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An innovative approach to reproduce the biodeterioration of industrial cementitious products in a sewer environment. Part I: Test design

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ABSTRACT

A test method to evaluate biogenic resistance of cementitious pipe products intended for sewer networks is presented. It consisted in inoculating pipes with a highly diverse microbial consortium (urban wastewater treatment plant), and trickling a feeding solution containing a safe and soluble reduced sulfur source, thiosulfate, over the inoculated surface in order to select a sulfur-oxidizing activity. Thiosulfate was used in the form of an aqueous solution, which facilitated the monitoring of (i) the bacterial activity by sulfur mass balances in the liquid phase, and thus quantification of acid production, and of (ii) the leaching of cementitious ions. Cement-based linings made of (i) blast furnace slag cement and (ii) calcium aluminates cement were tested. Results showed the selection of sulfur-oxidizing bacteria and the production of biogenic acid. Differences were shown between the linings in terms of Ca and Al dissolution. Biomass characterization highlighted the influence of the lining composition on colonization.

1. Introduction

Local biological production of hydrogen sulfide (H_2S), together with its chemical and biological oxidation, leads to major deterioration of concrete in sewer structures world-wide [1–3]. Concrete corrosion by the oxidation of H_2S is observed in all sewers of all countries but at different rates depending on the local environment and the cementitious products used [4,5].

These concrete deteriorations lead to major problems during the collection and treatment of wastewaters, firstly by the dysfunctions of wastewater treatment plants caused by the increase of the inlet flows due to clear water (rainwater, groundwater) intrusion and, secondly, by wastewater exfiltration into the groundwater. The financial investment needed for restoration is considerable [2,6].

In sewer networks, deteriorations of cementitious materials are primarily related to the production of hydrogen sulfide (H_2S) in stagnant zones, its volatilization and its condensation at the top of pipe-walls [7]. On fresh concrete (with surface pH ranging from 11 to 13 [8]), the chemical acid–base reactions with H_2S and with CO_2 in the air first decrease the pH at the surface to about 9 [9]. The chemical oxidation of H_2S in contact with cementitious materials in a moist environment produces sulfur compounds with different degrees of oxidation [10,11]. Sulfur-oxidizing bacteria (SOB) are able to grow by oxidizing these reduced sulfur compounds. The metabolic reactions in the biofilm produce acid (H^+) and sulfate (SO_4^{2-}) at the surface of the cementitious material [5–8]. Neutrophilic sulfur-oxidizing bacteria (NSOB) colonize and grow first, producing acid in contact with cementitious products, which progressively decreases the pH from 9 to 4 [8,14]. Afterwards, when the pH has decreased to values between 4 and 1, acidophilic sulfur-oxidizing bacteria (ASOB) replace NSOB [8,10,15]. This whole phenomenon is described as microbially induced concrete corrosion (MICC) [8,10,14].

The first step of biodeterioration of cementitious materials in sewer networks is considered to be biological acid production at the surface of the cementitious materials [8]. Decalcification of the cementitious matrix occurs because of the high solubility of calcic phases in an acid environment [8]. In a second step, the diffusion of exogenous sulfate into the matrix porosity and its reactivity towards some hydrates of the cement paste leads to secondary precipitations of gypsum ($CaSO_4{\cdot}2H_2O$) and expansive ettringite ($3CaO{\cdot}Al_2O_3{\cdot}3CaSO_4{\cdot}32H_2O$) [13,16].

Cementitious products used for sewer networks are currently qualified only by chemical tests (NF EN 598) although it is acknowledged that these tests are not representative of phenomena occurring in

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sewer environments [12,13,17]. Biological activity, occurring at the local scale, is a key factor in such deterioration. Moreover, several studies indicate differences between chemical and biological tests in terms of resistance to biogenic acidification for given cementitious materials [12,13,17].

In this context, several laboratory tests based on microbial transformations have been developed during recent decades to quantify the resistance of materials to MICC. Two different approaches have emerged, one based on a continuous process, such as the test developed at Hamburg University [17–19], and the other based on sequenced systems with immersion of the cementitious sample in biological culture, such as that developed at Ghent University [20–22], or that proposed at the University of Colorado at Boulder (USA) [15,23]. In most cases, the inoculum has been composed of a mix of pure strains of sulfur-oxidizing bacteria (including neutrophilic and acidophilic bacteria). However, the experiments carried out at Aalborg University [24] used real wastewater as the biological medium. H_2S was often used as a sulfur source, but other compounds such as thiosulfate [25] or elemental sulfur [26] have been used in some cases, with strong deterioration recorded on the cementitious materials tested. Cementitious specimens are often laboratory mortars or, in some cases, real sewer pipes (specific test design) [5,24]. However, for the biotests including an immersion period, the main step describing biological acid production limits the interaction between the mortars and the biological activity [20,23,25,26]. Moreover, no mass balances were possible in either approach, which limited the control and the analyses of the biological transformations of the sulfur compounds in contact with the cementitious materials. Work with a chosen inoculum based on the populations identified from the field samples collected in a sewer of Hamburg were representative of a local environment with concrete made of ordinary Portland cement [18,27]. However, in such tests, the role of the material as a selection factor for the biological activity was biased and some studies showed that other microorganisms, such as heterotrophic bacteria [16, 28–31] or archea [30] or fungus [32], and other sulfur-oxidizing bacteria than *Acidithiobacillus* genera even in extremely acidic environment [31] were established at the deteriorated concrete surface. Moreover, other studies indicated that some of the microbial species identified as being involved in the biogenic acid production had limited growth in the presence of aluminium for example, which supports the interest of taking the material composition into account [33,34].

Although available bio based tests are numerous, none of them cumulates all the requirements that, in our opinion, are crucial:

(i) the flux of sulfur compound should be high and its biological transformation performed at a high rate [17,24];
(ii) the quantification of biological acid production [17], sulfur mass balances and the characterization of the leachate composition must be feasible;
(iii) the inoculum should contain not only SOB but also heterotrophs [28,35], the latter playing a role in MICC. Selection of the right populations should be carried out during the first step of the test;
(iv) safe test conditions should be ensured (H_2S may lead to health problems [36] and its associated base may be unstable [37,38]);
(v) it should be possible to test both materials and ready-to-use pipe products; and
(vi) characterization analyses on the materials should clearly demonstrate that the alterations are representative of an in-situ phenomenon.

Based on these specifications, the main objective of this study (presented in the form of a two-part paper) was to propose an innovative test design intended for cementitious materials and/or industrial products. In this study, only industrial sewer pipe products were evaluated. In the first part of this work, the experimental device was evaluated in terms of sulfur oxidizing selection and biogenic acid production. The following issues were addressed using thiosulfate ($S_2O_3^{2-}$) as a substitute for H_2S: (i) characterization of the selection of SOB from an unspecific inoculum sampled at an activated sludge treatment plant; (ii) evaluation of reproducibility in terms of the selection of sulfur-oxidizing bacteria (using an activated sludge consortium sampled in another urban wastewater system); (iii) quantification of biogenic acid production over time by sulfur mass balances; (iv) analyses of lining leaching. In the second part [39], the representativeness of the transformations is assessed for two cementitious linings by chemical, mineralogical and micro-structural analyses.

Industrial sewer-pipes, consisting of ductile cast iron coated with mortars on their inner surface by an industrial centrifugation process (Saint-Gobain PAM), were tested. Two categories of linings made with different binders were subjected to intensive biogenic acid attacks under the same environmental conditions.

2. Materials and methods

2.1. Experimental set-up and analyses for the development of a sulfur-oxidizing activity in contact with cementitious linings

2.1.1. The pilot

The pilot was composed of two vertical parallel pipe-reactors, each mainly composed of a segment cut from a real sewer pipe. The description of the sewer pipes, produced by Saint-Gobain PAM, is presented in a Section 2.2. Fig. 1 presents a photograph of the laboratory pilot (Fig. 1(A)) and a descriptive diagram of the pilot (Fig. 1(B)).

The pipe segments were 200 mm long and 80 mm in internal diameter (0.05 m^2 of exposed lining surface). Each pipe-reactor was composed of the following: (i) a PVC double jacket on the outside of the segment to maintain the temperature at 20 ($\pm$1)°C by circulating thermostated water; (ii) two stainless steel cones, one at the inlet of the pipe and one at the outlet to collect the leaching solution in a flask. The stainless steel cones and the pipe segments were sealed by rubber rings and steel clamps (Fig. 1A—(1a) and (1b)).

A constant flow of a mineral solution (Fig. 1—(2)–(4)) was trickled onto the cement surface. Because of the vertical position of the pipe segment, the water ran down the pipe walls by gravity. To limit the impact of preferential paths of the trickling solution on the pipe walls, the orientation of the stainless-steel inlet cone was changed by 90° every day. The water inlet flow was fixed at 50 ml/h.

The pilot configuration could be directly used to test laboratory mortar samples cast in a cylindrical shape.

2.1.2. Choice of sulfur substrate

Fig. 2, adapted from Islander et al. [10] illustrates the chemical and biological reactions involved in the oxidation of the sulfur compounds and leading to acid and sulfate production. Several sulfur compounds are involved in the biogenic acid production. Elemental sulfur (S^0) and thiosulfate ($S_2O_3^{2-}$) are first produced by chemical oxidation of H_2S [10,38,40]. Afterwards, S^0 and $S_2O_3^{2-}$ are oxidized into biogenic acid and sulfate through biological reactions. The pathways of these reactions are pH dependent. The thiosulfate oxidation pathway is reported to be predominant at moderate pH while, at low pH, elemental sulfur oxidation is the main pathway (Fig. 2) [10]. In an acid environment, H_2S can also be directly oxidized into biogenic acid [41]. The biological oxidation of thiosulfate can involve several polythionates ($S_xO_6^{2-}$) as intermediates. In particular, tetrathionate ($S_4O_6^{2-}$) has been identified as a biological intermediate for several SOB. The global biological oxidation of thiosulfate is described by two main pathways in the literature [42]: (i) direct oxidation into sulfate through the Kelly–Friedrich pathway, also called the PSO pathway and described in Eq. (1) for the *Paracoccus* genera [43,44] and (ii) oxidation into sulfate through the S_4I pathway (for tetrathionate $S_4O_6^{2-}$ intermediate), where tetrathionate is first formed during the

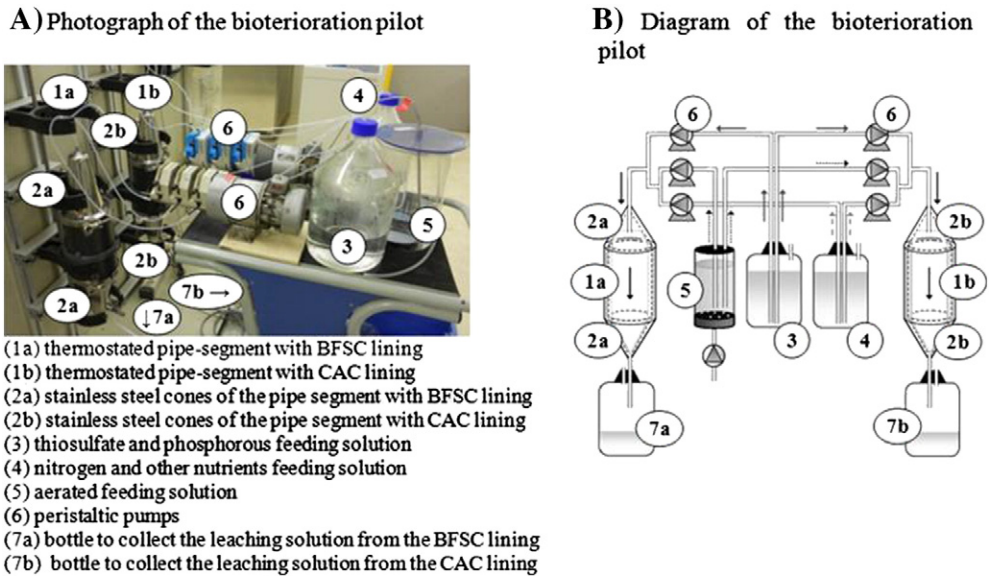

Fig. 1. Biodeterioration test pilot. A) Photograph. B) Descriptive diagram.

thiosulfate oxidation (Eq. (2)) and then oxidized into sulfate (Eq. (3)).

$$S_2O_3^{2-} + 2O_2 + H_2O \rightarrow 2SO_4^{2-} + 2H^+ \quad (1)$$

$$2S_2O_3^{2-} + 0.5O_2 + H_2O \rightarrow S_4O_6^{2-} + 2OH^- \quad (2)$$

$$S_4O_6^{2-} + 3.5O_2 + 3H_2O \rightarrow 4SO_4^{2-} + 6H^+ \quad (3)$$

Controlling the transformation of sulfur compounds (Fig. 2) is a key factor for selecting SOB, and thus simulating and intensifying the biogenic acid production. H_2S is a toxic compound [7], highly reactive in an aerobic environment at alkaline and neutral pH conditions (conditions of fresh cement paste) [37,38,45]. It was recently showed in the literature that the abiotic oxidation of H_2S could be influenced by the chemical/mineralogical nature of the support [46], indicating a possible way of differentiation between two materials. Nevertheless, sulfur mass balances were not carried out in these experiments, which prevents any quantitative conclusion regarding the role of the abiotic oxidation of H_2S. Vollertsen et al. [47] highlighted the role of elemental sulfur in sewers to maintain SOB activity in absence of H_2S. They revealed that the biological acid production got slower in absence of H_2S while elemental sulfur was used as only substrate. H_2S is an interesting substrate in sewer network, with intermittent sulfur providing, due to the immobilization of sulfur substrate by the precipitation of elemental sulfur at the concrete surface. However, for a test design, with sulfur provided continually, and with the objective of the quantification of the sulfur-oxidizing biological activity, its toxicity, on the one hand, and its highly abiotic reactivity, on the other hand, limit the quantitative analyses in time with currently no clear gain in terms of differentiation and intensification. Thiosulfate ($S_2O_3^{2-}$) was thus chosen as the source of reduced sulfur — instead of H_2S — to secure the system and to facilitate the quantification and control of biogenic acid production over time. The choice of thiosulfate is supported by several studies describing (i) the formation of thiosulfate as a natural intermediate [38,40], and (ii) the ability of many sulfur-oxidizing bacteria to grow on thiosulfate [42,43].

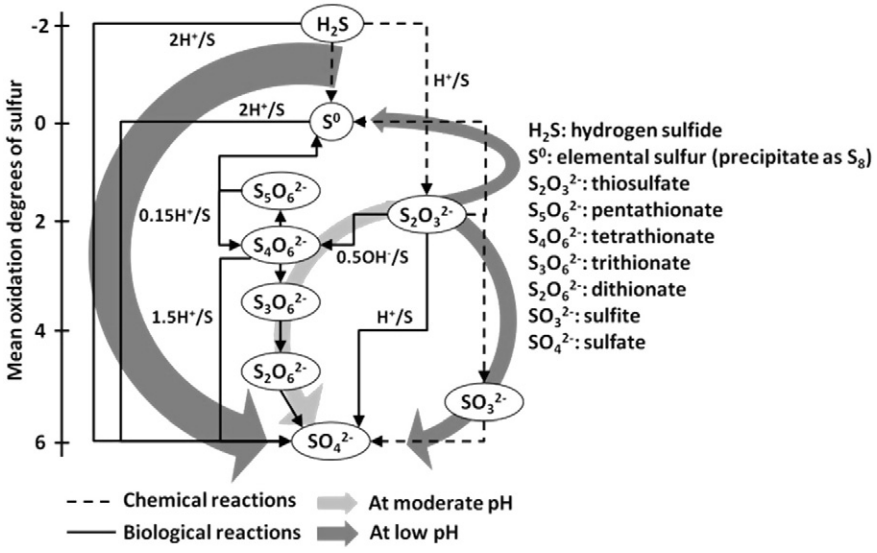

Fig. 2. Reduced sulfur oxidation by biotic and abiotic reactions with the associated acid production for one oxidized atom of sulfur. Adapted from Islander et al. [10].

Before the biological experiments with the two linings, an abiotic experiment was performed on CAC lining (CAC for Calcium Aluminates Cement) to evaluate the stability of thiosulfate as a sulfur source and the impact of the system on cementitious lining in the absence of biological activity.

2.1.3. Nutrient supplies for sulfur-oxidizing activity

For both pipe reactors (Fig. 1(B)), nutrients were supplied by trickling a mix of three feeding solutions into the inlet stainless steel cone, to produce a liquid phase that could be considered as a condensate at the surface of the exposed linings (reactive zone at the top of a pipe in real sewers) [7,48]. The sulfur supply was prepared by dissolving $Na_2S_2O_3$ crystals. In this study, to intensify the sulfur-oxidizing activity, the concentration of thiosulfate at the inlet of the pipe reactors was regularly increased. Five periods (labelled (i) to (v)) were distinguished (Table 1).

All the feeding solutions were prepared by dissolving mineral salts in deionized water to avoid any sulfate and calcium addition. Solution (3) (Fig. 1) provided thiosulfate ($Na_2S_2O_3$), phosphorous ($NaPO_3)_3$, manganese ($MnCl_2{\cdot}4H_2O$) and other trace elements (such as boron oxide, cobalt, zinc, molybdenum oxide, copper, and nickel). Solution (4) (Fig. 1) provided nitrogen (NH_4Cl), magnesium ($MgCl_2{\cdot}6H_2O$) and iron ($FeCl_3$). "Solution" (5) (Fig. 1) was composed of deionized water only and served to ensure the targeted dilution rate. Nitrogen and phosphorous sources were provided in separate solutions to avoid any biological growth in the feeding solutions themselves. For each period, all the nutrient concentrations in the feeding solutions were based on the sulfur atom concentration provided at the inlet of the pipe reactor. The N, P, Mg, Fe and Mn contents were as follows: $[N]_{in} = 0.56 \times [S]_{in}$, $[P]_{in} = 0.22 \times [S]_{in}$, $[Mg]_{in} = 0.046 \times [S]_{in}$, $[Fe]_{in} = 0.008 \times [S]_{in}$, $[Mn]_{in} = 0.0008 \times [S]_{in}$. All the feeding solutions were in contact with the atmosphere, and thus contained the dissolved oxygen necessary for the biological oxidation of the reduced sulfur compounds. Every week, all supply tanks were emptied, washed and filled with new feeding solutions. At the same time, all the silicon tubing used for the connections was changed (to avoid any biofilm accumulation).

2.1.4. Inoculum

Two activated sludge samples, named AS1 and AS2 were collected at two real urban wastewater treatment plants (WWTP) near Toulouse, France, and used for cement surface inoculation. The WWTP are conventional plants treating urban WW to eliminate COD (chemical oxygen demand), nitrogen, etc. The study carried out with AS1 was performed on BFSC lining and CAC lining for 107 days. The study with AS2 was performed with CAC lining for 240 days.

The inoculations were made once, at the beginning of the each experiment. The inocula were obtained after centrifugation (4500 rpm) of 400 ml of the activated sludge (at 4 g VSS/l (volatile suspended solid) corresponding to an initial inoculum of 1.6 g VSS (31.9 g VSS·m^{-2})). The inocula were deposited on the surface of the cementitious linings with a brush.

2.1.5. Chemical analyses of the leaching solution

20 ml of leaching solution was regularly sampled at the outlet of the pipe segments (every 3 or 4 days). After pH measurement (glass electrode (SCHOTT)), the solution samples were filtered at 0.2 μm and analysed before the end of the day to determine $S_2O_3^{2-}$, SO_4^{2-}, Ca^{2+} and Al^{3+} concentrations.

The concentrations of SO_4^{2-} and $S_2O_3^{2-}$ were measured by anionic chromatography (DIONEX: IC25, IonPacTM AS19, at 30 °C with eluent generator cartridge EGC III KOH). The dissolved calcium concentration was quantified by cationic chromatography (DIONEX: ICS 2000, IonPac CS12, at 30 °C with eluent generator cartridge EGC III MSA for methanesulfonic acid). The concentration of the total dissolved aluminium was measured by inductively coupled plasma–optical emission spectrometry (ICP–OES, Perkin Elmer Optimum DV 7000).

Fig. 2 shows that there are numerous possible intermediates in the oxidation of thiosulfate into acid and sulfate. Soluble COD measurements (NFT 90.101) were performed punctually to evaluate the transformation of reduced sulfur compounds. The thiosulfate oxidation converted into COD is described by Eq. (1) showing that 1 g of thiosulfate theoretically needs 0.57 g of O_2. For other soluble reduced sulfur compounds (labelled as polythionates $S_xO_6^{2-}$), the COD equivalent ratio consumes 0.5 g O_2/g of tetrathionate ($S_4O_6^{2-}$), 0.625 g O_2/g of pentathionate ($S_5O_6^{2-}$), 0.333 g O_2/g of trithionate ($S_3O_6^{2-}$) and 0.1 g O_2/g of dithionate ($S_2O_6^{2-}$).

COD measurements were combined with direct measurements of thiosulfate and sulfate concentrations in the leaching solution to check COD mass balances, and also sulfur mass balances in the absence of analytical equipment to measure polythionate concentrations directly.

2.1.6. Identification of populations

At the end of the experiments, different biomass samples were collected directly on the cementitious materials, deposited in 2 ml sterile plastic Eppendorf tubes, then frozen in liquid nitrogen and kept at −80 °C. The samples were then analysed by 16S rRNA tag-encoded pyrosequencing to identify the microbial populations. Total DNA was extracted with MoBio Ultra Clean Soil isolation kit following the manufacturer's instructions (MoBiol laboratories, Inc., USA). The V1–V3 region of 16S rRNA gene was amplified using the primers 27F (5′-AGR GTT TGA TCM TGG CTC AG-3′) and 519R (5′-GTN TTA CNG CGG CKG CTG-3′) [49]. A single-step 30-cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) were used under the following conditions: 94 °C for 3 min; then 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min; and final elongation at 72 °C for 5 min. PCR products for each sample were mixed to obtain equal molar DNA for sequencing. Pyrosequencing was performed on a 454 GS FLX system according to the manufacturer's instructions (Roche). Sequence analysis was performed with Mothur [50] as follows: (1) sequences were depleted of barcodes and primers; (2) short sequences (<200 bp) and ambiguous sequences were removed; (3) sequences were denoised and chimeras were removed; (4) operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity) [51]; OTUs were taxonomically classified using a naive Bayesian approach [52] and SILVA 111 SSU database.

2.1.7. Biomass quantification on each lining

At the end of the experiment, the surfaces of both cementitious materials were washed with deionized water to detach the biofilm while avoiding any abrasive effect on the surface lining. For both linings, the solution with the detached biofilm was concentrated using centrifugation (4 °C, 4500 rpm). Dry volatile matter was measured on each total sample to quantify the biological matter stabilized on each lining after 107 days of test.

2.2. Sewer pipe specimens

Fig. 3 shows a longitudinal section of a Saint-Gobain PAM sewer pipe (inner diameter 80 mm), comprising a ductile cast-iron tube and the

Table 1
Equivalent sulfur concentration in the feeding solution per period.

Day 0 to day 22 (i)	Day 22 to day 37 (ii)	Day 37 to day 51 (iii)	Day 51 to day 80 (iv)	Day 80 to day 107 (v)
Concentration of sulfur atom at the inlet of the pipe-reactors ($[S]_{in}$)				
1.08×10^{-3} mol S-$S_2O_3^{2-}$/l	1.62×10^{-3} mol S-$S_2O_3^{2-}$/l	2.67×10^{-3} mol S-$S_2O_3^{2-}$/l	4.22×10^{-3} mol S-$S_2O_3^{2-}$/l	6.32×10^{-3} mol S-$S_2O_3^{2-}$/l

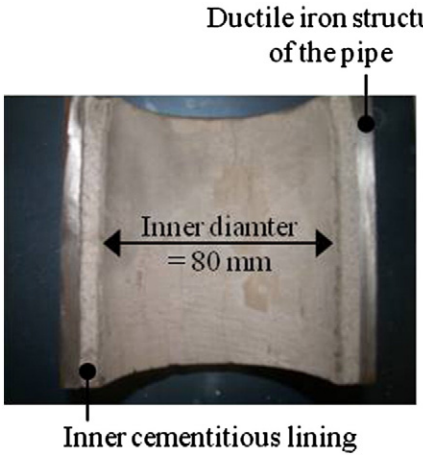

Fig. 3. Longitudinal section of a sewer-pipe: ductile cast-iron structure with mortar inner lining — here CAC mortar (produced by Saint-Gobain PAM).

inner cementitious lining (mortar) (Fig. 3). The cementitious lining is used to protect the ductile iron-cast structure of the sewer pipe (chemical and sacrificial protection against aggressive environments). The lining was 6 mm thick. The inner lining was produced by mortar centrifugation on the inner surface of ductile iron pipes. Details on the composition of the cement mortars are given in [39].

2.3. Cementitious materials

Documentation file FD P 18-011, complementary to the NF 206-1 standard, recommends CEM III/A or B, CEM V/A or B for an aggressive chemical acid environment (XA3). In this normative context, the cementitious linings tested in this study were mortars made of (i) blast-furnace-slag cement (BFSC) (corresponding to CEM III), and (ii) calcium-aluminate cement (CAC), which shows interesting behaviour in an acid environment, particularly against biogenic acid [13,17,53]. Both mortar linings were produced with siliceous aggregates. The relative resistance to biogenic acid attacks of the CAC paste is explained by (i) a higher acid neutralization capacity due to the hydrate composition and its evolution [54], and (ii) a still discussed bacteriostatic effect due to aluminium dissolution [34] and/or the surface properties [55].

BFSC and CAC linings were chosen to evaluate the test design for their actual use in sewer networks [12], their strong difference in terms of hydrated compositions [56] and the documentation existing on their evolution when subjected to biogenic acid attacks [17,18,22].

Table 2 gives the compositions of oxides of the hydrated cement paste for each lining obtained from EPMA analysis carried out on 150 points analysed randomly on control specimens [39]. The table also gives the standard deviation of the EPMA measurement in the corresponding operating conditions [57].

It should be noted that, due to the production of inner linings by a centrifugation process, the surface of the lining in contact with the biological medium was composed only of hydrated cement paste [39].

3. Results

3.1. Sulfur-oxidizing activity on cementitious linings

Figs. 4 and 5 show the evolution over time of the leaching solutions collected from the runoff from CAC lining (Fig. 4) and BFSC lining (Fig. 5). The thiosulfate inlet and outlet fluxes and the sulfate outlet fluxes (all quantified in moles of sulfur atoms per day) and the pH of the leaching solutions are presented. The increases in the thiosulfate inlet fluxes defined the five periods during the experiments (i to v).

First, a rapid acidification was observable on both linings during period (i): pH dropped from about 9 to 4 in approximately 17 days (Figs. 4 and 5). This rapid acidification was linked to the transformation of the thiosulfate feed into sulfate (Eq. (1)). The increase in sulfate production in the leaching solutions on both linings confirmed the selection and the growth of a sulfur-oxidizing activity from the activated-sludge consortium used as inoculum. During the first 60 days (until the middle of period (iv)), for both linings: (i) the increase in the thiosulfate feed resulted in an increase in sulfate production; (ii) the pH of the leaching solution stabilized between day 17 and day 60, although the sulfate production was increasing.

From day 60 (period (v)), the behaviour of the two linings differed. For the CAC lining, except for days 72 to 74, the totality of the thiosulfate supplied was consumed even though the feeding rate was increased significantly. During the same time, a stabilization of the sulfate production was recorded, suggesting a constant production of acid, indicated by a slight decrease in the pH of the leaching solution (Fig. 4). For the BFSC lining during the same period, although the pH decreased globally, the pH of the leaching solution appeared more unstable than for the CAC leachate, with some periods of basification (variations of up to 2 pH units; mean pH value = 4.63 ± 0.78) (Fig. 5).

3.2. Populations selected and biomass colonization

At the end of the tests, biofilm samples were collected at several locations on the lining surfaces (two samples for each lining, one at the entrance of the pipe, one in the middle of the pipe) for pyrosequencing. Results are shown in Fig. 6 (ref. AS2). The experiment was repeated once on a CAC lining (duration of the test: 240 days) using another activated sludge as the inoculum (ref. AS1). The results of microbial population characteristics obtained at the end of this second run (4 samples from same locations as in the first experiment) have been added to Fig. 6. The microbial composition of the activated sludge before inoculation in the pipes is also given. The error bars indicate the deviation between samples of the same pipe reactor. Activated sludges used as inocula (AS1 and AS2) were mainly composed of heterotrophic bacteria (92% ± 8%) and nitrifiers (8% ± 6%); sulfur-oxidizing bacteria were not detectable. At the end of the tests (AS1 and AS2 experiments), for both linings (CAC and BFSC), SOB were well represented in the biofilm, with a proportion of total microbial function ranging from 20% to 50% depending on the material and the experiment. The proportion of heterotrophic bacteria was reduced by about 50% during the experiments whatever the lining, but these bacteria were still present. This proportion of

Table 2
Oxide compositions of the control cement paste for the BFSC lining and the CAC lining from EPMA profiles (mean of 150 points).

CaO	SiO_2	Al_2O_3	Fe_2O_3	MgO	SO_3	K_2O	TiO_2	Na_2O	Other oxides + H_2O
Cement oxide composition for BFSC lining (%)									
42.81	22.05	6.12	0.42	2.06	4.52	0.22	0.36	0.05	21.39
Cement oxide composition for CAC lining (%)									
29.40	3.84	41.81	1.13	0.13	0.04	0.06	1.01	0.04	22.55
Standard deviations [57]									
CaO	SiO_2	Al_2O_3	Fe_2O_3	MgO	SO_3	K_2O	TiO_2	Na_2O	
4.1%	3.7%	1.2%	0.7%	0.37%	1.1%	0.06%	0.08%	0.07%	

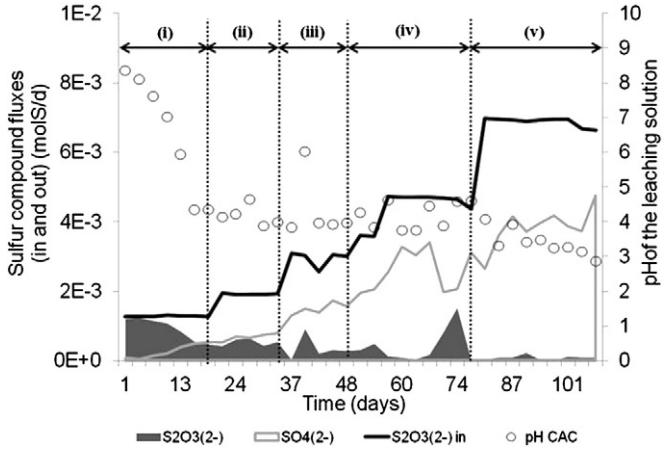

Fig. 4. Time courses of inlet and outlet sulfur compound fluxes and of the pH of the leaching solution for the experiment on the CAC lining.

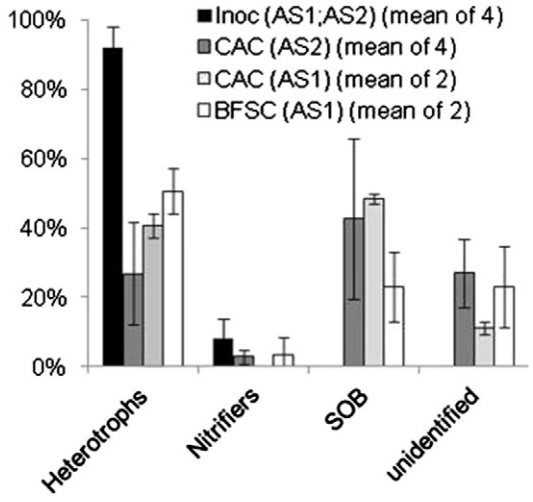

Fig. 6. Evolution of the bacterial consortia, observed by functional abundance after population identification by pyrosequencing. The inoculum (mean of AS1 and AS2) is compared (i) to consortia developed from AS2 inoculum at the surface of CAC lining after 240 days of exposure to thiosulfate environment (four samples), (ii) to consortia developed from AS1 inoculum at the surface of the linings (CAC and BFSC) after 107 days of exposure (two samples per lining).

heterotrophic bacteria is in good agreement with that reported on deteriorated concretes in sewer networks [16,28,35]. For CAC AS1 and BFSC AS1, further identification of SOB genera indicated the predominance of *Thiomonas* species (CAC/AS1 = 98% ± 2%; BFSC/AS1 = 78% ± 22%; CAC/AS2 = 11% ± 11%) and *Thiobacillus* species (CAC/AS1 = 2% ± 2%; BFSC/AS1 = 22% ± 22%; CAC/AS2 = 89% ± 11%) (not all the species were identifiable with the databases used). For CAC/AS2 compared to CAC/AS1 (with a double culture duration) the *Thiobacillus* species were identified as the major SOB. SOB were detected in each sample whatever the sample location on the linings or the activated-sludge used as inoculum.

Fig. 7 shows some photos of the inoculated pipes (BFSC and CAC lining) during the experiments. The day 0 photos show the pipes just after the inoculation. Photos at day 107 show the inside of the pipes at the end of the experiments.

For both linings, biological matter was observable at the end of the experiment. However, although they were exposed to the same thiosulfate fluxes and same feeds, the developed biofilm showed different features in terms of quantity and colonized surface area. Visually (Fig. 7) the biomass on CAC lining appeared to be less dense than that on BFSC lining. These observations were confirmed by the measurement of the volatile dry matter for each lining at the end of the experiment: 330 mg VSS (volatile suspended solid) for the BFSC lining and 80 mg VSS for the CAC lining. Differences in the water trickling may explain the differences in the final colonized surface areas, but not the differences in the biomass quantity. Moreover, the biofilm in contact with the CAC lining appeared to be dryer than the biofilm developed on the BFSC lining. However, although the composition of the lining seemed to have an impact on the biological colonization of the surface, the above results show that sulfur-oxidizing activity can occur on both linings.

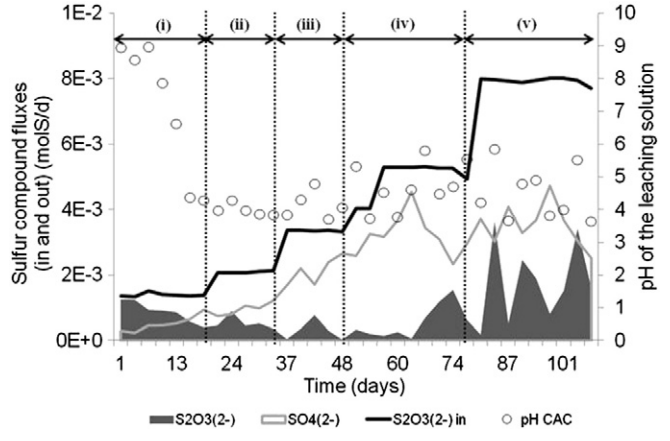

Fig. 5. Time courses of inlet and outlet sulfur compound fluxes and of the pH of the leaching solution for the experiment on the BFSC lining.

3.3. Analysis of the sulfur transformations based on sulfur mass balances

As shown in Figs. 4 and 5, the conversion of thiosulfate to sulfate was never complete whatever the lining, although the thiosulfate supplied was sometimes totally consumed. The incomplete sulfur mass balances (around 45% of sulfur loss for both linings during period (v) (Figs. 4 and 5)) can be explained by (i) the diffusion and entrapment after secondary precipitation of some sulfate inside the cementitious matrix, and/or (ii) the formation of some non-detected reactional intermediates during the thiosulfate oxidation as described in Fig. 2.

Whatever the lining, the COD mass balances (Fig. 8(A)) performed on 17 samples close rather well considering the uncertainty of 10% related to the COD analyses (Fig. 8(B), (C)). This means that the sulfur masses were also balanced in the soluble phase. Thus, if diffusion and secondary sulfate salt precipitations occurred, they involved a very small fraction of the sulfate produced.

The remaining soluble COD not quantified as thiosulfate indicates the formation of oxidizable sulfur intermediates. From Fig. 2, various intermediates can be suspected. i.e. tetrathionate $S_4O_6^{2-}$, pentathionate $S_5O_6^{2-}$, trithionate $S_3O_6^{2-}$ and dithionate $S_2O_6^{2-}$. However, making the hypothesis, in a first approach, that only one of these compounds was formed, and taking the theoretical COD/mass of each compound ratio into account, only tetrathionate or pentathionate would be eligible to achieve the sulfur mass balances in the liquid phase (103% ± 12% for $S_4O_6^{2-}$ or 98% ± 11% for $S_5O_6^{2-}$ on the CAC lining). As previously mentioned, tetrathionate was an identified intermediate in some oxidative pathways [42,43]. Thus, the thiosulfate feed, with an activated-sludge consortium as inoculum on both linings, led to the selection of a two-step sulfur-oxidizing activity producing biogenic acid. It may be assumed that thiosulfate was first oxidized into tetrathionate (Eq. (2)) and then tetrathionate was oxidized into biogenic acid composed of proton and sulfate (Eq. (3)).

The achieving (10% uncertainty) of the sulfur mass balances indicates that the kinetics of the biogenic acid production can be evaluated from the sulfate production measured against time in the leaching solution. This was verified for the two linings considered. Fig. 9 shows the

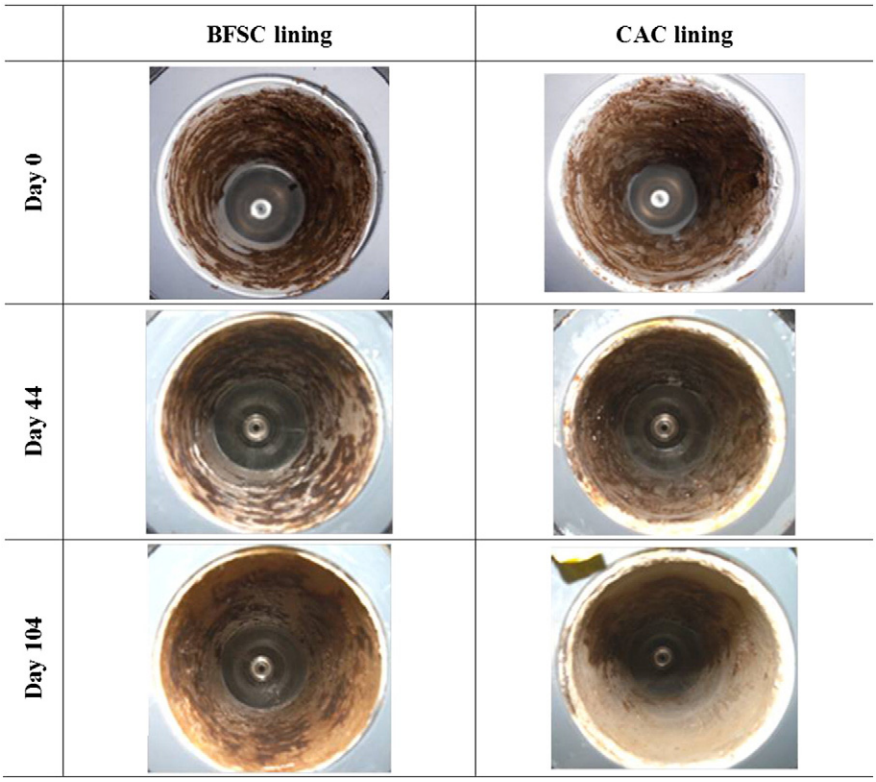

Fig. 7. Photos of the biofilms in contact with BFSC and CAC linings during the tests.

surface rates of sulfate production and of calcium dissolution in the leaching solution for the two inoculated linings. The results obtained on abiotic CAC lining are also provided.

Under abiotic conditions, thiosulfate appeared stable and a negligible amount of sulfate was produced (Fig. 9(A)) with no acidification (pH of the leaching solution ranged between 7.5 and 8.5 (data not shown)). The amount of dissolved calcium was also small (Fig. 9(B)). Under biotic conditions, the sulfate production had similar features for both linings. It increased exponentially in the first 60 days and then stabilized although the supply of thiosulfate was increased. This feature is rather common and it illustrates the growth of SOB and their colonization of the lining surface. Under the same feeding conditions, after 50 days, the surface rate of sulfate production was 33% higher on BFSC lining (0.052 ± 0.006 mol $SO_4^{2-} \cdot m^{-2} \cdot day^{-1}$) than on CAC lining ($0.038 \pm 0.004$ mol $SO_4^{2-} \cdot m^{-2} \cdot day^{-1}$) (Fig. 9(A)). Thus, during the development of the sulfur oxidizing activity, a higher acidification potential was recorded for the microbial community developed on BFSC lining than for that developed on CAC lining.

Concerning the calcium dissolution rate, three phases can be described for both linings:

(i) from day 0 to day 30, the dissolution rate was stable for both linings, with a higher dissolution rate for the BFSC lining (6.4×10^{-3} mol $Ca \cdot m^{-2} \cdot day^{-1}$) than for the CAC lining (2.6×10^{-3} mol $Ca \cdot m^{-2} \cdot day^{-1}$) (Fig. 9(B));

(ii) from day 30 to day 60, the rate of calcium dissolution at the surface increased. This phase matched an increase in the production rate of sulfate (Fig. 9(A)) itself provoked by an increase in the thiosulfate loading from 0.038 mol $S \cdot m^{-2} \cdot day^{-1}$ to 0.094 mol $S \cdot m^{-2} \cdot day^{-1}$. During this phase, the rate of calcium dissolution was higher on BFSC lining ($8.0 \times 10^{-3} \pm 8 \times 10^{-4}$ mol $Ca^{2+} \cdot m^{-2} \cdot day^{-1}$) than on CAC lining ($3.8 \times 10^{-3} \pm 4 \times 10^{-4}$ mol $Ca^{2+} \cdot m^{-2} \cdot day^{-1}$) (Fig. 9(B)); and

(iii) from day 60 to the end of the tests, the rate of calcium dissolution was quite similar for both linings (BFSC = 0.028 ± 0.004 mol $Ca^{2+} \cdot m^{-2} \cdot day^{-1}$; CAC = 0.026 ± 0.004 mol $Ca^{2+} \cdot m^{-2} \cdot day^{-1}$), corresponding to a similar production rate of sulfate (Fig. 9(A)).

Fig. 10 presents the dissolution rate of aluminium in the leaching solution for both inoculated linings. The dissolution of aluminium was not detectable for the BFSC lining because of its low content in the cement matrix and/or the specific behaviour of aluminous oxides [13,54]. For the CAC lining, the aluminium dissolution was very stable during the first 60 days (2×10^{-4} mol $Al^{3+} \cdot m^{-2} \cdot day^{-1}$) (Fig. 10). It was much lower than the calcium dissolution rate during the same period (20 fold lower for an Al content of twice the Ca content in the cement paste). From day 60, the aluminium dissolution accelerated, which matched the stable biogenic acid production, starting at the middle of period (iv). The dissolution rates then yielded $3.0 \times 10^{-3} \pm 3 \times 10^{-4}$ mol $Al^{3+} \cdot m^{-2} \cdot day^{-1}$ during the last 10 days.

3.4. Main results for the cementitious linings (from Part II of this article)

In the second part of this work [39], the chemical and microstructural changes of the linings were analysed by SEM observations and EDS analyses, using electron probe micro-analysis and X-ray diffraction. The analysis showed a decalcified zone for both linings at the surface in contact with the biological activity, confirming the analysis of Ca carried out in the leaching solutions. The decalcified depth was greater for the BFSC lining (700 μm partially decalcified) than for the CAC lining (200 μm). Moreover, inside the cementitious linings, two different behaviours were observed, especially in terms of the secondary precipitations. The BFSC lining presented a network of microcracks, with a major crack at 500 μm deep inside the cementitious lining. Ettringite was identified in the cracks. In contrast, no secondary precipitation of ettringite was observed in the CAC lining, the specimen was exempt of cracks and aluminous hydroxide ($Al(OH)_3$) gel was identified in the decalcified zone.

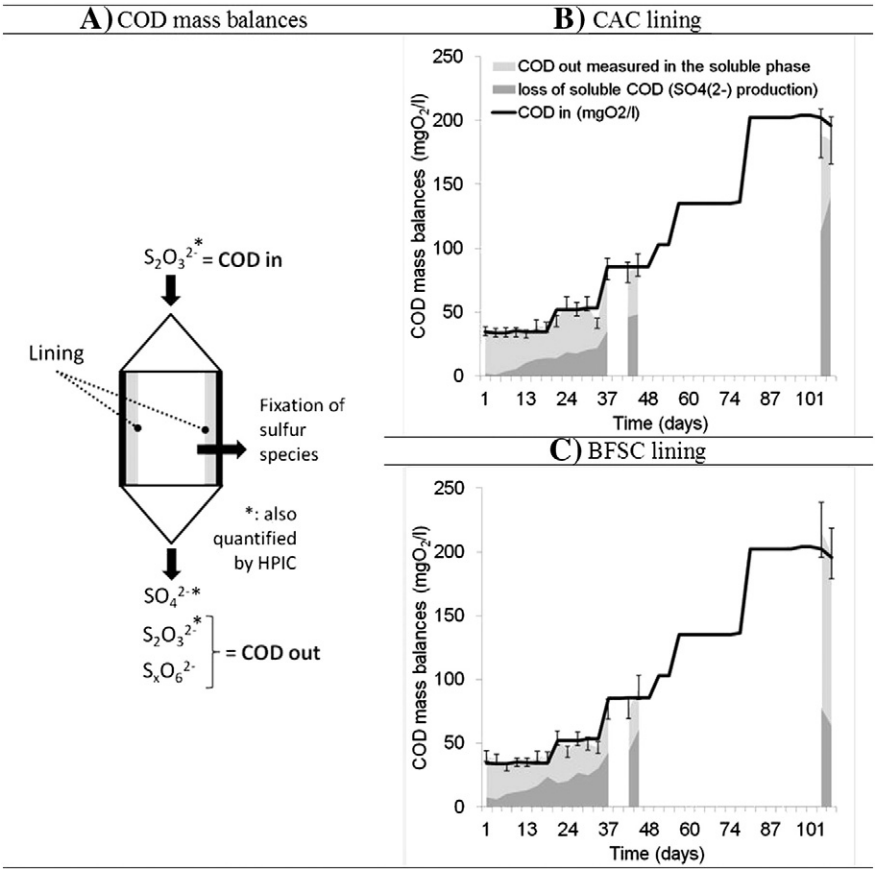

Fig. 8. (A) Schematic diagram of the COD mass balance for one pipe reactor. It includes the sulfate production as a loss of soluble COD. (B) and (C) give the results of the COD mass balances of 17 samples for the CAC lining and the BFSC lining respectively.

4. Discussion

This work aimed to propose a new experimental protocol for evaluating the resistance of cement materials and industrial products (pipes) to MICC (Microbially Induced Concrete Corrosion). The specifications of the test were as follows:

- Whatever the cement material, the test had to allow the implementation of efficient heterotrophic and autotrophic microbial activities leading to high biogenic acid production concomitant with the oxidation of reduced sulfur compounds into sulfate.
- The test had to be sensitive enough to discriminate among materials or products in terms of resistance to MICC.
- The test had to be reproducible and rapid enough to give the desired response in less than 6 months.

The reproducibility of the experiments has not been studied in this article. It will be the subject of future works. The study focused on the analysis of the following: (i) the selection of the sulfur-oxidizing function as a representative parameter of biological activity in sewers, (ii) the use of thiosulfate as a substitute for H_2S, (iii) biogenic acid production, (iv) the identification of the main processes represented, compared to the literature, and (v) the qualitative and quantitative differences between the two linings. The first, second and fourth aspects were analysed with a view to validating the representativeness of the

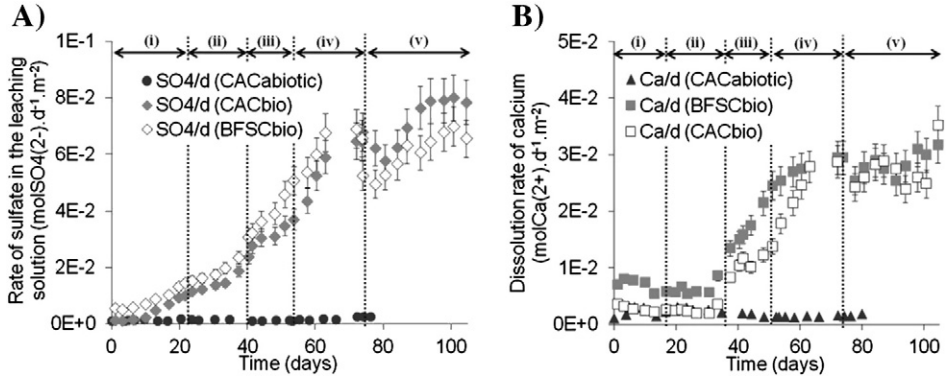

Fig. 9. Rate of sulfate production on the surface (A) and of calcium dissolution in the leaching solution (B) in biotic conditions for CAC and BFSC linings and in abiotic conditions for CAC lining.

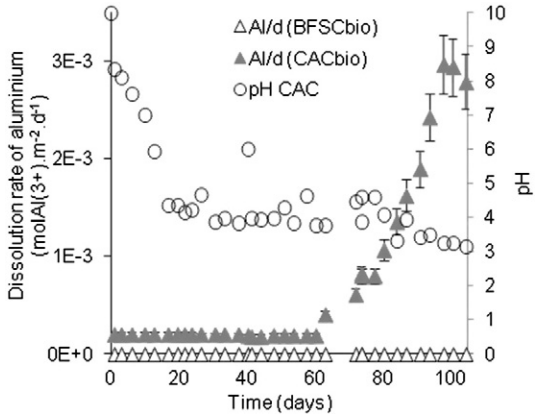

Fig. 10. Dissolution rate of aluminium in the leaching solution for both inoculated linings (BFSC and CAC).

test; the third was examined in order to quantify its efficiency. The fifth aspect also gave information on the capacity of the test design to differentiate between two cementitious linings.

4.1. The microbial selection

The selection of SOB from AS as inoculum was ensured by the use of thiosulfate as the sulfur source and the decrease of the pH of the medium in contact with the cementitious linings [31]. The pH, initially around 9, decreased to values ranging from 3 to 4 and the high sulfate production demonstrated intense metabolic activity leading to biogenic acid. Biofilm enrichment in sulfur-oxidizing bacteria (SOB) was confirmed by bacteria identification using pyrosequencing. Molecular identification showed that SOB were not detectable in the AS samples whereas they represented at least 23% ± 10% (with AS1 on BFSC lining) and up to 48% ± 1% (with AS1 on CAC lining) of the relative abundance (defined as the number of sequences identified for a genus relative to the total of sequences identified in the sample) at the end of the two experiments. SOB were found whatever the location at the surface of the linings, which demonstrated the capacity of these bacteria to grow and colonize the whole surface of the two. The SOB communities were represented by a combination of *Thiomonas* and *Thiobacillus* genera as described in the literature for some corroded surfaces [18,28] (*Thiomonas intermedia* being formerly named *Thiobacillus intermedius*), and used as inoculum for the main testing methods proposed in the literature [12,17,20,23,26]. In this study, SOB were systematically associated with heterotrophic bacteria, which made up 11 to 55% of the total bacterial abundance depending on the lining and the culture duration. Heterotrophic bacteria certainly grow on cell lysis products and participate in the biofilm structure and activities. They may have had an effect on the O_2 mass transfer and they were responsible for local CO_2 production. Both these effects may have had a significant impact on the material degradation and local autotrophic growth. The presence of these heterotrophic bacteria has been described in the majority of field observations [16,18,28–31,35]. Moreover, no nitrification was observable in the present study with the thiosulfate feed when the pH of the leaching solution reached acid values below 4, although significant amounts of nitrifiers were present in the AS inocula and some were still detected at the end of the experiment (Fig. 6) as confirmed by some metagenomic analysis realized on field samples [30]. It was demonstrated (data not shown) that the absence of nitrification was due to the rapid acidification provided by the high sulfo-oxidizing activity occurring in the biofilm soon after inoculation. This was achieved by imposing a high thiosulfate feeding rate and no nutrient limitation. Therefore, the use of AS inocula characterized by high diversity may help to develop well adapted, highly resistant bacterial populations at the surface of cementitious materials, including SOB and heterotrophic bacteria with the conservation of a high diversity.

4.2. Interest of thiosulfate as a sulfur source

The use of thiosulfate in the feeding solution, a soluble, chemically stable compound in the experimental conditions, enabled the sulfo-oxidation rate to be easily controlled and enabled more direct and reliable quantification of the acid production in contact with the linings. Mass balances could be carried out using sulfate analyses coupled to COD analyses. The increase in the SOB activity and thus in the kinetics of the biogenic acid production by the biofilm was simply performed by adjusting the thiosulfate concentration in the feed solution Moreover, the use of thiosulfate as a substitute for H_2S led to a safer test that was easier to set up and operate.

4.3. Intensity of the biogenic acid production

The efficiency of the test in terms of aggressive conditions must be evaluated by the quantifying the acid production per square metre exposed and comparing it with the time needed to reach significant values. In this work, a steady state in the acid production was reached at 0.072 mol $H^+ \cdot m^{-2} \cdot day^{-1}$ at 20 °C after only 2 months. For comparison, after 18 months, Nielsen et al., simulating sewer pipes exposed to an aggressive environment, quantified a maximal H_2S consumption rate of 200 mg $S \cdot m^{-2} \cdot h^{-1}$ on OPC concrete pipes for pulses of 1000 ppm_v of H_2S [58]. Using the hypothesis of total production of sulfuric acid [36], a maximal acid production of 0.288 mol $H^+ \cdot m^{-2} \cdot day^{-1}$ at 15 °C can be extrapolated, i.e. four times that reached in this study, but with an experiment duration 9 times as long. On the other hand, Ehrich et al., used concrete coupons exposed during one year in a simulation chamber, with a continuous H_2S feed at a concentration of 10 ± 5 ppm and at 30 °C. They found a production after 5 months of around 0.130 mol $H^+ \cdot m^{-2} \cdot day^{-1}$ on a CAC mortar leading to a weight loss of 13.1% and around 0.175 mol $H^+ \cdot m^{-2} \cdot day^{-1}$ on a BFSC mortar leading to a weight loss of 25.3% [17]. By comparing the weight losses of the tested coupons with samples taken from severe corroded sewer pipes of the city of Hamburg, Ehrich et al. estimated after one year of exposure an accelerator factor of 24 for their test [17].

Thus, in the present study carried out using activated sludge as the inoculum, thiosulfate permitted the rapid selection of sulfur-oxidizing bacteria, leading to intense biogenic acid production in contact with cementitious materials.

4.4. Representativeness of the test design

Identification of the main processes occurring at the surface of the material and inside the material is of great interest for assessing the capacity of the test to represent real processes involved in pipe degradation. In this work, BFSC and CAC linings were tested for their different behaviour towards biogenic acid attacks in sewers in terms of degradation mechanisms and resistance (lower resistance identified for BFSC materials) [13,17]. The fate of the sulfate produced is a key factor in the description of MICC in sewers [8,14]. In this study, although the sulfur mass balances achieved in the leaching solution revealed low sulfate penetration inside the cementitious matrix, the analyses of materials (presented in second part of this study) highlighted some major differences between the two linings [39]. Secondary precipitations of ettringite occurred in BFSC lining, leading to microcracks. In contrast, no crack was observed for the CAC lining (formation of $Al(OH)_3$ in the decalcified layer was only observed). Moreover, the decalcification front was deeper in the BSFC lining (700 μm) than in the CAC one (200 μm). It should be noted that the quite low degraded layer thicknesses obtained may be attributed to the particular microstructure of the linings, which were very dense owing to the centrifugation process [39]. The delay in the aluminium dissolution compared to the calcium

dissolution confirmed the preservation of the aluminium inside the CAC lining. These measurements were in accordance with some literature observations of CAC materials under acid environment [13,54]. Therefore, the main processes of biogenic acid attack were reproduced [8,10,12,14], with a selection of sulfur oxidizing bacteria from a wastewater environment leading to a biological acid production at the surface of the cementitious materials, and to chemical and microstructural changes, the nature of which was highly dependent on the mortar composition.

4.5. Differences between CAC and BFSC lining against biogenic acid attack

In terms of differentiation between the two linings, this study pointed out interesting phenomena concerning calcium leaching on the one hand, and the impact of the material on the biomass characteristics on the other. In the first 50 days of the experiment, the acid production was 33% higher on BFSC lining than on CAC lining, which was in accordance with the literature describing the first steps of MICC [17], and qualitatively confirmed the effect of the CAC paste [13,16]. During the last 50 days, when the pH of the leaching solutions reached values between 3 and 4, the acid production was equivalent on both linings. This acid production led to the release of calcium in the leaching solution. The calcium dissolution rates were equivalent for both linings, with a ratio between moles of calcium released and moles of acid produced close to 0.5, although the CaO content was higher in the BSFC than in the CAC lining (Table 2). This means that, during the stronger acid production phase, the relation between acid production and calcium dissolution rates was only stoichiometric, and was independent of the mineralogical composition of the cement matrix.

Moreover, it was shown that the amounts of biofilm mass were different at the end of the experiments. For the same surface area, 330 mg VSS was measured on the BFSC lining but only 80 mg VSS was found on the CAC lining. Qualitatively, the biofilm developed on the CAC surface appeared dryer than the biofilm developed on the BFSC surface. These differences may be explained by some differences in the chemical compositions of the cement pastes [17] and their evolution [13] and differences in surface properties as material roughness, porosity and hydrophobicity are the key factors influencing bacterial adhesion and biofouling [59–61]. A bacteriostatic effect of the aluminium proposed by some authors [13,17] could also explain the observed differences. However, during the first steps of the biogenic acid attack (first 50 days), differences between the sulfur-oxidizing activity on each lining were recorded, with a stronger activity on the BFSC lining than on the CAC lining (Fig. 9). During this period less than 1 mg Al^{3+}/l was measured in the leaching solution of the exposed CAC lining. The local concentrations of dissolved aluminium at the interface of the cementitious linings and the microbial communities must be higher than those measured in the leaching solution. However, the speciation calculation of amorphous $Al(OH)_3$ with an aqueous solution (based on Minteq thermodynamic constant database) estimated Al^{3+} concentration of about 1.32×10^{-3} mg Al^{3+}/l at pH 4. At pH 3, the same calculation gave a concentration of 1.32 mg Al^{3+}/l. In contrast, the literature indicated that minimum concentrations in the range 50–750 mg Al^{3+}/l were necessary to obtain a significant growth inhibition of some SOB species [30]. These results support the hypothesis that other factors than inhibition by Al^{3+} could explain the delayed increase in the SOB activity during the first step of the biogenic acid attack in the case of CAC lining. A similar delay has been observed by Ehrich et al. on CAC mortars [17]. The CAC lining apparently induced a direct impact on microbial activity, at least in the first step of the biogenic acid attack before any aluminium dissolution had taken place [53]. Thus, the selection and enrichment of well adapted microorganisms on the whole surface of the lining occurred rapidly in the first step of the test. It was imposed by the test operating conditions and apparently also by the material composition and structure, showing the interest of working with an inoculum having high diversity.

5. Conclusions

A new test was designed and implemented with thiosulfate ($S_2O_3^{2-}$) as the sulfur source. The test design (i) considered the role of the composition of the cementitious products as a selection factor on the microbial communities by the use of a highly diverse inoculum sampled from an urban activated sludge; (ii) quantified the acid produced in contact with the materials according to time; and (iii) quantified the dissolution rates of calcium and aluminium released by the cementitious products over time.

The use of thiosulfate allowed the reduced sulfur compound fed onto the surface of the cementitious lining to be controlled, and permitted the measurement of biogenic acid fluxes directly produced. The biogenic acid production was immediate on both linings tested, with efficiency in the calcium dissolution recorded after 40 days of exposure at 20 °C. The biogenic acid production reached its maximum on both linings after 60 days in the experimental conditions (20 °C), and was stable during the last 50 days.

Differences in behaviour between the CAC and BFSC linings tested were highlighted in the first step of the biogenic acid attack. After 50 days, the biogenic acid production quantified in contact with the linings was 0.052 ± 0.006 mol $H^+ \cdot m^{-2} \cdot day^{-1}$ on the BFSC lining and 0.038 ± 0.004 mol $H^+ \cdot m^{-2} \cdot day^{-1}$ on the CAC lining; the calcium dissolution recorded was $8.0 \times 10^{-3} \pm 8 \times 10^{-4}$ mol $Ca^{2+} \cdot m^{-2} \cdot day^{-1}$ on the BFSC and $3.8 \times 10^{-3} \pm 4 \times 10^{-4}$ mol $Ca^{2+} \cdot m^{-2} \cdot day^{-1}$ on the CAC lining. Finally, on BFSC lining, after 15 weeks, the biofilm maintained at the surface was denser than that on the CAC lining. The little leaching of Al^{3+} highlighted the specific effect of the initial microstructure of the CAC lining on the biological activity, even though the mechanisms involved in the lower bioreceptivity of this lining need further investigation. Moreover, the role of the surface properties of the mortars (roughness, porosity, hydrophobicity, etc.) must not be forgotten.

The second part of this article will focus on the analysis of the behaviour of BFSC and CAC linings in terms of chemical and microstructural evolutions, especially for the transport and reactions inside the cementitious matrix of the sulfate produced, in order to evaluate the representativeness of the transformations induced by the use of thiosulfate as the sulfur source.

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