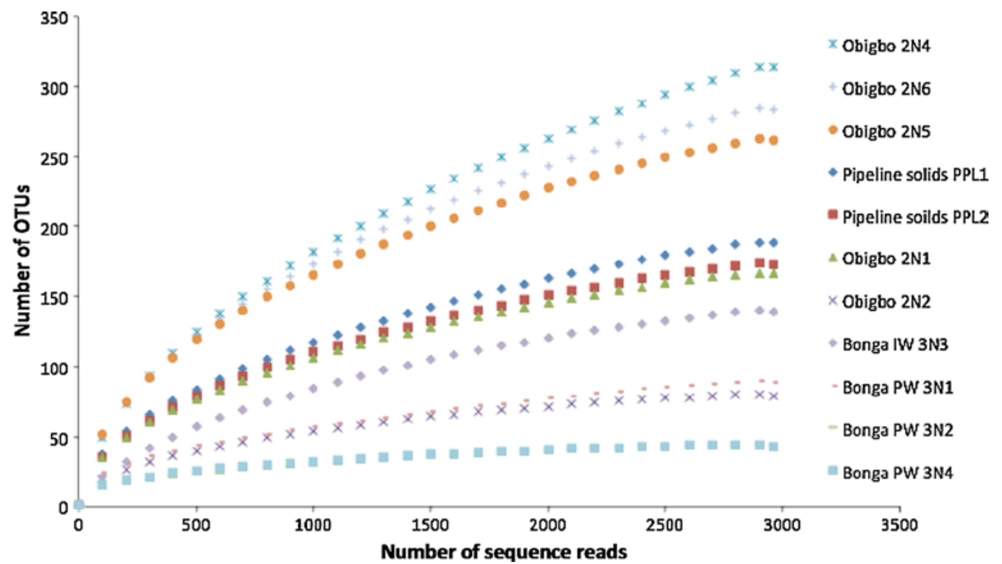


**Fig. 1** Rarefaction curves for the 16S rRNA sequence libraries indicated in Fig. 2 and Table 3. The curves are normalized to the same number of sequences as obtained for sample 2N2 (Table 3; 3074). The data indicate that Obigbo samples have on average more OTUs than Bonga samples. Bonga samples 3N2 and 3N4 had near-identical rarefaction curves causing the data for 3N2 to be invisible

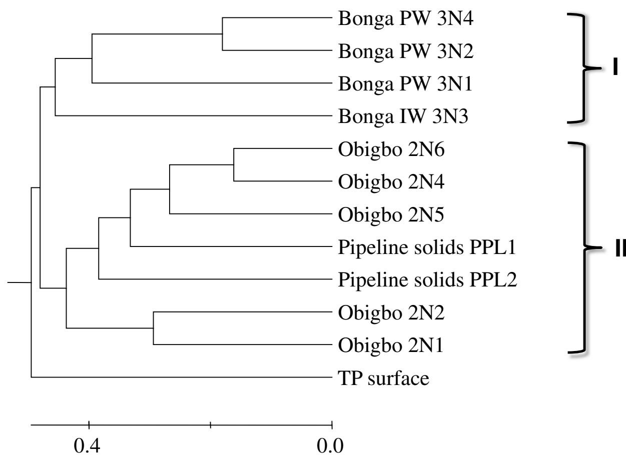


**Fig. 2** Relational tree for microbial community compositions derived by pyrosequencing. Compositions for samples from the Obigbo and Bonga fields formed distinct clusters I and II, as indicated. The composition for a tailings pond surface water sample (TP surface) was used as the outgroup to root the tree. The *scale* indicates the fraction of sequence divergence

larger experiment, in which incubations were labeled CR1 to CR8. The Obigbo samples are therefore referred to as injection water 2N3/CR6 and produced water 2N5/CR4, whereas the Bonga samples are referred to as injection water 3N3/CR1 and produced water 3N1/CR2. General corrosion rates were determined by weight loss of coupons suspended in unamended samples under anaerobic conditions with a headspace of 90 % (v/v) $N_2$ and 10 % (v/v) $CO_2$. Following incubation for 4 weeks significant blackening, indicating FeS formation, was evident in the incubations with Bonga samples (Fig. 3c, d) but not in those with the Obigbo samples (Fig. 3a, b). Filtration of samples through a 0.2-μm filter decreased sulfide formation and associated corrosion in Bonga produced water sample 3N1/CR2 (Fig. 3d), but not in Bonga injection water sample 3N3/CR1 (Fig. 3c). Corrosion rates, obtained from the measured weight loss of the carbon steel coupons, were (0.045 ± 0.010) and (0.041 ± 0.004) mm/year for the unfiltered and filtered tests in Fig. 3c and (0.049 ± 0.004) and (0.020 ± 0.019) mm/year for the unfiltered and filtered tests in Fig. 3d.

At Obigbo near-zero general corrosion rates were observed of (0.0024 ± 0.0013) and (0.0004 ± 0.0002) mm/year for the unfiltered and filtered injection water (groundwater) sample 2N3/CR6 (Fig. 3a) and (0.0014 ± 0.0006) and (0.0066 ± 0.0064) mm/year for the unfiltered and filtered produced water sample 2N5/CR4 (Fig. 3b). Hence, these rates were approximately 20-fold lower than those observed at Bonga.

## Discussion

As indicated in the "Introduction", preventing or eliminating reservoir souring, defined as the microbial production of sulfide, may decrease the risk of MIC to the metal infrastructure that receives, separates, and transports the mixture of produced water and oil [32–34, 36]. Because SRB are considered the primary culprits in MIC, monitoring their presence through dilution counting in a lactate- and sulfate-containing medium is done routinely in the industry. A problem in equating the presence of high numbers of lactate-utilizing SRB with high MIC risk is that these organisms can grow fermentatively in the absence of sulfate and that other microbes can also contribute to MIC. This includes fermentative, acid-producing bacteria (APB), as

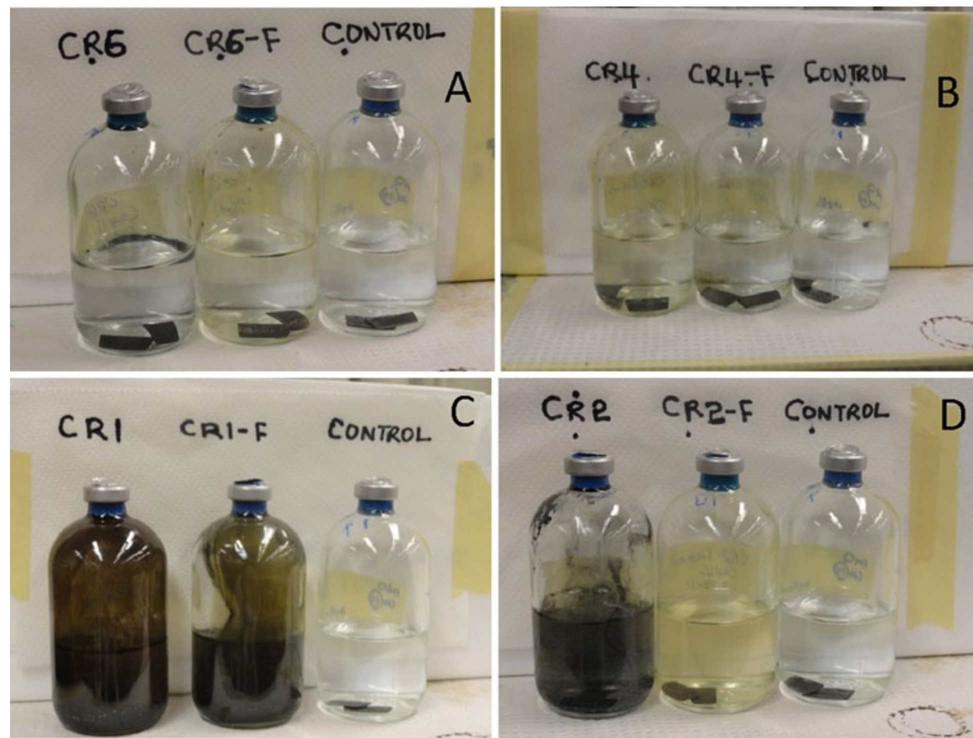


**Fig. 3** Weight loss corrosion tests with Obigbo and Bonga samples. Duplicate iron coupons were incubated under anaerobic conditions (90 % $N_2$, 10 % $CO_2$) with 70 ml of sample, either used as is or passed through a 0.2-µm filter (F). The control contained 70 ml of filtered, deionized water. **a** CR6, Obigbo groundwater 2N3; **b** CR4, Obigbo produced water 2N5; **c** CR1, Bonga injection water 3N3; **d** CR2, Bonga produced water 3N1. The photographs were taken following 4 weeks of incubation

well as other microbes that can use the reducing power of steel for their metabolism such as the methanogens. Hence, MIC is likely catalyzed by a microbial consortium [15], the composition of which is best determined by molecular methods such as pyrosequencing. A disadvantage of molecular methods is that they have a long turnaround time and that they cannot be easily implemented in the field. A goal of analysis of microbial communities in MIC sites is thus to identify key players, which can then be quantified through more rapid PCR assays to determine MIC risk.

In the current work we have used both cultivation-based and molecular methods to characterize souring and associated MIC risk in two Nigerian oil fields. Among four samples from the Bonga field (injection water 3N3 and produced waters 3N2, 3N1, and 3N4) we found the highest count of SRB ($5 \times 10^5$/ml) and the highest SRB activity in produced water sample 3N1 (Table 2). The pyrosequencing survey indicated that this sample contained the highest fractions of anaerobic taxa, including the methylotrophic methanogen *Methanolobus* (Table 3, entry 8: 5.5 %) and the SRB *Desulfovibrio* (entry 46: 2.0 %) and *Desulfuromonas* (entry 47: 3.3 %). Hence, all methods pointed to 3N1 as having the highest souring potential. Near-zero fractions of the thermophilic SRA *Archaeoglobus* (Table 3, entry 1) indicate limited souring potential in the bulk of the reservoir, where the temperature is in excess of 60 °C.

All Bonga samples had significant hNRB and soNRB activity (Table 2). One might be tempted to relate this to the addition of 45 ppm of calcium nitrate to the injection water to prevent souring at Bonga [13, 14]. Injection water sample 3N3 was collected prior to the nitrate injection point and no residual nitrate was detected in the produced water samples, indicating nitrate to be reduced. The presence of high fractions of the hNRB *Deferribacteraceae* in all three produced waters and of the hNRB *Petrobacter* and the soNRB *Nitratifractor* in injection water 3N1 (Table 3, entries 21, 41, 52) may have resulted from nitrate injection [6, 7, 18, 24]. However, the ability to reduce nitrate is widely distributed among heterotrophic bacteria and hNRB activity is routinely found in samples from oil fields, irrespective of whether these are subjected to nitrate injection. In contrast, soNRB activity is limited to specialized chemolithotrophic bacteria, which derive energy for growth from the oxidation of sulfide or sulfur with nitrate or oxygen. These have significant activity at Bonga, but are largely absent from Obigbo, which is not injected with nitrate (Table 2).

SRB appear to contribute to corrosion in Bonga samples under lab conditions (Fig. 3c, d). At Bonga the topsides

injection water facilities are also treated twice weekly for 2 h with 450 ppm of the biocide tetrakis(hydroxymethyl) phosphonium sulfate (THPS). General corrosion rates of 0.045–0.049 mm/year as found here are considered moderate [12]. Hence the data indicate that mitigation measures at Bonga (nitrate injection and biocide treatment) succeed in limiting souring and associated corrosion. Our data do not provide information on the risk of pitting corrosion, which is likely best evaluated by examining corrosion in the field.

Despite the near-absence of sulfate from produced waters at Obigbo, four of these (2N1, 2N4, 2N5, and 2N6) had a significant SRB count ($10^4$–$10^5$/ml). All had SRB activity with lactate and sulfate and significant hNRB activity with VFA and nitrate (Table 2). In view of the low sulfate concentrations of these waters and the absence of nitrate, which is not injected at Obigbo, these SRB and hNRB are unlikely to grow as sulfate- and nitrate-reducers at the sampling sites. In the absence of sulfate or nitrate, water-mediated fermentation of oil organics to methane and $CO_2$ becomes a dominant process [8, 9, 21, 36, 38]. At Obigbo this is facilitated by the high bicarbonate concentration of produced waters (Table S1). Pyrosequencing indicated the presence of microbes catalyzing this process, including fermentative, syntrophic bacteria (Table 3, entries 22–31 and 49–51), acetotrophic methanogens (Table 3, entry 7), and hydrogenotrophic methanogens (Table 3, entries 2, 3, 5, and 6). The last of these could also be involved in MIC, using hydrogen from steel to reduce $CO_2$ to methane [4, 15, 20, 30]. As a result of the different water chemistry (Table 1, Table S1) and reservoir conditions (62 °C for Bonga and 40 °C for Obigbo), the microbial community compositions in samples from the Obigbo field differ significantly from those in the Bonga field (Fig. 2; Table 3).

The injection and produced waters from Obigbo had near-zero corrosion rates and did not turn black during corrosion tests in agreement with their low sulfate concentration (Fig. 3a, b; Table 1, Table S1). However, the pipeline solids removed from two failed pipeline sections had very different chemical and microbial characteristics. This included high concentrations of sulfate (Table 1, 29 and 14 mM), as well as high counts of SRB ($10^8$–$10^9$/g) in the pipeline solids extracts. The origin of these high sulfate concentrations is not clear. Perhaps these result through adsorption of low aqueous concentrations. Because SRB were only a minor community component (Table 3, entries 44–48), the actual number of microbes in these solids may be much higher than $10^8$–$10^9$/g with the majority of the cells being the fermentative and syntrophic bacteria and methanogens that were main community components in both samples of pipeline solids. For example, assuming a density of these solids of 1 g/ml and an average cell volume of 3 $\mu m^3$, 30 % of the volume of these solids would be biomass at $10^{11}$ microbes/g. Methanogenic hydrocarbon degradation, facilitated by a continuous flow of water and hydrocarbon, may be a key activity in these pipeline solids. This may lead to high concentrations of acetic acid (Table 1), which can contribute to corrosion [28]. Likewise, the occurrence of iron sulfides in pipeline deposits may accelerate SRB-mediated corrosion [5]. Since the pipeline solids obtained in this study were scraped from pipeline failures, their chemical and microbial composition must have contributed to high rates of under-deposit corrosion. In view of the low general corrosivity of Obigbo waters it is clear that future research on corrosion failures should focus on the mechanisms that may cause high under-deposit pitting corrosion rates and how these can be prevented.

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