J Ind Microbiol Biotechnol (2008) 35:1481–1491
DOI 10.1007/s10295-008-0450-6

ORIGINAL PAPER

The use of magnesium peroxide for the inhibition of sulfate-reducing bacteria under anoxic conditions

Yu-Jie Chang · Yi-Tang Chang · Chun-Hsiung Hung

Received: 24 March 2008 / Accepted: 30 July 2008 / Published online: 20 August 2008

Abstract Sulfate-reducing bacteria (SRB), which cause microbiologically influenced material corrosion under anoxic conditions, form one of the major groups of microorganisms responsible for the generation of hydrogen sulfide. In this study, which is aimed at reducing the presence of SRB, a novel alternative approach involving the addition of magnesium peroxide (MgO_2) compounds involving the use of reagent-grade MgO_2 and a commercial product (ORC^{TM}) was evaluated as a means of inhibiting SRB in laboratory batch columns. Different concentrations of MgO_2 were added in the columns when black sulfide sediment had appeared in the columns. The experimental results showed that MgO_2 is able to inhibit biogenic sulfide. The number of SRB, the sulfide concentration and the sulfate reducing rate (SRR) were decreased. ORC^{TM} as an additive was able to decrease more effectively the concentration of sulfide in water and the SRB-control effect was maintained over a longer time period when ORC^{TM} was used. The level of oxidation–reduction potential (ORP), which has a linear relationship to the sulfide/sulfate ratio, is a good indicator of SRB activity. As determined by fluorescence in-situ hybridization (FISH), most SRB growth was inhibited under increasing amounts of added MgO_2. The concentration of sulfide reflected the abundance of the SRB. Utilization of organic matter greater than the theoretical SRB utilization rate indicated that facultative heterotrophs became dominant after MgO_2 was added. The results of this study could supply the useful information for further study on evaluating the solution to biocorrosion problems in practical situations.

Keywords Sulfate-reducing bacteria (SRB) · Magnesium peroxide · Oxidation–reduction potential (ORP) · Fluorescence in-situ hybridization (FISH)

Y.-J. Chang (✉)
Department of Safety Health and Environmental Engineering, Tungnan University, Shen-Keng, Taipei 222, Taiwan
e-mail: yujchang@gmail.com

Y.-T. Chang
Department of Microbiology, Soochow University, Shih Lin, Taipei 111, Taiwan

C.-H. Hung
Department of Environmental Engineering, National Chung-Hsing University, Taichung 402, Taiwan

Introduction

Sulfate-reducing bacteria (SRB) are a group of phylogenetically diverse anaerobes that perform the dissimilatory reduction of sulfur compounds including sulfate, sulfite, thiosulfate and even sulfur to form sulfide. SRB are the major biological source of biogenic sulfide, which causes microbiologically influenced corrosion (MIC) in anoxic habitats. Many serious MIC problems in industrial activities are related to SRB activity, including metal corrosion in oil reservoirs [20, 47] and concrete pipe corrosion in sewers [12, 50]. Although the complex mechanisms of biocorrosion are observed by various SRBs [13], inhibiting the activity of SRB might provide an effective method to control biocorrosion. In southwestern Taiwan, there are many stainless steel groundwater wells that are used to monitor water level and quality and these become corroded due to the presence of biogenic sulfide in the water [6, 8]. SRB can grow in organic matter containing solutions under various anoxic conditions, including natural systems such as in the sediment of the Black Sea and in industrial processes [18, 36]. Many

food processing, pharmaceutical, petroleum and pulp/paper industrial processes generate wastewater with a high concentration of hydrogen sulfide (H_2S). Appropriate strategies for controlling sulfide caused bio-corrosion, therefore, are required in subsurface environments.

The SRB-controlling technology has been reported in many literatures under various SRB-related problems especially on many industry activities. The addition of disinfectant chemicals to restrain the growth of SRB has been one of the more effective processes. For example, SRB in oil recovery reservoirs have been controlled by the use of bactericides such as glutaraldehyde, molybdate ions, nitrite or nitrate [11, 20, 32, 33]. Hydrogen peroxide (H_2O_2) below 100 mg L^{-1} is often used to control the production of H_2S by SRB in water due to the chemical's non-toxic characteristics [44]. However, applying an improper substance to control SRB corrosion may result in unforeseen problems. Some chemicals like H_2O_2 are not active for very long and this results in increased labor costs because of the need for frequent dosage. Chemical compounds might be effective for the SRB control but may interfere with other chemical/biological reactions. Many commercial products used as SRB-inhibitors contain heavy metals or other toxic substances like nitrite and molybdate and these could result in significant environmental pollution [15, 33].

Magnesium peroxide (MgO_2) is used in this study in an attempt to inhibit SRB corrosion. The advantages of MgO_2 as an oxygen additive to aqueous systems are that it is harmless to decrease the sulfide concentration that uses a chemical mechanism. In abiotic oxidation, sulfide might be converted to sulfate after the addition of oxygen released from MgO_2. Sulfide also is decreased due to the formation of a precipitate with metals at high aqueous pH due to the gradual entry into solution of the MgO_2. In the biotic process, dissolved oxygen is able to promote oxidation of sulfide to sulfate by any sulfur oxidation bacteria present [39, 40]. The released oxygen is also able to inhibit SRB activity and therefore sulfate reduction because this requires strictly anoxic conditions [24, 40]. However, there is still very little information available on the possible applications of MgO_2 to the industrial control of MIC corrosion. The details of the mechanisms by which MgO_2 are able to act to control SRB needs more evaluation. For example, the influence on water quality parameters of adding MgO_2 needs to be evaluated. Furthermore changes in the bacterial community, especially the SRB population, needs to be studied in order to understand the relationship between microbial activity and water quality parameters.

The objective of this study therefore is, for the first time, to study MgO_2 as a corrosion control agent when it acts as a potential inhibitor of SRB activity in laboratory columns. Different amounts of MgO_2 additives, including reagent grade MgO_2 and the commercial product (ORC^{TM}) MgO_2, were used for SRB-control in this study and these were compared to a control without added MgO_2. Variations in the water quality such as total organic carbon (TOC), dissolved oxygen (DO), pH and ORP after adding MgO_2 were also investigated during the experiments. The dynamics of the microbial communities in the experiments were monitored by fluorescence in-situ hybridization (FISH), which has been widely used to detect the different species and the diversity of various groups of bacteria in various environments, including aquatic environments [19], activated sludge [48], biofilm systems [29] and industrial wastewater [3]. In this study, the signal detected by the phylogenetic probe EUB 338 represented the presence of microorganisms from the Domain Bacteria (Eubacteria). The phylogenetic probe ARCH 915 was used to measure the presence of the Domain Archaea. The specific probe SRB385 was applied to monitor the presence of SRB in this study, belonged to Domain Bacteria. Following the probe match program of RDPII at Michigan State University, USA, most SRB are coverage of 73.3% Order *Desulfovibrionales*, 40.6% Order *Desulfobacterales* and these can be detected by the SRB385 probe under controlled conditions [2, 34, 39]. Based on this, a positive signal with this probe reflects the presence of SRB in this study. Only a limited number of non-sulfate reducing bacteria, including several gram-positive bacteria such as *Clostridium* spp., can be detected by SRB385 [30] and the numbers of such bacteria should be low enough not to affect the results of this study. These results provide a preliminary study that will help the development of a new process for controlling SRB-induced corrosion during industrial activity.

Materials and methods

SRB enrichment and inoculation

A semi-batch incubation process was used for SRB enrichment. Flasks containing SRB and enriched-SRB medium were incubated at room temperature in the dark for 2 months. Black sulfide sediment in the flasks gradually appeared during incubation and this provided evidence of SRB growth. The SRB source was a mixture of water samples from several observation wells in the area around Tainan and Chiayii in the southwestern Taiwan where anoxic biocorrosion problems had been identified [8]. The SRB numbers were measured before column incubation to confirm the presence of SRB in the mixture and the SRB were found to range from 3.81×10^4 to 8.37×10^4 cells mL^{-1} by the FISH method (see following section). The enriched-SRB medium per liter of water was made up of Na-lactate 50% solution (4.0 mL, Showa), $MgSO_4 \cdot 7H_2O$

(0.5 g, Merck), $FeSO_4 \cdot 7H_2O$ (0.5 g, Merck), KH_2PO_4 (0.5 g, Merck), NH_4Cl (0.5 g, Merck), Na_2SO_4 (3.0 g, Merck), $CaCl_2 \cdot 2H_2O$ (0.1 g, Merck), Na-thioglycolate (0.0805 g, Showa), yeast extract (1.0 g, Merck) and ascorbic acid (0.36 g, Merck). The incubation columns contained abundant organic compounds and excess sulfate. The solution was supplied at above 2,500 mg L^{-1} TOC with carbon energy source present in the enriched-SRB medium set up to match the typical values described by Hulshoff Pol et al. [21]. Sulfate was used as the electronic acceptor for the SRB.

Column experiments

A series of one-liter polypropylene columns were used (with a diameter of 7.5 cm and a height of 39 cm) for the bench-scale batch experiments. Each experiment started with 20 ml of log phase SRB inoculation culture at an optical density of 0.7 at 660 nm (OD_{660}) added to each column. All columns were then filled with 1 L of culture medium for SRB growth. The culture medium per liter of water contains Na-lactate 50% solution (5.0 mL, Showa), $MgSO_4 \cdot 7H_2O$ (22.5 mg, Merck), K_2HPO_4 (21.75 mg, Merck), NH_4Cl (17.0 mg, Merck), Na_2SO_4 (640 mg, Merck), $CaCl_2 \cdot 2H_2O$ (27.5 mg, Merck), KH_2PO_4 (8.5 mg, Merck), $Na_2HPO_4 \cdot 7H_2O$ (33.4 mg, Merck) and $FeCl_3 \cdot 6H_2O$ (0.25 mg, Merck). The final pH of medium was in the range of 7.30–7.60. Lactate was selected as the carbon source (TOC = 2,500 mg L^{-1}) for the SRB growth because it is the best understood SRB pathway [17]. At the beginning of the experiments, nitrogen was injected into columns for 5 min creating an anoxic environment for SRB growth. Aluminum foil was used to seal the columns preventing light or air entering during incubation. When black sulfide sediment had appeared in the columns after 1–2 weeks of incubation, different concentrations of MgO_2 were added to inhibit the production of sulfide in the columns. Table 1 illustrates the detailed experimental design for addition of the chemicals in this study. Column 1 was set up as the control and did not have any added MgO_2. Reagent grade MgO_2 (10 g, 24–28% purity, Fluka, Switzerland) was added to column 2 (total concentration = 1%, w/v). Column 3 had 20 g of reagent grade MgO_2 added (total concentration = 2%, w/v). Finally, 10 g of commercial MgO_2 (1% w/v) called ORC™ (Regenesis, USA) was added to column 4. ORC™ is mainly composed of MgO_2. When the sulfate in columns was completely used up over the experimental period, more Na_2SO_4 (Table 1) was added to the columns with the aim of supplying the SRB bacteria with sufficient sulfate over the full incubation time. Hence sulfate was not a limiting factor for SRB growth in these experiments. All incubations were at room temperature, which was 26.0 ± 2.0 °C on average.

Water quality monitoring

Water quality parameters for the column experiments were monitored and recorded continuously for 60 days. At the beginning of the experiments, the frequency of sampling was once a week; later, it was twice a week. DO, pH and ORP were directly measured using electrodes at 17.4 cm below the liquid medium level of experimental columns. DO was measured by a DO meter (ATI ORION model 835) and probe (CellOX325, WTW). pH and ORP were measured by a pH/mv/TEMP meter (SP-701, Suntex) with an ORP probe (InLab® 501 Redox, METTER TOLEDO) and pH probe (InLab® 439/120 pH, METTER TOLEDO). Water parameters, including sulfide, sulfate and TOC analysis of the columns, were measured using 10 mL samples from the same location in columns, which were filtered through a 0.22-μm PVDF filter (Millipore) to remove any impurities. Each sample was replaced by 10 mL of fresh medium. Sulfide was determined by the methylene blue method using a Beckman DU® 530 spectrophotometer at 665 nm. Measurement kits, Cat. No. 1816-32 and No. 1817-32 were supplied by the manufacturer (HACH, USA). Sulfate was determined by ion chromatography analyzer using an Agilent 1100 system equipped with a 302C4.6 column (VYDAC), and an ion-suppressed CD-5 conductivity detector (Shodex). The

Table 1 Experimental design in terms of chemical additives in this study

Chemical additives	Purpose	Run			
		Column 1	Column 2	Column 3	Column 4
MgO_2	Oxygen supply for restraining SRB	None	1% MgO_2 (7th day)	1% MgO_2 (6th day) 1% MgO_2 (11th day)	1% MgO_2 (10th day) 1% ORC™ (24th day)
Lactate	Carbon source for SRB	2,500 mg L^{-1} (0th day)			
Na_2SO_4	Sulfate source for SRB	0.64 g (0th day) 0.20 g (31th day) 0.20 g (47th day)	0.64 g (0th day) 0.20 g (31th day) 0.20 g (47th day)	0.64 g (0th day) 0.20 g (47th day)	0.64 g (0th day) 0.20 g (47th day)

Table 2 Oligonucleotide probes used in this study

Probe name[a]	Name[b]	Target group	Target Site (rRNA positions)[c]	Probe sequence from 5′ to 3′	FA%[d] in Situ	NaCl[e]	Reference
EUB338	S-D-Bact-0338-a-A-18	Domain Bacteria	16S (338–355)	GCT-GCC-TCC-CGT-AGG-AGT	0	20	[3]
ARCH915	S-D-Arch-0915-a-A-20	Domain Archaea	16S (915–934)	GTG-CTC-CCC-CGC-CAA-TTC-CT	20	20	[43]
SRB385	S-*-Srb-0385-a-A-18	Most Desulfovibrionales and other SRB	16S (385–402)	CGG-CGT-CGC-TGC-GTC-AGG	30	30	[2]
NONEUB338	–	Negative control	–	ACT-CCT-ACG-GGA-GGC-AGC	20	0	[29]

[a] From the probeBases website [26]

[b] From the Oligonucleotide Probe Database [1]

[c] 16S rRNA position according to *Escherichia coli* numbering

[d] Formamide concentration in the hybridization buffer

[e] Washing buffer NaCl concentration

mobile phase was 4 mM phthalic acid (pH 4.9) at a flow rate 2.0 ml min^{-1}. Total organic carbon (TOC) is estimated by the wet oxidation method on a TOC Model 1010 Analyzer (OI Co., USA). The concentration of the sample for analysis was adjusted to below 10 mg L^{-1} by dilution. The sample was injected into the instrument where it was acidified by 5% H_3PO_4 and purged off inorganic carbon. Next, 0.42 M $Na_2S_2O_8$ was added and this oxidant quickly reacts with organic carbon in the sample at 100 °C to form carbon dioxide. The carbon dioxide from the oxidized organic carbon was purged from the solution and detected by a non-dispersive infrared (NDIR) detector that had been calibrated by potassium hydrogen phthalate (KHP) to display directly the mass of carbon dioxide detected.

Bacterial community analysis

Fluorescent probes targeting SRB were used to monitor SRB abundance in order to evaluate the influence of the different amount of MgO_2 additives on SRB numbers. For FISH, the sample was first filtered through 1.2-μm pore-size glass microfibre filters (GF/C, Whatman®) to remove any autofluorescent impurities or by-products that might confuse the experiments. Cells from the samples were collected by filtration through a 0.22-μm pore-size polycarbonate filter (Critical®, USA) and transferred to gelatin-coated slides by the addition of 5 μL of distilled deionized water to each well after which the filter was gently pressed onto the gelatin for a few seconds [4]. These air-dried filters fall onto the slides very easily. After filtration, the samples were fixed in 2 ml of paraformaldehyde-PBS (4%) for 60 min at 4 °C. Hybridization buffer (0.9 M NaCl, 0.01% sodiumdodecylsulfate (SDS), 20 mM Tris–HCl, 5 ng/μL HPSF-grade probes, pH 7.2) was added to dilute the cell sample, and then that sample was hybridized at 46 °C for 3 h. The probes were labeled with a CY3™ florescent tag (MWG-Biotech, German) at the 5′ end. Formamide was added to the buffer for probes that required more stringent hybridization conditions. Table 2 describes the selected oligonucleotide probes, hybridization conditions and relevant references. After hybridization, the cells were washed twice in washing buffer (variable NaCl, 20 mM Tris–HCl, pH 8.0, 0.01% SDS, pH 8.2) at 48 °C for 20 min. They were stained with polyphosphate for 30 min with 1 μg mL^{-1} DAPI (4′-6′-diameidino-2-phenylindole)/ddH_2O. Since DAPI can react with both DNA and RNA from the cells, the DAPI-positive cells represent the total bacterial number in the sample. Cells were visualized using a Zeiss Axioscope 2 plus epifluorescent microscope with two fluorescent filters, Zeiss No. 2 (G365 nm, FT395 nm, LP397 nm) for DAPI and Zeiss No. 15 (G546/12 nm, FT580 nm, LP590 nm) for Cy3. The microscope was attached to a charged coupled device camera (Axio Cam HR, Zeiss®). The total cell counts involved 500–1,500 bacteria

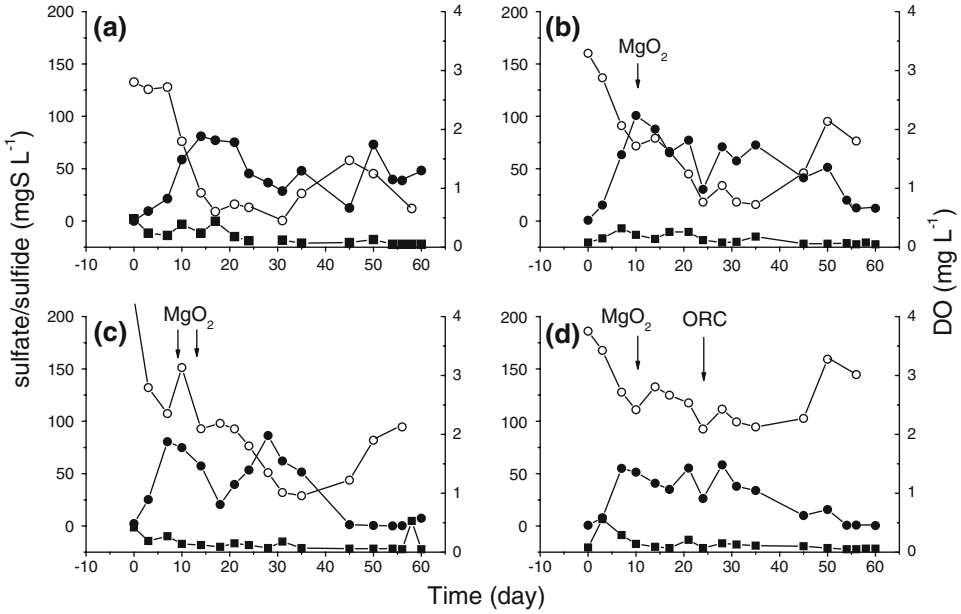

Fig. 1 Variation in concentrations of sulfate (*open circle*), sulfide (*closed circle*) and DO (*square*) with time in various columns: **a** column 1 (control), **b** column 2 (1% MgO_2), **c** column 3 (2% MgO_2), **d** column 4 (1% MgO_2/1% ORC^{TM})

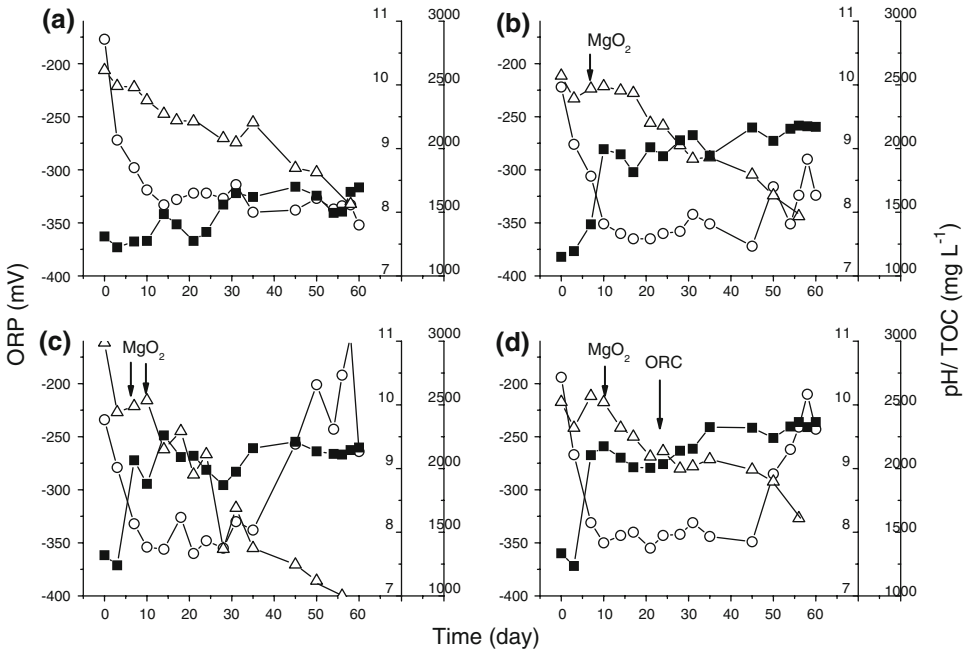

Fig. 2 Variation in concentrations of ORP (*circle*), TOC (*triangle*) and pH (*square*) with time in various columns: **a** column 1 (control), **b** column 2 (1% MgO_2), **c** column 3 (2% MgO_2), **d** column 4 (1% MgO_2/1% ORC^{TM})

per sample. Microscopic analysis included manual counting of the cells from at least ten photographs of duplicate samples.

Results

Control experiment

The variation in column 1 for sulfate concentration, sulfide concentration and water quality parameters are shown in Figs. 1a and 2a. In order to eliminate the effect of extreme values (very large or small) during the experiments, the medians of the water quality parameters are calculated. The average pH of column 1 gradually increased to its highest value (8.39) on day 60 and this can be ascribed to SRB activity. This is because SRB growth used lactate as the carbon source and the electron donor consumed hydrogen ions (H^+) from the solution as the sulfate was reduced to sulfide [31]. DO and ORP remained at 0.32 ± 0.12 mg L^{-1} and -324 ± 15 mV, respectively, which indicates that the column remained anoxic. An obvious decrease in sulfate and TOC were detected before day 31 and day 47, when extra sulfate was added. The potential for SRB biocorrosion can be indicated by either the sulfate reduction rate (SRR) or the sulfide concentration. Table 3 shows the sulfate reduction

Table 3 Water quality parameters and TOC utilization rate in experimental columns

Run	Column 1 (control)	Column 2 (1% MgO_2)	Column 3 (2% MgO_2)	Column 4 (1% MgO_2 and 1%ORC™)
Time (day)	7–17	7–31	14–35	24–45
pH	7.68 ± 0.21 (7.54–7.97)[a]	8.95 ± 0.44 (7.81–9.21)	9.18 ± 0.26 (8.74–9.52)	9.31 ± 0.25 (9.07–9.65)
ORP	−324 ± 15 (−298 to −333)	−359 ± 20 (−306 to −365)	−348 ± 13 (−326 to −360)	−343 ± 7 (−331 to −349)
Sulfide (mg-S L^{-1})	67.9 ± 27.2 (21.3–81.0)	68.0 ± 21.1 (30.3–100.7)	53.5 ± 20.2 (20.5–86.3)	33.9 ± 17.6 (10.0–58.4)
Sulfate (mg-S L^{-1})	51.6 ± 53.4 (128.0–9.0)	55.7 ± 28.1 (18.2–91.1)	76.4 ± 29.7 (28.9–98.0)	99.4 ± 7.5 (92.6–111.6)
DO (mg L^{-1})	0.32 ± 0.12 (0.20–0.44)	0.18 ± 0.09 (0.08–0.32)	0.12 ± 0.04 (0.07–0.18)	0.11 ± 0.03 (0.07–0.10)
Sulfate reducing rates (mg-S L^{-1} day^{-1})	11.95	3.08	3.80	0.30
TOC utilization rate (mg L^{-1} day^{-1})	26.08	24.86	43.20	4.37
Calculated TOC utilization rate[b] for sulfate reduction (mg L^{-1} day^{-1})	8.96	2.31	2.85	0.09

[a] The parentheses presented the range between minimum values and maximum value during the selected time

[b] The Calculated TOC utilization rate is given relative to the sulfate reducing rates

rate (SRR) and the TOC consumption rate could be calculated to be 11.95 mg L^{-1} day^{-1} and 26.08 mg L^{-1} day^{-1} in Figs. 1a and 2a, respectively, using linear regression. Sulfide increased by the growth of SRB after each sulfate addition. For example, sulfide was measured as 67.9 ± 27.2 mg L^{-1} from day 7 to day 17 after sulfate was added on the first day.

Figure 3a illustrates the changes in the bacterial community present in column 1. Eubacteria were the major bacterial Domain present and occupied 69.2 ± 13.3% of the total population throughout the experimental period (Table 4). The proportion of Archaea was low at 7.4 ± 2.6%. A specific SRB community gradually became predominant in the control column, which demonstrated that suitable simulated circumstances for SRB enriched had been set up. The proportion of SRB increased from 11.9% on day 1 up to 60.5% on day 14, while the SRB/Eubacteria ratio increased from 18.2 to 90.8%. The trend in SRB growth corresponded to the increase in sulfide, which increased from 0.07 mg L^{-1} on day 1 to 81 mg L^{-1} on day 14. The SRB median number was calculated to be 1.19×10^6 cells mL^{-1}. The SRR for each SRB in this column was calculated to be 3.14×10^{-14} mole day^{-1} SRB $cell^{-1}$, which is similar to the activity of SRB in marine sediment under anoxic conditions [46].

Column experiments with MgO_2 addition

The addition of different amount reagent MgO_2 had the effect of inhibiting the SRB population and this can be clearly seen in column 2 (1% MgO_2) and in column 3 (2% MgO_2). The sulfate concentration, sulfide concentration and water quality parameters are showed in Figs. 1b, 2b for column 2 and Figs. 1c, 2c for column 3. Table 3 shows the median observations for the water quality parameters. DO and ORP remained anoxic at 0.09 ± 0.32 mg L^{-1} and −359 ± 20 mV for column 2 and 0.12 ± 0.04 mg L^{-1} and −348 ± 13 mV for column 3, respectively. The addition of different amounts of MgO_2 changed the pH of the columns. The pH increased to 8.95 ± 0.44 in column 2 and 9.18 ± 0.26 in column 3, due to the releasing OH^- into the aqueous environment by the MgO_2. Good SRB growth control was obtained by the use of the MgO_2 additive. Sulfate in column 2/column 3 was obviously decreased during day 1 to day 35. Sulfide had also decreased in column 2/column 3 from day 10 to day 45. The decrease in sulfate and production of sulfide seemed to stop after day 50 in column 2 and after day 35 in column 3. The calculated SRRs of column 2/column 3 are obviously lower than that in column 1 (shown in Table 3). The consumed TOC was calculated to be 24.86 mg L^{-1} day^{-1} in column 2 and 43.20 mg L^{-1} day^{-1} in column 3, which indicates that the obvious consumption of organic matter still occurred.

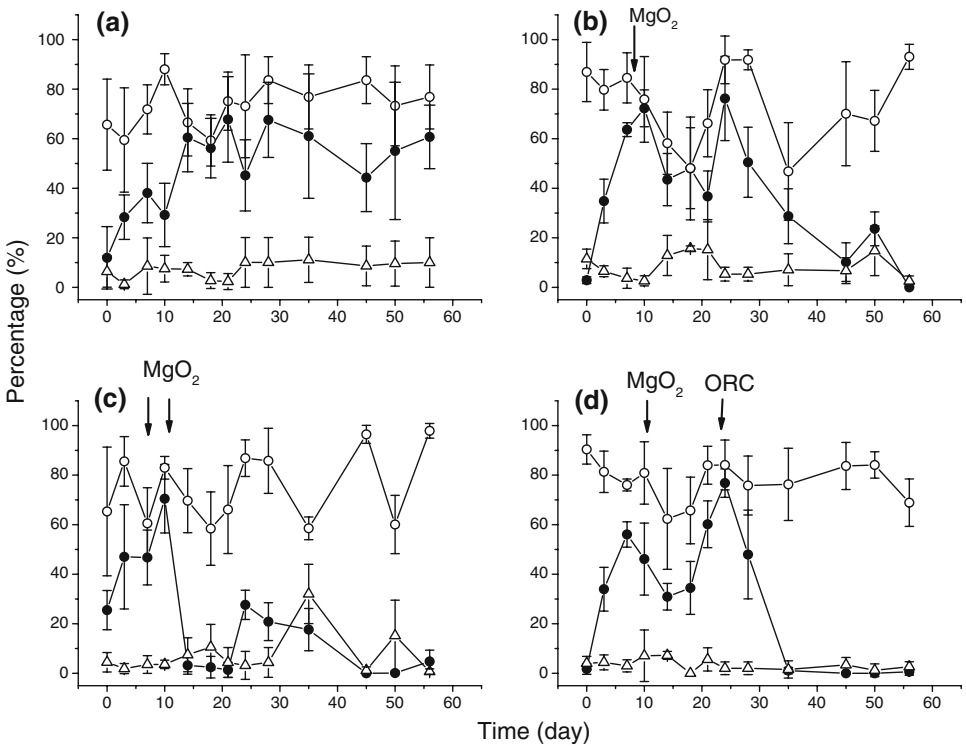

Fig. 3 Variation in the bacterial community detected by FISH with time for various columns: **a** column 1 (control), **b** column 2 (1% MgO_2), **c** column 3 (2% MgO_2), **d** column 4 (1% MgO_2/1% ORC^{TM}). SRB385 (*closed circle*), EUB338 (*open circle*), ARCH915 (*triangle*)

Figure 3c shows that Eubacteria was the major bacterial species, 75.9 ± 17.0% in column 2 and 67.9 ± 12.7% in column 3. The percentage of Archaea was very low at 5.3 ± 5.7% in column 2 and 10.0 ± 11.0% in column 3. The greater amount of MgO_2 in column 3 was able to control the SRB more effectively. The SRB slowly decreased and were undetectable (0%) by day 56 in column 2 and by day 45 in column 3. After adding 1% MgO_2, the median percentage of SRB was 50.5 ± 15.0%. The SRB/Eubacteria ratio was 66.5%. After adding 2% MgO_2, the median percentage of SRB dropped to 10.4 ± 11.3%. The SRB/Eubacteria ratio was 15.3%. Theoretically, microorganisms are sensitive to changes in surrounding pH and the rise in pH due to the addition of MgO_2 could have had an effect over the short term. However, the pH change did not affect SRB growth in this study because the SRB percentage did not decrease immediately after MgO_2 addition, while there was an immediate pH increase. Delgado et al. [12] found the SRRs of the SRB were highest over a pH range from 6.0 to 8.5. Goeres et al. [16] also found that non-alkaliphilic SRB produced hydrogen sulfide at pH 9.3 and even survived at pH 10.2. These results help to explain why SRB were not significantly affected in this study by pH.

Column experiment for ORC^{TM} addition

ORC^{TM}, contained by the food-grade phosphate [42, 45, 49], usually is applied to many organic pollutants as an in-situ bioremediation method for soil and groundwater and it provides oxygen for biodegradation over a long period. The targets have included total petroleum hydrocarbons [14], BTEX [23] and MTBE [5]. Figures 1d and 2d illustrate the variation of water quality parameters of sulfate and sulfide in column 4. A higher pH of 9.31 ± 0.25 was obtained, compared to the other columns that had reagent grade MgO_2 added. The reason for this is that as ORC^{TM} dissolves in water, it slowly and steadily releases both MgO_2 and MgO, which produce hydoxil ion (OH^-) resulting in a higher pH in column 4 [23]. Anoxic conditions were maintained and the DO and ORP values were 0.11 ± 0.03 mg L^{-1} and −343 ± 7 mV, respectively. The addition of ORC^{TM}, which retained higher sulfate (99.4 ± 7.5 mg L^{-1}), resulted in faster sulfide inhibition than the other columns. The concentration of sulfide decreased initially to 33.96 ± 17.6 mg L^{-1} and then underwent a gradual further decrease to below 1.0 mg L^{-1} on day 54, which shows that the treatment produced an effectively controlled level of sulfide. Chang et al. [7] demonstrated that the addition of ORC^{TM} was able to lower the concentration of hydrogen sulfide under SRB enrichment. Overall, 8 mg O_2 L^{-1} day^{-1} is by released 1% ORC^{TM} (w/v) in a batch reactor and 0.4% (w/v) ORC^{TM} was able to lower the production of biogenic sulfide under anoxic conditions for a period longer than 40 days. In this study, the SRR and utilized TOC in column 4 were as low as 0.12 mg L^{-1} day^{-1} and 4.37 mg L^{-1} day^{-1}, respectively, which is far lower than for columns 1, 2 and 3 over the same period (Table 3).

Eubacteria still were the most common bacterial species in column 4 and ranged 62.3–90.4% as shown in Fig. 3d. Archaea was detected with a low-ratio median of 1.6 ± 1.2%. After adding 1% (w/v) regent grade MgO_2 over the period from the day 7 to the day 18, the microbial activities of SRB were reduced from 56.1 to 34.5%. The high SRB/Eubacteria ratio of the SRB was even close to 100% on day 24, which means that the 1% reagent grade MgO_2 was not really very effective at controlling the SRB under this column. However, SRB were obviously inhibited after adding ORC™. The percentage of SRB sharply decreased from 76.8% on day 24 to 1.1% on day 35. After this time point, SRB could not be detected (0%) due to the ORC™'s persistent characteristic of releasing O_2.

Discussion

The influence of organic matter on the growth of SRB

The presence of organic matter played an important role in inhibiting the SRB when MgO_2 was added. The SRB utilize organic matter as a carbon source when reducing sulfate to sulfide. Rinzema and Lettinga [38] developed a theoretical consumption of organic matter during the reduced reaction of SRB. A chemical oxygen demand (COD)/sulfate ratio above 0.67 means there is enough organic matter for the reduction of sulfate to sulfide completely. Based on the theory of Rinzema and Lettinga, the calculated TOC utilization ascribed to the biogenic sulfate reduction is as follows: column 1 > column 2 ≒ column 3 > column 4 and this is calculated in Table 3. However, when the actual rates of TOC consumption are compared, the order is: column 3 > column 1 ≒ column 2 > column 4. The TOC consumption was obviously greater than the theoretical calculation and was still high when there was only a very small percentage of SRB present after the addition of MgO_2. This indicated that the SRB was not the only bacterial species consuming organic matter in the columns. Other bacteria were able to become predominant and use the organic matter in the columns as a carbon and/or energy source.

The percentage of TOC consumption by SRB related to the total TOC consumption is compared as following: column 1 (34.4%) > column 2 (9.3%) > column 3 (6.6%) > column 4 (2.1%), calculated by the data in Table 3. It is indicated that the proportion of TOC consumed during biogenic sulfate reduction in columns 2 and 3 is far smaller than in column 1, the control. It is possible that organic matter is metabolized by facultative microorganisms and such bacteria (especially fermentative bacteria) are usually able to uptake simple organic compounds such as formate, acetate, lactate, ethanol and hydrogen than can be used by SRB for growth [9]. Many heterotrophic bacterial species might be present in this study those are able to utilize organic matter as carbon source. Among these, Campylobacter and *Wolinella succinogenes* from the Eubacteria and *Pyrococcus furiosus* belonging to Archaea are able to generate hydrogen sulfide into environment [37]. Khanal and Huang [24] described how facultative bacteria can utilize TOC when oxygen is supplied under anoxic conditions; this is because facultative bacteria compete strongly with each other and have a low half-velocity constant for the Michaelis–Menten equation. Since the ORP and DO values remained at anoxic levels in the experimental columns, MgO_2 must release oxygen slower than the microorganisms consume it. Oxygen released from MgO_2 therefore provided the terminal electron acceptor for the facultative bacteria. The facultative bacteria belonging to the Eubacteria or Archaea would seem to utilize most of the organic matter and become the dominant species under the more oxidative state created by the addition of MgO_2 (Table 4). Maier [28] indicated that a reduction potential of +0.81 V is achieved for oxygen respiration compared to −0.22 V for sulfate reduction when the oxidation potential is the same. The redox potential difference between the two reactions automatically causes an increase in facultative bacteria and a decrease in SRB in the experimental columns. However, the low consumption of organic matter in column 4 might be due to inhibition caused by the addition of ORC™. ORC™ seems to control hydrogen sulfide production by SRB and TOC utilization by facultative bacteria at the same time. Another possible reason for this is that some SRB species might utilize other compounds for the terminal electron acceptor in the same environment but with a different redox state, such as oxygen and nitrate, both of which have a higher redox state (redox potential = −1.28 and −0.83 V, respectively) and sulfate with a lower redox state (redox potential = −0.25 V) [25]. The utilization of TOC as a carbon source by the SRB will then occur. However, the very low percentage of SRB that was detected after adding MgO_2 suggests that this mechanism is not predominant in this study (Table 4).

The significance of ORP on the inhibition of SRB

The increase in ORP seems to be strongly related to the inhibition of SRB numbers. Figure 2 shows the ORP was theoretically decreased as the SRB incubation time increased [22, 27], so the increase in ORP that is caused by MgO_2 addition may be a result of a fall in SRB activity. Thus, the increase in ORP would seem to be caused by the inhibition of SRB, rather than by the inhibition of SRB when the ORP was increased. This inference is supported by the fact that ORP always rises after the SRB become

Table 4 The percentage structure of the bacterial community in each experimental column[a]

Run	Column 1 (control)	Column 2 (1% MgO_2)	Column 3 (2% MgO_2)	Column 4 (1% MgO_2 and 1%ORC^{TM})
Time (day)	7–18	7–28	14–35	35–50
EUB338 (%)	69.2 ± 13.3	75.9 ± 17.0	67.9 ± 12.7	83.7 ± 4.4
ARCH915 (%)	7.4 ± 2.6	5.3 ± 5.7	10.0 ± 11.0	1.6 ± 1.2
SRB385 (%)	48.7 ± 15.6	50.5 ± 15.0	10.4 ± 11.3	0.4 ± 0.6

[a] The median observation and standard deviation

undetectable. Sass et al. [40] and Cypionka [10] suggested that SRB are able to reduce oxygen levels in order to maintain the inhabitability of their environment, so the ORP becomes free to increase after the SRB become extinct.

The ORP is a good index by which to interpret the effectiveness of SRB control. Relative changes in ORP are thus suitable for evaluating the effect of various SRB-inhibition strategies. Delgado et al. [12] indicated that ORP is the most important factor related to the logarithm of the sulfide/sulfate ratio according to the following equation:

$$\mathrm{ORP(mV)} = -355 - 52.68 \log \frac{[S^{2-}]}{[SO_4^{2-}]} \quad (1)$$

The variations in ORP show the same tendency as the formation of hydrogen sulfide due to SRB activity, although different amounts of MgO_2 were applied. Figure 4 shows that there is a good linear regression relationship that follows Eq. 1 when the experimental data from this study is used. The more dominant SRB will enhance sulfate reduction to sulfide and cause the ORP to become more negative. Once the SRB are inhibited by MgO_2, the reduction reaction is deceased in the columns. Postgate [35] found that biological sulfide production does not occur when the ORP exceeds −100 mV. Khanal and Huang [24] also found that the sulfide was completely removed when the ORP was elevated from −280 mV to −180 mV. Experimental columns in this study, the ORP exceeds around −300 mV, the decrease in microbial density is slowed considerably and the sulfide concentration never recovers thereafter, perhaps because of SRB decay or inhibition due to the released oxygen [40, 41]. Measurement of the ORP value might help to determine the optimal dosage of MgO_2 that needs to be used for control of biocorrosion by SRB. However, the ORP might be affected by various other factors such as pH. Thus the optimal dosage of MgO_2 that needs to be added to control the SRB will not necessarily be entirely dependent on the ORP value alone.

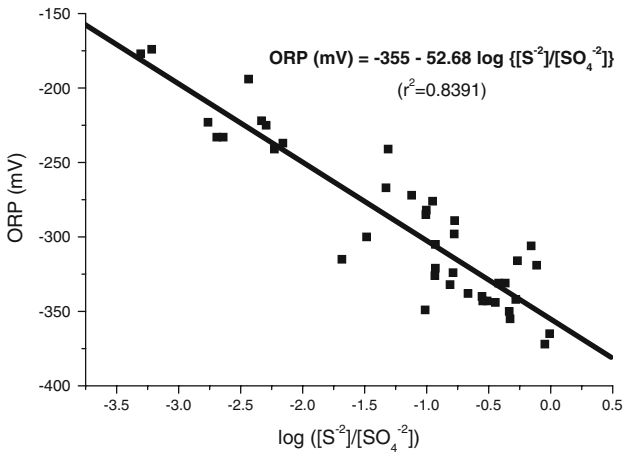

Fig. 4 The relationship of ORP and the ratio of sulfide/sulfate in this study (experimental sample numbers = 40)

Conclusions

SRB activity under anoxic condition can be inhibited by the addition of reagent grade and commercial MgO_2. The effect on water quality, such as decreased sulfide, and SRR can be observed in solution. The bacterial community was varied after the control of SRB growth. There was an increase in TOC utilization rates, which seems to indicate that the oxygen released by the MgO_2 replaces sulfate as the terminal electron acceptor and this allows facultative heterotrophic bacteria to become the dominant species through aerobic respiration. ORC^{TM} would seem to be capable of some unknown chemical mechanism that allows release of oxygen over a longer time period than reagent grade MgO_2. SRB and facultative heterotrophic bacteria were controlled very effectively by ORC^{TM}. Based on the ecology, MgO_2 can specifically control SRB and it is clear that using MgO_2 for SRB control is friendlier to the survival of bacteria in the natural environment. However, adding MgO_2 to control biocorrosion in groundwater wells needs to be very carefully considered because there is an addition of more Mg^{2+} to the groundwater. The taste of the groundwater may deteriorate if the groundwater is a drinking water source. In terms of harmlessness and an environmental friendly treatment, the application of MgO_2 for hydrogen sulfide control is worthy of further research. Further laboratory studies to demonstrate the validity of this approach in a practical situation would complement the results described here. The changes in the bacterial community, including the reversibility of SRB inhibition, should be investigated, especially after all the added

oxygen is consumed and when TOC remains available. The facultative heterotrophs present after treatment need to be identified to the species level and perhaps even further.

Acknowledgments The authors wish to thank Mr. Cheng-Yan Lin for his analysis work. This study is financially supported by the National Science Council of the Republic of China, Taiwan under the Contract No. NSC94-2211-E-236-001.

References

1. Alm EW, Oerther DB, Larsen N, Stahl DA, Raskini L (1996) The oligonucleotide probe database. Appl Environ Microbiol 62:3557–3559
2. Amann R, Stromley J, Devereux R, Key R, Stahl D (1992) Molecular and microscopic identification of sulfate-reducing bacteria in multispecies biofilms. Appl Environ Microbiol 58:614–623
3. Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl Environ Microbiol 56:1919–1925
4. Amann RI, Krumholz L, Stahl DA (1990) Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J Bacteriol 172:762–770
5. Buzea DC, DeStefanis EJ (1999) Accelerated bioremediation as an alternative to conventional remedial technologies. In: Alleman BC, Leeson A (eds) In situ bioremediation of petroleum, hydrocarbon and other organic compounds, vol 5. Battelle Press, Columbus, pp 427–432
6. Chang W-L (1994) Fractal geometry of biological and chemical aggregates in damaged wells. The fifth international conference on statistical methods for the environmental sciences, Taiwan
7. Chang Y-J, Chang Y-T, Chen H-J (2007) A method for controlling hydrogen sulfide in water by adding solid phase oxygen. Bioresour Technol 98:478–483
8. Chang Y-J, Chang Y-T, Huang H-K, Chen W-F (2004) Stagnant water variation in observation wells in Taiwan-site survey and purging investigation. J Chin Inst Environ Eng 14:25–32
9. Chapelle FH (2001) Ground-water microbiology and geochemistry, second edn. Wiley, New York
10. Cypionka H (2000) Oxygen respiration by Desulfovibrio species. Annu Rev Microbiol 54:827–848
11. Davidova I, Hicks MS, Fedorak PM, Suflita JM (2001) The influence of nitrate on microbial process in oil industry production waters. J Ind Microbiol Biotech 27:80–86
12. Delgado S, Alvarez M, Rodriguez-gomez LE, Aguiar E (1999) H_2S generation in a reclaimed urban wastewater pipe. Case study: Tenerife (Spain). Water Res 33:539–547
13. Dinh HT, Kuever J, Mußmann M, Hassel AW, Stratmann M, Widdel F (2004) Iron corrosion by novel anaerobic microorganisms. Nature 427:829–832
14. Duffy BE, Oudijk G, Guy JH (1999) Enhanced aerobic bioremediation of petroleum UST releases in Puerto Rico. In: Leeson A, Alleman BC (eds) In situ bioremediation of petroleum hydrocarbon and other organic compounds. Battelle Press, Columbus, pp 319–324
15. Flick EW (1993) Corrosion inhibitors: an industrial guide, second edn. Noyes Publications, Park Ridge
16. Goeres DM, Nielsen PH, Smidt HD, Frolund B (1998) The effect of alkaline pH conditions on a sulphate reducing consortium from a Danish district heating plant. Biofouling 12:273–286
17. Gottschalk G (1986) Bacterial metabolism, second edn. Springer-Verlag, New York
18. Hamilton WA (1985) Sulphate-reducing bacteria and anaerobic corrosion. Annu Rev Microbiol 39:289–305
19. Hicks RE, Amann RI, Stahl DA (1992) Dual staining of natural bacterioplankton with 4′,6-diamino–2-phenylindole and fluorescent oligonucleotide probes targeting kingdom-level 16S rRNA sequences. Appl Environ Microbiol 58:2158–2163
20. Hubert C, Nemati M, Jenneman G, Voordouw G (2003) Containment of biogenic sulfide production in continuous up-flow packed-bed bioreactors with nitrate or nitrite. Biotechnol Prog 19:338–345
21. Hulshoff Pol LW, Lens PNL, Stams AJM, Lettinga G (1998) Anaerobic treatment of sulphate-rich wastewater. Biodegradation 9:213–224
22. Iverson WP (2001) Research on the mechanisms of anaerobic corrosion. Int Biodeter Biodeg 47:63–70
23. Kao CM, Borden RC (1994) Enhanced aerobic degradation of gasoline contaminated aquifer by oxygen-releasing barriers. In: Hinchee RE, Alleman BC, Hoeppel RE, Miller RN (eds) Hydrocarbon bioremediation. Lewis Publishing, Boca Raton, pp 262–266
24. Khanal SK, Huang J (2003) ORP-based oxygenation for sulfide control in anaerobic treatment of high-sulfate wastewater. Water Res 37:2053–2062
25. Krekeler D, Teske A, Cypionka H (1997) Strategies of sulfate-reducing bacteria to escape oxygen stress in a cyanobacterial mat. FEMS Microbiol Ecol 25:89–96
26. Loy A, Horn M, Wagner M (2003) ProbeBase—an online resource for rRNA-targeted oligonucleotide probes. Nucleic Acids Res 31:514–516
27. Lyew D, Sheppard J (2001) Use of conductivity to monitor the treatment of acid mine drainage by sulphate-reducing bacteria. Water Res 35:2081–2086
28. Maier RM, Pepper IL, Gerba CP (2002) Environmental microbiology. Academic Press, San Diego
29. Manz W, Amann R, Ludwig W, Wagner M, Schleifer KH (1992) Phylogenetic oligodeoxynucleotide probes for the major subclasses of proteobacteria: problems and solutions. Syst Appl Microbiol 15:593–600
30. Manz W, Eisenbrencher M, Neu TR, Szewzyk U (1998) Abundance and spatial organization of Gram-negative sulfate-reducing bacteria in active sludge investigated by in situ probing with 16S rRNA targeted oligonucleotides. FEMS Microbiol Eco 25:43–61
31. Marchal R, Chaussepied B, Warzywoda M (2001) Effect of ferrous ion availability on growth of a corroding sulfate-reducing bacterium. Int Biodeter Biodeg 47:125–131
32. Nemati M, Jenneman GE, Voordouw G (2001) Mechanistic study of microbial control of hydrogen sulfide production in oil reservoirs. Biotech Bioeng 74:424–434
33. Nemati M, Mazutinec TJ, Jenneman GE, Voordouw G (2001) Control of biogenic H_2S production with nitrite and molybdate. J Ind Microbiol Biotech 26:350–355
34. Okabe S, Itoh T, Satoh H, Watanabe Y (1999) Analysis of spatial distributions of sulfate-reducing bacteria and their activity in aerobic wastewater biofilms. Appl Environ Microbiol 65:5107–5116
35. Postgate JR (1984) The sulphate-reducing bacteria, second edn. Cambridge University Press, Cambridge
36. Poulton SW, Krom MD, van Rijn J, Raiswell R (2002) The use of hydrous iron (III) oxides for the removal of hydrogen sulphide in aqueous systems. Water Res 36:825–834
37. Prescott LM, Harley JP, Klein DA (2005) Microbiology, sixth edn. McGraw Hill, New York
38. Rinzema A, Lettinga G (1988) Anaerobic treatment of sulfate containing waste water. In: Wise DL (ed) Biotreatment systems, vol 3. CRC Press Inc, Boca Raton, pp 65–109

39. Santegoeds CM, Ferdelman TG, Muyzer G, de Beer D (1998) Structural and functional dynamics of sulfate-reducing populations in bacterial biofilms. Appl Environ Microbiol 64:3731–3739
40. Sass AM, Eschemann A, Kühl M, Thar R, Sass H, Cypionka H (2002) Growth and chemosensory behavior of sulfate-reducing bacteria in oxygen-sulfide gradients. FEMS Microbial Ecol 40:47–54
41. Schäfer D, Ebert M, Köber R, Plagentz V, Dahmke A (2006) Kinetics of oxygen release from ORC. Bioremediation J 10:71–82
42. Schmidtke T, White D, Woolard C (1999) Oxygen release kinetics from solid phase oxygen in Arctic Alaska. J Hazard Mater B6:157–165
43. Stahl DA, Amann R (1991) Development and application of nucleic acid probes in bacterial systematics. Wiley, Chichester
44. Tomar M, Abdullah T (1994) Evaluation of chemicals to control the generation of malodorous hydrogen-sulfide in wastewater. Water Res 28:2545–2552
45. US EPA (2000) Engineered approaches to in situ bioremediation of chlorinated solvents: fundamentals and field applications, EPA 542-R-00-008, Washington DC
46. Vester F, Ingvorsen K (1998) Improved most-probable-number method to detect sulfate-reducing bacteria with natural media and a radiotracer. Appl Environ Microbiol 64:1700–1707
47. Voordouw G, Armstrong SM, Reimer MF, Fouts B, Telang AJ, Shen Y, Gevertz D (1996) Characterization of 16S rRNA genes from oil field microbial communities indicates the presence of a variety of sulfate-reducing, fermentative, and sulfide-oxidizing bacteria. Appl Environ Microbiol 62:1623–1629
48. Wallner G, Erhart R, Amann R (1995) Flow cytometric analysis of activated sludge with rRNA-targeted probes. Appl Environ Microbiol 61:1859–1866
49. White C (1993) A study of the impact of monitoring well purging and filtering techniques on metals concentrations in groundwater samples from the auburn road landfill site in Londonderry, N.H. In: Kerr RS (eds) Ground water sampling—a workshop summary, US EPA, Dallas, Texas, EPA 600-R-94-205
50. Yamanaka T, Aso I, Togashi S, Tanigawa M, Shoji K, Watanabe T, Watanabe N, Maki K, Suzuki H (2002) Corrosion by bacteria of concrete in sewerage systems and inhibitory effects of formates on their growth. Water Res 36:2636–2642