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ENVIRONMENTAL BIOTECHNOLOGY

Design features of offshore oil production platforms influence their susceptibility to biocorrosion

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Abstract Offshore oil-producing platforms are designed for efficient and cost-effective separation of oil from water. However, design features and operating practices may create conditions that promote the proliferation and spread of biocorrosive microorganisms. The microbial communities and their potential for metal corrosion were characterized for three oil production platforms that varied in their oil-water separation processes, fluid recycling practices, and history of microbially influenced corrosion (MIC). Microbial diversity was evaluated by 16S rRNA gene sequencing, and numbers of total bacteria, archaea, and sulfate-reducing bacteria (SRB) were estimated by qPCR. The rates of ^{35}S sulfate reduction assay (SRA) were measured as a proxy for metal biocorrosion potential. A variety of microorganisms common to oil production facilities were found, but distinct communities were associated with the design of the platform and varied with different locations in the processing stream. Stagnant, lower temperature (<37 °C) sites in all platforms had more SRB and higher SRA compared to samples from sites with higher temperatures and flow rates. However, high (5 mmol L^{-1}) levels of hydrogen sulfide and high numbers (10^7 mL^{-1}) of SRB were found in only one platform. This platform alone contained large separation tanks with long retention times and recycled fluids from stagnant sites to the beginning of the oil separation train, thus promoting distribution of biocorrosive microorganisms. These findings tell us that tracking microbial sulfate-reducing activity and community composition on off-shore oil production platforms can be used to identify operational practices that inadvertently promote the proliferation, distribution, and activity of biocorrosive microorganisms.

Keywords Offshore oil production platforms · Biocorrosion · Microbially influenced corrosion · Sulfate-reducing bacteria

Introduction

The activity of microorganisms in oil reservoirs and processing facilities can have undesirable effects on oil quality and the infrastructure of the processing facility. Offshore oil-producing platforms are subject to microbially influenced corrosion (MIC; or biocorrosion) in addition to other types of corrosion. Platform operators act to mitigate the damage caused by MIC through the use of biocides or other control chemicals, together with operational factors such as controlling the rate of fluid flow (Skovhus and Eckert 2014). Untargeted, continuous biocide application to control MIC is not a desirable or cost-effective option, especially with increased regulatory scrutiny over the use of environmentally hazardous chemicals (Bradley et al. 2011). A more effective and less expensive approach for MIC primarily driven by sulfate reduction would be to apply treatments to areas identified to have the greatest sulfate-reducing activity and, therefore, at the greatest risk of MIC. The biological production of

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sulfide, common in environments where marine waters are routinely encountered, is of special concern because sulfide is associated with oil souring and the corrosion of metal equipment (Birkeland 2004; Duncan et al. 2009; Ferris et al. 1992).

Microorganisms contributing to MIC on an oil production platform may originate from several possible sources, including the reservoir itself. A combination of culture-independent and culture-based approaches revealed that high-temperature oil fields contain thermophilic bacteria and archaea (L'Haridon et al. 1995; Magot et al. 2000; Head et al. 2003; Gittel et al. 2009; Guan et al. 2014; Nazina et al. 2007; Youssef et al. 2009) that are most likely indigenous to the reservoirs. But when determining strategies to mitigate MIC in an oil-water separation system, we need to look beyond the origin of biocorrosive microorganisms and perform a comprehensive examination of the design and operation of the platform to determine which of its features promote microbial activity and proliferation. Factors that influence microbial community composition and activity on the platform include physiochemical conditions such as the availability of electron donors and terminal electron acceptors, temperature, flow rate, and salinity, which can vary along the oil-water separation processing stream. Operational procedures (water recycling back into production lines, exposure to air, and retention time of fluids) or specific design features (closed drains and loops) may create areas with an increased risk of MIC (Shaw et al. 2016). Thus, once inoculated with biocorrosive microorganisms, the design and operation of the oil production facility itself might play a crucial role in maintaining and distributing these biocorrosive microorganisms.

We investigated the distribution of sulfate reduction activity and the diversity of microbial communities at three offshore oil-producing platforms located off the west coast of Africa. The platforms differed in their design: the two first platforms were based on a series of separation vessels with short retention times (within 10 min) while the third one involved separation tanks with much longer retention times (several hours) and recycling of large volumes at the system entrance. The objective of this study was to evaluate the impact of these different system designs on the microbial diversity and activity levels. It was of particular concern to determine the major source of sulfate-reducing microorganisms on the platforms. Two scenarios were evaluated: (1) thermophilic SRB are brought up from the high-temperature reservoirs and continually inoculate the platform or (2) mesophilic SRB originate from surface water or injected seawater but proliferate on board in stagnant sites and are distributed throughout the platform during fluid recycling. Sequencing of 16S ribosomal RNA (rRNA) gene libraries in samples taken from locations throughout the processing facilities revealed differences in community composition at the three platforms, and specific design features, e.g., vessels that collected oily fluids and held them at low temperatures, signaled sites of high SRA, demonstrating how operational features and platform design affected the microbial communities and their activities.

Experimental procedures

Collection of samples

Samples from three offshore oil-producing platforms (assets A, B, and C; Fig. 1) located on the Atlantic shelf of Africa in the Gulf of Guinea were collected September–October 2013 at various sites along the production lines. Samples for sulfate reduction assays were collected into sterile bottles and closed without a headspace. Samples for molecular analysis were filtered on site (30 to 250 mL; 0.45-μm pore size) and the filters were preserved with either ethanol (60% *v*/*v*) or DNAzol® (DN127, Molecular Research Center, Cincinnati, OH), depending on the availability of preservative at each platform. The effect of preservation method was tested at some sites by collecting duplicate samples and preserving them with either DNAzol or ethanol (Fig. S1). Samples B09 and B10 consisted of 5 g of sand collected from the sand pot of the desander and preserved with DNAzol®. Samples were received 1 to 2 weeks after collection and stored at −80 °C prior to DNA extraction. Note that not all assays were performed on every sample.

Sulfate reduction assay (SRA)

The rates of sulfate reduction in samples were measured using a radiotracer technique (Ulrich et al. 1997). In brief, samples (10 mL, in triplicate) were dispensed into sterile anoxic bottles in an anaerobic chamber and supplemented with 2 μCi $Na_2{}^{35}SO_4$ per bottle from an anoxic sterile stock solution. The $^{35}SO^{2-}{}_4$ amendments did not measurably change the original concentration of sulfate in the sample (Ulrich et al. 1997). The bottles were incubated from 2 to 5 days at temperatures close to the respective ambient temperatures and, in some instances, in parallel at a lower temperature. Following the incubation period, the pool of total reduced inorganic sulfur compounds was extracted by chromium reduction, volatilized by strong acid, and trapped in 10% Zn-acetate solution. The amount of ^{35}S in an aliquot of the trap solution was quantified by scintillation counting.

Anion analysis

Particulates, including microbial biomass, were removed from liquid samples by filtration through a polyethersulfone filter (0.22-μm pore size) prior to anion analysis. Samples were divided for chloride and sulfate analyses and serially diluted 10-fold with 18.2 MΩ H_2O. Dissolved halides were removed by precipitation using the Dionex OnGuardII Ag and Na

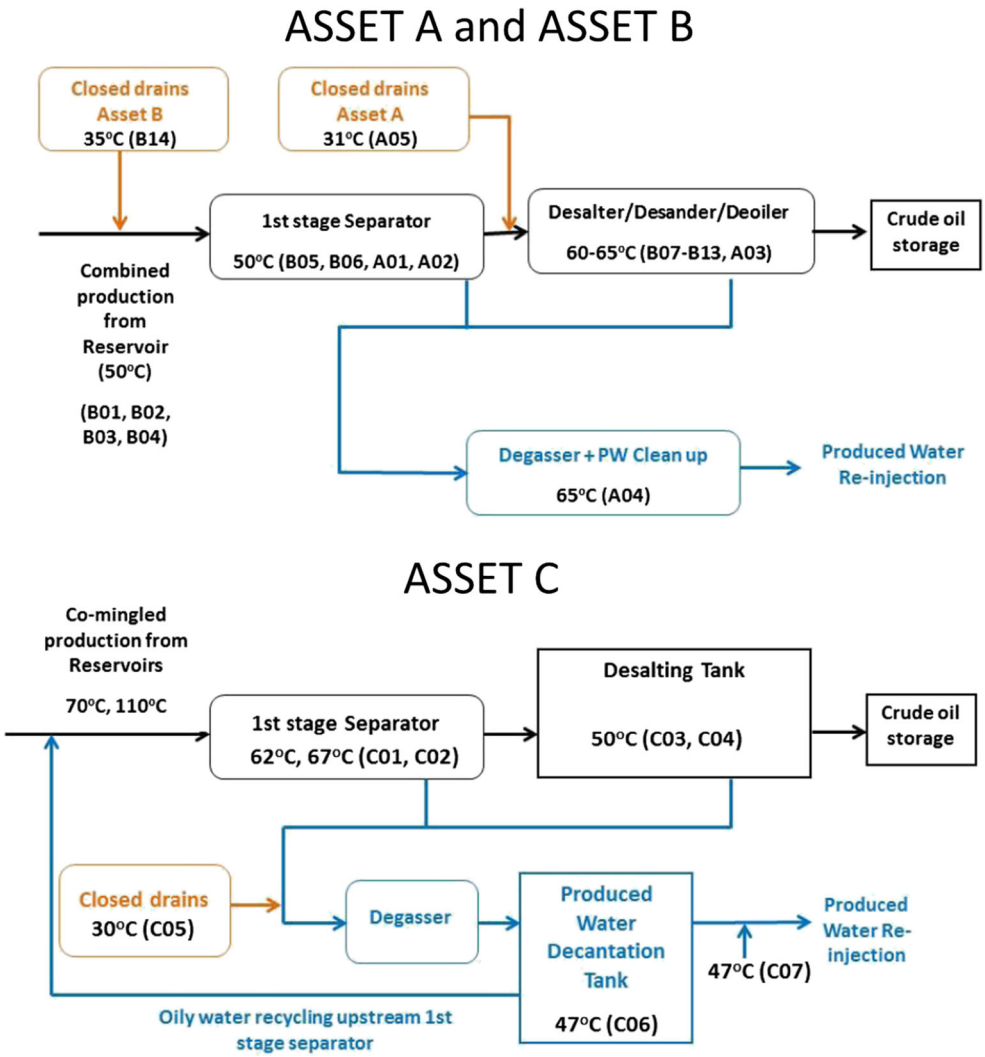

Fig. 1 Schematic diagram of operations on the oil production platforms (assets) showing the major vessels, process pathways, location, and temperature of samples taken for analyses. Not all types of analyses were performed on all samples. Diagram of assets A and B. Although fluids from the closed drains vessels are ultimately routed back to the production line either before (asset B) or after (asset A) the 1st stage separator, fluids can remain in the closed drain vessels for over a day. Diagram of asset C. The contents of the closed drain vessel flow into the degasser prior to the produced water decantation tank, which in turns recycles oily water back to the 1st stage separator

cartridges (Dionex, Thermo Scientific Inc.) assembled in series as described by the manufacturer. Chloride and sulfate concentrations were determined by suppressed-ion chromatography using a Dionex ICS-1000 equipped with AS4A-SC Guard and analytical columns (4 mm) and AERS-500 suppressor (27 mA) operated isocratically (2.0 ml min^{-1}) with a carbonate (1.8 mM)/bicarbonate (1.7 mM) buffer mobile phase. Analyte concentrations were determined relative to external standards for each sample.

DNA extraction

Filters or other solid samples, together with preservative fluids, were placed in 50-mL sterile centrifuge tubes with 0.5 to 1 mL nuclease-free water, 10 μL Proteinase K (>600 mAU/ml, Qiagen, Germantown, MD), and 10 μL 10% sodium dodecyl sulfate (Sigma-Aldrich, St. Louis, MO), vortexed briefly at maximum speed, and incubated at 50 °C for 30 min. The RNA lysis buffer and RNA dilution buffer (250 μL each) from the Maxwell®16 Tissue LEV Total RNA purification kit (Promega, Madison, WI) were added to the tube containing the filter and vortexed briefly at maximum speed. Fluid from the tube was split between two Maxwell cartridges and DNA was extracted using the Maxwell instrument programed to operate in DNA mode (Oldham et al. 2012). The two extractions were pooled, and DNA was quantified by fluorometry using the Qubit® dsDNA HS Assay (Life Technologies, ThermoFisher Scientific, Carlsbad, CA).

16S rRNA library preparation and sequencing

A 16S rRNA gene library was amplified by PCR from each DNA extraction. The primers used in the initial amplification generated amplicons that spanned the V4 region of the 16S rRNA gene between positions 519 and 802 (*E. coli* numbering), and produced a PCR amplicon of about 300 bp in length. The forward primer (M13L-519F: 5′-**GTA AAA CGA CGG CCA G**CA CMG CCG CGG TAA-3′) contains the M13 forward primer (in bold), followed by the 16S-specific sequence (underlined). The reverse primer (785R: 5′-TAC NVG GGT

ATC TAA TCC-3′) was taken directly from the primer "S-D-Bact07850b-A-18" in Klindworth et al. (2013). Each 25 μL PCR reaction consisted of 1× Taq buffer with $(NH_4)_2SO_4$ (Fermentas, Glen Burnie, Maryland), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 μM of the forward and reverse primer, 0.625 U of Taq DNA polymerase (Fermentas), and 2 μL of extracted DNA. Thermal cycling was carried out in a Techne TC-512 thermal cycler (Techne, Burlington, NJ) using the following conditions: initial denaturation for 3 min at 96 °C; 30 cycles of 30 s at 96 °C, 45 s at 52 °C and 45 s at 72 °C; and a final extension for 10 min at 72 °C. Duplicate PCR reactions for each sample were combined and purified using Ampure® XP paramagnetic beads (Beckman Coulter, USA) according to manufacturer's protocols. A second 6 cycle PCR was used to add a unique 12 bp barcode (Hamady et al. 2008) to each amplicon library using a unique forward primer containing the barcode+M13 forward sequence (5′-3′) and the 785R primer. The resulting barcoded PCR products were purified using Ampure® XP paramagnetic beads (Beckman Coulter), quantified using the Qubit® HS assay (Life Technologies, Carlsbad, CA, USA), pooled in equimolar amounts, and concentrated to a final volume of ≈80 μL using two Amicon® Ultra-0.5 mL 30 K Centrifugal Filters (Millipore). The final pooled library was then submitted to the Oklahoma Medical Research Foundation Genomics Facility (Oklahoma City, OK, USA) for sequencing on the MiSeq platform using PE250 V2 chemistry. All sequences were deposited in the short read archive under accession no. SRX1989442.

Sequence analysis

The sequence reads were merged using PEAR (Zhang et al. 2014), de-multiplexed in QIIME (Caporaso et al. 2010b), and UPARSE (Edgar 2013) was used to filter the sequences by quality and assign operational taxonomic units (OTUs) at a cutoff of 97% sequence similarity. After de-replication in UPARSE, taxonomy was assigned using the RDP Naïve Bayesian classifier (Wang et al. 2007) and the SILVA database (Release 111) (Pruesse et al. 2007). All OTUs were aligned with pyNAST (Caporaso et al. 2010a) against an aligned version of the SILVA r111 database, and filtered to remove uninformative bases. Finally, a tree was produced with FastTree (Price et al. 2010) for β-diversity analyses. The β-diversity was estimated using the weighted UniFrac index (Lozupone and Knight 2005).

Quantitative PCR

The number of bacterial and archaeal 16S rRNA gene copies was estimated using the primers 27F and 338R (bacteria) or A8F and 344R (archaea) as previously described (Stevenson et al. 2011). Estimates of the copy number of a gene coding for an enzyme essential for sulfate reduction, adenosine-5′-phosphosulfate reductase (*aprA*), were assayed with primers RH1apsF and RH2apsR (Ben-Dov et al. 2007). Thermal cycling, data acquisition, and analyses were carried out with the StepOnePlus™ Real-Time PCR System and StepOne Software v2.1 (Life Technologies, Carlsbad, CA). Cycling conditions were described in Stevenson et al. (2011) and Ben-Dov et al. (2007), respectively. For each qPCR run, a 1:10 dilution series of a control DNA plasmid containing a bacterial or archaeal 16S rRNA gene sequence or *aprA* gene sequence was used to generate a 7-point standard curve. Standards were assayed in duplicate and samples assayed in triplicate.

Results

Site description

The three oil production platforms (assets A, B, and C) were located off the western coast of Africa in the Gulf of Guinea. A schematic diagram of the production lines at assets A, B, and C is depicted in Fig. 1. Assets A and B had reservoir temperatures of 50 °C and 60 to 70 °C, respectively. The reservoir oil serviced by asset A was 30.6° API gravity while the oil produced at asset B was heavier (23.68° API gravity). Asset C serviced a light crude oil (36° API) from two different formations with estimated reservoir temperatures of 70 and 110 °C. The fluids from assets A and B at almost all sampling points were higher in salinity than seawater (60–103 g L^{-1} NaCl, Table 1). The salinity of fluids sampled from asset C was similar to seawater (21.8–32.6 g L^{-1}), except for the sample from the oil-water separator handling fluids from the 110 °C formation (141 g L^{-1}, C01, Table 1). The level of sulfate in all assets was low (20 to 192 μM, Table 1) compared to levels in seawater but sufficient to support maximum rates of sulfate reduction (Dalsgaard and Bak 1994; Ingvorsen and Jørgensen 1984).

Process description on A and B assets

The oil-water separation process follows a three-phase separation design based on a succession of vessels connected by metal tubing ("process piping"). A simplified version of the processing system is presented in Fig. 1. The sample label designations are ordered according to their relative position in the processing stream.

Pipelines and risers transport oil, gas, and water from the reservoirs to the separation vessels on the platforms. The water produced during the 1st separation phase and the subsequent desalting, desanding, and deoiling operations is collected into the degassing vessel where additional removal of oil (and other contaminants) occurs via a hydrocyclone. The average fluid retention time in all these vessels is quite low:

Table 1 Geochemical characterization of samples

Sample label	Sample origin (Asset)	Sample description	T (°C)	Salinity (g/L)	Sulfate (μM)
A01[a]	A	1st stage separator	50	NI	NI
A02	A	1st stage separator	50	NI	NI
A03	A	Desalter	60	36.9	168
A04	A	PW cleanup (hydrocyclone)	65	60.8	180
A05	A	Closed drain vessel	31	78.9	192
B01	B	Riser AR	50	96	82
B02	B	Riser AL	50	79.5	65
B03	B	Riser BL	50	103	74
B04	B	Riser BR	50	NI	NI
B05	B	1st stage separator A	50	NI	NI
B06	B	1st stage separator B	50	NI	NI
B07	B	Desalter	60	75	67
B08	B	Desalter	65	NI	NI
B09	B	Desander A	60	NI	NI
B10	B	Desander B	65	67.3	34
B11	B	Desander B	65	67.3	NI
B12	B	Deoiler A	60	62.9	71
B13	B	Deoiler B	65	60	NI
B14	B	Closed drain vessel	35	102	54
C01	C	1st stage separator	67	141	86
C02	C	1st stage separator	62	44.7	44
C03	C	Desalting tank A	50	22.66	20
C04	C	Desalting tank C	50	25.46	39
C05	C	Closed drain	30	32.6	30
C06	C	PW decantation tank	47	22.55	87
C07	C	Downstream PW Decantation tank	47	21.8	87

Salinity: g/L NaCl

NI no information, *PW* produced water

[a] Not all assays (qPCR, 16S sequencing, SRA) were performed on every sample

approximately 10 min per vessel. The separated water is re-injected into the reservoir or discharged into the sea. Crude oil obtained through the processing stream is stored in cargo tanks until it is transported.

A network of drains consisting of the closed drains network and hazardous open drains network runs throughout the platform and receives liquids occasionally drained from process vessels or spilled. The only recirculated fluids (e.g., oil and/or water) on assets A and B are the fluids collected by the drains network and gathered into the closed drains vessel. All these collected fluids can remain stagnant for several days at ambient temperature before their re-routing upstream to the oil-water separator at the locations depicted in Fig. 1. It is a common practice in the oil industry to recycle any hydrocarbon-laden fluids back into the oil/water separation process to be treated. For assets A and B, the volume of fluids recycled from the closed drains is quite low—50m^3 day^{-1} maximum returning to a vessel with a total water flow rate of up to 700 m^3 h^{-1}. In other words, at the separator inlet, the fluids coming from the closed drains account for <0.3% of the volume and these are the only fluids recirculated to the start of the separation chain. Note that the ambient temperature in the closed drains vessel (30 °C) is lower than elsewhere in the process stream (Table 1).

Process description on asset C

Asset C production facilities are quite different, with up to 20% of the separated produced water being recirculated upstream of the 1st stage separators and longer retention times throughout the process. Crude oil desalting on asset C is performed in tanks with much larger volumes than the successive process vessels used on assets A and B, to improve water settling and oil-water separation efficiency (Fig. 1). The

produced water is then routed to a large (7000 m^3) produced water decantation tank, and sent for additional purification using hydrocyclones before being re-injected into the reservoir. The average retention time in asset C desalting tanks and produced water decantation tanks can vary from 5 to 10 h which is much longer than the average 10 min retention time for assets A and B. The oily water phase of the produced water decantation tank is recycled upstream of the 1st stage separator—up to 200 m^3 h^{-1}, i.e., as much as 20% of the total inlet fluid (oil + water) at the 1st stage separator can come from the produced water decantation tank. As noted, the relatively low concentration of electron acceptor is unlikely to limit the biological sulfate reduction rate, but the amount of sulfide generated is a function of the large volume of production fluids fluxing through the system. Finally, closed drains containing the same types of oily fluids as on A and B facilities are also recirculated into the asset C decantation tank.

Corrosion evaluation

Asset A experienced no significant corrosion in the process piping connecting the processing vessels. The fluids in the processing vessels have an estimated pH value of 6.0–6.2 (pH calculations based on ISO 15156, Annex D 2009) and a low predicted CO_2 corrosiveness: 0.1 to 0.3 mm $year^{-1}$ expected if no preventive action was taken (operator in-house software calculation).

In asset B, the estimated pH values are lower (5.5–5.8) with a higher predicted corrosion rate if no mitigation is applied (high—1 to 2 mm $year^{-1}$, and 2 to 4 mm $year^{-1}$ in an erosion-corrosion scenario, NACE Standard SP0775-2013). Important internal corrosion issues were experienced on the topsides of this asset, but they occurred at areas of high fluid velocity where MIC was unlikely. The actual corrosion rates experienced were generally in the range predicted by the in-house model in the case of no mitigation. The profile and aspect of the internal pipe wall after inspection was either typical of generalized CO_2 corrosion or flow-oriented erosion-corrosion grooves (Chilingar et al. 2008). This strongly suggests that the corrosion experienced was mostly related to the high CO_2 content causing generalized corrosion and erosion-corrosion in areas of high velocity, because of a lack of mitigation efficiency.

Asset C also had a predicted corrosion rate considered high (1 to 2 mm $year^{-1}$) if no action was taken, with an even lower calculated pH (5.3) than on asset B. The experienced corrosion was higher (4 to 6 mm $year^{-1}$), but in this case, it was not considered to have been caused by a high velocity; instead, very rapid localized and "pitting type" corrosion was experienced at several locations on the process piping. Interestingly, many of these were on vertical sections, with sufficient fluid velocity predicted to prevent a significant development of a microbial biofilm (Chilingar et al. 2008). However, dissolved hydrogen sulfide was present in the produced water decantation tank in levels as high as 5 mmol L^{-1}. The origin of the H_2S and the cause of severe localized corrosion experienced on asset C are still under investigation but it is suspected that the high H_2S levels present in the decantation tank may be partly responsible for the localized corrosion experienced, by causing preferential corrosion from an active iron sulfide contaminated surface.

Numbers of bacteria, archaea, and sulfate-reducing bacteria (SRB) as estimated by qPCR

Estimated copies of bacterial 16S rRNA genes ranged from 10^4 mL^{-1} in the 1st stage separator of asset C (C01) to more than 10^9 mL^{-1} in the closed drain vessel in asset B (B14) and more than 10^9 g^{-1} sand from the asset B desander (B10, Fig. 2, Table S1). Estimated copies of *aprA*, a surrogate for SRB numbers, likewise varied widely, from less than 200 mL^{-1} in various risers and 1st stage separators to 2×10^5 mL^{-1} (B14) and 1×10^6 g^{-1} (B10) in asset B (Table S1). Members of the archaea made up a very low percentage, representing 1% or less of all samples (based on 16S rRNA gene primers specific for archaea, Table S1).

Of the three samples from asset A assayed by qPCR, the closed drain vessel (A05, ~10^8 mL^{-1}) had the highest abundance of bacterial 16S gene copies (Fig. 2, Table S1). The number of SRB, quantified as copies of the gene coding for APS (*aprA*), was estimated to range from 4.7×10^2 mL^{-1} (A04) to ~10^4 mL^{-1} (A05).

Asset B samples allowed us to track numbers throughout the process stream. Risers (B02, B03, B04), which convey fluids and gases from the reservoir, had lower estimated numbers of bacteria (<10^7 mL^{-1}) while samples taken downstream in the processing unit (desalting/desander/deoiler) generally had higher numbers of bacteria and SRB. Note that the closed drains vessel (B14), which holds collected fluids, was estimated to have high numbers of bacteria and SRB (>10^9, >10^5 mL^{-1} respectively, Fig. 2, Table S1).

Asset C also showed a general increase in the bacterial numbers and SRB along the processing stream. Similar to the results from asset A, the number of bacteria in asset C samples, as estimated by 16S rRNA gene copies, was lowest in a sample originating closest to the reservoir (C01, 1×10^4 mL^{-1}, 1st stage separator), higher in the desalting tanks (C03 4×10^6, C04 6×10^6), still higher in the closed drain vessel (C05, 5×10^7 mL^{-1}) which feeds into the produced water decantation tank by way of the degasser, and highest in the produced water decantation tank itself (C06, 5×10^8 mL^{-1}, Fig. 2, Table S1).

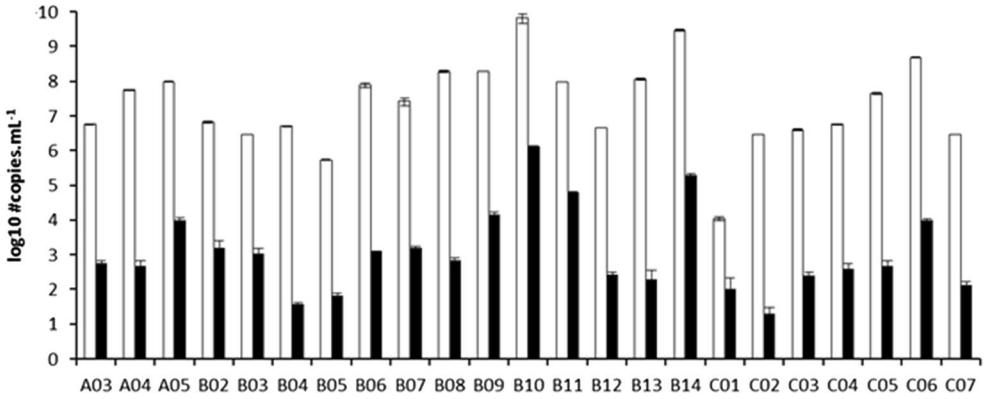

Fig. 2 Copy number of 16S rRNA genes $mL^{-1}$of sample for bacteria (*white bars*) and *aprA* genes (*black bars*) based on qPCR analysis. *Error bars* indicate standard deviation of means (n = 3). Samples A03–A05, B02–B15, and C01–C07 were collected from assets A, B, and C, respectively. A05, B14, and C05 are samples from the low-temperature closed drains vessels

Sulfate-reducing activity

The rates of sulfate reduction were determined on a variety of samples taken from the processing facilities of all three platforms and referenced to those of sterile controls (e.g., "baseline" values). Relatively high rates of sulfate reduction (e.g., 100-fold higher than baseline) were detected in the closed drain (A05) of asset A at ambient atmospheric temperature (31 °C) while samples from the desalter (A03, 60 °C) and produced water cleanup (A04, 65 °C) had sulfate reduction activity near baseline levels (Table 2).

In asset B, the rates of sulfate reduction in samples taken from three risers (B01, B02, B03) at the ambient temperature (50 °C) did not exceed that of the baseline (Table 3), except for B02, which was approximately threefold higher than the baseline. Rates near baseline were also detected in the samples from desalter A (B07) and deoiler A (B12) at 60 °C. The only high-temperature sample with higher rates (e.g., >10-fold baseline) of sulfate reduction was the sample from desander B (B10), at 65 °C. However, the desander sample consisted of a fluid and sand slurry and so was not directly comparable to the fluid samples. As with the closed drain sample from asset A, a relatively high sulfate reduction rate (>10-fold baseline) was observed at 35 °C in the asset B closed drain (B14).

The low rates of sulfate reduction in asset B risers suggested that thermophilic sulfate reducers transported with the fluids from the deep reservoir do not substantively contribute to sulfide production in this particular offshore production facility. Alternatively, heat tolerant mesophilic organisms and true mesophiles could be carried from the hot subsurface to above the ground facilities where they have a better opportunity to proliferate. To test whether mesophilic SRB were indeed present in the risers, we incubated samples from two risers at 37 °C. However, sulfate reduction activity remained low in the B01 sample and decreased to baseline levels in the B02 sample (Table 3).

The rates of sulfate reduction in asset C samples taken from high-temperature sites were generally low and often did not exceed baseline values. However, samples taken from a site with a lower temperature were definitely associated with higher sulfate reduction rates. The closed drain vessel C05 had the lowest ambient temperature (31 °C) and its rate of sulfate reduction exceeded that of the other samples incubated at ambient temperature by 20-fold. The SRA assay for the produced water decantation tank sample (C06), downstream from the closed drains vessel, was run in parallel at two different temperatures: at the ambient temperature of 47 °C and also at a lower temperature equal to the estimated atmospheric temperature of 30 °C. As summarized in Table 4, the rates of sulfate reduction for sample C06 at 30 °C were more than 50-fold higher than that measured at 47 °C incubations. This demonstrates that lower ambient temperatures markedly increase the rate of sulfate reduction from the resident microbial community in this sample. A detailed examination of the relationship between temperature and rate of sulfate reduction, however, was beyond the scope of this project.

Table 2 Sulfate reduction activity along production lines in asset A

Sample	T °C ambient	T °C incubation	SRA µmolS/L/d
A03 desalter	60	60	1.9 ± 0.8[a]
A04 PW cleanup	65	65	1.9 ± 0.26
A05 closed drain vessel	31	31	121 ± 11
Baseline[b]			1.2 ± 0.02

SRA sulfate reduction assay, *PW* produced water

[a] Average and 1 standard deviation of three replicates

[b] Baseline sulfate reduction was estimated from three sterilized samples

Table 3 Sulfate reduction activity along production lines in Asset B

Sample	T °C ambient	T °C incubation	SRA μmolS/L/dμ
B01 riser AR	50	50	0. 29 ± 0.04[a]
		37	0.29 ± 0.04
B02 riser AL	50	50	0.64 ± 0. 055
		37	0.18 ± 0.006
B03 riser BL	50	50	0.22 ± 0.04
B07 desalter A	60	60	0.186 ± 0.01
B10[b] desander B	65	65	3.9 ± 0.001
B12 deoiler A	60	60	0.23 ± 0.02
B14 closed drain vessel	35	35	3.4 ± 0.02
Baseline[c]			0.237 ± 0.08

SRA sulfate reduction assay, *PW* produced water

[a] Average and 1 standard deviation of three replicates

[b] B10 was fluid slurried with sand

[c] Baseline sulfate reduction was estimated from seven sterilized samples

Diversity of microbial communities among and within assets A, B, and C

Sequencing of 16S rRNA gene libraries was used to assess the bacterial and archaeal diversity in samples from the three offshore oil-producing platforms. The 58,276 sequences obtained from the 22 samples clustered into 420 operational taxonomic units (OTUs), which were binned at 97% similarity. The smallest library contained 167 sequences and the largest contained 9094, with a mean of 2649 and a median of 2035 (Table S2). The archaea formed a small percentage of the sequences in each asset, with no sequences being affiliated with those of the *Archaeoglobi*, the only known sulfate-reducing archaea.

Samples collected from asset A had temperatures ranging from 50 to 65 °C, with the closed drain site at 31 °C (Table 1). No significant corrosion was noted on this asset (asset operator, personal communication) and indeed it had low numbers of SRB relative to other assets based on qPCR and a low relative abundance of sequences of known sulfate reducers. Members of the *Clostridiaceae* and unclassified *Clostridiales* were the most abundant taxa in two of the sites in this asset (Fig. 3), with OTUs most closely related to members of the genus *Caminicella* as the most abundant taxon (42 and 65% relative abundance for A03 and A04, respectively, Table S3). The biocorrosion potential of *Caminicella* and other dominant taxa described below is summarized in Table S4.

The low-temperature closed drain sample (A05) had a distinctive microbial community compared to A03 and A04, with a markedly lower relative abundance of *Caminicella* (8.3%) and a higher relative abundance of the genus *Marinobacter* (*Alteromonadales*, 22.6%, other sites <2%) and of the genus *Arcobacter* (*Campylobacterales*, 8.3%, Hubert et al. 2012; Roalkvam et al. 2015).

Temperature from asset B (Table 1) ranged from 50 °C for samples taken closest to the formation and from the 1st stage separator, 60–65 °C from the desalting/deoiling vessels later in the processing stream, and 35 °C at the closed drain vessel. The overall density of microorganisms in some samples from asset B was higher relative to the other assets (Fig. 2, Table S1). Although *Clostridiales* formed a large proportion of the asset B samples (Fig. 3), asset B sites contained a somewhat lower relative abundance of *Caminicella* (highest was 25%, B06, Table S3) than asset A samples A03 and A04. However, three asset B samples contained OTUs most closely related to the genus *Garciella* (Order *Clostridiales*) at >10% (B02, B06, B07). Other taxa at greater than 10% relative abundance included OTUs most closely related to members of the genera *Thermoanaerobacter* (*Thermoanaerobacterales*, Roh et al. 2002; B07, B08, B12, B13—desalter and deoiler), *Pelobacter* (Lovley et al. 1995; sulfur/iron-reducer, *Desulfuromonadales*, B05, B06, B07, B08, B09, B13, B14—from the 1st stage separator on throughout the system), *Arcobacter* (B09, B10, B11,

Table 4 Sulfate reduction activity in asset C

Sample	T °C ambient	T °C incubation	SRA μmolS/L/d
C01 1st stage separator	67	65	0.3 ± 0. 03[a]
C02 1st stage separator	62	65	0.145 ± 0.015
C03 desalting tank A	50	50	0.675 ± 0.01
C04 desalting tank C	50	50	0.455 ± 0.01
C05 closed drain	30	31	15 ± 0.37
C06 PW decantation tank	47	47	0.36 ± 0. 02
		30	28 ± 3
C07 downstream PW decantation tank	47	47	0.34 ± 0.03
Baseline[b]			0.15 ± 0.06

SRA sulfate reduction assay, *PW* produced water

[a] Average and 1 standard deviation of three replicates

[b] Baseline sulfate reduction was estimated from seven sterilized samples

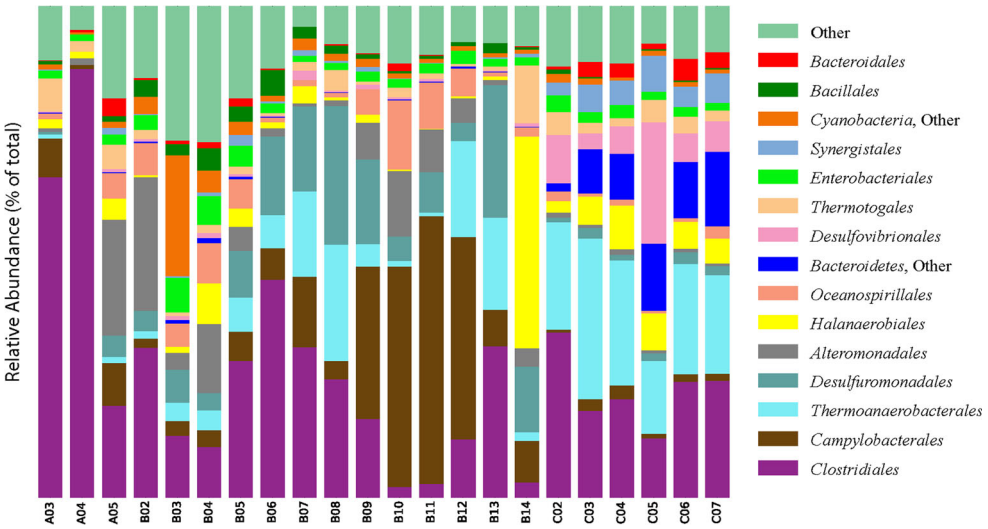

Fig. 3 Comparison of microbial communities based on 16S rRNA gene sequences, showing the relative abundance (%) of different bacterial orders. A05, B14, and C05 are samples from the low-temperature closed drains vessels. Samples C02–C07 have salinity near that of seawater, and all other samples have much higher salinity (Table 1)

B12, all >24%), *Sulfurospirillum* (Kodama et al. 2007), and *Marinobacter* (AB02, B04, B10, B11). The closed drain vessel sample B14 was noteworthy for a high relative abundance of members of the genus *Halanaerobium* (43%, *Halanaerobiales*, Ravot et al. 1997; Cluff et al. 2014). Sample B14 also contained the highest relative abundance of members of the genus *Geotoga* (11%, *Thermotogales*) in the set of samples.

Asset C (Fig. 1b, Table 1), in common with the other two assets, contained samples with temperatures ranging from 47 °C to 62 °C with the exception of the closed drains vessel sample (30 °C, C05). However, the salinity of most asset C samples was similar to seawater, lower than that of the other assets. Members of the *Clostridiales*, *Thermoanaerobacterales*, and *Desulfovibrionales* were the most numerically dominant families found in asset C, but the genera prevalent in asset C differed from the dominant genera in assets A and B. The most abundant *Clostridiales* in asset C samples were *Desulfotomaculum* (sulfate-reducing, Birkeland 2005; >7% in all C samples except C05) and unclassified *Clostridiales* while members of the genus *Caminicella* composed only 1% of the community. Members of the genus *Halocella* (Simankova et al. 1993) were the dominant members of the *Halanaerobiales*, comprising 5 to 8% of the community in all samples from asset C except C02 (1st stage separator, Table S3). Among the *Thermoanaerobacterales* in asset C were sequences

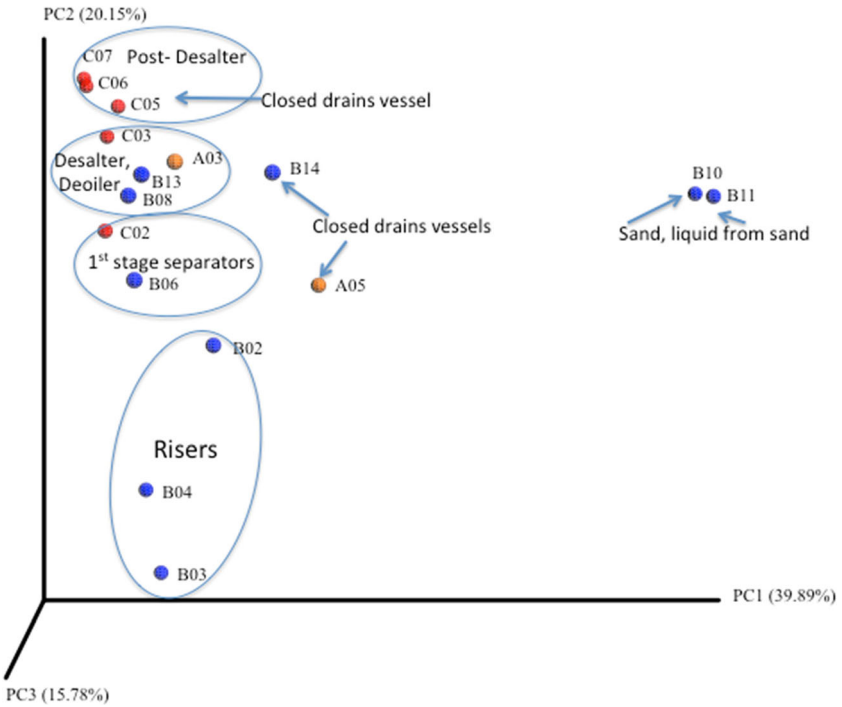

Fig. 4 Principal coordinate analysis of weighted UniFrac values between asset A (*yellow*), asset B (*blue*), and asset C (*red*) based on 16S rRNA gene sequence datasets of samples containing 1000 or more sequences. The axes are scaled by the percentage of the variance they explain. Samples associated with solid materials (B10, B11) group together as do samples taken from a particular portion of the separation system

affiliated with *Thermoanaerobacter* and also those similar to *Thermacetogenium* (Table S3). Sequences affiliated with the genus *Desulfonauticus* (sulfate-reducing, *Desulfovibrionales*) reached highest relative abundance (26%) in the closed drain sample (C05) and were found at >3% relative abundance in all asset C samples but at <1% in samples from assets A and B.

Comparisons of the community structure for the libraries comprising 1000 or more sequences from each asset show a separation between samples associated with solid surfaces (e.g., sand, B10, B11) versus fluid samples (Fig. 4). Fluid samples themselves from the same asset appear to cluster according to their position in the processing platform. Processing alters the fluid chemistry of the production water stream by incrementally removing oil, gas, and diluting out salt. The closed drains samples from assets A and B, which are low temperature (31 °C, 35 °C) but with salinity greater than that of seawater, cluster separately from the other samples and from the closed drains of asset C (C05), which has a salinity close to that of seawater.

Discussion

Many of the microorganisms in high relative abundance on each asset are associated with oil reservoirs, oil production systems, and/or implicated in cases of MIC (Duncan et al. 2009; Grabowski et al. 2005; Head et al. 2003; Lenhart et al. 2014; Magot et al. 2000; Miranda-Tello et al. 2003; Ravot et al. 1997, summarized in Table S4). This is not surprising since offshore oil platforms provide habitats conducive to the proliferation of certain microorganisms (Shaw et al. 2016). Sulfate and iron are potential major terminal electron acceptors, as well as oxygen if there are sites with leaks from the atmosphere, while heterotrophic fermentative microorganisms are typical members of anaerobic communities associated with the production of petroleum (Magot et al. 2000; Ollivier and Cayol 2005). Sources of carbon and nutrients include the petroleum hydrocarbons as well as a wide variety of chemicals used in oil production (corrosion inhibitors, scale inhibitors, emulsion breakers, etc., Videla et al. 2000; Duncan et al. 2014) and organic material from injected seawater. Despite the availability of petroleum hydrocarbons as an electron donor few typical groups of anaerobic hydrocarbon degraders were found (Rabus et al. 2016). Rather, facultatively anaerobic members of the *Marinobacter* and *Marinobacterium* genera (*Gammaproteobacteria*) that are capable of aerobic hydrocarbon degradation (Gauthier et al. 1992; Grimaud 2010; Yakimov et al. 2005) were found in abundance in assets A and B (Table S4). *Marinobacter* and *Marinobacterium* were absent from asset C; instead, there is a high relative abundance of OTUs most closely related to WCHB1-69 (*Bacteroidetes; Sphingobacterales*) and a lower abundance of other *Bacteroidetes* (Table S3). WCHB1-69 has been found in aquifers contaminated with hydrocarbons and solvents (Dojka et al. 1998) and also associated with the degradation of algae under anaerobic conditions (Morrison et al. 2017). Marine *Bacteroidetes* degrade biopolymers such as proteins and chitins, part of the dissolved organic matter in oceans (Bauer et al. 2006). The design of asset C promotes greater efficiency of oil removal, which may in turn have given an advantage to biopolymer-degrading *Bacteroidetes* over hydrocarbon-degrading *Gammaproteobacteria*.

A variety of types of microorganisms have been implicated in contributing to MIC in the oil and gas industry: acetogens, fermentative, thiosulfate-reducing, sulfate-reducing, iron-reducing, sulfide-producing archaea, and methanogens, among others (Liang et al. 2014; Skovhus et al. 2017). In addition, certain lithotrophic sulfate-reducing bacteria are able to perform "electrical microbially influenced corrosion" (EMIC) by accepting electron directly from iron (Enning et al. 2012). As shown in Table S4, a variety of microorganisms with the potential to enhance corrosion were found in all three assets. Asset C, however, is noteworthy for having both sites of pitting corrosion and high levels of hydrogen sulfide. The latter suggests we should focus on the sulfide production potential of each asset's microbial communities. Based on both qPCR and 16S RNA gene sequencing, assets A and B have few OTUs identified as those of sulfate-reducing microorganisms (all samples less than 1% relative abundance, Tables S3 and S4). These assets instead are characterized by a high relative abundance of fermentative microorganisms capable of sulfide production from sulfur, thiosulfate, or organic-sulfur compounds such as cystine (Tables S3 and S4). Over 40% of the sequences from the closed drains vessel in asset B (B14) were classified as members of the genus *Halanaerobium*. Some species of *Halanaerobium* can produce hydrogen sulfide from thiosulfate (Zeikus et al. 1983; Cayol et al. 1994; Ravot et al. 1997, 2005), but none can reduce sulfate. Assets A and B were also noteworthy because they contained a high relative abundance of sequences most closely related to those of *Caminicella*, a thermophile which can produce hydrogen sulfide in the presence of elemental sulfur, cystine, or thiosulfate (Alain et al. 2002). All these organisms can also grow fermentatively using acetate and other volatile fatty acids. Aside from the closed drains on asset A (and B to a lesser extent), there was no observation of hydrogen sulfide in any other vessel from assets A or B (operator personal communication). The asset A closed drains showed a very high SRA (Table 2) but the sulfide generated is most likely not detected anywhere else in the asset because the closed drains fluids are significantly diluted as they get recycled.

In asset C, sulfate-reducing bacteria (e.g., members of the genera *Desulfonauticus*, *Desulfotomaculum*, and *Thermoacetogenium)* constituted a substantial proportion of the microbial community. *Desulfotomaculum* was at a relative abundance between 7 and 11% for four of the 5 asset C samples and *Thermoacetogenium* at >5% for all asset C samples while *Desulfonauticus* was at 26% in the closed drains (C05).

The type strains of the two described species of the genus *Desulfonauticus* (*D. submarinus* and *D. autotrophicus*, *Desulfovibrionales*) are moderately thermophilic sulfate reducers isolated from oil-production water and marine hydrothermal vents (Audiffrin et al. 2003; Mayilraj et al. 2009). They do not form spores and cannot grow under conditions of high salinity, such as found in many samples from assets A and B (Table 1). Likewise, *Thermoacetogenium phaeum* is not highly halotolerant, with an upper limit of growth at 4.5% NaCl (Hattori et al. 2000). Thus, the salinity levels in assets A and B may promote highly halotolerant groups such as members of the genus *Halanaerobium* while excluding *Desulfonauticus* and *Thermoacetogenium*.

The estimated number of SRB in asset C as determined by qPCR was much lower than would be predicted from the relative abundance of *Desulfonauticus* in16S RNA gene libraries multiplied by the estimated number of bacteria in asset C samples. However, commonly used primers for *dsrAB* (as noted by Vigneron et al. 2016) and *aprA* (this study) contain multiple mismatches to the corresponding sequences of *D. submarinus* and *D. autotrophicus*, leading to underestimation of their abundance by qPCR. Recalculating the estimated number of SRB by multiplying the total number of bacteria (from qPCR) by the relative abundance of *Desulfonauticus* (from 16S data) gives an estimated number of SRB in the closed drains of asset C (C05) of 10^7 mL^{-1}, far higher than the SRB estimate for asset A closed drains (A05, 10^4 mL^{-1}) or asset B closed drains (B14, 2×10^5 mL^{-1}).

The high numbers of SRBs in the asset C closed drains vessel suggest that SRBs proliferate on board the platform in stagnant sites and are then distributed through recycling fluids. However, an alternative hypothesis for the origin of SRBs is that thermophilic SRB are brought up from the high-temperature reservoirs and continually inoculate the platform. Parallel incubations for SRA at different temperatures were employed to aid in diagnosing the origin of sulfate-reducing microorganisms. Samples from the asset B risers (our closest point of sampling to the reservoir) incubated in parallel at the ambient temperature of 50 °C and at atmospheric 30 °C had similarly low SRA values, which argues that neither mesophilic nor moderately thermophilic bacteria from the asset B reservoir were contributing substantially to sulfate reduction activity on this platform. The temperature of asset C reservoirs (110 and 70 °C) makes the reservoir fluids unlikely to be a major continuing source of *Desulfonauticus* at least since the highest temperature that permits growth for the two described species is around 60 °C and neither species forms spores (Audiffrin et al. 2003; Mayilraj et al. 2009). Therefore, we suggest that proliferation of *Desulfonauticus* occurs on the platform, especially within the closed drains vessel, where it reaches a relative abundance of 26%.

In summary, on assets A and B, stagnation occurs for several days in mesophilic conditions that can be favorable for bacterial proliferation. However, the stagnating volumes are small and the fraction of fluids recirculated is low. On asset C, however, the fraction of fluids recirculated is much higher overall: any microbe entering the separation process from the reservoir or from the closed drains could in fact remain in circulation for a significant amount of time. Because of this, the asset C design is considered as inducing a higher risk of microbial proliferation compared to A and B. In addition, our study found that SRB genera *Desulfonauticus* and *Thermoacetagenium* were present in high numbers in the asset C closed drains vessel, and there was active reduction of sulfate to sulfide at that site (Table 4), thus confirming that asset C was at increased risk for MIC.

This study illustrates that the design of oil processing facilities in addition to operational practices such as recycling fluids from closed drains, large volume processing tanks with long retention times, differences in temperature, and/or salinity along production lines, all shape microbial communities. Biocorrosive microorganisms most likely proliferate in "low" temperature sites (e.g., <37 °C) experiencing long retention times, as evidenced by the high numbers of bacteria estimated by qPCR in closed drains samples. In another study of an offshore processing system, closed drains vessels were shown to contain the greatest numbers of SRBs, as demonstrated using single serial dilutions, ATP and qPCR (Shaw et al. 2016). Design features that prevent the distribution of microorganisms from similar vessels could help to mitigate MIC. In summary, knowledge of how the design of oil separation processes affect microbial microenvironments can be used to guide operational practices and to target problem sites to help control MIC.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no competing interests.

Ethical approval This research does not contain any studies with human participants or animals.

References

Alain K, Pignet P, Zbinden M, Quillevere M, Duchiron F, Donval JP, Lesongeur F, Raguenes G, Crassous P, Querellou J, Cambon-Bonavita MA (2002) *Caminicella sporogenes* gen. nov., sp. nov., a novel thermophilic spore-forming bacterium isolated from an East-Pacific Rise hydrothermal vent. Int J Syst Evol Microbiol 52:1621–1628. doi:10.1099/00207713-52-5-1621

Audiffrin C, Cayol JL, Joulian C, Casalot L, Thomas P, Garcia JL, Ollivier B (2003) *Desulfonauticus submarinus* gen. nov., sp. nov., a novel sulfate-reducing bacterium isolated from a deepsea

hydrothermal vent. Int J Syst Evol Microbiol 53:1585–1590. doi:10.1099/ijs.0.02551-0

Bauer M, Kube M, Teeling H, Richter M, Lombardot T, Allers E, Würdemann CA, Quast C, Kuhl H, Knaust F, Woebken D, Bischof K, Mussmann M, Choudhuri JV, Meyer F, Reinhardt R, Amann RI, Glöckner FO (2006) Whole genome analysis of the marine Bacteroidetes '*Gramella forsetii*' reveals adaptations to degradation of polymeric organic matter. Environ Microbiol 8:2201–2213. doi:10.1111/j.1462-2920.2006.01152.x

Ben-Dov E, Brenner A, Kushmaro A (2007) Quantification of sulfate-reducing bacteria in industrial wastewater, by real-time polymerase chain reaction (PCR) using *dsrA* and *apsA* genes. Microb Ecol 54: 439–451. doi:10.1007/s00248-007-9233-2

Birkeland NK (2004) The microbial diversity of deep subsurface oil reservoirs. In: R. Vazquez-Duhalt R, Quintero-Ramirez R (eds) Studies in surface science and catalysis. Elsevier, Vol. 151 pp 385–403 doi:10.1016/S0167-2991(04)80155-1

Birkeland N-K (2005) Sulfate-reducing bacteria and archaea. In: Ollivier B, Magot M (eds) Petroleum microbiology. ASM Press, Washington, D.C., pp 35–54

Bradley GJ, McGinley HR, Hermsen NL (2011) A global perspective on biocides regulatory issues. OTC 21806. Offshore Tech Conference, Houston, TX, USA, 2–5 May 2011

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R (2010a) PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26:266–267. doi:10.1093/bioinformatics/btp636

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsuenenko T, Zaneveld J, Knight R (2010b) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. doi:10.1038/nmeth.f.303

Cayol J-L, Ollivier B, Lawson Anani Soh A, Fardeau M-L, Ageron E, Grimont PAD, Prensier G, Guezennec J, Magot M, Garcia J-L (1994) *Haloincola saccharolytica* subsp. *senegalensis* subsp. nov., isolated from the sediments of a hypersaline lake, and emended description of *Haloincola saccharolytica*. Int J Syst Bacteriol 44: 805–811

Chilingar, George V. Mourhatch, Ryan Al-Qahtani, Ghazi D (2008) Fundamentals of corrosion and scaling—for petroleum and environmental engineers. Gulf Publishing Company. Online version available at:http://app.knovel.com/hotlink/toc/id:kpFCSFPEE3/fundamentals-corrosion/fundamentals-corrosion

Cluff MA, Hartsock A, MacRae JD, Carter K, Mouser PJ (2014) Temporal changes in microbial ecology and geochemistry in produced water from hydraulically fractured Marcellus shale gas wells. Environ Sci Technol 48:6508–6517. doi:10.1021/es501173p

Dalsgaard T, Bak F (1994) Nitrate reduction in a sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, isolated from rice paddy soil: sulfide inhibition, kinetics, and regulation. Appl Environ Microbiol 60:291–297

Dojka MA, Hugenholtz P, Haack SK, Pace NR (1998) Microbial diversity in a hydrocarbon- and chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation. Appl Environ Microbiol 64: 3869–3877

Duncan KE, Gieg LM, Parisi VA, Tanner RS, Tringe SG, Bristow J, Suflita JM (2009) Biocorrosive thermophilic microbial communities in Alaskan North Slope oil facilities. Environ Sci Technol 43:7977–7984. doi:10.1021/es9013932

Duncan KE, Perez-Ibarra BM, Jenneman G, Busch Harris J, Webb R, Sublette K (2014) The effect of corrosion inhibitors on microbial communities associated with corrosion in a model flow cell system. Appl Microbiol Biotech 98:907–918. doi:10.1007/s00253-013-4906-x

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996–998. doi:10.1038/nmeth.2604

Enning D, Venzlaff H, Garrelfs J, Dinh HT, Meyer V, Mayrhofer K, Hassel AW, Stratmann M, Widdel F (2012) Marine sulfate-reducing bacteria cause serious corrosion of iron under electroconductive biogenic mineral crust. Environ Microbiol 14: 1772–1787. doi:10.1111/j.1462-2920.2012.02778.x

Ferris FG, Jack TR, Bramhill BJ (1992) Corrosion products associated with attached bacteria at an oil field water injection plant. Can J Microbiol 38:1320–1324

Gauthier MJ, Lafay B, Christen R, Fernandez L, Acquaviva M, Bonin P, Bertrand J-C (1992) *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. Int J Syst Bacteriol 42:568–576

Gittel A, Sørensen KB, Skovhus TL, Ingvorsen K, Schramm A (2009) Prokaryotic community structure and sulfate reducer activity in water from high-temperature oil reservoirs with and without nitrate treatment. Appl Environ Microbiol 75:7086–7096. doi:10.1128/AEM.01123-09

Grabowski A, Nercessian O, Fayolle F, Blanchet D, Jeanthon C (2005) Microbial diversity in production waters of a low-temperature biodegraded oil reservoir. FEMS Microbiol Ecol 54:427–443

Grimaud R (2010) Chapt. 34. Marinobacter. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer-Verlag, Berlin, pp 1290–1295. doi:10.1007/978-3-540-77587-4_90

Guan J, Zhang BL, Mbadinga SM, Liu JF, Gu JD, Mu BZ (2014) Functional genes (dsr) approach reveals similar sulphidogenic prokaryotes diversity but different structure in saline waters from corroding high temperature petroleum reservoirs. Appl Microbiol Biotechnol 98:1871–1882. doi:10.1007/s00253-013-5152-y

Hamady M, Walker JJ, Harris JK, Gold NJ, Knight R (2008) Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. Nat Methods 5:235–237. doi:10.1038/nmeth.1184

Hattori S, Kamagata Y, Hanada S, Shoun H (2000) *Thermacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. Int J Syst Evol Microbiol 50(4):1601–1609

Head IM, Jones DM, Larter SR (2003) Biological activity in the deep subsurface and the origin of heavy oil. Nature 426:344–352

Hubert CR, Oldenburg TB, Fustic M, Gray ND, Larter SR, Penn K, Rowan AK, Seshadri R, Sherry A, Swainsbury R, Voordouw G, Voordouw JK, Head IM (2012) Massive dominance of *Epsilonproteobacteria* in formation waters from a Canadian oil sands reservoir containing severely biodegraded oil. Environ Microbiol 14:387–404. doi:10.1111/j.1462-2920.2011.02521.x

Ingvorsen K, Jørgensen BB (1984) Kinetics of sulfate uptake by freshwater and marine species of *Desulfovibrio*. Arch Microbiol 139:61–66

ISO 15156-2:2009(en) (2009) Petroleum and natural gas industries—materials for use in H_2S-containing environments in oil and gas production—Part 2: cracking-resistant carbon and low-alloy steels, and the use of cast irons. Annex D: Recommendations for determining pH. pp. 37–41. International Organization for Standardization. https://www.iso.org/obp/ui/#iso:std:iso:15156:-2:ed-2:v1:en Accessed June 7, 2016

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids res 41:e1. doi:10.1093/nar/gks808

Kodama Y, Ha LT, Watanabe K (2007) *Sulfurospirillum cavolei* sp. nov., a facultatively anaerobic sulfur-reducing bacterium isolated from an underground crude oil storage cavity. Int J Syst Evol Microbiol 57: 827–831. doi:10.1099/ijs.0.64823-0

L'Haridon S, Reysenbach A-L, Glenat P, Prieur D, Jeanthon C (1995) Hot subterranean biosphere in a continental oil reservoir. Nature 377: 223–224

Lenhart TR, Duncan KE, Beech IB, Sunner JA, Smith W, Bonifay V, Biri B, Suflita JM (2014) Identification and characterization of microbial biofilm communities associated with corroded oil pipeline surfaces. Biofouling 30:823–835. doi:10.1080/08927014.2014.931379

Liang R, Grizzle RS, Duncan KE, McInerney MJ, Suflita JM (2014) Roles of thermophilic thiosulfate-reducing bacteria and methanogenic archaea in the biocorrosion of oil pipelines. Frontiers in Microbiology: Microbial Physiology and Metabolism 5:89. doi:10.3389/fmicb.2014.00089

Lovley DR, Phillips EJ, Lonergan DJ, Widman PK (1995) Fe(III) and S^0 reduction by *Pelobacter carbinolicus*. Appl Environ Microbiol 61: 2132–2138

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71: 8228–8235. doi:10.1128/AEM.71.12.8228-8235.2005

Magot M, Ollivier B, Patel BK (2000) Microbiology of petroleum reservoirs. Antonie van Leeuwenhoek 77:103–116

Mayilraj S, Kaksonen AH, Cord Ruwisch R, Schumann P, Spröer P, Tindall BJ, Spring S (2009) *Desulfonauticus autotrophicus* sp. nov., a novel thermophilic sulfate-reducing bacterium isolated from oil-production water and emended description of the genus *Desulfonauticus*. Extremophiles 2:247–255. doi:10.1007/s00792

Miranda-Tello E, Fardeau ML, Sepúlveda J, Fernández L, Cayol JL, Thomas P, Ollivier B (2003) *Garciella nitratireducens* gen. nov., sp. nov., an anaerobic, thermophilic, nitrate- and thiosulfate-reducing bacterium isolated from an oilfield separator in the Gulf of Mexico. Int J Syst Evol Microbiol 53:1509–1514. doi:10.1099/ijs.0.02662-0008-0212-4

Morrison JM, Murphy CL, Baker K, Zamor RM, Nikolai SJ, Wilder S, Elshahed MS, Youssef NH (2017) Microbial communities mediating algal detritus turnover under anaerobic conditions. Peer J 5: e2803. doi:10.7717/peerj.2803

NACE Standard SP0775-(2013) (formerly RP0775). Preparation, installation, analysis, and interpretation of corrosion coupons in oil field operations; NACE International: Houston, TX, 2005; Item No. 21017

Nazina TN, Grigor'ian AA, Shestakova NM, Babich TL, Ivoĭlov VS, Feng Q, Ni F, Wang J, She Y, Xiang T, Luo Z, Beliaev SS, Ivanov MV (2007) Microbiological investigations of high-temperature horizons of the Kongdian petroleum reservoir in connection with field trial of a biotechnology for enhancement of oil recovery. Mikrobiologiia 76:329–339

Oldham AL, Drilling HS, Stamps BW, Stevenson BS, Duncan KE (2012) Automated DNA extraction platforms offer solutions to challenges of assessing microbial biofouling in oil production facilities. AMB Express 2:60. doi:10.1186/2191-0855-2-60

Ollivier B, Cayol J-L (2005) The fermentative, iron-reducing, and nitrate-reducing microorganisms. In: Ollivier B, Magot M (eds) Petroleum microbiology. ASM Press, Washington, D.C., pp 71–88

Price MN, Dehal PS, Arkin AP (2010) FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One 5: e9490. doi:10.1371/journal.pone.0009490

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glöckner FO (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 35:7188–7196. doi:10.1093/nar/gkm864

Rabus R, Boll M, Heider J, Meckenstock RU, Buckel W, Einsle O, Ermler U, Golding BT, Gunsalus RP, Kroneck PM, Krüger M, Lueders T, Martins BM, Musat F, Richnow HH, Schink B, Seifert J, Szaleniec M, Treude T, Ullmann GM, Vogt C, von Bergen M, Wilkes H (2016) Anaerobic microbial degradation of hydrocarbons: from enzymatic reactions to the environment. J Mol Microbiol Biotechnol 26:5–28. doi:10.1159/000443997

Ravot G, Magot M, Ollivier B, Patel BKC, Ageron E, Grimont PAD, Thomas P, Garcia J-L (1997) *Haloanaerobium congolense* sp. nov., an anaerobic, moderately halophilic, thiosulfate-reducing bacterium from an African oilfield. FEMS Microbiol Lett 147:81–88. doi:10.1111/j.1574-6968.1997.tb10224.x

Ravot G, Casalot L, Ollivier B, Loison G, Magot M (2005) rdlA, a new gene encoding a rhodanese-like protein in *Halanaerobium congolense* and other thiosulfate-reducing anaerobes. Res Microbiol 156:1031–1038. doi:10.1016/j.resmic.2005.05.009

Roalkvam I, Drønen K, Stokke R, Daae FL, Dahle H, Steen IH (2015) Physiological and genomic characterization of *Arcobacter anaerophilus* IR-1 reveals new metabolic features in Epsilonproteobacteria. Front Microbiol 6:987. doi:10.3389/fmicb.2015.00987

Roh Y, Liu SV, Li G, Huang H, Phelps TJ, Zhou J (2002) Isolation and characterization of metal-reducing *Thermoanaerobacter* strains from deep subsurface environments of the Piceance Basin, Colorado. Appl Environ Microbiol 268:6013–6020. doi:10.1128/AEM.68.12.6013-6020.2002

Shaw MP, Hoffmann H, Home M (2016) Case study: comparison of microbial monitoring techniques used in the field and how their complementarity can be harnessed to build a full picture of the microbial life in the field. In: SPE International Oilfield Corrosion Conference and Exhibition, Aberdeen, Scotland, UK, 9–10 May, SPE-179936-MS

Simankova MV, Chernych NA, Zavarzin GA (1993) *Halocella cellulolytica* gen. nov., sp. nov., a new obligately anaerobic, halophilic, cellulolytic bacterium. Syst Appl Microbiol 16:385–389. doi:10.1016/S0723-2020(11)80270-5

Skovhus TL, Eckert RB (2014) Practical aspects of MIC detection, monitoring and management in the oil and gas industry. Paper #3920, Corrosion 2014, San Antonio TX, USA, March 9–13, 2014

Skovhus TL, Lee JS, Little BJ (2017) Predominant MIC mechanisms in the oil and gas industry. Chapt. 4, pp. 75–86 In Skovhus TL, Enning E, and Lee JS (eds.) Microbiologically Influenced Corrosion in the Upstream Oil and Gas Industry. Routledge. doi:10.1201/9781315157818-5

Stevenson BS, Drilling HS, Lawson PA, Duncan KE, Parisi VA, Suflita JM (2011) Microbial communities in bulk fluids and biofilms of an oil facility have similar composition but different structure. Environ Microbiol 13:1078–1090. doi:10.1111/j.1462-2920.2010.02413.x

Ulrich GA, Krumholz LR, Suflita JM (1997) A rapid and simple method for estimating sulfate reduction activity and quantifying inorganic sulfides. Appl Environ Microbiol 63:1627–1630

Videla HA, Guiawet PS, Saravia SG, Allegreti P, Furlong J (2000) Microbial degradation of film forming inhibitors and its possible effects on corrosion inhibition performance. In: NACE Corrosion 2000 (Paper no. 00386), Houston, TX, NACE International, 2000

Vigneron A, Alsop EB, Chambers B, Lomans BP, Head IM, Tsesmetzis N (2016) Complementary microorganisms in highly corrosive biofilms from an offshore oil production facility. Appl Environ Microbiol 82: 2545–2554. doi:10.1128/AEM.03842-15

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267. doi:10.1128/AEM.00062-07

Yakimov MM, Denaro R, Genovese M, Cappello S, D'Auria G, Chernikova TN, Timmis KN, Golyshin PN, Giluliano L (2005) Natural microbial diversity in superficial sediments of Milazzo Harbor (Sicily) and community successions during microcosm enrichment with various hydrocarbons. Environ Microbiol 7(9):1426–1441

Youssef N, Elshahed MS, McInerney MJ (2009) Microbial processes in oil fields: culprits, problems, and opportunities. In: Allen I, Laskin SS, Geoffrey MG (eds) Adv Appl Microbiol, vol 66. Academic Press, Burlington, pp 141–251

Zeikus JG, Hegge PW, Thompson TE, Phelps TJ (1983) Isolation and description of *Haloanaerobium praevalens* gen. nov. and sp. nov., an obligately anaerobic halophile common to Great Salt Lake sediments. Curr Microbiol 9:225–234. doi:10.1007/BF01567586

Zhang J, Kobert K, Flouri T, Stamatakis A (2014) PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30:1–7. doi:10.1093/bioinformatics/btt593