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Use of carbon steel ball bearings to determine the effect of biocides and corrosion inhibitors on microbiologically influenced corrosion under flow conditions

Tijan Pinnock[1] · Johanna Voordouw[1] · Gerrit Voordouw[1]

Abstract

A consortium of sulfate-reducing bacteria consisting mostly of *Desulfovibrio*, *Desulfomicrobium*, and *Desulfocurvus* from oil field produced water was cultivated in a chemostat, receiving medium with 20 mM formate and 10 mM sulfate as the energy and 1 mM acetate as the carbon source. The chemostat effluent, containing 5 mM sulfide and 0.5 mM of residual acetate, was passed through 1-ml syringe columns with 60 carbon steel ball bearings (BBs) of 53.6 ± 0.1 mg each at a flow rate of 0.8 ml/h per column. These were treated every 5 days with 1.6 ml of 300 ppm of glutaraldehyde (Glut), tetrakis(hydroxymethyl)phosphonium sulfate (THPS), benzalkonium chloride (BAC), or Glut/BAC, a mixture of Glut and BAC. Alternatively, BBs were treated with 33% (v/v) of a water-soluble (CR_W) or an oil-soluble (CR_O1 or CR_O3) corrosion inhibitor for 20 s after which the corrosion inhibitor was drained off and BBs were packed into columns. The effluent of untreated control columns had no acetate. Treatment with the chemically reactive biocides Glut and THPS, as well as with Glut/BAC, gave a transient increase of acetate indicating decreased microbial activity. This was not seen with BAC alone indicating it to be the least effective biocide. Relative to untreated BBs (100%), those treated periodically with Glut, THPS, BAC, or Glut/BAC had a general weight loss corrosion rate of 91, 81, 45, and 36% of the untreated rate of 0.104 ± 0.004 mm/year, respectively. Single treatment with corrosion inhibitors decreased corrosion to 48, 2, and 1% of the untreated rate for CR_W, CR_O1 and CR_O3, respectively. Analysis of the distribution of corrosion rates from the weight loss of individual BBs ($N = 120$) indicated the presence of a more slowly and a more rapidly corroding group. BAC treatment prevented emergence of the latter, and this quaternary ammonium detergent appeared most effective in decreasing corrosion not because of its biocidal properties, but because of its corrosion inhibitory properties.

Keywords Corrosion rate · Sulfate-reducing bacteria · Carbon steel · Flow · Microbial community · Biocide · Oil field

Introduction

Corrosion is a serious and expensive problem to which microorganisms contribute through microbiologically influenced corrosion (MIC). It affects both flowing and non-flowing systems, like pipelines and oil storage tanks, respectively, and is often caused by sulfate-reducing bacteria (SRB), although other bacteria have also been shown to contribute (Cord-Ruwisch and Widdel 1986; Jack 2002; Alberta Energy Regulator 2013). The study of MIC in non-flowing, stagnant systems can be done in serum bottles in which carbon steel coupons are incubated with a field sample or a microbial culture in medium for a defined length of time (typically 1 month). The general corrosion rate is then determined by weight loss of the coupons, and pitting corrosion is evaluated by surface examination (Beech et al. 1994; Peng and Park 1994; Miranda et al. 2006; Dall'Agnol et al. 2014).

Although serum bottle corrosion experiments are easy to perform and correctly model corrosion in static systems such as storage tanks, MIC in the oil and gas industry often occurs in pipelines under flow conditions. Replicating a flowing system has been done by the use of continuous culture vessels

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(chemostats) (Angell et al. 1997) or other flow cell reactors (Lee and Characklis 1993; Stipaničev et al. 2013; Duncan et al. 2014). While these flow system experiments rarely replicate the flow velocities in oil transmission pipelines, the data obtained from these experiments are more representative than those obtained from static serum bottle corrosion experiments.

The detrimental effects of MIC in the oil and gas industry can be controlled by the use of corrosion inhibitors and/or biocides. Corrosion inhibitors are mixtures of chemicals, which include surfactants (Kelland 2009), whereas biocides usually have only one or two active components. The biocides used in this study included glutaraldehyde (Glut; Fig. S1), which kills by cross-linking amino groups of proteins and nucleic acids (Ganzer et al. 2001; Videla 2002; Greene et al. 2006; Bartlett and Kramer 2011; Stipaničev et al. 2013). We also used tetrakis(hydroxymethyl)phosphonium sulfate (THPS; Fig. S1), a commonly used quaternary phosphonium salt, which kills microorganisms by cleaving disulfide bonds in the cell wall (Videla 2002; Kahrilas et al. 2015) and the quaternary ammonium biocide benzalkonium chloride (BAC; Fig. S1). This positively charged surfactant solubilizes cell membranes and is often used together with other biocides such as Glut. Due to their amphiphilic nature, quaternary ammonium compounds like BAC can also act as film-forming corrosion inhibitors (Schaeufele 1984; Enzien et al. 1996). Hence, BAC may act both as a biocide and as a corrosion inhibitor. Biocide treatment of flowing systems is more complex than treating stagnant systems. Because the volumes of transported fluid are large, applying a high concentration of biocide for a limited duration (a batch) is most often done (Videla 2002).

Carbon steel coupons and removable studs, which are flush with the pipeline wall often in a dedicated side-stream, are frequently used for corrosion monitoring under flow conditions (Videla 2002). We have recently advocated the use of carbon steel BBs as corrosion probes (Pinnock and Voordouw 2016; Sharma et al. 2016; Voordouw et al. 2016). Use of three to five of these per static corrosion assay allowed accurate determination of general corrosion rates. Their uniform size (53.6 ± 0.1 mg with a diameter of 0.236 cm, although different sizes are available) also permits evaluation of the corrosion rate distribution. Packing carbon steel BBs into small columns (60/ml) allows the evaluation of corrosion under flow conditions (Voordouw et al. 2017). Its inhibition by batches of biocides or corrosion inhibitors and the microbial communities involved are presented here.

Materials and methods

Growth medium and SRB inoculum

Modified Coleville Synthetic Brine medium K (CSBK) contained (g/l) NaCl (1.5), KCl (0.1), KH_2PO_4 (0.05),

NH_4Cl (0.08), $CaCl_2·2H_2O$ (0.08), $MgCl_2·6H_2O$ (0.2), Na_2SO_4 (1.42, 10 mM), sodium acetate (0.082, 1 mM), and sodium formate (1.36, 20 mM). Following autoclaving and cooling under 90% N_2 and 10% CO_2 (N_2-CO_2), the following were added per liter: 15 ml of 1 M $NaHCO_3$, 1 ml of trace elements solution, 1 ml of selenite-tungstate solution, and 1 ml of 1 M Na_2S (Widdel and Bak 1992). The pH was then adjusted to between 7.2 and 7.6 with 2 M HCl. Produced water (20 ml) from the Medicine Hat Glauconitic C oil field in Southern Alberta (Voordouw et al. 2009) was used to inoculate 160-ml serum bottles with 100 ml of CSBK medium and a 40-ml headspace of N_2-CO_2. These were closed with butyl rubber stoppers and crimped with aluminum rings. Once SRB had grown, 10% (v/v) of these cultures were used to inoculate continuous culture vessels (chemostats).

### Biocides and corrosion inhibitors

Biocides were dissolved in an autoclaved salt solution containing (g/l) NaCl (1.5), KCl (0.1), and Na_2SO_4 (1.42). Glut (50% w/w) was from Sigma-Aldrich (St. Louis, MO), THPS (76.5% w/w) from Dow (Midland, MI), and BAC from INC Biomedicals Inc. (Aurora, OH). These biocides were dissolved to a final active concentration of 300 mg/l (300 ppm). Glut/BAC contained 150 ppm Glut and 150 ppm BAC in autoclaved salt solution. CR_W was mixed in a ratio of 1 part (v/v) corrosion inhibitor to 2 parts (v/v) autoclaved salt solution. Corrosion inhibitors CRO_1 and CRO_3 were mixed in a ratio of 1 part (v/v) corrosion inhibitor to 2 parts (v/v) diesel. Carbon steel BBs were soaked in diluted corrosion inhibitor for 20 s. The solution was then drained off and the BBs were loaded into the columns.

Corrosion under flow conditions

BBs made from ASTM a36 carbon steel (Thomson Precision Balls) were purchased from Grainger, Lake Forest, USA. Most were 53.6 ± 0.1 mg with a calculated diameter of 0.236 cm; some were from another batch of 55.0 ± 0.3 mg with a diameter of 0.238 mm. BBs were sanded by rolling them between sheets of 400 grit sandpaper and then treated with a modified version of the NACE SP0775-2013 protocol. The BBs were immersed in 6 M HCl with 5.3 g/l N,N′-dibutylthiourea for 2 min, followed by immersion in 1.2 M sodium bicarbonate for 2 min. They were then rinsed once with deionized water and twice with acetone and then dried under a stream of N_2.

A schematic of the experimental setup is shown in Fig. 1. A Gilson (Middleton, WI) Minipuls® 3 peristaltic pump was used to pump CSBK medium from a 2-l reservoir into the chemostat. The chemostat was a 160-ml borosilicate serum bottle with one port on the top and one port on the side near the bottom of the bottle. Both ports were closed with butyl

Fig. 1 Schematic of experimental setup. CSBK medium was pumped from the medium reservoir into a stirred chemostat using a peristaltic pump; effluent from the chemostat was pumped into multiple syringe columns with carbon steel BBs, using a second peristaltic pump. Effluent from the columns was collected in bottles. An N_2-CO_2 filled syringe maintained atmospheric pressure in the medium reservoir
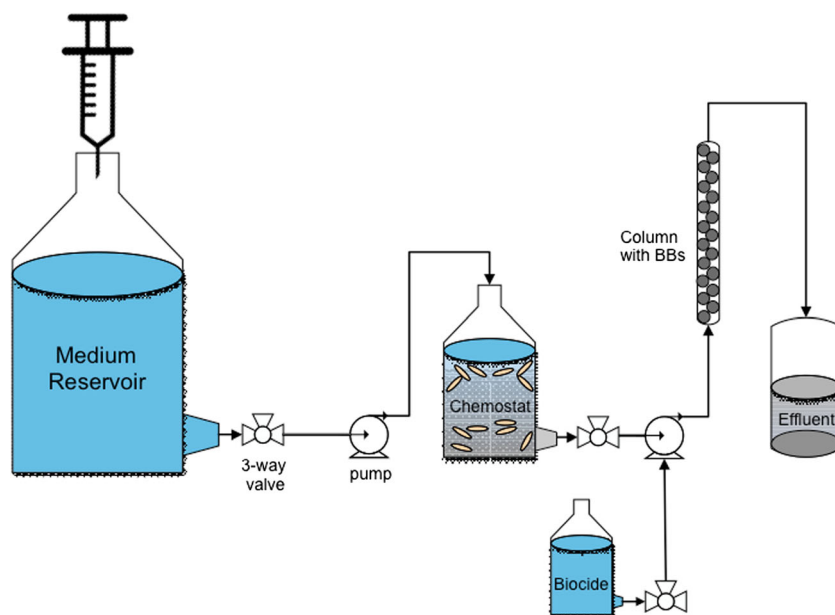
rubber stoppers and sealed with aluminum rings. The stoppers were perforated with 21-gauge needles connected to 0.76-mm inner diameter PVC tubing (Gilson, USA). Fresh medium was pumped into the chemostat through the top port, and the growing culture ($V = 100$ ml) was pumped out through the side port at the same flow rate of 4.65 ml/h, giving a dilution rate of 0.0465 h^{-1}. The chemostat culture was stirred constantly and kept at 30 °C through the use of a heated stirring plate. For most experiments, the chemostat culture was pumped into six 1-ml Luer-Lok™ Tip syringes (BD, Franklin Lakes, NJ), each containing 60 pre-treated BBs at a flow rate of 0.78 ml/h per column. Effluent was collected from the top of each up-flow column in 250-ml Nalgene® bottles. Biocide treatment was done by changing the column inflow from the chemostat to 300 ppm biocide in salt solution for 2 h at the same flow rate every 5 days. Untreated control columns were injected with salt solution. Note that all of these operations were carried out on a lab bench in air and that a certain amount of oxygen ingress cannot be excluded. Following 45 days of flow, the BBs were removed, washed to remove biomass for DNA isolation, and then treated with the modified NACE SP0775-2013 procedure to remove corrosion products. Cleaned and dried BBs were weighed three times with a NewClassic MF analytical scale (Mettler Toledo, Switzerland) both collectively and individually to determine the general corrosion rate (CR) and weight loss distribution, respectively. CR was calculated as:

$$CR = 87600 \times \Delta W / ATD \text{ mm/year}$$

where ΔW is the weight loss (g), A is the area (cm^2), T is the incubation time (h), D is the density of carbon steel (7.85 g/cm^3), and 87,600 is a constant for conversion of centimeters per hour into millimeters per year.

X-ray diffraction (XRD) analysis of corrosion products obtained from untreated control columns after 45 days was done by DNX Materials Analysis in Calgary, AB. Once the BBs were removed from the untreated control columns, a small sample of the corrosion product was put into a 1.5-ml microfuge tube and left open to dry in an anoxic (N_2-CO_2) hood overnight. XRD analysis was performed on the sample the following day.

Determination of the concentration of analytes and most probable numbers

Approximately 700 μl of the chemostat or of the six column effluents was collected each day in 1.5-ml microfuge tubes, containing 5 μl of 1 M $ZnCl_2$ as a sulfide trap. Aliquots of 5 μl from each sample were used to determine the sulfide concentration with the diamine method (Cline 1969) using a Shimadzu UV-1800 spectrophotometer (Japan). Sulfate concentrations were determined by anion high-performance liquid chromatography (HPLC), using a conductivity detector (Waters 432, USA) and an IC-PAK anion column (4 × 150 mm, Waters, USA) with borate/gluconate buffer at a flow rate of 2 ml/min. Acetate and formate were determined by HPLC using an instrument equipped with a Waters 2487 UV detector (USA) and an organic acid column (Prevail, 250 × 4.6 mm, USA) eluted with 25 mM KH_2PO_4 buffered to pH 2.5 at a flow rate of 1 ml/min.

Viable SRB were enumerated by a miniaturized MPN method, using 48-well cell culture plates. The entire procedure was done in an anaerobic hood (Forma Scientific) with an atmosphere of 5% (v/v) H_2, 10% CO_2, and 85% N_2. A 1-ml sample was removed anaerobically from the chemostat; 0.1 ml of this sample was inoculated into 0.9 ml of Postgate medium

B, which was serially diluted tenfold to 10^{-12} in the same medium in triplicate wells (Shen and Voordouw 2015). The medium contained 17 mM sulfate, 22 mM lactate, and ferrous iron. The plates were immediately covered with a Titer-Tops membrane and incubated at 32 °C in the anaerobic hood for 4 weeks. Wells were scored as positive when a black FeS precipitate was evident.

DNA isolation and sequencing

At the end of each experiment, a 10-ml sample was taken from the chemostat and centrifuged at $14000 \times g$ for 10 min at 4 °C. The cell pellet was stored at -20 °C until DNA extraction. To isolate DNA, BBs were removed from a column and placed in a 15-ml polypropylene screw cap tube (Sarstedt Inc., Newton, NC). Sodium phosphate buffer (600 μl/tube) from the MP Biomedicals FastDNA® Spin Kit for Soil (Solon, OH) was added, and the tubes were vortexed for 20 s. The supernatant was removed and transferred to a 1.5-ml microfuge tube (Diamed, Mississauga, ON). This extraction procedure was repeated, and the combined supernatants from each column were centrifuged at $14000 \times g$ for 10 min at 4 °C. The cell and corrosion product pellets were stored at -20 °C. The FastDNA® Spin Kit for Soil was used to extract genomic DNA from these. Purified DNA was adjusted to a concentration of 5 ng/μl.

Genomic DNA was amplified through 25 PCR cycles using primers 926Fi5 and 1392Ri7, as described elsewhere (Menon and Voordouw 2018). The purified PCR products were subjected to another 10 cycles of PCR with the barcoded Illumina primers P5-S50X-OHAF and P7-N7XX-OHAF. PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) and were then sequenced with an Illumina MiSeq sequencer (Energy Bioengineering Group, Department of Geosciences, University of Calgary). The 300PE reads from both ends were merged using PEAR 0.9.6 with a 50-bp overlap and were further processed with a 420-bp cutoff of amplicon size using MetaAmp, a 16S rRNA data analysis pipeline, developed by the Energy Bioengineering Group, Department of Geosciences, University of Calgary. MetaAmp was also used for bioinformatic analysis (Dong et al. 2017). Raw read sequences have been submitted to NCBI Sequence Read Archive (SRA) under accession numbers SRR5907373 to SRR5907381.

Results

Effect of biocides and corrosion inhibitors on microbial physiology

Duplicate columns were injected with the effluent of a chemostat in which oil field SRB were grown with formate

and sulfate as the energy source and with acetate and CO_2 as the carbon source. Most (> 90%) of the 20-mM formate in the medium was used in the chemostat. This caused the sulfate concentration in the chemostat to drop from 8.5 to 4 mM and the sulfide concentration to increase from 1 to 5 mM from day 1 to day 15, after which these were rather constant. Results for the effluents of columns 1 to 6, which were injected with column effluent and were (1, 2) untreated or (3, 4) treated with Glut or (5, 6) with THPS, are shown in Figs. 2a, S2A, and S3A. The columns were treated ten times with 1.6 ml of 300 ppm Glut or 300 ppm THPS for 2 h over the 45-day course of the experiment, as indicated. The sulfate concentrations of the chemostat and the column effluents were similar from day 15 onwards, indicating little sulfate reduction in the columns (Fig. S2A; Table 1, 0.35–0.41 mM). However, the sulfide concentrations in the column effluents were much lower than those in the chemostat, 0.5 mM on day 15 increasing to 1.5 mM on day 45 (Fig. S3A). Interestingly, the sulfide
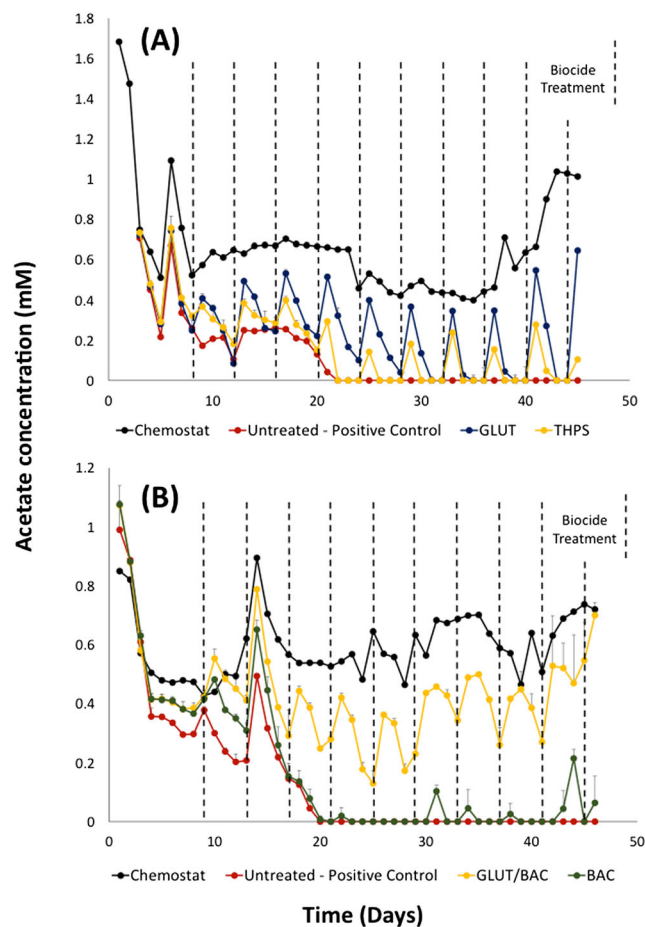

Fig. 2 Concentration of acetate in the chemostat and in effluents of BB-containing columns as a function of time. Data are shown for **a** columns that were (1, 2) untreated, (3, 4) treated with Glut, or (5, 6) treated with THPS and for **b** columns that were (7, 8) untreated, (9, 10) treated with Glut/BAC, or (11, 12) treated with BAC. The average concentrations for duplicate columns are shown. Biocide treatments are indicated by the vertical dotted lines

Table 1 Average concentrations of sulfate and sulfide for the chemostat and column effluents over the 45-day monitoring period. The total iron weight loss (ΔW_Fe) and the average concentration of iron produced by corrosion (Fe_corr) are also indicated

Columns	Treatment	Sulfate (mM)	ΔSulfate (mM)[1]	Sulfide (mM)	ΔSulfide (mM)[1]	ΔW_Fe (mg)	Fe_corr (mM)[2]
Chemostat		4.51		4.76			
1, 2	Untreated	4.16	0.35	0.84	3.92	206.7	2.21
3, 4	Glut	4.16	0.35	0.77	3.99	193.0	2.07
4, 6	THPS	4.10	0.41	0.98	3.78	173.1	1.85
Chemostat		4.50		4.60			
7, 8	Untreated	4.20	0.30	0.80	3.80	216.4	2.32
9, 10	Glut/BAC	4.04	0.46	1.82	2.78	73.0	0.78
11, 12	BAC	4.34	0.16	1.9	2.70	92.6	0.99

[1] Difference of sulfate or sulfide concentration in the chemostat and in the column effluent

[2] Concentration of iron produced by corrosion in two columns: $1000 \times (\Delta\text{W\_Fe}/55.8)/(2 \times 18.6 \times 45)$; note that this is a calculated number. Most of the corroded iron remains attached to the BBs and is removed by acid treatment

concentrations in the effluent of columns treated with Glut and THPS peaked following each treatment, something not seen in the control columns, which were injected with saline-sulfate solution only (Fig. S3A). Average concentrations of sulfide in the chemostat and in the effluent of the untreated, the Glut-treated, and the THPS-treated columns were 4.76, 0.84, 0.77, and 0.98 mM, respectively (Table 1).

Use of acetate in the chemostat caused its concentration to drop to 0.4–0.6 mM from days 10 to 40 (Fig. 2a). The acetate concentration in the untreated control column effluents was zero from day 20 onwards (Fig. 2a). However, in columns treated with Glut, upward spikes in effluent acetate of 0.4 mM were observed following each treatment. The peak concentrations of these spikes were close to the concentration of the chemostat effluent, indicating that acetate use in the columns had almost halted. This effect was less pronounced after each treatment with THPS, which gave acetate spikes of 0.2 mM (Fig. 2a).

Results for columns 7 to 12, which were injected with chemostat effluent and were (7, 8) untreated or (9, 10) treated with Glut/BAC or (11, 12) with BAC, are shown in Figs. 2b, S2B, and S3B. Sulfate concentrations in the chemostat averaged 4.5 mM (Table 1) with a decreasing trend from days 12 to 35 and an increasing trend from days 35 to 45 (Fig. S2B). The reasons for this are not known. The time-averaged sulfate concentration in the chemostat was 4.5 mM for both sets of experiments (Table 1). Sulfate concentrations in the column effluents tracked these changes quite precisely, indicating little sulfate reduction in the columns (Fig. S2B; Table 1, 0.16–0.46 mM). The sulfide concentration in the chemostat averaged 4.6 mM (Table 1, Fig. S3B), whereas those in the column effluents were again lower, averaging 0.80, 1.82, and 1.90 mM for untreated, Glut/Bac-treated, and BAC-treated columns, respectively (Table 1, Fig. S3B). Sulfide concentrations in the effluent of Glut/BAC-treated columns spiked after each treatment. This was not seen for BAC-treated columns (Fig. S3B).

Average acetate concentrations in the effluent of untreated columns were 0 mM from day 17 onwards. Zero acetate concentrations were also observed in the effluent of BAC-treated columns with small upward spikes after some biocide treatments (Fig. 2b). Acetate concentrations in the effluents of Glut/BAC-treated columns were much higher spiking to 0.4–0.6 mM after each biocide treatment (Fig. 2b).

Treatment of BBs with corrosion inhibitors, prior to packing them in columns, did not affect physiological parameters. The sulfate, sulfide, and acetate concentrations in the column effluents were similar for columns with untreated BBs and for columns treated with corrosion inhibitor (Fig. S4). Sulfate concentrations in the chemostat and column effluents were very similar (Fig. S4A), but the sulfide concentrations were very different, on average 5.2 and 0.75 mM, respectively (Fig. S4B). The average acetate concentrations in the chemostat and column effluents were 0.7 and 0.4 mM, respectively (Fig. S4C). Hence, microbial processes decreasing the sulfide and acetate concentrations were not affected by pre-treatment of BBs with corrosion inhibitors.

Corrosion rates of BBs under flow conditions

The average CRs for groups of 60 BBs in duplicate columns 1 to 24 subjected to flow conditions for 45 days are summarized in Table 2. The averages for untreated columns 1 and 2, 7 and 8, and 13 and 14 were 0.101 ± 0.004, 0.107 ± 0.005, and 0.0962 ± 0.0004 mm/year, respectively, which is a moderate MIC rate (Al-Shamari et al. 2013). Columns 23 and 24 injected with sterile CSBK for 45 days had an average CR = 0.020 ± 0.002 mm/year (Table 2). Hence, the presence of SRB and the associated production of sulfide increased the CR fivefold. Periodic treatment with biocides decreased the CR by 6, 17, 65, and 56% for Glut, THPS, Glut/BAC, and BAC, respectively (Table 2).

Table 2 Average general CRs of carbon steel BBs in columns 1 to 24, treated as indicated. Averages are for two columns with 60 BBs each

Treatment	Untreated	Glut	THPS	Untreated	Glut/BAC	BAC
Columns	1, 2	3, 4	5, 6	7, 8	9, 10	11, 12
$CR_{Av} \pm SD$ (mm/year)	0.101 ± 0.004	0.095 ± 0.003	0.084 ± 0.003	0.107 ± 0.005	0.037 ± 0.002	0.047 ± 0.002
%	100	94.1	83.2	100	34.6	43.9
Treatment	Untreated	CR_W	CR_O1	CR_O3	Diesel	Sterile CSBK
Columns	13, 14	15, 16	17, 18	19, 20	21, 22	23, 24
$CR_{Av} \pm SD$ (mm/year)	0.0962 ± 0.0004	0.050 ± 0.010	0.002 ± 0.002	0.001 ± 0.000	0.081 ± 0.002	0.020 ± 0.002
%	100	52.0	2.1	0.6	84.2	20.8

Relative to the untreated control (columns 13, 14; 0.0962 ± 0.0004 mm/year; 100%), a single treatment of BBs with corrosion inhibitors CR_W, CR_O1, or CR_O3 decreased the CR by 48, 98, and 99%, respectively (Table 2). An experiment in which BBs were treated with diesel only and then packed into columns gave an average corrosion rate of 84% of that of the untreated value (Table 2). Thus, exposure to diesel did not contribute much to the large decrease in CR seen with CR_O1 and CR_O3. Relative to unexposed BBs (Fig. 3a), BBs not treated with corrosion inhibitor and subjected to flowing SRB culture for 45 days showed significant pitting corrosion and noticeable changes in the entire surface topography (Fig. 3b). Carbon steel BBs treated with CR_W had localized corrosion (Fig. 3c) with large areas remaining unaffected. BBs treated with CR_O1 (Fig. 3d) and CR_O3 (Fig. 3e) showed no visible signs of corrosion and looked very similar to BBs, which were not exposed to flowing SRB culture (Fig. 3a).

Residual weights of individual BBs were determined for columns, which were either untreated or treated with biocides. The weight distribution is presented in Fig. 4 for 120 BBs in each panel. In the absence of biocide treatment, a more slowly and a more rapidly corroding BB group was seen (Table 3: N_{slow} is 65 and 66; N_{fast} is 55 and 54). Treatment with Glut or THPS decreased the number of rapidly corroding BBs to 45

and 39, respectively, whereas treatment with Glut/BAC and BAC greatly decreased this group to 10 and 8 BBs, respectively (Table 3). The average corrosion rate calculated from the weight loss of the two groups of BBs (Table 3: CR_{AV}) agreed well with the corresponding values in Table 2. Hence, treatment with BAC prevented a rapidly corroding group from emerging.

XRD analysis of the corrosion products in the untreated columns indicated that these consisted of 35–45% (w/w) of mackinawite (FeS), 30–40% (w/w) of pyrrhotite ($Fe_{1-x}S$; $x = 0$–0.2), and 15–25% of unidentified material. Importantly, $FeCO_3$ did not appear to be a major corrosion product.

Microbial community compositions

The microbial communities in the chemostats serving columns 1–6, 7–12, and 13–20 were dominated by *Deltaproteobacteria*, predominantly SRB of the genera *Desulfovibrio*, *Desulfomicrobium*, and *Desulfocurvus*. Together these comprised 92, 88, and 91% of the total reads obtained for these chemostat communities, respectively (Figs. 5 and S5; Table S1), indicating that these SRB were capable of growth in medium with formate and sulfate as the energy and with acetate and CO_2 as the carbon source. The MPN of lactate-utilizing SRB, which includes many *Desulfovibrio* and

Fig. 3 Effect of corrosion inhibitors on BBs exposed to corrosive flow conditions. BBs were left untreated or were treated with corrosion inhibitors prior to being packed in columns. Images are of BBs prior to (**a**) or following exposure to corrosive flow conditions (**b**–**e**). The latter were untreated (**b**), treated with water-dissolved CR_W (**c**), treated with oil-dissolved CR_O1 (**d**), or treated with oil-dissolved CR_O3 (**e**)
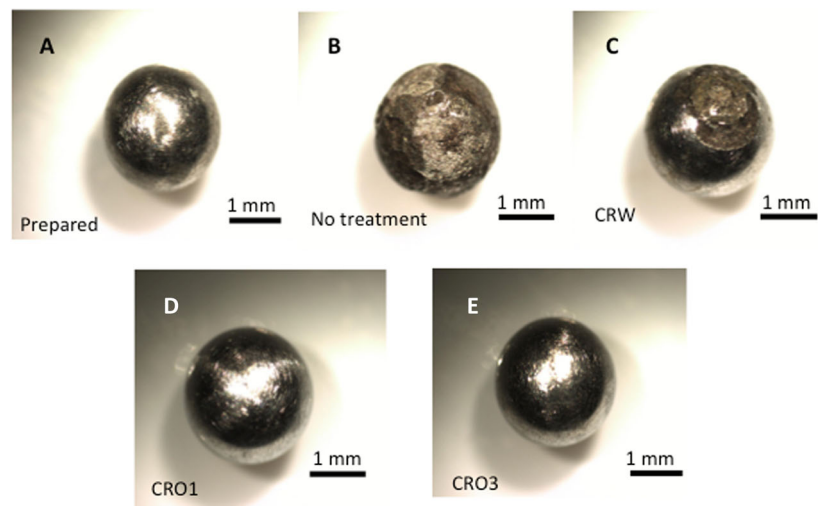
Fig. 4 Distribution of residual weights of 120 BBs, following exposure to flow conditions for 45 days. Data are for duplicate columns 1 to 12 with 60 BBs each, which were (1, 2) untreated (**a**), (3, 4) treated with Glut (**b**), (5, 6) treated with THPS (**c**), (7, 8) untreated (**d**), (9, 10) treated with Glut/BAC (**e**), and (11, 12) treated with BAC (**f**). The number N of BBs with residual weights in intervals of 0.5 mg from 46.0 to 54.0 mg is plotted against the residual BB weight. The average residual BB weight ± SD is given for each distribution. The average residual BB weights for a more slowly (↓) and a more rapidly corroding group (↓) is indicated (Table 3)
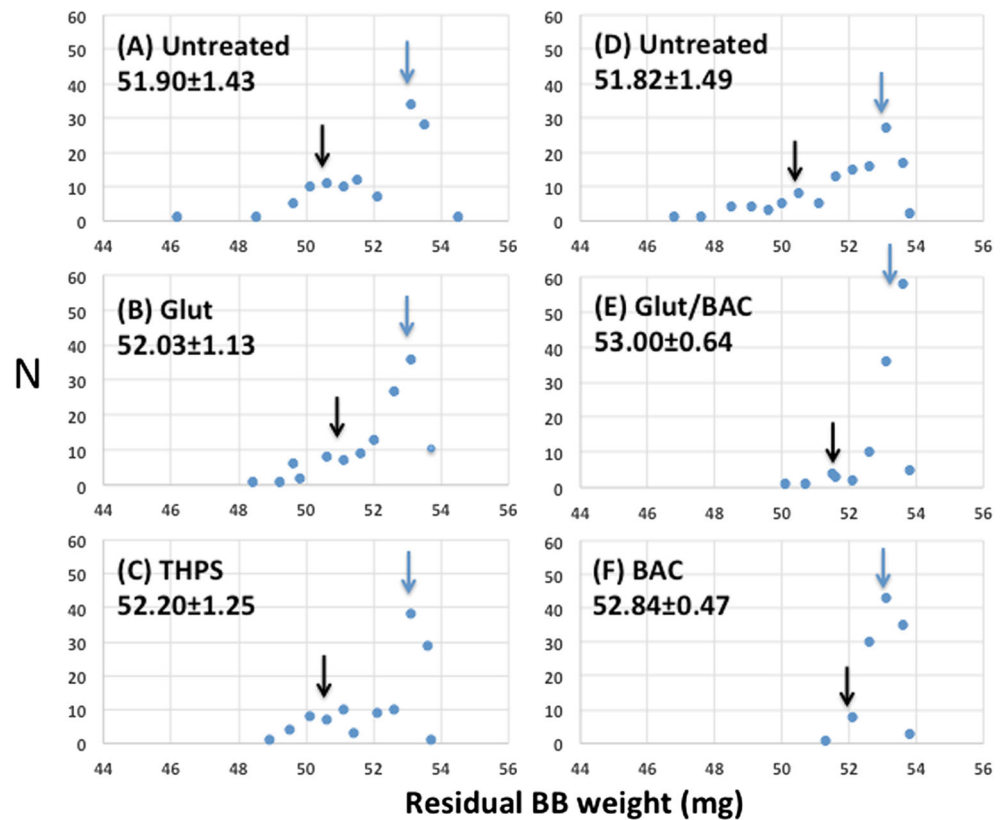
Desulfomicrobium species, in the chemostats was 10^8/ml. High fractions of *Deltaproteobacteria* were also found in the corrosion products of all columns 1 to 20. These varied from 22 to 93% (Figs. 5 and S5; Table S1) and consisted of these same three genera. The community compositions in duplicate columns with the same treatment were always most similar, as seen in the dendrograms in Figs. 5 and S5. Microbial communities in Glut-treated columns (3, 4) were distinct from THPS-treated columns (5, 6) and untreated columns (1, 2) (Fig. 5a–c). Likewise, microbial communities in Glut/BAC-treated columns (9, 10) were distinct from BAC-treated columns (11, 12) and untreated columns (7, 8) (Fig. 5d–f). Communities in columns with BBs treated with diesel-dissolved corrosion inhibitors formed a clade, which was distinct from communities in columns that were untreated or treated with the water-soluble corrosion inhibitor CR_W (Fig. S5).

Table 3 Distribution of residual weights (W_{res}) of carbon steel BBs in columns 1 to 12 over slowly and rapidly corroding groups, using W_{res} = 52.0 mg as the cutoff

| Treatment	Untreated	Glut	THPS	Untreated	Glut/BAC	BAC
Columns	1, 2	3, 4	5, 6	7, 8	9, 10	11, 12
N_{slow} (> 52.0 mg)[1]	65	75	81	66	110	112
W_{res} (Av ± SD)[2]	53.03 ± 0.41	52.76 ± 0.32	52.98 ± 0.37	52.88 ± 0.42	53.16 ± 0.36	52.92 ± 0.39
CR_{slow} (mm/year)[3]	0.034	0.050	0.037	0.043	0.026	0.040
N_{fast} (<52.0 mg)[1]	55	45	39	54	10	8
W_{res} (Av ± SD)[2]	50.56 ± 0.97	50.80 ± 0.92	50.59 ± 0.82	50.51 ± 1.28	51.30 ± 0.54	51.82 ± 0.22
CR_{fast} (mm/year)[3]	0.179	0.165	0.177	0.182	0.135	0.105
CR_{fast}/CR_{slow}	5.3	3.3	4.8	4.3	5.2	2.6
CR_{Av} (mm/year)[4]	0.100	0.093	0.082	0.105	0.035	0.045

[1] Number of BBs in the slow and fast corroding groups

[2] Average residual weight of BBs in the slow and fast corroding groups

[3] Corrosion rate derived from the average residual weight of BBs in the slow and fast corroding groups

[4] $CR_{AV} = (N_{slow} \times CR_{slow} + N_{fast} \times CR_{fast})/(N_{slow} + N_{fast})$; $N_{slow} + N_{fast} = 120$

Fig. 5 Taxonomic profile of microbial communities in chemostat effluents and in BBs of columns. Data are shown for columns that were untreated (1, 2, 7, 8), treated with Glut (3, 4), treated with THPS (5, 6), treated with Glut/BAC (9, 10), or treated with BAC (11, 12). Shown are the Bray-Curtis dendrograms with the bars representing a sequence divergence of 0.05 (**a**, **d**), the presence of phyla other than *Proteobacteria* (**b**, **e**), and of classes within the phylum *Proteobacteria* (**c**, **f**)
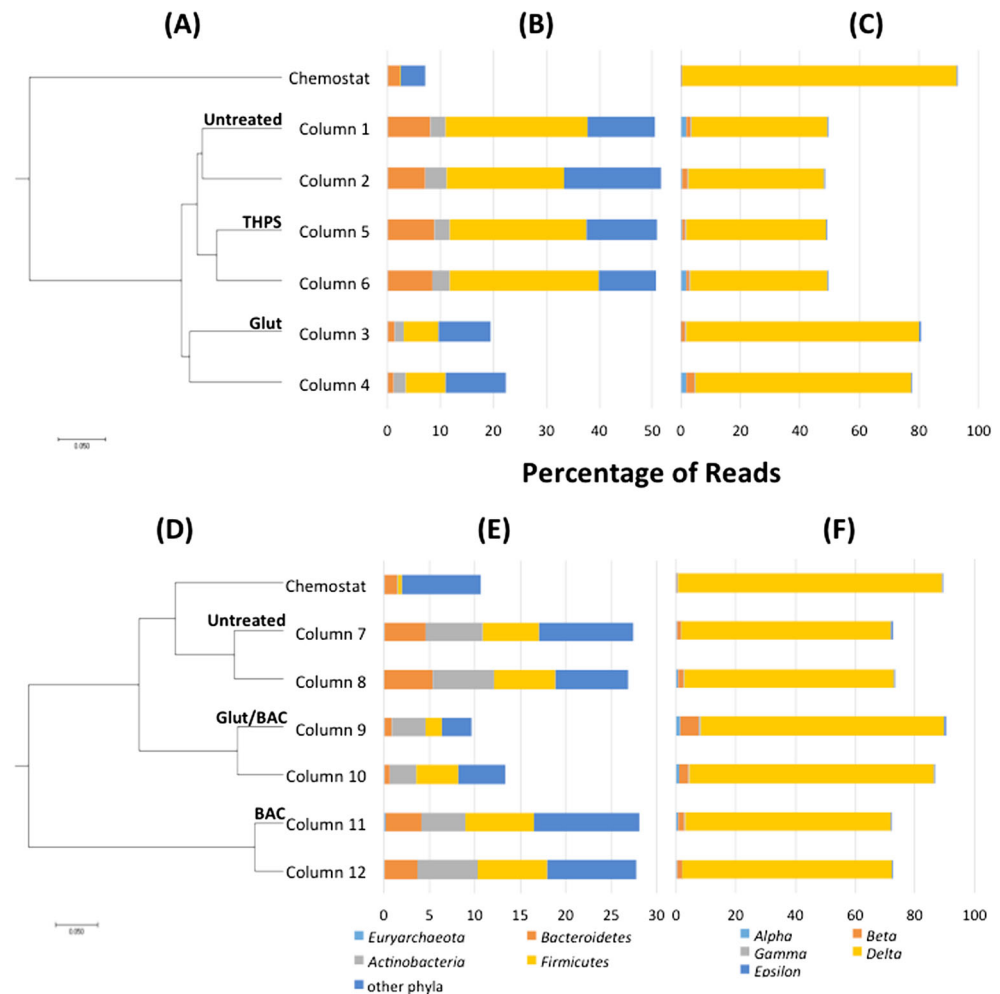
Discussion

Injection of a high concentration (300 ppm) of biocide for 2 h every 5 days transiently increased the sulfide and acetate concentrations in the effluents of BB-containing columns, which had a pore volume (PV) of 0.4 ml and a flow rate of 0.78 ml/h (2 PV/h). At this flow rate, planktonic chemostat microbes were removed within 30 min, and the biocide treatment targeted the sessile population attached to the BBs. Likewise, biocide was removed, and the planktonic chemostat community was re-established in the columns within 30 min of resumption of injection of chemostat effluent. Because the time scale of transient increases of sulfide and acetate concentrations was in days not hours (Figs. S3 and 2), it follows that these reflect the partial killing and subsequent regrowth of the sessile microbial communities attached to the BBs. These were distinct from the planktonic chemostat community (Table S1, Fig. 5). However, *Deltaproteobacteria* of the genera *Desulfovibrio*, *Desulfomicrobium*, and *Desulfocurvus* were major components in both types of communities. These SRB are often found in sulfidogenic, corrosive, biofilms, and their involvement in corrosion is well documented (Leu et al. 1999; Dinh et al.
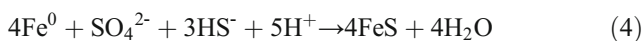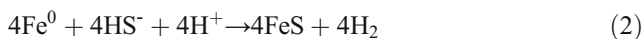
2004; Auvinen et al. 2009; Enning et al. 2012; Islam and Karr 2013; Duncan et al. 2014). Recently, bacteria from the genus *Desulfocurvus* have also been associated with the corrosion of carbon steel (Chang et al. 2015).

MIC of iron by SRB can be through electrical MIC (EMIC) or chemical MIC (CMIC) mechanisms (Enning et al. 2012; Enning and Garrelfs 2014). In EMIC, SRB use four metallic iron (Fe^0) as electron donor for reduction of a sulfate to sulfide. Assuming that one of the ferrous ions formed precipitates as FeS and that the other three react with bicarbonate, which was abundant in our medium, to form $FeCO_3$ gives the following microbially catalyzed reaction (Enning et al. 2012; Enning and Garrelfs 2014):

$$4Fe^0 + SO_4^{2-} + 3HCO_3^- + 5H^+ \rightarrow FeS + 3FeCO_3$$
$$+ 4H_2O \tag{1}$$
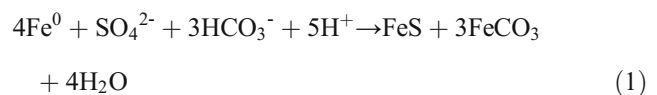
One of the diagnostic features of EMIC is that the corrosion scale has only 25% FeS and 75% $FeCO_3$, as predicted by Eq. (1). This is inconsistent with the corrosion scale formed on the BBs, which had little $FeCO_3$ and had 60–85% of FeS.

Corrosion scale dominated by iron sulfides, as found here, is typical for anaerobic SRB-mediated CMIC scenarios (Little et al. 2006; Enning et al. 2012; Enning and Garrelfs 2014). In CMIC, SRB use organic electron donors to reduce sulfate to sulfide. In the chemostat, SRB oxidized approximately 20 mM formate to CO_2 to reduce 5 mM sulfate to sulfide. In the columns, part of this sulfide reacted chemically with Fe^0 to form FeS and H_2, as in Reaction (2). The H_2 formed was then also used as electron donor for sulfate reduction in the microbially catalyzed Reaction (3), giving overall Reaction (4):

$$4Fe^0 + 4HS^- + 4H^+ \rightarrow 4FeS + 4H_2 \qquad (2)$$

$$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O \qquad (3)$$

$$4Fe^0 + SO_4^{2-} + 3HS^- + 5H^+ \rightarrow 4FeS + 4H_2O \qquad (4)$$

Assuming that H_2 production and consumption are tightly coupled, Eq. (4) predicts that its inhibition by biocide addition will transiently increase the sulfide concentration in the columns' effluent. For untreated columns, we estimate the concentration of corroded iron, calculated by dividing the amount (mmol) of corroded iron by the volume of column effluent collected over the 45-day period of the experiment, to be 2.3 mM (Table 1: Fe_corr). If all Fe^0 corrosion in the columns was caused by CMIC according to Eq. (4), then the sulfate concentration would decrease by 0.6 mM in the effluent of untreated columns. If Reaction (4) is transiently inhibited by the addition of a biocide, a maximal increase in the sulfide (HS^-) concentration of 1.7 mM is expected. The results indicate that in untreated columns, the sulfate concentration decreased by 0.3 to 0.35 mM (Table 1). In columns treated with Glut or THPS, transient increases of 1 to 2 mM sulfide were routinely observed (Fig. S3). However, more sulfide was removed during fluid flow through untreated columns (Table 1, 3.8–3.9 mM) than the 1.7 mM expected from Eq. (4). The reasons for this are currently unclear. We have considered the possibility that the experimental setup (Fig. 1) is subject to O_2 ingress causing sulfide to be removed by oxidation with O_2 to elemental sulfur through chemical or microbially catalyzed reactions. The latter can be catalyzed by phylum/class *Epsilonproteobacteria/Campylobacteraceae* (An et al. 2016). These was only a minor component of the chemostat community (Table S1, entries 23, 55, and 73, 0.01–0.07%), but was an increase although still minor component of the sessile BB community in all columns (Table S1, entries 23, 55, and 73, 0.03–0.66%), except in columns 13 and 14 (6.5%). Overall, both the chemostat and BB-attached communities were dominated by anaerobic taxa indicating limited O_2 ingress (Table S1).

Columns treated with BAC or Glut/BAC had a lower concentration of corroded Fe^0 (Table 1, 0.8 to 1 mM). This indicates that in the presence of BAC, Reaction (2) of sulfide with iron was partially prevented, increasing the sulfide concentration in the column effluent (Table 1, 1.8–1.9 mM) compared with columns that were untreated or treated with Glut or THPS (Table 1, 0.77–0.98 mM). No transient increases in sulfide concentration were observed following treatment with BAC (Fig. S3B).

The microbial community in the chemostat used on average 0.4 mM of the 1-mM acetate present in the inflowing medium. Acetate use decreased from days 40 to 45 in one of the chemostats (Fig. 2a). This was not reflected in a difference in microbial community composition, determined at the end of the experiment, which indicated dominance of the same three genera of SRB in both chemostats (Table S1). SRB of the genera *Desulfovibrio*, *Desulfomicrobium*, and *Desulfocurvus* require acetate as a carbon source when these are grown chemolithotrophically with H_2 or formate as electron donor for sulfate reduction. This is because these genera synthesize pyruvate from acetate and CO_2 under these conditions (Badziong and Thauer, 1978). In the columns, the complete depletion of acetate was caused by its continued use by these same SRB, because Reaction (4) is also chemolithotrophic, requiring continued use of acetate as a carbon source. Acetate use could also involve activity of *Desulfuromonas* (Roden and Lovley 1993), which was more prevalent in the columns than in the chemostats (Table S1: entries 6 and 41). *Clostridium* species, which can also metabolize acetate, were also much more prevalent in the columns than in the chemostat (Table S1). Whatever the mechanism of acetate use, this was microbially catalyzed. Injection of biocide caused spikes in the acetate concentration, especially with Glut and THPs and to a much lesser degree with BAC (Fig. 2). In the case of Glut, the acetate concentration almost reached its value in the chemostat effluent (Fig. 2a), indicating that microbial use of acetate was transiently almost completely inhibited. In the case of BAC, little inhibition of acetate use was seen indicating that BAC was the least effective biocide.

Our results indicate that batch-wise application of Glut or THPS was not very effective in preventing SRB-mediated corrosion (Table 2), whereas application of Glut/BAC or BAC was more effective. In view of the ineffectiveness of Glut by itself, we assume that BAC was the more active corrosion-preventing component in Glut/BAC. Thus, batch-wise application of BAC, which was the least effective biocide as judged by inhibition of acetate use (Fig. 2), was most effective in decreasing corrosion. Repeated batch-wise BAC treatment was similarly effective as a single application of the water-soluble CR_W. But the diesel-dissolved CR_O1 and CR_O3 were much more effective than BAC (Table 2).

Microbial processes decreasing the sulfide and acetate concentrations were not affected by pre-treatment of BBs with corrosion inhibitors (Fig. S4). Microbial communities

in BBs treated with diesel-dissolved corrosion inhibitors CR_O1 and CR_O3 had high fractions of *Pseudomonas*, which were much lower in all other column effluents (Table S1: entries 26, 52, and 69). This organism is a known hydrocarbon degrader (Obuekwe et al. 2008). The observation that different microbial control agents, or corrosion control compounds, affect the microbial communities involved in corrosion differently has been documented (Chang et al. 2015).

The effect of film-forming corrosion inhibitors with surfactant properties on corrosion and on the microbial community on coupons subjected to flow of an SRB-containing medium has also been studied by Duncan et al. (2014). Corrosion inhibitors were added continuously and coupons were not treated prior to their placement in the flow cell, i.e., similar to the methodology used by Harris et al. (2010). Corrosion inhibitors were found to decrease the general corrosion rate but to increase the pitting corrosion rate in both studies (Harris et al. 2010; Duncan et al. 2014), possibly because scale and/or biomass particles prevented parts of the surface to be contacted by corrosion inhibitor. No pitting was observed on the surface of BBs treated with CR_O1 and CR_O3 in this study, likely because these were treated prior to their placement in the flow cell. Similar to the results reported here (Figs. 5 and S5), Duncan et al. (2014) found that microbial communities on coupons in replicate flow cells had similar compositions and that these were affected by the treatment. For example, the abundance of *Beta-* and *Gammaproteobacteria* increased in the presence of oil-soluble corrosion inhibitors similar to the increase in *Gammaproteobacteria* of the genus *Pseudomonas* in our study.

The differential of the pitting corrosion rate, defined as the corrosion rate of the deepest pit, to the general corrosion rate in these studies (Harris et al. 2010; Duncan et al. 2014) was a factor 10 to 100, i.e., much more than the difference in general corrosion rates observed for fast and slowly corroding BBs, which was a factor 2.6 to 5.2 (Table 3: CR_{fast}/CR_{slow}). Position in the column is one of the factors, which determines whether BBs corrode fast or slow. BBs at the inlet which are subjected to the highest concentrations of sulfide corroded more rapidly, but we have not yet investigated this in detail.

The flow system presented here offers a simple way to study the effect of treatment on SRB-mediated corrosion under flow conditions. General corrosion rates can be determined with great precision and their distribution can be determined. Among the biocides, we found that BAC was most effective in decreasing the corrosion rate by preventing the emergence of a more rapidly corroding group and this appeared to be caused more by its film-forming corrosion inhibitor properties than by its biocidal properties.

Compliance with ethical standards

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no conflict of interest.

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