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Biogenic sulfuric acid attack on different types of commercially produced concrete sewer pipes

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ABSTRACT

Laboratory experiments were conducted to compare the degradation of low and high quality concrete under conditions simulating sewer pipes with and without bacteria. Small concrete samples were exposed to hydrogen sulfide, multiple species of bacteria found in corroding sewer pipes and artificial wastewater. Experiments without bacteria were used as controls. The corrosion rates of the concrete samples exposed to bacteria over 227 days were 0.08 mm/yr (millimeters per year) for the concrete from a domestic manufacturer with moderate strength and a lower water–cement ratio (Low-w/c) versus 0.208 mm/yr for the concrete samples from a foreign country with low strength and a higher water–cement ratio (High-w/c). The (Low-w/c) concrete was more resistant to the biodegradation even though a lower pH was attained for its bioactive systems. Experiments showed the influence of biogenic sulfuric acid production on short term corrosion rates.

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1. Introduction

Rapid deterioration of concrete structures in sewage collection systems has a major impact on environmental health and safety in countries with warm climates. American Society of Civil Engineers (ASCE; Reston, VA) conducted an evaluation of the wastewater infrastructure in the United States in 2005. Reported poor performance was based on information from the U.S. Environmental Protection Agency (EPA) that approximately $3.2\times10^9\,m^3$ of untreated wastewater and storm water were released annually as combined sewer overflows, along with another 11.4×10^6 to $37.9\times10^6\,m^3$ of untreated wastewater released each year as sanitary sewer overflows. ASCE's report also cites a 2002 EPA estimate that the United States will need 390 billion dollars during the next 20 years to repair and replace the existing wastewater infrastructure and construct new facilities, including collection systems. Similar damage to the infrastructure has also been reported in such countries as Germany [1], Japan [2,3] and Belgium [4]. Thorough understanding of major factors contributing to infrastructure deterioration is absolutely essential, and microbiological activity is an important contributor.

Microorganisms that produce sulfuric acid accelerate the deterioration of concrete sewer pipes in a process termed “Microbially Induced Concrete Corrosion” (MICC). The theory describing MICC has been under development since 1900, when Olmstead and Hamlin first mentioned it in the literature [5]. In their study, a concrete sewer pipe was converted to a white pasty material. This conversion was theorized to be a result of the chemical attack by hydrogen sulfide. Sulfate reducing bacteria (SRB, e.g., *Desulfovibrio* sp.) are present in the bottom part of the pipelines and produce hydrogen sulfide under anaerobic conditions by reducing oxidized sulfur compounds. In 1945, five strains of *Thiobacillus* were isolated from the surface of corroding concrete and their ability to produce acid and association with concrete corrosion were confirmed [6]. Others have extensively studied the corrosion process in sewer pipes and pipeline junctions [3,7]. These tests demonstrated that the highest deterioration occurred when high concentrations of hydrogen sulfide, moisture and oxygen were present, and these high concentrations were found to increase *Thiobacillus* activity.

Research to simulate and investigate the microbial corrosion process as well as abiotic attack has been performed in a variety of studies, using different chambers. Sand et al. [1] built a chamber in Hamburg for microbiological corrosion tests with control of temperature, aqueous nutrients and humidity. They measured corrosion rate based on mass loss of concrete specimens. Mori, et al. [2,3] also used a simulation chamber; in their experiments, the corrosion rate was determined by measuring the decrease of cross section of mortar specimens.

MICC progresses through three distinct stages which must be understood before modeling of MICC can be accurate enough for practical use. In the first stage, chemical reactions with CO_2 and other acidic gasses in the headspace of sewers cause the pH of moisture,

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condensing on virgin concrete materials, to drop from values of about 13 to about 9 [8]; this pH drop is the result of abiotic processes, and no microorganisms have been associated with the initial stage of MICC. H_2S diffuses into the surface layer of the concrete and is abiotically oxidized to produce thiosulfuric and polythionic acids, both of which contribute to the neutralization of the concrete surface [8]. Studies have recently characterized the rate and extent of this initial chemical pH reduction for three different concrete mixes [9]. The first stage usually completes its pH decline within 1 to 8 weeks after incubation in the presence of common sewage headspace gasses.

The second stage of MICC is the colonization on the moist pipe crown surfaces by aerobic, autotrophic sulfur oxidizing microorganisms (SOM), the inoculum for which comes from the sewage [10]. Sewage splashes onto the pipe surfaces above the water level at frequent intervals, and from this inoculation a population of Neutrophilic Sulfur Oxidizing Microorganisms (NSOM) establishes itself above the sewage flow. This population will then utilize the reduced sulfur compounds emitted from the sewage and produce elemental sulfur and polythionic acids that further lower the pH of the concrete surface to below neutral conditions [8]. As the pH of the concrete drops, NSOM are theorized to continue colonization, producing even more acid until low pH (4–5) limits their growth. Beyond this, an Acidophilic Sulfur Oxidizing Microorganism (ASOM) population is established, which continues the oxidation of H_2S into sulfuric acid and can sustain this autotrophic oxidation at pH levels below 3. There is very little quantitative information to help in understanding the second stage of the corrosion process, which may be the rate-limiting step in MICC. The theory explained above has been put forth based on the pH growth preferences of ASOM and NSOM isolates cultured from sewer environments and the end products of their metabolism.

The third stage of concrete corrosion requires the establishment of acidophilic microorganisms (ASOM) in the biofilm; their subsequent growth and acid production is the actual cause of sustained concrete corrosion due to solubilization. $CaSO_4$ can react with Ca aluminate present in the cement mixture and ettringite can be formed. Ettringite $((CaO)_3 \cdot Al_2O_3 \cdot (CaSO_4)_3 \cdot 32H_2O)$ is an expansive product and can lead to increased internal pressure resulting in small cracks. It changes into gypsum $((CaSO_4) \cdot 2H_2O)$ when the concentration of sulfate is increasing and pH is decreasing [11]. Gypsum and ettringite may be removed by the flow of sewage thereby accelerating the corrosion process [3]. The ASOM are presumed to move into the concrete with the corroding front [12], but there is very little information to delineate this stage in the process.

Peccia et al. and Hernandez et al. [13,14] developed fluorescently labeled oligonucleotide probes complimentary to the 16sRNA of selected members of the genus *A. thiooxidans*, (reclassified as *Acidithiobacillus* [15]), *Thiobacillus ferrooxidans* and the entire *Acidiphilium* genera. With these probes, fluorescent *in situ* hybridizations (FISH) were used to enumerate these bacteria in homogenized biofilm samples taken from the corroding crowns of concrete sewer collection systems in Houston, Texas. As judged by FISH analyses with species-specific probes and a domain-level probe that recognizes all bacteria (Eub338), *T. ferrooxidans* and *Acidithiobacillus thiooxidans* comprised between 12% and 42% of the total active bacteria present in corroding concrete samples; *Leptosprillium* spp. was absent. These results are consistent with the narrow diversity of 16sRNA clone libraries compiled from acidic biofilm samples taken from corroding sewers in Toronto, Canada, Las Vegas, NV and Boulder, CO [14].

The objective of the experiment was to compare MICC of a domestic sewer pipe with a lower quality pipe material obtained from a foreign country. The experimental approaches described in this paper simulated conditions found in sewer collection systems with the use of artificial wastewater, hydrogen sulfide containing headspace and inoculation with *Thiobacillus* species. The corrosion attack was modeled as a cyclic process, where batches of artificial wastewater were changed when the system reached stable conditions.

2. Materials and methods

2.1. Materials

2.1.1. Concrete samples

Several concrete samples were obtained *in situ* and from companies which produce sewer collection pipelines. Two different types of concrete were selected for the experiments, named for this study as (High-w/c) and (Low-w/c) to signify relative difference in compositions. The (High-w/c) concrete is a higher-weight, low quality material that was obtained from a foreign country. Tables 1 and 2 show the reported composition of cement and the concrete types, and Fig. 1 shows a visual comparison of the concrete samples. Most of the values in the tables were reported as typical of locally manufactured pipes or provided by the manufacturer, but not verified. We note that the reported w/c ratio of the (High-w/c) concrete does not match the calculated value. The concrete samples were cut with a water-cooled diamond saw into rectangular shapes with dimensions of 5 × 2 × 2 cm. Bulk densities were measured as 1.883 ± 0.058 g/mL and 2.362 ± 0.029 g/mL for the (High-w/c) and (Low-w/c) concrete samples, respectively. Each block was tied with a thin inert plastic string on the top so the sample could be suspended partially submerged in synthetic wastewater. Natural colors with epoxy glue (black, red, green and white) were used to identify each side of the concrete sample.

2.1.2. Microorganisms

The microorganisms were pure cultures of sulfur oxidizers, isolated from sewer collection systems. *A. cryptum* (ATCC 33463)—a heterotrophic acidophilic bacteria, was of particular interest in these

Table 1
Chemical and compound composition of cement, %, as provided by manufacturer.

	Type I	Type II
CaO	64.4	63.6
SiO_2	20.9	21.7
Al_2O_3	5.2	4.7
MgO	2.8	2.9
Fe_2O_3	2.3	3.6
SO_3	2.9	2.4
C_3S	55	41
C_2S	19	29
C_3A	10	7
C_4AF	7	15
Insol residue	0.2	0.4
Loss on ignition	1	0.8

Table 2
Composition and characteristics of original concrete.

Concrete source	Foreign	Domestic
Label	High-W/C	Low-W/C
Cement type	I	II
Composition per m^3		
Cement (kg)	368^r	320^p
Rock 3/8 in (kg)	593^r	654^p
Rock 3/4 in (kg)	89^r	555^p
Sand (kg)	926^r	846^p
Water (L)	155^r	105^p
Admixture (L)	0.44^r	1.65^p
Air (%)	7.5^r	2^p
W/C ratio	$0.42^c/0.53^r$	$0.33^c/0.34^p$
Density (kg/m^3)	$2380^c/2360^m$	$1970^c/1880^m$
Diameter (m)	0.304^r	$0.304–0.914^p$
Compressive strength 28 day (MPa)	$20.6–27.5^r$	$27.5–41.3^p$
Water absorption (%)	–	$8.5–9^p$

^{m}Measured, ^{c}calculated, ^{p}provided, and ^{r}reported as typical local characteristics.

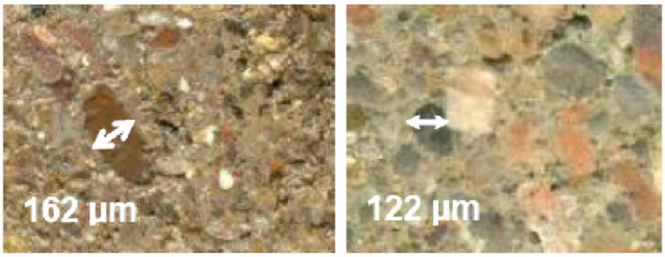

Fig. 1. Optical comparison of concrete samples (High-w/c) (left) and (Low-w/c) (right). The dimension of one of the aggregate pieces has been shown in each picture.

studies because of its frequent occurrence in sewer collection systems and the capacity to grow at low pH, providing good environmental conditions for the activity of Sulfur Oxidizing Microorganisms. *Acidiphilium cryptum* was grown in LHET2 medium [16].

Thiobacillus neopolitanus (ATCC 23641) and *Thiobacillus thioparus* (ATCC 23646) were used as the autotrophic, neutrophilic sulfur oxidizing strains. These were grown in S6 medium for *Thiobacillus* NSOM with pH adjusted to 7.5. *A. thiooxidans* (ATCC 8085), a strongly acidophilic, obligatory autotrophic Thiobacilli, was grown in medium S6 for *Thiobacillus* ASOM. All cultures were obtained from the American Type Culture Collection (ATCC) and grown in media according to methods from the microbial growth media handbook [16]. Prepared media were distributed to sterile flasks as needed. The growth of all these microorganisms was at room temperature (23 ± 2 °C).

2.1.3. Culture media

Concrete blocks were incubated in a 0.5% artificial wastewater. The solution contained per liter: trypton oxoid 0.175 g, meat extract 0.095 g, $NaHCO_3$ 0.265 g, urea 0.23 g, NaCl 0.055 g, $CaC1_2$ 0.0265 g, $MgSO_3 \cdot 7H_2O$ 0.0175 g, $K_2HPO_4 \cdot 3H_2O$ 0.001835 g [17]. For controls, a solution of artificial wastewater with 1 g/L of sodium azide was used. The azide was added to prevent growth of bacteria in the abiotic controls. pH of solutions was between 7 and 8. Tests were performed in two cycles. A new test cycle started when the liquid in the test flask was fully replaced.

2.1.4. H_2S-atmosphere

Hydrogen sulfide was produced by adding 0.043 M Na_2S and 0.02 M HCl solutions using a peristaltic pump. The solutions were mixed and the gas product was removed from the generator chamber using an air pump (GAST, model DOA-P161-AA) to ensure a constant and continuous flow rate. This procedure allowed feeding H_2S/air-mixture into corrosion chambers, which contained between 300 and 600 ppm of H_2S.

2.2. Methods

2.2.1. Simulation test

Based on previous optimizations [18] where concrete was exposed to *Thiobacillus* and sulfur–sodium thiosulfate as substrates, the resistance of different types of concrete to biodeterioration, Table 3, was studied using artificial wastewater to simulate conditions in the real sewer collection systems. The experimental designs were similar to those previously described by [1,3,19,20].

Each reactor was a glass Erlenmeyer flask with a total internal volume of 1.0 L, a liquid volume of 600 mL, and a gas volume of 400 mL. A single concrete specimen 5 × 2 × 2 cm was suspended vertically along the longest dimension in each reactor using a nylon fishing line and half-submerged in the 600 mL of liquid at the bottom of the reactor. Concrete samples were exposed to wastewater which contained active cultures to determine the effects of their metabolism on different mixtures. Observations were performed at 25 °C for 227 days in systems containing these corrosion-inducing cultures. Separate reactors had a common inlet of hydrogen sulfide and a common outlet with two sampling ports. The reactors were placed on a B1–1000 multi-position magnetic stirrer set at speed 3, Fig. 2. A control for each concrete type was implemented by a concrete block suspended in inactivated artificial wastewater.

Inoculums of SOM and heterotrophs were added at the beginning of each cycle. There was initially significant heterotrophic growth and little SOM activity evident using full-strength artificial wastewater (data not shown). After 80 days, the samples were removed and the media replaced with 5% artificial wastewater. Therefore, data from the first 80 days have been omitted from the Results section. Approximately every 10 days, liquid samples were taken with a syringe using a sample port at the top of each reactor. The total liquid volume removed per cycle was less than 10% of the original reactor's volume. The media were changed, when stable conditions for pH, INT (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl terazolium chloride) and sulfate were reached. Thus the same concrete specimen was corroded in sequential experimental cycles.

2.2.2. Concrete corrosion rate measurements

The corrosion rate of the concrete blocks was measured using scanner-based image analysis—a non-destructive method which was developed to track changes in concrete samples (details described in [24]). Deposits and microbial slimes were physically removed from concrete surface, and the 4 sides of the concrete scanned at 3200 dpi resolution. The digital images were processed with Matlab™ (Image Processing Toolbox). A calibration curve, that correlated shadow length (in number of pixels) to the depth (in centimeters), was used to estimate the depth of the pits by measuring the lengths of shadows. A script in Matlab™ selected 1.95 by 1.38 cm^2 area, filtered the image to the gray scale, and quantified the average of depths of pits per time to estimate corrosion rates. The error in this method was primarily due to the angle of the incident light. In this research the angle was about 85°; therefore, most of the features in the surface were taken into account. The thickness of the concrete block was determined by the mean value of 4 measurements for each concrete sample. For each concrete composition and condition, the mean values of the thickness of three samples were determined.

Gravimetric measurements were performed, where the excess of media was first removed from the samples and then the samples were weighed immersed in a 250-mL glass beaker containing 180 mL of ultra pure Milli-Q water. This provided more accurate estimate for weight of the sample because the concrete dried rapidly when exposed to the atmosphere. Earlier tests found that the (Low-w/c) concrete weight loss due to air drying was at an approximate rate of 14.89 mg/s and at 113.24 mg/s for (High-w/c) samples.

Table 3
Summary of experimental variables.

Name/replicates	Bacterial culture	Comments
WW–(Low-w/c)/2	None (artificial wastewater + azide)	Control
SOM–(Low-w/c)/3	NSOM + ASOM + heterotroph: *T. neopolitanus* (ATCC 641) *T. thioparus* (ATCC 23646) *A. thiooxidans* (ATCC 8085) *A. cryptum*, (ATCC 33463)	
WW–(High-w/c)/3	None (artificial wastewater + azide)	Control
SOM–(High-w/c)/3	NSOM + ASOM + heterotroph: *T. neopolitanus* (ATCC 641) *T. thioparus* (ATCC 23646) *A. thiooxidans* (ATCC 8085) *A. cryptum*, (ATCC 33463)	

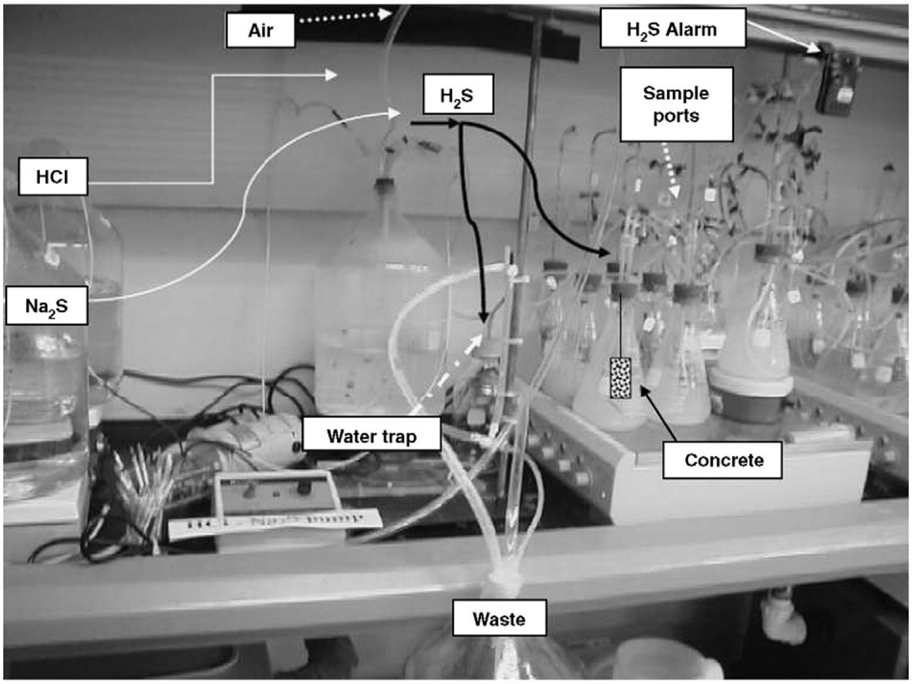

Fig. 2. Photograph of the experimental setup.

3. Results

3.1. Hydrogen sulfide

Differences in the inlet and effluent H_2S concentrations from the experimental system are presented in Fig. 3. There was relatively high diffusion of gas into concrete blocks at the beginning of the experiment (550 ppm), which decreased to 190 ppm once the concrete reached its apparent saturation equilibrium, at less than or equal to 100 ppm for the first cycles. For the second cycle, an initial diffusion of 220 ppm was detected followed by a stable period with diffusion of 50 ppm. The incubation of concrete specimens in an atmosphere with hydrogen sulfide resulted in formation of sulfur-substrates by oxidation on the surface of concrete.

3.2. Dehydrogenase activity

INT activity results are summarized in Fig. 4. The results only include bacteria in suspension and not associated with concrete surfaces. In cycle 1, rapid increase in activity occurred in 10 days, and then reached a stationary phase. In cycle 2, a higher activity was reached over 45 days and then decreased. (Low-w/c) concrete with different bacteria showed that the inoculated systems had much higher INT activity than controls, that showed little evidence of bacterial growth; whatever activity was present was likely due to the heterotrophic bacteria in the concrete sample. The INT activity measured in the controls indicated that 1 g/L azide was not completely successful in eliminating bioactivity. Overall, a paired *t*-test did not show a significant difference ($p = 0.67$ and $p = 0.27$) with respect to the controls (SOM—(Low-w/c):WW—(Low-w/c)) and (SOM—(High-w/c):WW—(High-w/c)), respectively.

As shown in Fig. 4, the bacteria applied to (High-w/c) concrete induced an exponential increase in activity over 20 days, then reverted to stationary phase for a short period of time and finally exhibited a decay in activity. Very little growth occurred during the second exposure cycle. There was apparently higher bacterial activity associated with the (High-w/c) concrete during the first cycle and with declining activity during the second one, but these were not statistically significant ($p = 0.08$ cycle 1, $p = 0.21$ cycle 2).

3.3. MPN

Because the INT method quantifies total dehydrogenase activity an MPN method was used in conjunction to distinguish the relative activity of different microbial populations. Fig. 5 presents the results of the enumeration of the (Low-w/c) concrete systems exposed to NSOM, ASOM and heterotrophic bacteria. The results correlate with the pH

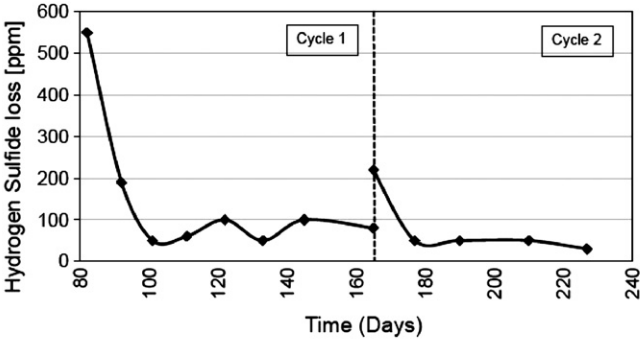

Fig. 3. Transfer of hydrogen sulfide between the headspace and the concrete, showing the total diffusion in the system in both cycles.

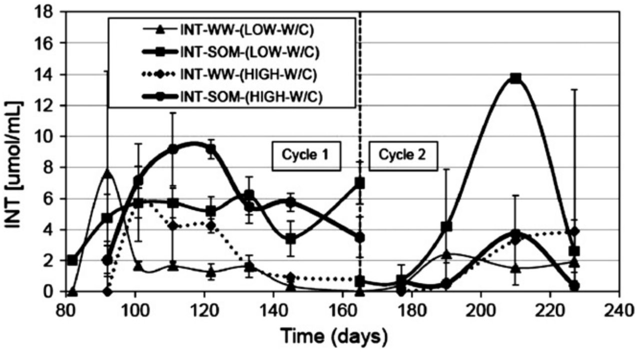

Fig. 4. Average formazan production due to activity of microbial populations in each system.

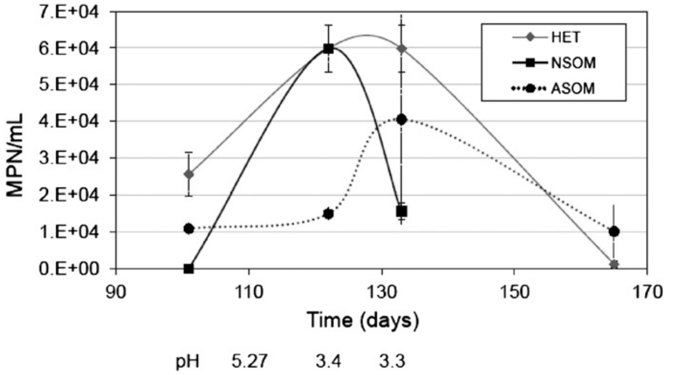

Fig. 5. Cycle 1, concentration of NSOM, ASOM and heterotrophic bacteria measured as Most Probable Number and pH over time.

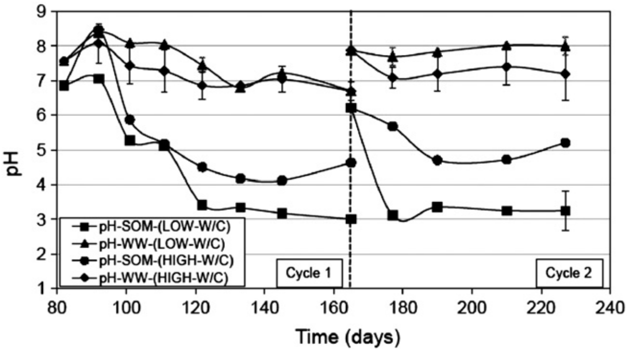

Fig. 7. Profiles of pH measurements of the solution during two cycles.

presented at the bottom of the graphs, where heterotrophic bacteria could grow at pH around 5 as well as neutrophilic bacteria, and are associated with a pH drop which then results in the enhanced growth of ASOM. The INT was compared with MPN's, showing a poor correlation as the INT seemed to indicate a slightly higher activity, Fig. 4.

3.4. TOC, DO, and pH

The decrease of total organic carbon (TOC) in the respective systems is shown in Fig. 6. There was a rapid TOC decrease in media containing SOM—in 10 days there was a decrease from 7 to 2 ppm. There was organic carbon uptake, likely associated with *A. cryptum* inoculated at the beginning of the cycle. Similar TOC uptake trends were observed in the second cycle. A paired *t*-test did show a significant difference in the TOC, ($p=0.44$, and $p=0.36$) with the controls for (SOM—(Low-w/c):WW—(Low-w/c)) and (SOM—(High-w/c):WW—(High-w/c)), respectively.

Den Ende, et al. [26] demonstrated that with a low ratio of oxygen/sulfide (<1.6), there was not enough oxygen to oxidize all sulfide completely to sulfate, therefore *Thiobacillus* oxidized part of the sulfide to sulfur and thiosulfate. The presence of sulfur was observed on the concrete surface, and the dissolved oxygen data demonstrated that the oxygen concentration in the liquid media was in the range of 3 to 6 mg/L. Dissolved oxygen levels were lower in the bacteria-seeded conditions (average DO = 4.76 mg/L) as compared to corresponding controls (average DO = 5.35 mg/L). No statistically significant difference was present during cycle 1 between the different types of concrete ($p=0.68$ and 0.05), respectively.

Fig. 7 shows the profile of pH measurements as an additional indication of biological activity. The pH of the solution inoculated with SOM dropped from 6.87 to 3.42 over 40 days followed by a period of slowly declining pH for the next 45 days. This pH drop was consistent with the production of sulfuric acid by the *Thiobacillus* sp. In the second cycle the pH dropped even faster, from 6.22 to 3.12 in 12 days. Compared to the biokinetic studies [27], the pH drop in both cycles using SOM relates to the activity of *Thiobacillus* species, as the pH is favorable for the species except *A. thiooxidans*, which grows, but the growth conditions were not optimal. The NSOM activity was likely due to *T. thioparus* based on the final pH being as low as 4.5.

The different types of concrete that were exposed to the same groups of bacteria showed similar pH reduction trend. However, the minimum pH of 4.12 with (High-w/c) concrete was significantly higher than that associated with (Low-w/c) concrete. A significant difference in the pH change occurred due to the concrete type based on a paired *t*-test ($p=0.011$ and $p=0.0077$).

The controls showed a slight decreasing trend of pH. This could be explained by the H_2S from the headspace reacting with the concrete. Roberts, et al. [10] determined the rate of pH reduction in concrete samples incubated in hydrogen sulfide gas concentrations of 50, 100 and 250 ppm. They found a correlation between pH and hydrogen sulfide, with a reduction of 4.34×10^{-5} pH/day. When the concrete was exposed to concentrations higher than 50 ppm in the headspace, the initial pH reduction was completed in a period of 60–90 days. In our experiments concentrations in the headspace were between 300 and 600 ppm and these concentrations dropped the pH more rapidly; the pH change rate in the experiments in the wastewater control in cycle 1 was 0.01 pH/day. Overall, a paired *t*-test showed a significant difference ($p=1.6\times10^{-4}$, and $p=5\times10^{-4}$) using the controls for (WW—(Low-w/c):SOM—(Low-w/c)), and (WW—(High-w/c):SOM—(High-w/c)).

3.5. Sulfate

One of the main byproducts of SOM activity is sulfate. Profiles of the measured concentrations of sulfate in solution are presented in Fig. 8. For the (Low-w/c) concrete inoculated with all bacterial species,

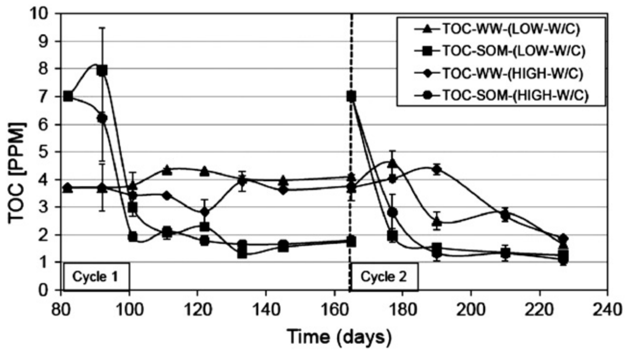

Fig. 6. Change in total organic carbon content in the liquid media when using SOM, controls and different types of concrete.

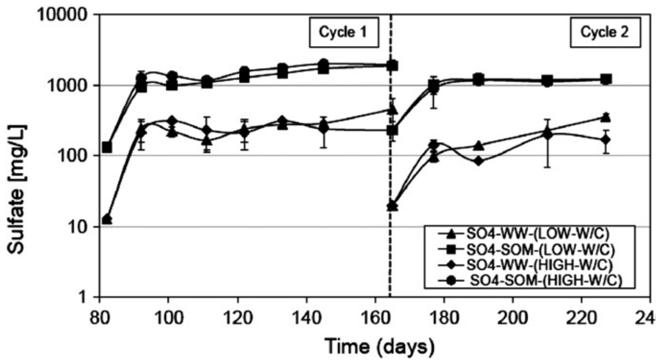

Fig. 8. Oxidation processes of hydrogen sulfide during corrosion. The profile of sulfate released during two cycles using different types of concrete is presented.

the sulfate concentration increased after 10 days to 939 mg/L, reaching the maximum of 1.85 g/L. For the control samples, the sulfate concentrations started at 12 mg/L and increased to 237 mg/L; this was likely due to the abiotic oxidation of the hydrogen sulfide added to the system. Control samples had no significant difference comparing the slopes; those systems had no bacterial activity and consequently were not considerable in the sulfate production in the solution. Overall, a paired *t*-test did not show a significant difference ($p = 3.8 \times 10^{-4}$, and $p = 2 \times 10^{-4}$) using the controls (WW—(Low-w/c):SOM—(Low-w/c)) and (WW—(High-w/c):SOM—(High-w/c)). An emerging pattern could be observed during the second cycle for the concrete exposed to SOM. The sulfate concentration profile was statistically different from cycle 1 with both concrete types ($p = 0.003$ and $p = 0.07$).

The potential formation of sulfate (from sulfide) was calculated based on stoichiometry and compared with the measured sulfate. The actual sulfate concentration was 12.2% of the predicted maximum for the low pH pathways (hydrogen sulfide–sulfur–sulfate) and 35% of the moderate pH pathway (hydrogen sulfide–thiosulfate–sulfate). These results indicate that sulfate may react in the formation of polythionic acids and may also react with calcium of the concrete forming gypsum.

3.6. Calcium

The amounts of calcium liberated in bioactive systems were significantly higher than for the controls. The maximum calcium release was 840 mg/L in the first cycle and 1060 mg/L in the second cycle for SOM.

On the other hand, a maximum of 600 mg/L calcium was released under the control conditions. This indicates that the control samples were not affected by the sodium azide solution. Overall, a paired *t*-test did show a significant difference except for the (High-w/c) concrete ($p = 1.5 \times 10^{-6}$, and $p = 0.079$) using the controls for (WW—(Low-w/c):SOM—(Low-w/c)) and (WW—(High-w/c):SOM—(High-w/c)), respectively. The different types of concrete exposed to SOM indicate that the released concentration of calcium was higher for the (Low-w/c) concrete in cycle 2, but similar in cycle 1, Fig. 9. A comparison between the two different concrete types was difficult due to the likely different initial calcium contents. These data reflected microbial activity, but did not provide quantitative information for the reduction of thickness in the sample. The calcium concentration profile from the different types of concrete was not statistically significant based on a paired *t*-test in the cycle 1 ($p = 0.07$ and $p = 0.0027$).

3.7. Concrete thickness changes

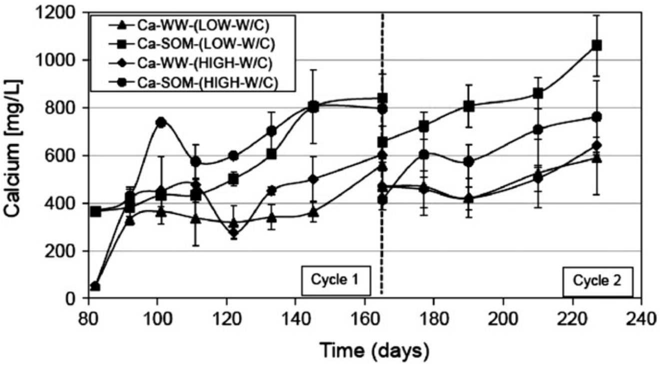

Fig. 9. Amount of calcium released during two cycles from (Low-w/c) versus (High-w/c) concrete exposed to SOM and heterotrophic bacteria.

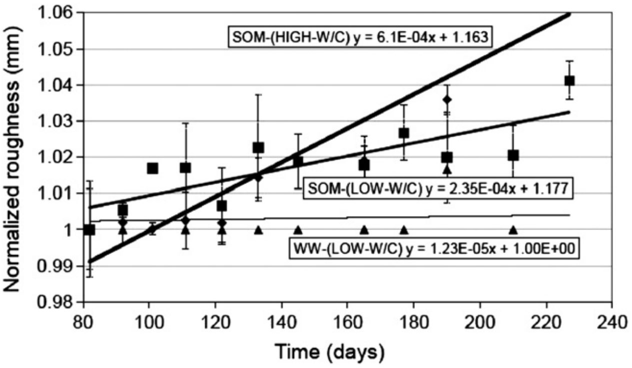

Fig. 10. Change of average surface roughness of (High-w/c) and (Low-w/c) concretes exposed to SOM and control.

The changes in thickness, represented by changes in surface roughness versus time of concrete samples, are given in Fig. 10. This information validates the scanner-based image analysis with the model based on the concrete properties (density and surface area); the latter was calculated from the scanned image using Matlab™ and the average value for the replicates was used,

$$CR = \frac{1}{(SA) \times \rho} \times m, \tag{1}$$

where *CR* is the concrete corrosion rate typically given in (mm/yr), *SA* is the surface area (cm^2), ρ is the density of concrete (g/cm^3), and *m* is the loss of weight (g/day).

The measurements showed difference in corrosion rates based on the concrete compositions and the inoculated microorganisms. The thickness was presented as the average of at least 8 surface area measurements, when processing 3 concrete samples. The surface area subjected to the analysis per sample was 10.76 cm^2 from the bottom part of the concrete sample, which was exposed to the liquid media; this provided enough information to validate the deterioration of the material. This method was made reliable by taking the average value of four thickness measurements for every concrete sample.

The (Low-w/c) concrete had a gradual deterioration upon prolonged exposure to SOM; the loss of thickness of 0.08 mm/yr occurred over 215 days. Control samples exposed to artificial wastewater and water demonstrated very small loss of cement compared to the active systems at 4.42×10^{-3} and 3.78×10^{-3} mm/yr, respectively.

There was a difference between corrosion rates of the concrete samples from (High-w/c) and (Low-w/c) batches. With (High-w/c) concrete, a rapid deterioration occurred due to the fall-out of aggregate particles, which led to an increase of the roughness. The aggregate was observed in the beaker at the end of both cycles. The corrosion rates are 0.208 and 0.0146 mm/yr for the SOM and the control, respectively. For (Low-w/c) concrete, the deterioration was slow, as the process occurred layer by layer. This deterioration increased with the fall-out of aggregates opening more surface of cement to the acid. The corrosion rates of these samples were 0.08 mm/yr and 4.42×10^{-3} mm/yr for the control.

The biogenic concrete corrosion is a slow process with corrosion rates typically ranging from 1 mm/yr to 5 mm/yr [2]. Thus several years are required to investigate the durability of new materials under normal conditions. Therefore research has been performed by creating optimal conditions for the bacterial growth that may not be always present *in situ*, so that the corrosion rates can be accelerated. Our study differs from the typical laboratory approach in the way the artificial wastewater was used and in the concentrations of hydrogen sulfide and sulfates that were similar to the ones found in the field. Our results were in the range of those measured in the field studies (Table 4).

The biogenic sulfuric acid corrosion is a very complex process, due to microbiological (populations, biomass, and substrate available) as

Table 4
Corrosion rates *in situ* and chamber studies.

Author	Sulfate concentration (mg/L)	Site of measurement	Corrosion rate (mm/yr)
Ayoub G, et al. [28]	30–80	Tripoli and Jounich	0.004
ASCE, [29]	20–130	Sacramento Central Trunk	0.342
Ayoub G, et al. [28]	900–1005	Kassis and Coniche Al-Manara	0.1
This study	1858.58	Lab study	0.08 (Low-w/c) and 0.208 (High-w/c)
Monteny J, et al. [4]	2700	Rotterdam (Netherlands)	0.8
de Belie N, et al. [31]	2000–4000	Lab study	1.6–4.3

well as mechanical factors such as the ettringite formation which is removed by the sewage flow [3]. As a result researchers in [3] have used highly enhanced temperature and nutrients for the microbial activity using sulfur media that allows sulfate concentration higher than 3 g/L. A simulation chamber was used to investigate the effects of nutrients on the corrosion of the concrete [3]; the duration of experiments was 6 months and a hydrogen sulfide concentration of 400 ppm was used. The corrosion rate was determined by measuring the thickness of the concrete samples. The corrosion rate of the specimens exposed to basal medium was 3.8 mm/yr. Another experiment was developed, where a demonstration plant was built with a pipe diameter of 15 cm and a length of 20 m. Concentration of hydrogen sulfide ranged from 25 to 300 ppm, and after 6 months a corrosion rate between 3.8 and 7.6 mm/yr was found.

With the information obtained in the present study, a corrosion rate for the conditions is presented in Fig. 11. An increase in surface roughness was determined, where the *t*-test presented a significant difference when using various types of concrete and controls ($p = 7 \times 10^{-4}$ and $p = 0.034$).

Two models were used for fitting the experimental corrosion rates for the (Low-w/c) concrete exposed to SOM. One of the models is based on the correlation of alkalinity and the water absorption (as a measure for concrete porosity) [31] and the other according to Pomeroy's model [30], which considers factors related to the acid formation, sulfide release and alkalinity of the pipe material.

The equation selected by de Belie [31] has the following form,

$$C = \frac{c_1}{alk} + c_2 \times W, \qquad (2)$$

where C is the degradation depth (mm), alk is the alkalinity (g/g), W is the water absorption (%), and c_1 and c_2 are model coefficients.

The best fit values obtained for the coefficients were $c_1 = 2$ and $c_2 = -0.04$. The fit based on Eq. (2) had a correlation coefficient of $r = 0.95$ compared with $r = 0.75$ for the Pomeroy model. The de Belie model included the water absorption, therefore the higher water absorption points to a lower concrete quality and faster deterioration.

The concrete weight loss was determined as an additional parameter to judge concrete deterioration. The measurements showed an increase at the beginning of the experiment due to the liquid saturation of the pores in the concrete; this provided for an increase in the internal pressure in the concrete. It seemed that during this process there were no differences in parameters that could be related to biodegradation. When the concrete reacted with the sulfate, material loss, weight decrease and small cracks were observed in the samples.

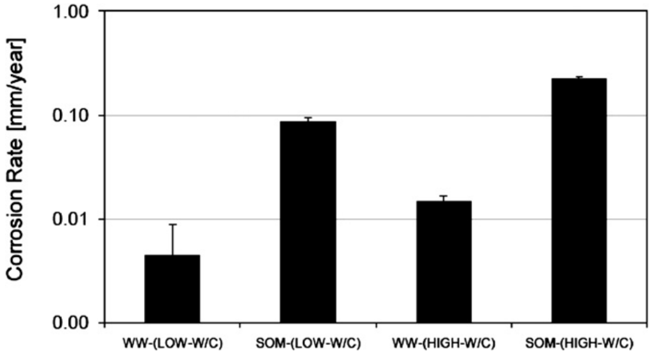

Fig. 11. Comparison of corrosion rates of different types of concretes.

The weight measurements are shown in Fig. 12. For the (Low-w/c) concrete in SOM conditions, a weight loss of 0.55 g (1.27%) was detected; for the (High-w/c) concrete a weight loss of 0.53 g (1.68%) was determined. The slope of the normalized weight loss profile of SOM—(Low-w/c) was -5×10^{-5} and 1×10^{-5} for SOM—(High-w/c), respectively. There was statistically significant difference in cycle 2 ($p = 0.17$ and $p = 0.0026$). The irregular plots of weight likely corresponded to the variable level of immersion of the concrete in the media and therefore differ in the moisture content in the upper-portion of the concrete.

Corrosion in concrete samples has been previously characterized by loss of weight. Sand W, et al. [1] corroded test blocks by periodically spraying with Thiobacilli cultures; they found that severely corroded specimens had a weight loss of almost 6% after 15 months. Another simulation was performed by Hormann K, et al. [32], where they investigated the resistance of several concrete types; they found that concrete specimens with high alumina cement lost 3–4% of weight after 5 months, compared to ordinary Portland cement specimens that lost 18–31% of weight. Over the same 5-month period, Schmidt M, et al. [19] demonstrated that the biogenic sulfuric acid attack caused 3 to 4% loss of weight.

Overall, a paired *t*-test showed a significant difference except for the (High-w/c) concrete ($p = 2.7 \times 10^{-21}$ and $p = 0.62$) using the controls for (WW—(Low-w/c):SOM—(Low-w/c)) and (WW—(High-w/c):SOM—(High-w/c)), respectively. Increasing the volume fraction of coarse aggregates increased the weight loss of the concrete exposed to sulfuric acid attack due to higher fall-out.

3.8. X-ray diffraction

Results of the X-ray diffraction tests, performed only on a dehydrated sample of the (Low-w/c) concrete surface, indicated a

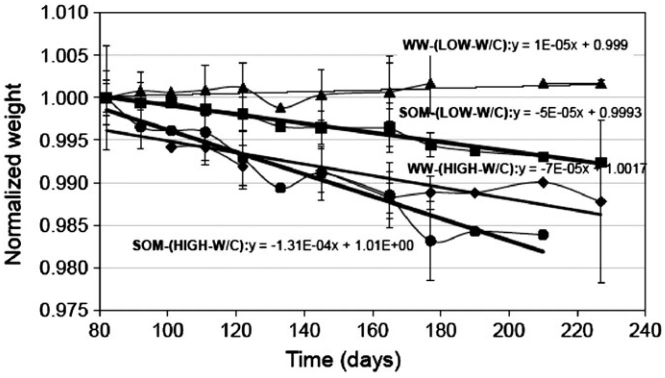

Fig. 12. Change in weight of different types of concrete exposed to SOM and control during two cycles.

Table 5
Summary of factors associated with the concrete corrosion.

Concrete type	Condition	Min pH	Max TOC (ppm)	Max formazan (μmol/mL)	Corrosion rate (mm/yr)	Max sulfate (mg/L)	Max Ca^{+2} (mg/L)	Weight loss %
Low-w/c)	ww	6.71	4.6	7.59	0.0044	453.0	589.5	0.17
	SOM + H	3.01	7.96	13.72	0.080	1858.5	1061.4	0.76
High-w/c)	ww	6.7	4.36	11.68	0.0146	309.9	640.7	1.24
	SOM + H	4.12	7.01	9.15	0.208	1991.4	804.8	1.78

SOM + H: Sulfur Oxidizing Microorganisms and Heterotrophs.

presence of sulfur and gypsum, but no ettringite. Further reaction of gypsum with calcium aluminate phases in the cementitious matrix could form ettringite, which had more volume increase than that of gypsum thus leading to more micro- and macro-cracking. Additionally sulfuric acid decomposed the cementitious matrix by decalcifying calcium silicate hydrate (C–S–H), therefore contributing to the strength loss [33].

Mori T, et al. [3] reported the dependence of formation of crystals on the pH; gypsum was produced on the surface of concrete materials at pH levels less than 3. Sulfur was the result of abiotic oxidation of hydrogen sulfide at low pH; it was observed on the surface of the concrete after 40 days.

3.9. Discussion

The deterioration of concrete is associated with sulfate production, calcium release, weight loss and thickness decrease. Table 5 summarizes results discussed previously in this paper.

The key parameters of the hypothetical mechanism for the second cycle have been plotted together in Fig. 13 for the results observed in the (Low-w/c) concrete inoculated with SOM. A pH drop from 5.27 to a minimum of 3.0 was associated with the increased number of NSOM cells. This pH drop allowed acidophilic bacteria to grow.

The damage in sewer collection systems is a slow process, therefore an accelerated biogenic sulfuric acid attack was another parameter included. The experimental period of 227 days was a short period compared to collection systems and test protocols. The difference in composition of the concrete, sewage characteristics, aerobic/anaerobic conditions, temperature, turbulence in the sewage flow, as well as species present provides complexity in comparing the corrosion rate of the experimental set up with the corrosion rate *in situ*. Literature shows that differences in atmospheric content and *Thiobacillus* species on concrete surfaces occur in corroded sewer systems all over the world [34].

The experimental system was organized in such a way as to demonstrate the measurements of pH, sulfate formation, and loss of material by weight in the accelerated approach that was similar to the conditions found in sewer collection systems. There were statistically significant differences between the corrosion responses of concrete compositions. Future setups can be further optimized by doing more field studies. This will provide a comparison between the experimental procedures and experiments *in situ*.

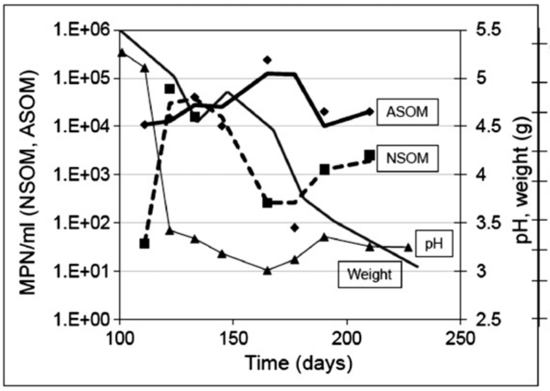

Fig. 13. Experimental parameters describing the progression of MICC in (Low-w/c) concrete exposed to SOM and heterotrophic bacteria.

Two approaches have been adopted to extend the service life of concrete structures exposed to sulfuric acid attack: improving the chemical resistance of concrete using different mixture proportioning and incorporating innovative materials, and preventing the growth of the bacteria producing sulfuric acid using biocides [35].

4. Conclusions

- The measured corrosion rate of the (Low-w/c) concrete in the simulated MICC conditions was 0.08 mm/yr, significantly lower than the (High-w/c) concrete 0.208 mm/yr. The greater corrosion of the low strength, low quality foreign concrete is logical. The results are comparable with previous studies performed in the field and correlate well with the sulfate concentration.
- The obtained data fit the proposed mechanism for MICC. The increase of NSOM cells dropped the pH from 5.27 to a minimum of 3.0 allowing acidophilic bacteria to grow. In this study, the pH and sulfate amounts provided for a low corrosion rate. The correlation between pH and the bacterial activity, by means of sulfate and corrosion rate, supports the hypothetical mechanism for the microbially induced concrete corrosion.

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Appendix A. Supplementary methods

A.1. Microbial analyses

INT (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl terazolium chloride) was used to quantify the respiratory activity of the microorganisms [21]. The Most Probable Number (MPN) technique was used to assess the number of active sulfur oxidizing microorganisms that could be cultured from a concrete corrosion system. Analyses were performed using 8 replicate samples of each of 14 dilutions and the MPN's of active organisms were assessed using information in [22].

The liquid media in the experiments were LHET2 medium for *A. cryptum*, S6 medium for *Thiobacillus* NSOM, and S6 medium for *Thiobacillus* ASOM. The media were sterilized for 15 min at 121 °C and a dilution series was inoculated for each of the different cultures that were distributed between 96 wells and incubated at 25 °C for 7 days. Resazurin concentration of 25 mg/L was used as internal redox indicator. Upon reduction, resazurin changes color from blue to pink to clear as oxygen becomes limited within the medium [23].

A.2. Chemical analyses

The pH was measured routinely with an Accumet 13–620–300 pH electrode (Fisher Scientific) connected to an Orion model 525A pH meter. Dissolved oxygen (DO) was measured using a YSI DO probe and meter (model 58 YSI Incorporated, Yellow Springs, OH). Sulfate was quantified using a Dionex DX-300 ion chromatography system with method 4110B described in [25] and USEPA method 300.0B. Samples were filtered through a 0.2 µm nylon syringe filter (Life Science Products) and stored in a 4 °C refrigerator prior to analysis. Sodium bicarbonate/sodium carbonate was used as eluent at a flow rate of 1 mL/min with a sample injection volume of 200 µL. An AG14 and AS14 guard and analytical columns (Dionex Corporation), respectively, were used to separate the anions, and the suppressed conductivity was used to measure sulfate, which was eluted at an average retention time of 9 min. The dissolved total organic carbon (TOC) in the samples was measured using a Sievers 800 Total Organic Carbon Analyzer. Samples were filtered through a 0.45 µm porous size membrane (HVLP, Millipore, Bedford, MA). Samples with high TOC were diluted to reach less than 10 ppm of total organic carbon. Three drops of phosphoric acid were added as a preservative in addition to lowering the pH to less than 2. The method follows 5310 C Persulfate-Ultraviolet Oxidation method [25]. The total calcium in the media was quantified by using a Dionex DX-300 ion chromatography system (Dionex Corporation, Sunnyvale, CA) with method described in Dionex for IONPAC CS12. A standard solution was prepared from a 1000 ppm calcium chloride ($CaCl_2$) stock solution at the following concentrations: 10, 30, 50, 70, 100 mg/L Ca^{2+}. Samples were filtered through a 0.2 µm nylon syringe filter (Life Science Products) and stored in a 4 °C refrigerator prior to analysis. 20 mN Methanesulfonic acid was used as eluent at a flow rate of 1.2 mL/min with a sample injection volume of 50 µL. An AC12 analytical column (Dionex Corporation) was used to separate the cations, and the suppressed conductivity was used to measure calcium. Hydrogen sulfide was quantified by the MSA AUER Gas-Tester. This method was based on the reaction of hydrogen sulfide with lead forming lead sulfide. The detection range was from 0.2 to 4000 ppm. The hydrogen sulfide gas, which was not oxidized, went to a neutralization unit containing sodium acetate [1 N]; the same was done with the liquid from the hydrogen sulfide generator. This produced a safer effluent, which was zinc sulfide.

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