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1 **Resistance to biodeterioration of aluminium-rich binders in sewer network**  
2 **environment: study of the possible bacteriostatic effect and role of phase reactivity**

3

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9

10 **Keywords:** Durability (C); Calcium aluminate cement (D); Portland cement (D); Granulated Blast-  
11 Furnace Slag (D); Waste management (E)

12

13 **Abstract**

14

15 This paper aims to better understand the mechanisms explaining the superior resistance of calcium  
16 aluminate cement (CAC) materials compared to Portland cement (PC) based materials in sewer  
17 networks. The bacteriostatic effect of CAC materials on Sulfur Oxidising Bacteria (SOB) is often  
18 argued in the literature as a possible mechanism explaining their better resistance. Reactor tests  
19 conducted on SOB demonstrated their ability to acclimatise to high aluminium contents. More  
20 generally, using a laboratory biodeterioration protocol reproducing aggressive conditions, the nature of  
21 the material (CAC or PC with different slag contents) did not significantly affect the SOB selection.  
22 Moreover the CAC materials seemed to favour the development of a higher SOB activity (and so acid  
23 production) than PC-based systems, leading to more aggressive conditions. Finally, the resistance of

24 CAC materials to biodeterioration appeared to be mainly linked to the intrinsic resistance of phases  
25 initially present or precipitated during the deterioration process.

26

## 27 **1. Introduction**

28

29 The local biological production of H<sub>2</sub>S released in the aerial part of sewer networks and its biological  
30 oxidation into H<sub>2</sub>SO<sub>4</sub> are partly the cause of deterioration of their cementitious materials [1,2]. In very  
31 aggressive conditions (humidity, temperature, hydraulic constants, H<sub>2</sub>S levels etc.) and with a  
32 conventional concrete formulation, the lifespan of concrete structures may be reduced to below 10  
33 years [3,4]. Such biodeterioration leads to major problems in wastewater collection and treatment,  
34 implying significant rehabilitation costs [5,6].

35 The biodeterioration of concrete in sewer networks is the result of a series of complex biological and  
36 chemical reactions: (i) In stagnant zones (in organic-rich wastewater), sulfate-reducing bacteria (SRB)  
37 reduce sulfate into H<sub>2</sub>S in anaerobic and nitrate depletion conditions [7]. This compound degases into  
38 the headspace of the networks and condenses on the upper part of the cementitious coating by  
39 absorption and diffusion. The chemical reactions of the H<sub>2</sub>S - and the CO<sub>2</sub> - on a hardened concrete  
40 (with surface pH ranging from 11 to 13.5 [2]) induce a decrease in the pH at the material surface to  
41 around 9 [8]. The abiotic reactions of H<sub>2</sub>S with the cementitious matrix produce several sulfur  
42 compounds with different degrees of oxidation [9]. These precursors (thiosulfate (S<sub>2</sub>O<sub>3</sub>), elemental  
43 sulfur (S<sup>0</sup>), etc.) are sources of electrons for bacterial development. The pH conditions and the sulfur  
44 compounds create a suitable environment for sulfur-oxidising bacteria (SOB) to develop. With the  
45 transformation by SOB of the reduced sulfur into H<sup>+</sup> and SO<sub>4</sub><sup>2-</sup> as final oxidation products [2,10],  
46 successive populations of SOB develop during the pH decrease. The first bacterial succession  
47 concerns the neutrophilic sulfur-oxidising bacteria (NSOB) which results in a progressive pH decrease  
48 from 9 to 4 [2,11]. However, starting from pH 4, the environmental conditions are no longer suitable  
49 for the growth of NSOB and, hence, acidophilic sulfur-oxidising bacteria (ASOB) – the second  
50 bacterial succession – develop [2,9,12]. The development of ASOB is considered as the main active

51 stage of biodeterioration due to the high production of sulfuric acid [2,9,13]. In this range of pH  
52 *Acidithiobacillus Thiooxidans* is reported as the predominant aerobic bacterial species responsible for  
53 the acidification [3,13]. The attack of the cementitious material by the aggressive agent produced by  
54 the microbial activity is usually called biogenic acid attack. Grengg et al. [14] showed the presence of  
55 bacteria deep inside the deteriorated area of concrete exposed to sewer conditions in situ. Moreover  
56 these authors demonstrate the role in the biodeterioration process of *Acidithiobacillus ferrooxidans*  
57 using Fe as an energy source in anaerobic areas far from the surface of the concrete.

58 The deterioration of the cementitious matrix by biogenic acid is a dual attack by acid ( $H^+$ ) and by  
59 sulfate ( $SO_4^{2-}$ ). Decalcification and dissolution of the main calcium-based phases (portlandite (noted  
60 CH) and C-A-S-H for PC-based material and  $3CaO \cdot Al_2O_3 \cdot 6H_2O$  (katoite or  $C_3AH_6$  in cementitious  
61 notation) for CAC-based material lead to the formation of silica or alumino-silica rich deteriorated  
62 areas on the upper part of the material in contact with the biofilm [2,15]. The so called siliceous gel  
63 observed for PC systems is mainly amorphous and the alumina gel for CAC is mainly composed of  
64  $Al(OH)_3$  phases (noted  $AH_3$  using cementitious notations)[16]. On the other hand, the diffusion of  
65 sulfur compounds in the porosity of the cement matrix and their reaction with hydrated phases induces  
66 the precipitation of sulfate based phases (often identified as gypsum and ettringite) [17,18].

67 The combination of these phenomena is commonly called Microbial Induced Concrete Corrosion  
68 (MICC) even though the term “corrosion” might be reconsidered in a biogenic acid attack. The  
69 biodeterioration of the cementitious materials combines biological, physical and chemical reactions  
70 and occurs in a complex system associating microorganisms, the material and the surrounding  
71 environment (sulfur substrate level, temperature, humidity, hydraulic constant, etc.). [4,17].

72 Therefore, accelerated biodeterioration protocols are needed in order to obtain data in several months  
73 and to dissociate the different key parameters of the resistance of materials to biogenic acid attack.  
74 [19,20], [21,22], A test was developed at the University of Toulouse (called BAC test for Biogenic  
75 Acid Concrete test) [23,24] for this purpose, which uses the development of a biofilm on the surface of  
76 a cementitious material fed by trickling tetrathionate solution over it. The use of a liquid sulfur  
77 substrate instead of  $H_2S$  – often used in laboratory tests – allows the feeding and the leaching solution

78 to be analysed and thus the biological transformation of the sulfur and the progressive leaching of the  
79 cementitious matrix to be monitored through mass balance [25]. In the BAC test, the inoculum is  
80 composed of sludge from a waste water treatment plant. The use of a diversified inoculum takes the  
81 biodiversity into account while selecting the sulfur-oxidising function.

82 Calcium Aluminate Cement (CAC) based materials have demonstrated a higher resistance to a  
83 biogenic acid attack than PC-based materials, in both on-site and laboratory conditions [15,17,25,26].

84 A range of hypotheses have been emitted by different authors to account for this better resistance.  
85 They can be divided into two categories: the physicochemical resistance of the material, i.e. its  
86 capacity to resist the chemical attack because of the chemical and physical properties of the original  
87 and newly formed phases, and the biological resistance, i.e. the ability of the material to influence or  
88 hinder the biological activity, leading to less severe attack on the material. In the first category, many  
89 authors attribute the higher durability of CAC-based materials to: (i) the higher neutralisation capacity  
90 of these materials compared to PC-based materials, which would neutralise a larger amount of  
91 biogenic acid, (ii) the greater stability of original hydrated phases (including  $C_3AH_6$  and/or phases  
92 newly formed through acid attack ( $AH_3$ ), compared to calcium silicate phases (CH, C-A-S-H and  
93 silica gel), and (iii) the low porosity of  $AH_3$  gel of biodeteriorated CAC matrix, which would slow  
94 down transport processes [15,19,26–28]. Concerning the biological resistance, the bacteriostatic effect  
95 of aluminium is often mentioned. It is thought that the release of  $Al^{3+}$  by the CAC matrix inhibits the  
96 SOB activity, leading to a decrease in biogenic sulfuric acid production, and that the neutralisation  
97 capacity of CAC mortar would limit the SOB growth by depriving them of their ideal growth  
98 conditions and so decreasing the biogenic sulfuric acid production [12,17,19,26,29]. However, no real  
99 consensus and no direct evidence or robust demonstration of the validity of the different hypotheses  
100 are to be found in the literature so far.

101 The aim of the present work was to better understand the mechanisms explaining the better resistance  
102 of CAC materials compared to PC-based materials in sewer network conditions, and to assess each  
103 previous hypothesis. The possible bacteriostatic effect of aluminium was assessed by studying the  
104 influence of soluble aluminium on SOB activity in reactor conditions. Then, the influence of the

105 nature of the material (composition, neutralisation capacity, etc.) on the SOB biofilm settlement,  
106 development and activity during biodeterioration was examined using a laboratory test (BAC test).  
107 Finally, the influence of the chemical species produced by the biological activity on the material was  
108 studied, in order to understand the relationship between the physicochemical characteristics of a  
109 cementitious material (composition, porosity, etc.) and its resistance to deterioration.

110 To understand the influence of physicochemical characteristics of cementitious binders and, in  
111 particular, the role of aluminium on the biotic activity, materials containing various aluminium  
112 contents and with different aluminium mineralogical forms were selected. PC-based materials with  
113 different substitution rates of ground granulated blast-furnace slag (GGBS) (from 0 to 95%) and CAC-  
114 based materials with two different types of thermal curing were cast.

115

## 116 **2. Materials and testing procedure**

117

### 118 **2.1 Preparation and characterisation of cement pastes**

119 Cement paste specimens were made with a white PC, CEM I 52.5N CE CP2 (Lafargeholcim, Le Teil,  
120 France) substituted with different amounts of commercial superfine GGBS (Ecocem Ltd, Ireland)  
121 from 0 wt.% to 95 wt.%. CAC (Secar 51, Kerneos, France) based samples were also cast (Tables 1  
122 and 2). The chemical compositions in Table 1 were obtained by XRF analysis. The Blaine surface was  
123 evaluated with the method described in the standard NF EN 196-6. Phase quantification data in Table  
124 2 were obtained by quantitative XRD with Rietveld refinement. A white PC was chosen in this study  
125 (i) to limit the presence of iron, which can influence the development of some acidophilic bacteria and.

126 Water intrusion porosimetry was performed on cement samples before exposure to the BAC Test. The  
127 samples were kept in a vacuum for 4 hours to remove any air entrapped in pores. Afterwards, the pores  
128 of the samples were filled with water under vacuum conditions for 24 hours. The samples were then  
129 removed from the water and hydrostatically weighed before being dried at 105 °C. The samples were

130 weighed daily until the difference between two successive weighings, 24 hours apart, was less than  
 131 0.05%. The porosity and the apparent density were then calculated [30].

132

133

**Table 1. Oxide composition of PC, GGBS and CAC**

	<b>PC</b>	<b>GGBS</b>	<b>CAC</b>
CaO	66.6	43.3	37.9
SiO <sub>2</sub>	22.0	37.1	5.1
Al <sub>2</sub> O <sub>3</sub>	2.3	11.1	52.1
MgO	0.5	6.5	0.3
Fe <sub>2</sub> O <sub>3</sub>	0.3	0.6	1.6
TiO <sub>2</sub>	<0.1	0.5	2.1
K <sub>2</sub> O	0.5	0.2	0.4
SO <sub>3</sub>	3.4	0.2	<0.1
other oxides	4.8	0.5	0.5
loss on ignition	3.8	<1.5	/
Blaine	4250	7500	3870

134

135

**Table 2. Mineralogical composition of PC and CAC.**

<b>Phases:</b>	<b>C<sub>3</sub>S</b>	<b>C<sub>2</sub>S</b>	<b>C<sub>3</sub>A</b>	<b>C<sub>4</sub>AF</b>	<b>CaSO<sub>4</sub>.0.5H<sub>2</sub>O</b>	<b>Calcite</b>
PC (wt.%)	58	28	6	1	2.5	5
<b>Phases:</b>	<b>CA</b>	<b>C<sub>12</sub>A<sub>7</sub></b>	<b>C<sub>2</sub>AS</b>	<b>C<sub>2</sub>S</b>	<b>Q-phase</b>	<b>Perovskite</b>
CAC (wt.%)	67.3	0.4	20.1	5.0	2.4	4.2

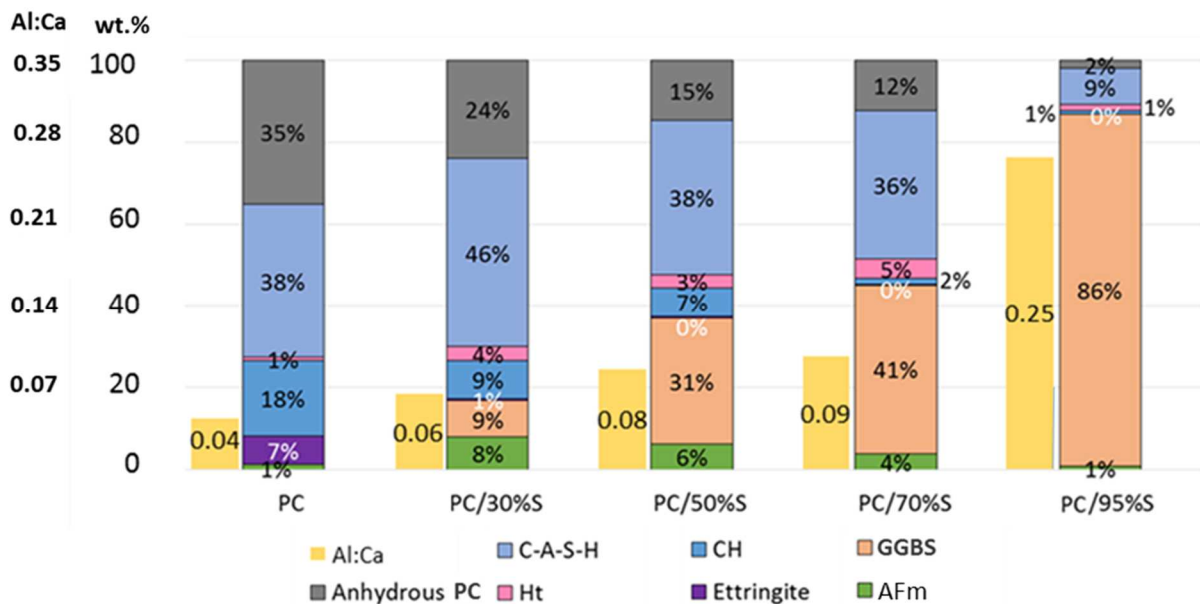
136

137 Cement paste specimens were prepared with a water/binder (w/b) ratio of 0.4 for PC-GGBS binders  
 138 and 0.3 for CAC binders. In EN-196-1 the w/b ratio is 0.5 for PC-based mortar, and in EN-14647 the  
 139 w/b ratio is 0.4 for CAC mortar. Specimens were cast in rectangular plastic moulds with dimensions

140 5\*4\*1 cm<sup>3</sup> and demoulded after 24 hours of hydration. PC-based specimens were stored at 20 °C in  
 141 sealed plastic bags for 90 days. Two types of thermal curing were used for CAC specimens in order to  
 142 evaluate the influence of conversion of metastable hydrates on the resistance of the material to  
 143 biogenic acid attack. After casting, a first series of CAC was cured for 16 hours at 70 °C (98% RH)  
 144 followed by 8 hours at 20 °C (98% RH) to ensure that conversion took place (the specimens were  
 145 called CAC-70°C). A second series of CAC was not exposed to any thermal curing and was kept at 20  
 146 °C (called CAC-20°C). CAC specimens were stored at 20 °C in sealed plastic bags for 90 days.

147 After curing, the cast face located on the bottom of the mould (5\*4 cm<sup>2</sup>) was selected for exposure to  
 148 the BAC-test whereas the other faces were protected from acid attack with a special epoxy resin  
 149 coating (EUROKOTE 48-20 from BS COATING). The longitudinal edges (each 5 cm long and 3 mm  
 150 thick) of the uncoated and further exposed cast surface were also coated with the epoxy in order to  
 151 guide the liquid dripping on the sample during the BAC test. These guides were later used as a  
 152 reference for measuring depth of deterioration.

153 The mineralogical features of the OPC-GGBS hydrated binders (Figure 1) at 90 days before exposure  
 154 to the BAC-test were determined by mass balance based on the method described by Yu et al. [31].

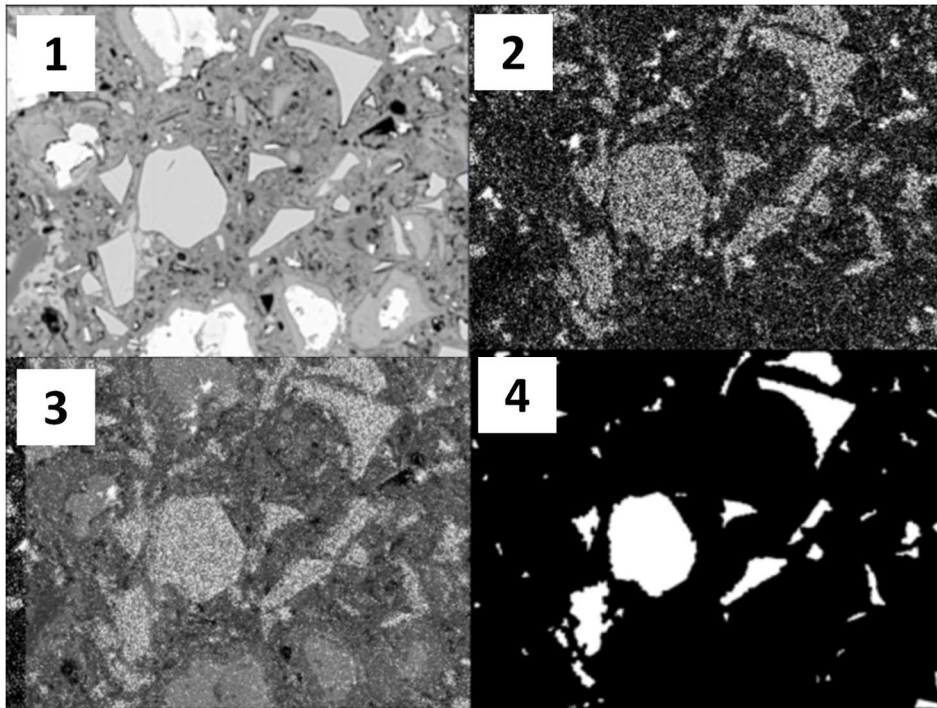


155  
 156 **Figure 1. Phase assemblage estimation of PC with 0%, 30%, 50%, 70% and 95% of GGBS sample after 90 days'**  
 157 **hydration. On the left, Al:Ca molar ratio in the binders.**



158 Mass-balance calculations were carried out to quantify approximately the amount of C-S-H and  
159 poorly-crystalline hydrates such as AFm. Actually, a direct quantification of these phases by TGA,  
160 NMR ( $^{27}\text{Al}$  for example), SEM or XRD coupled with Rietveld refinement is very difficult, if  
161 impossible.

162 The amount of anhydrous clinker and that of GGBS were measured by SEM-based methods (on the  
163 basis of Mouret et al. and Kocaba et al. approaches [32,33]). Quantitative analysis of backscattered  
164 electron images with image analysis (BSE and IA) coupled with EDX (i.e., energy dispersive x-ray  
165 spectroscopy) were done to quantify the residual anhydrous phases. Mg-EDS mapping were used to  
166 separate CH areas and GGBS grains showing closed grey levels. Figure 2 illustrates the measurement  
167 of the degree of GGBS reaction using SEM-EDX methods.



168

169 **Figure 2. The steps applied in measurement of the degree of slag reaction using SEM-EDX methods: (1) SEM**  
170 **image, (2) Mg-EDS Mapping, (3) Superimposition of BSE and EDS images and (4) Identification of the GGBS**  
171 **particulates (unreacted).**

172 As such, assuming standard stereological correlations for randomly distributed microstructures, the  
173 residual anhydrous clinker and GGBS can be determined. Thirty BSE images were acquired on plane  
174 polished paste sections using a JEOL JSM 6380 LV scanning electron microscope (SEM) at a  
175 magnification of 900x. The total amount of Ca, Si, Al, Mg, S and C released during the hydration was  
176 calculated, knowing the chemical composition of PC and GGBS. All the Mg was considered included  
177 in hydrotalcite ( $\text{Mg}_4\text{Al}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ ), and Al relative to the chemical formula was subtracted. All the Si  
178 was assumed to be incorporated in C-S-H, and Ca, Al, and S were subtracted based on the Ca/Si,  
179 Al/Ca, and S/Si ratios in the C-S-H measured by SEM-EDS pointing. The ettringite was quantified by

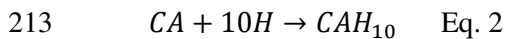
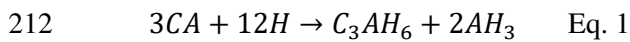
180 XRD-Rietveld, and Ca, Al and S were subtracted. The remaining S was considered included in  
181 monosulfate, subtracting Ca and Al. Finally, the remaining Ca was attributed to CH.

182 One of the main error of the mass-balance calculation is the evaluation of the quantity of monosulfate,  
183 because it is calculated from the amount of sulfate after assignment to C-S-H and to ettringite. In this  
184 study, the ettringite peak for OPC-GGBS systems is very low and in the error range of the method (no  
185 more than 1 wt.%). The accuracy of mass balance calculations of hydrates is then likely questionable.  
186 But this simplified mass balance calculation allows addressing the main correlations between major  
187 changes in phase assemblage and resistance to biodeterioration.

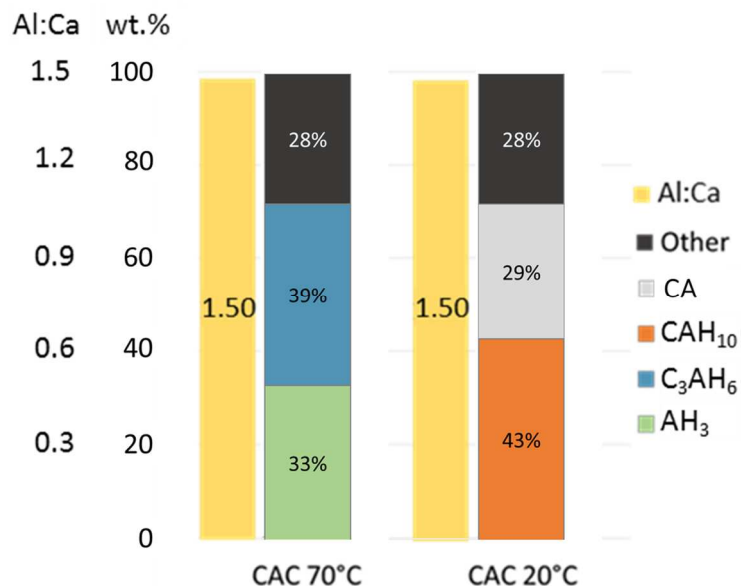
188 The relative proportion of C-A-S-H decreased and the Ca:Al ratio increased with higher substitution  
189 levels of GGBS. Hydrotalcite (Ht) and monosulfoaluminate (AFm) were present in small proportions.

190 The ettringite content was very low in GGBS blended systems, probably due to a sulfate deficit. The  
191 proportion of unreacted GGBS was high and increased with higher substitution levels.

192 XRD analysis have been done on solid samples of CAC 20°C and CAC 70°C before exposure to the  
193 biodeterioration test. The qualitative XRD analyses revealed the presence of  $C_3AH_6$  and  $AH_3$  for CAC  
194 70°C, and presence of  $AH_3$ ,  $CaO \cdot Al_2O_3 \cdot 10 \cdot (H_2O)$  (noted  $CAH_{10}$ ) (with traces of  $C_3AH_6$  for the system  
195 CAC 20°C). No  $2CaO \cdot Al_2O_3 \cdot 8(H_2O)$  (noted  $C_2AH_8$ ) was spotted in neither sample. A simplified phase  
196 assemblage has been calculated for both CAC 20°C and CAC 70°C systems. The CA phase is the  
197 major phase in CAC cements and the most reactive one (with  $C_{12}A_7$  which is present in a very low  
198 content, i.e. 0.4 wt.%). In the proposed phase assemblage, the CA phase is considered as the only  
199 anhydrous phase reacting during hydration. The contents of the other anhydrous phases of the CAC  
200 ( $C_2AS$ ,  $C_2S$ , Q-phase, perovskite) are supposed to be constant between 0 and 90 days. The residual  
201 quantity of anhydrous CA grains was determined by SEM image analysis based on BSE grey levels  
202 according to the same method as the quantification of anhydrous PC. Gosselin has already shown the  
203 ability of satisfactorily isolating the CA phase contribution on the grey-level histogram in SEM-BSE  
204 mode and the ability to accurately quantify this anhydrous phase by this method [34]. The residual  
205 content of CA was 0 wt.% and 29 wt.% for the CAC 70°C and CAC 20°C systems, respectively. The  
206 CAC 70°C system was thus significantly more hydrated than the CAC 20°C one. Equations Eq. 1 and  
207 Eq. 2 were used for mass balance calculations of hydrate formation for CAC 70°C and CAC 20°C,  
208 respectively [34]. These results are in accordance with experimental results of the literature showing  
209 that a 20°C curing of CAC cements favors the formation of  $CAH_{10}$  which is potentially metastable  
210 whereas the 70°C curing enhances the production of  $C_3AH_6$  and  $AH_3$  through the so-called process  
211 “conversion phenomena”.



214 The results of the mass balance calculation for the CAC 70°C and the CAC-20°C system are detailed  
 215 in Figure 3.



216

217 **Figure 3. Phase assemblage of CAC-20°C and CAC-70°C. On the left, Al:Ca molar ratio in the binders.**

218 AH<sub>3</sub> is only present in CAC-70°C, in a comparable mass proportion to C<sub>3</sub>AH<sub>6</sub> (respectively 33 and 39  
 219 wt. %). The main hydrated phase of the CAC-20°C system is CAH<sub>10</sub>

220

221 **2.2.2 Hydroxide potential of materials tested**

222

223 One of the literature hypotheses explaining the better resistance of CAC-based material is the higher  
 224 neutralisation capacity of CAC binders [15,19,27]. During the biodeterioration of the material, oxides  
 225 present in the matrix are dissolved, which induces the release of hydroxide. The theoretical amount of  
 226 hydroxides dissolvable in each tested material was calculated. This “hydroxide potential” depends  
 227 only on the chemical composition of the material and not on the phase assemblage. If all oxides in a

228 material are dissolved, the hydroxide potential corresponds to the molar amount of OH<sup>-</sup> that can be  
 229 released and is named PtOH (total OH<sup>-</sup>potential) in what follows.

230 In the first stage of acid attack, and biogenic acid attack in particular, the deterioration process  
 231 corresponds to the decalcification of the material, with dissolution of CH and C-A-S-H for PC-based  
 232 material and decalcification of C<sub>3</sub>AH<sub>6</sub> (and CAH<sub>10</sub>) into AH<sub>3</sub> for CAC-based material [15,24,35]. An  
 233 intermediate OH<sup>-</sup> potential, PiOH, corresponding to the first stage of the deterioration of a material,  
 234 i.e. the release of calcium (calculated only from the initial calcium oxide composition obtained by  
 235 XRF on anhydrous samples) from the decalcification of CH, C-A-S-H, C<sub>3</sub>AH<sub>6</sub>, and CAH<sub>10</sub>, can thus be  
 236 calculated. The PiOH corresponds to the neutralisation capacity when pH starts to decrease (formation  
 237 of new deterioration products) until all the initial phases have been dissolved. The dissolution  
 238 reactions used for this study are reported in Table 3. Potassium, sulfur and titanium oxides were not  
 239 taken into account because of their low contribution to the potential. It can be noted that SiO<sub>2</sub> did not  
 240 participate in the hydroxide potential calculation.

241

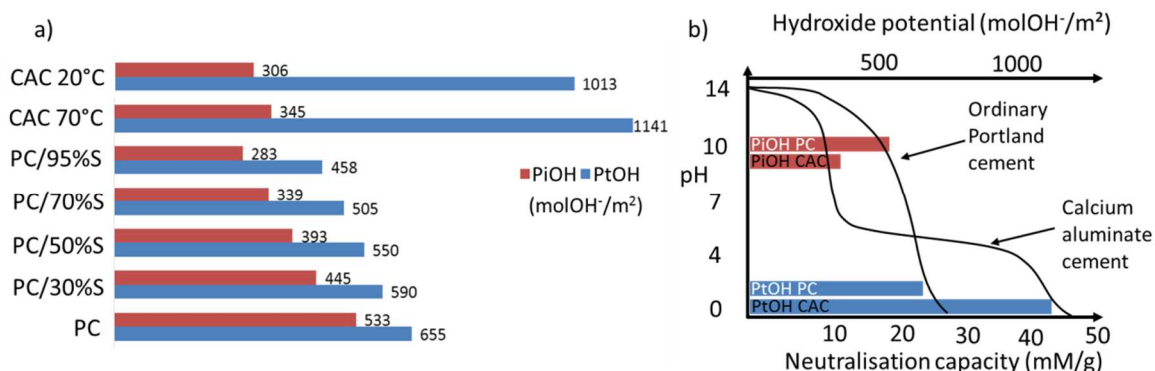
242 **Table 3. Dissolution reactions taken into account in the calculation of PtOH and PiOH.**

Oxide	Dissolution reaction
CaO	CaO + H <sub>2</sub> O -> Ca <sup>2+</sup> + 2OH <sup>-</sup>
SiO <sub>2</sub>	SiO <sub>2</sub> + 2H <sub>2</sub> O -> H <sub>4</sub> SiO <sub>4</sub>
Al <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub> + 3H <sub>2</sub> O -> 2Al <sup>3+</sup> + 6OH <sup>-</sup>
MgO	MgO + H <sub>2</sub> O -> Mg <sup>2+</sup> + 2OH <sup>-</sup>
Fe <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub> + 3H <sub>2</sub> O -> 2Fe <sup>3+</sup> + 6OH <sup>-</sup>

243

244 The OH<sup>-</sup> potential was calculated by summing the moles of OH<sup>-</sup> per gram of material, reported in mole  
 245 of OH<sup>-</sup> per volume of material (taking the density and porosity of each material into account [36]). It  
 246 was then converted into a molar amount of OH<sup>-</sup> for a given surface by multiplying by an arbitrary  
 247 thickness of the material (1 cm).

248 Figure 4 shows a) the PtOH and the PiOH of the different cement pastes tested and b) a comparison  
 249 with the neutralisation capacity calculated by Letourneux and Scrivener [37].



250  
 251 **Figure 4. a) Intermediate OH<sup>-</sup> potential (PiOH) and total OH<sup>-</sup> potential (PtOH) for PC-based material substituted**  
 252 **with 0 to 95% of GGBS and for CAC-based material (in molOH<sup>-</sup>/m<sup>2</sup>). b) Neutralisation capacity and hydroxide**  
 253 **potential of CAC and PC (adapted from Letourneux and Scrivener [37]).**

254  
 255 With increasing GGBS contents, the PiOH and PtOH of PC-based material decreased because of the  
 256 lower amount of CaO in GGBS than in PC. The intermediate potential was higher for PC-GGBS  
 257 pastes than for CAC pastes because of the higher CaO content. However, the PtOH of CAC-based  
 258 material was the highest. The large discrepancy between PiOH and PtOH for CAC matrix corresponds  
 259 to the dissolution of AH<sub>3</sub>, the product of CAC phase dissolutions because of acid attack. When pH  
 260 decreases to below 4, AH<sub>3</sub> starts dissolving and it releases 3 moles of OH<sup>-</sup> per mole of the molecule,  
 261 which explains the large increase of hydroxide potential at this pH. These results are in accordance  
 262 with the neutralisation capacity calculated by Letourneux and Scrivener [37] as represented in Figure  
 263 4b).

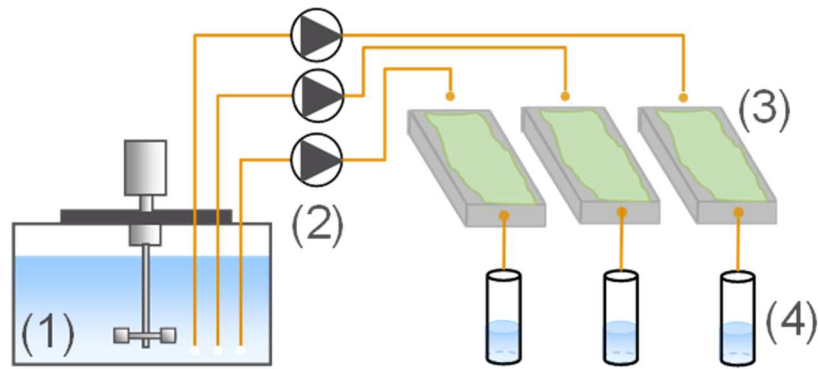
264  
 265 **2.4 Experimental set-up for cement paste biodeterioration using biofilm development**

266  
 267 A laboratory test developed at the University of Toulouse, the BAC test, was used to expose the  
 268 cementitious specimens to sewer-like environments in accelerated conditions. This test set-up was

269 designed to ensure the development of sulfur-oxidising microorganisms on the surface of a material,  
270 evolving over time and depending on the physicochemical characteristics of the material tested [23].  
271 For this purpose, a mineral solution of selected concentrations of soluble reduced sulfur was trickled  
272 over the surface of a material previously inoculated by a microbial consortium of NSOB and ASOB  
273 selected from a waste water treatment plant sludge (Toulouse, France). The mineral solution contained  
274 tetrathionate ( $S_4O_6^{2-}$ , reduced sulfur intermediate compound in the oxidative chain of sulfur) allowing  
275 the selection of a sulfur-oxidising activity in the biofilm, and leading to the production of acid and  
276 sulfate in contact with the surface of the material [24,25]. The test was monitored by the quantification  
277 of biogenic sulfate (and consequently acid) produced, and released calcium and aluminium in the  
278 leaching solution collected downstream of the specimen. The reproducibility and representativeness of  
279 the test has been demonstrated [24,25].

280 An optimised configuration of the test previously developed by Peyre Lavigne et al. [23] was used  
281 (Figure 5). Cement paste was preferred rather than mortar lining to focus on the reactivity of the  
282 cementitious phases of the material and to facilitate SEM and XRD analyses. Specimens were tilted by  
283 less than  $5^\circ$  in order to impose high retention time and to promote chemical equilibrium between the  
284 bulk, the biofilm and the cementitious material. The exposure time was slightly longer (4.3 months or  
285 133 days) in this study than in previous ones (3.7 months in [23]) because of the small amount of  
286 microorganisms inoculated on the material and the lower sulfurous substrate flow ( $\sim 5.81 \text{ molS/m}^2/\text{d}$  in  
287 this study vs. max.  $7.44 \text{ molS/m}^2/\text{d}$  in [23]), but the acid flow in contact with the material was three  
288 times higher ( $\sim 250 \text{ molH}^+/\text{m}^2$  on average in this study) inducing more calcium leaching per square  
289 meter.

290 Downstream of each exposed specimen, the solution was collected for 1 h twice a week ( $\sim 20 \text{ mL}$   
291 collected) in order to monitor the flow rates, pH (analysed with Schott sensor) and concentrations of  
292 sulfate produced, and calcium and aluminium leached (analysed by ion chromatography DX320-  
293 Dionex Thermofisher) (4).



294

295

Figure 5. Schematic diagram of the BAC test.

296

## 297 2.5 Experimental set up for sulfur-oxidising activity measurement

298

299 Sulfur-oxidising microorganisms grown in different conditions were exposed to various concentrations  
 300 of soluble aluminium in reactors in order to evaluate a possible bacteriostatic effect of this ion. For  
 301 the experiment in slightly acidic conditions (pH ~4), biofilms from WWS were developed on CAC  
 302 pastes (CAC condition) and on an “inert” plastic material (inert condition) using the BAC test in the  
 303 conditions described in section 2.4, for 2 months. The biofilms were removed using a water flow and  
 304 then mixed in order to disperse bacteria, and finally introduced into an open jacketed reactor of 300  
 305 mL. These two conditions were chosen in order to look at the influence of the nature of the material  
 306 environment on SOB possible selection and thus on different SOB populations. For the experiment in  
 307 a highly acid environment (pH ~2), a controlled 1.6 L open reactor was seeded with active sludge from  
 308 the wastewater treatment plant of Toulouse (France) and fed with nutritive solution. The sulfur-  
 309 oxidising activities were studied in gas open batch reactors (thermostated at 20 °C) using respirometry  
 310 (with online measurement of dissolved O<sub>2</sub> concentration (Hamilton sensor), pH (Schott sensor), and  
 311 temperature (Hamilton sensor)) [38]. Aeration and measurement of oxygen uptake rates (OUR) were  
 312 managed by sequentially bubbling pressurised air into the reactor. The aeration provided the O<sub>2</sub> and  
 313 the CO<sub>2</sub> necessary for the growth of chemolithotrophic bacteria as SOB. The feeding consisted of  
 314 successive pulses of a 0.2 M concentrated potassium tetrathionate solution (P2926-Aldrich).

315 For the experiments in a slightly acid environment, the pH was buffered at 4 by a CO<sub>2</sub> enrichment of  
316 pressurised air (~10%). For the experiment in the highly acid environment, the pH was not controlled,  
317 so a reference culture was operated in parallel.

318 Three different levels of aluminium concentration (0 mM, 50 mM and ~100 mM) were used in this  
319 study. Aluminium was added using AlCl<sub>3</sub> in order to keep the ionic strength modification to a  
320 minimum with the addition of inhibitory compound. During the biodeterioration of CAC and PC  
321 mortar using the BAC test (reproducing an accelerated aggressive deterioration), Peyre Lavigne et al.  
322 [25] measured a concentration of 0.5 mM of Al<sup>3+</sup>. Herisson et al. [26] measured total aluminium  
323 concentration of condensed water in a PVC tube planted in CAC concrete exposed to a sewer network.  
324 They obtained a concentration of Al<sup>3+</sup> of 125 mM. The range of concentration tested corresponds to  
325 concentrations that are possible in situ.

326 The activity was evaluated by measuring the bacterial yield and evaluating the maximum specific  
327 growth rate ( $\mu_{\max}$ ) during a growth with a minimum of a doubling of the SOB population. The  
328 bacterial yield, Yo/s (molO<sub>2</sub>/molS<sub>4</sub>O<sub>6</sub><sup>2-</sup>), was measured for every pulse of substrate by dividing the  
329 quantity of dioxygen consumed by the quantity of tetrathionate consumed. The  $\mu_{\max}$  was obtained by  
330 fitting a model developed on AQUASIM with the experimental OUR using the following equation  
331 (Eq. 3).

$$332 \quad \mathbf{OUR = A * \exp(\mu_{\max} * t)} \quad \mathbf{Eq. 3}$$

333 where OUR is the oxygen uptake rate in mgO<sub>2</sub>/L/h, A is a constant in mgO<sub>2</sub>/L/h,  $\mu_{\max}$  is the maximum  
334 specific growth rate in h<sup>-1</sup>, and t is the time in h.

335 The model was verified twice: with successive growth in pulses of sulfur and in constant excess of  
336 sulfur. The details of the device are reported in [36,39]. The accuracy on the  $\mu_{\max}$  and on the bacterial  
337 yield data were  $\pm 0.003 \text{ h}^{-1}$  and  $\pm 0.11 \text{ molO}_2/\text{molS}_4\text{O}_6^{2-}$ , respectively, representing relative errors of  
338 ~6% and ~3%, respectively. To evaluate the effect of aluminium chloride on the bacteria, the  $\mu_{\max}$  and  
339 the Yo/s with and without the compound were compared. The adaptation of bacteria to the compound  
340 was evaluated by successive growth over several days for a constant aluminium concentration.



341

## 342 **2.6 Analyses of cement pastes exposed to SOB activity in the BAC test**

343 At the end of the exposure period (4.3 months) of the cementitious specimens to the BAC test, 1 cm  
344 thick cement paste slices were cut along the cross section of each specimen, i.e. perpendicularly to  
345 their surface in contact with the biofilm (or interface). The chemical and mineralogical composition of  
346 the specimens was analysed as a function of the distance to the interface using scanning electron  
347 microscopy (SEM coupled with energy dispersive spectrometry (EDS) and X-ray diffraction (XRD)).

348 For SEM analyses, the slices were embedded in an epoxy resin (Mecaprex MA2 from Presi) in small  
349 moulds prior to polishing. The polishing was performed using a series of three silicon carbide  
350 polishing disks: P800-22 $\mu$ m, P1200-15 $\mu$ m, and P4000-5 $\mu$ m using the protocol described in [40]. After  
351 polishing, the sections were coated with carbon. Alteration patterns were observed with SEM (JEOL  
352 JSM-6380LV) in BSE mode. One control specimen was also analysed 90 days after casting for  
353 comparison. Chemical analyses were carried out using EDS (BRUCKER XFLASH 6130).

354 XRD analyses (D8 ADVANCE Brucker diffractometer - CuK $\alpha$  radiation ( $\lambda=1.54\text{\AA}$ )) were performed  
355 on bulk samples, with a progressive abrasion of the surface in order to characterise the different depths  
356 of deterioration using the protocol described in [41]. The acquisition was achieved with a step size of  
357 0.02 $^\circ$ , 0.25 sec per step, in the 2 theta range of 4 $^\circ$ -24 $^\circ$ . The mineralogy was identified with the help of  
358 the software EVA. XRD analyses of the biodeteriorated materials were carried at different depths in  
359 the sample i.e. 1) on the top of the deteriorated surface, 2) at a few hundred micrometres below the top  
360 surface and 3) in the sound area of the sample (at about 1.5 cm deep). The specimens were abraded  
361 with SiC abrasive paper (P800-22 $\mu$ m) between two successive analyses. The different depths were  
362 controlled by measurements with a calliper.

## 363 **2.7 Biofilm preparation for scanning electron microscopy observation**

364 A small prism of cement paste (3 mm \* 4 mm \* 2 mm) was positioned on the surface of each  
365 specimen tested during exposure to the BAC test. With the development of biofilm on the surface, the  
366 small piece of material was also covered by biofilm and deteriorated. After 133 days of exposure,

367 these pieces of material covered by biofilm were collected and prepared for observation. In order to  
368 observe the biofilm morphology, the water present in bacterial cells was removed without destroying  
369 the integrity of the cell walls. The protocol consisted of aldehydic gelification and dehydration.  
370 Samples were immersed in paraformaldehyde solution (4%) for 3 hours and washed for 8 times 10  
371 minutes in a saline phosphate buffer. Samples were then washed in successive baths of water/ethanol  
372 solution with increasing ethanol concentrations (15 minutes in 50% ethanol, 15 minutes in 75%  
373 ethanol and 30 minutes in 96% ethanol). Samples were then dried for 3 hours at 30 °C and fixed on  
374 specimen holders 0.5 cm in diameter, tilted at 45°. Samples were then covered by a thin layer of gold.  
375 Observations were made using SEM-FEG in SE mode (JSM 7100F TTLS). The bacterial structure  
376 seemed not to be altered by drying during SEM observations (walls of bacteria were not damaged).

377

### 378 **3. Results**

379 The influence of the material on the biofilm will be presented: (i) the influence of soluble  $\text{Al}^{3+}$  on SOB  
380 in reactor, to evaluate the bacteriostatic effect and (ii) the effect of the material on the biofilm  
381 morphology, composition and metabolism. Then, the effect of the biofilm on the material will be  
382 presented: (iii) the analyses of the leaching solution during the biodeterioration, providing leaching  
383 kinetics, and the observation of deterioration patterns together with deteriorated depths.

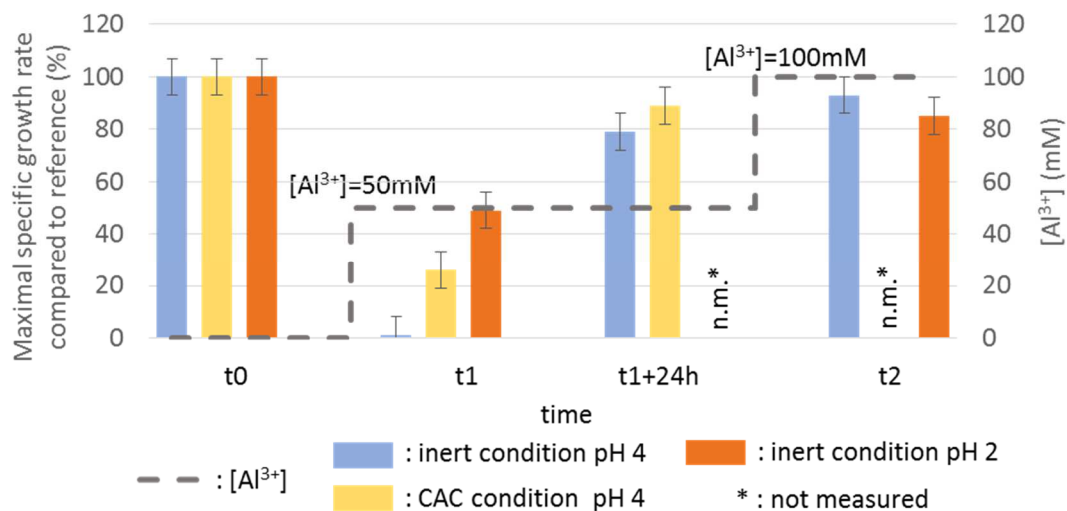
384

#### 385 **3.1 Influence of soluble $\text{Al}^{3+}$ on SOB**

386 The maximal specific growth rate ( $\mu_{\max}$ ), and the oxidation yield ( $Y_{O/S}$ ) were measured. Preliminary  
387 tests were performed up to 30 mM but they did not reveal any rapid influence of the injection of  
388 aluminium on the SOB oxygen uptake rate, so they are not presented here.

389 Figure 6 represents the evolution of  $\mu_{\max}$  measured without addition of soluble aluminium (0 mM, time  
390  $t_0$ ), after a first injection of  $\text{Al}^{3+} = 50$  mM at time  $t_1$ , and after a second injection leading to a final  
391 concentration of  $\text{Al}^{3+} = 100$  mM at time  $t_2$ . Maximum specific growth rate was measured immediately

392 after each injection of soluble aluminium, and 24h after the first addition of  $\text{Al}^{3+}$  (50 mM, t1+24h).  
 393 Values of t1 and t2 depended, among other parameters, on the bacterial growth rate and on the time  
 394 between two successive pulses of tetrathionate.  
 395



396  
 397 **Figure 6. Maximal specific growth rate for successive  $\text{Al}^{3+}$  additions, for different growth conditions. \*not**  
 398 **measured.**

399 Before any  $\text{Al}^{3+}$  addition to the medium (t0), the maximal specific growth rate was measured and was  
 400 then used to standardise further measurements. At t0,  $\mu_{\max}$  was thus equal to 100%. SOB developed in  
 401 all further growth conditions suffered a temporary decrease in  $\mu_{\max}$  with injection of 50 mM of  $\text{Al}^{3+}$   
 402 (t1). Figure 6 shows that, after 24 hours of acclimation to the aluminium injection (t1+24h), SOB were  
 403 no longer significantly affected by soluble aluminium in terms of maximum specific growth rate. This  
 404 was due to the acclimation of SOB to the chemical conditions. After the second injection of  $\text{AlCl}_3$   
 405 (total concentration of 100 mM of  $\text{Al}^{3+}$ ) at time t2, the  $\mu_{\max}$  was not significantly different from the  
 406 control growth. A high level of aluminium no longer modified the activity of SOB. It can be noted  
 407 that, for each injection of  $\text{AlCl}_3$ , the  $Y_{O/S}$  (not shown here)[36,39] increased significantly (by about  
 408 10%), meaning that the bacteria needed energy to adapt to their environment. After acclimation to the  
 409 injection of aluminium, SOB recovered the initial oxidation yield value and they no longer  
 410 overconsumed energy to survive.

411 The growth conditions of the biofilms used in the experiment significantly impacted  $\mu_{\max}$ , essentially  
412 after the first injection of  $\text{Al}^{3+}=50$  mM (time t1), whereas no significant effect was observed for other  
413 measurements. For SOB developed at pH=4 in inert conditions, bacteria were so disturbed right after  
414 the 1<sup>st</sup> injection (t1) that no  $\mu_{\max}$  could be measured. For SOB developed on the CAC samples,  
415 standardised  $\mu_{\max}$  was 23%, i.e. these SOB resisted better than those developed in inert conditions. The  
416 pre-acclimation of SOB to the aluminium and high salt concentration due to the contact between the  
417 biofilm and the CAC material induced a possible selection of SOB resistant to this environment. The  
418 pH conditions of the biofilm growth were also probably responsible for a selection of resistant  
419 microorganisms. Actually, SOB developed at pH=2 in inert conditions showed a lower decrease in  
420  $\mu_{\max}$  after injection of 50 mM of  $\text{Al}^{3+}$  than those developed on the same support at pH =4. It can be  
421 noted that the form of aluminium at pH<4 was more than 95% free  $\text{Al}^{3+}$  [42]. Therefore activity  
422 analyses for both pH levels were performed with the same inhibitory compound.

423 The experiment was also run at pH=2 using NaCl instead of  $\text{AlCl}_3$  with the same ionic force (data not  
424 shown)[36,39]. The modification of osmotic pressure induced in the solution by the modification of  
425 the ionic strength when NaCl was added provoked a temporary decrease of  $\mu_{\max}$ . So, the very short  
426 term effect of  $\text{AlCl}_3$  can be attributed to a modification of the osmotic pressure induced by a sudden  
427 modification in ionic strength, rather than by a specific effect of aluminium.

428 In conclusion, whatever the growth conditions, the experiment demonstrated an adaptation of  
429 microorganisms to their environment. Even though a short term effect was observed, soluble  
430 aluminium did not seem to have a significant effect on SOB activity after their acclimation.

431

## 432 **3.2 Influence of the nature of the material on the microbial development**

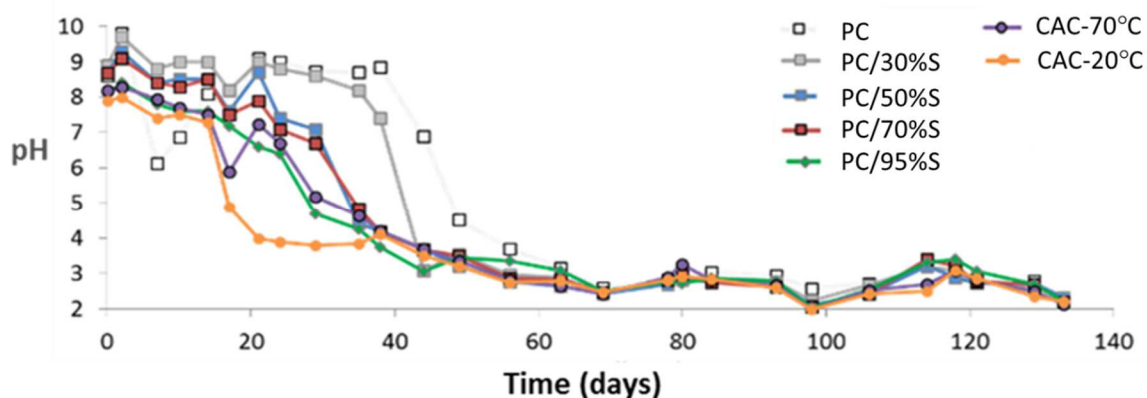
433

### 434 **3.2.1 Influence of the neutralisation capacity of the material on the pH evolution and sulfate** 435 **production (biological activity)**

436 The influence of the nature of the material on the SOB development was studied. The aim was to  
437 understand whether, as the literature assumes, the better resistance of CAC-based material could be  
438 explained by an effect of the solid material surface on SOB activity in the form of a biofilm i.e. on  
439 biogenic sulfuric acid production, for a given SOB inoculum.

440 PC-based pastes with substitutions of 0 w.t.%, 30 w.t.%, 50 w.t.%, 70 w.t.%, and 95 w.t.% of GGBS  
441 and CAC-based pastes (with curing condition at 20°C or 70°C) were exposed to the BAC test for 133  
442 days. The pH and the concentration of sulfate (expressing the sulfur-oxidising activity) of the leaching  
443 solutions trickled over the surface and collected downstream of each specimen (Figure 5) were  
444 monitored over time.

445 The evolution of pH with time is represented in Figure 7. The development of neutrophilic and then  
446 acidophilic SOB induced the production of sulfuric acid, decreasing the pH of the leaching solutions.



447

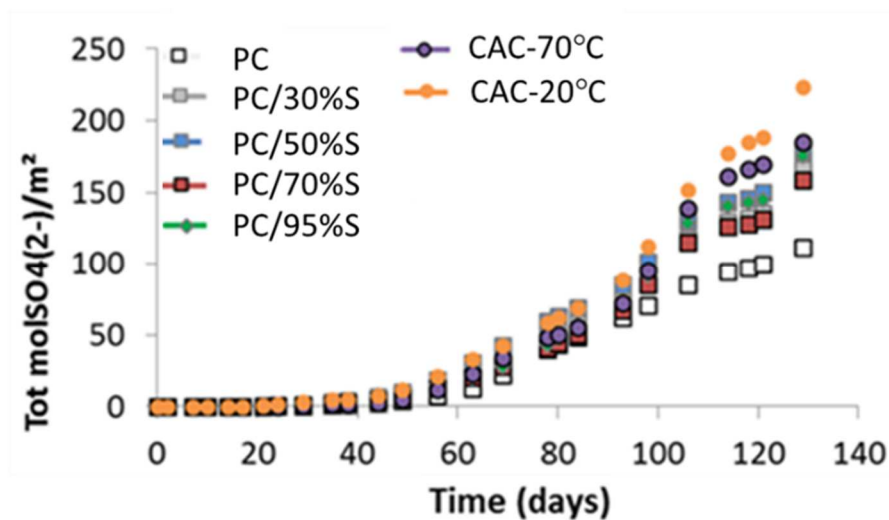
448 **Figure 7. Evolution of pH of the leaching solution with time during BAC test biodeterioration**

449

450 The evolution of the pH occurred in three phases. (i) One day after the beginning of the test, the pH  
451 was around 8 for CAC-based material and 8 to 10 for PC-based materials. Whatever the material  
452 composition, the pH remained high for at least 15 days. (ii) From day 15 and with a delay depending  
453 on the nature of the material, the pH decreased quickly due to the production of sulfuric acid by SOB.  
454 (iii) After the drop of the pH (ending at around 60 days for all the materials), leaching solutions were  
455 characterised by a relatively constant pH, around 2.5, until the end of the experiment (133 days).

456 Leaching solutions collected downstream of CAC-based materials showed an earlier decrease in pH  
457 than those of PC+GGBS based materials. With decreasing GGBS contents, the delay before the fall of  
458 pH increased. The 100% PC system showed the longest delay in pH fall (day 39).

459 Production of  $H^+$  is associated with the production of sulfate and is directly linked to the SOB activity.  
460 Figure 8 shows the evolution of sulfate concentration in the leaching solution for each material tested.  
461 It can be noted that no significant sulfate production was observed before 60 days had elapsed,  
462 corresponding to the end of the pH drop for all the materials.



463

464

Figure 8. Evolution of sulfate concentration in leaching solution

465

466 At the early stage, before the fall in pH, sulfate production was low, corresponding to phase (i) of the  
467 pH evolution. After acidification and development of ASOB (corresponding to phase (iii) of pH  
468 evolution), the production of sulfate increased almost linearly with time.

469 CAC-based materials were characterised by the highest cumulative sulfate flow at any time. Despite  
470 the higher neutralisation capacity of CAC-based material, acidophilic SOB activity was also favoured  
471 on CAC materials, with no evidence of a bacteriostatic effect of the material on the biogenic sulfuric  
472 acid production, in the conditions of the test. (Cumulated final values: PC, 111 mol/m<sup>2</sup>; PC/30%S, 166  
473 mol/m<sup>2</sup>; PC/50%S, 178 mol/m<sup>2</sup>; PC/70%S, 159 mol/m<sup>2</sup>; PC/95%S, 176 mol/m<sup>2</sup>; CAC-20°C, 224  
474 mol/m<sup>2</sup>; CAC-70°C, 185 mol/m<sup>2</sup>)

475

### 476 **3.2.2 Effect on the selection of bacterial population**

477

478 In order to study the influence of the binder composition on the selection of bacteria on the surface of  
479 the material, analyses of bacterial population were made on biofilm samples collected after 120 days  
480 of exposure to the BAC test (data not shown) [36]. Similar biodiversity was observed on PC and CAC  
481 materials with respectively 87% and 79% of *Acidithiobacillus thiooxidans* and around 7% of  
482 *Thiomonas Intermedia*. These bacterial population analyses revealed no differences in the predominant  
483 bacterial population according to the nature of the material.

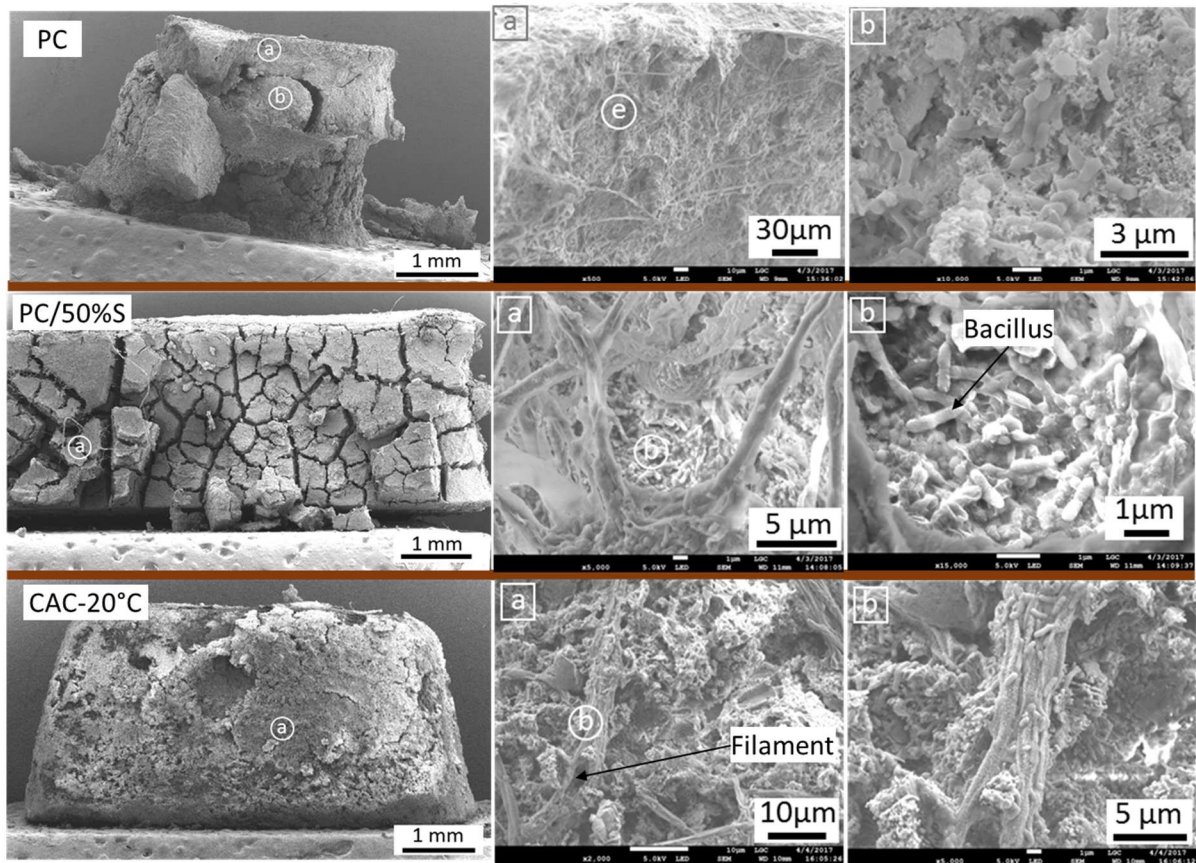
484

### 485 **3.2.3 Observation of the biofilm morphology**

486

487 In order to study the influence of the material composition on the biofilm morphology, biofilm  
488 developed on each of the materials tested using the BAC test were analysed as described in 2.7. After  
489 133 days of exposure, the coupons supporting the biofilm were removed, and the biofilm was fixed,  
490 dehydrated and then observed using SEM (Figure 9).

491



492

493 **Figure 9. SEM-FEG image in SE mode of biofilms developed on PC, PC/50%S and CAC-20°C cement paste after 133**  
 494 **days of exposure in the BAC test.**

495

496 The SEM images presented in Figure 9 reveal that biofilm morphology and covering were relatively  
 497 similar whatever the composition of the material. A large quantity of bacillus covered the surface of  
 498 the materials.. These bacteria were present in the cracks and under the freshly detached pieces of  
 499 materials. Filaments of  $\sim 1 \mu\text{m}$  diameter and of diameter  $>3\mu\text{m}$  were also present.

500

501 It could be observed that PCs were microcracked (Figure 9 left). Needle-shaped products crystallised  
 502 in microcracks may correspond to ettringite crystals, as suggested by EDS analyses (data not shown  
 503 [36]). The CAC surface remained more homogeneous with a smoother surface, which could indicate a  
 504 different behaviour toward the SOB attack from that of PC systems.

505



506 **3.3 Resistance of materials to biodeterioration: role of the chemical, mineralogical and**  
507 **microstructural characteristics of the materials**

508

509 The following experiment dealt with the characterisation of the resistance to biodeterioration of the  
510 materials tested using the BAC test. The evolution of chemical features of the leaching solution during  
511 biodeterioration will be presented, followed by microstructural analysis of deteriorated materials.

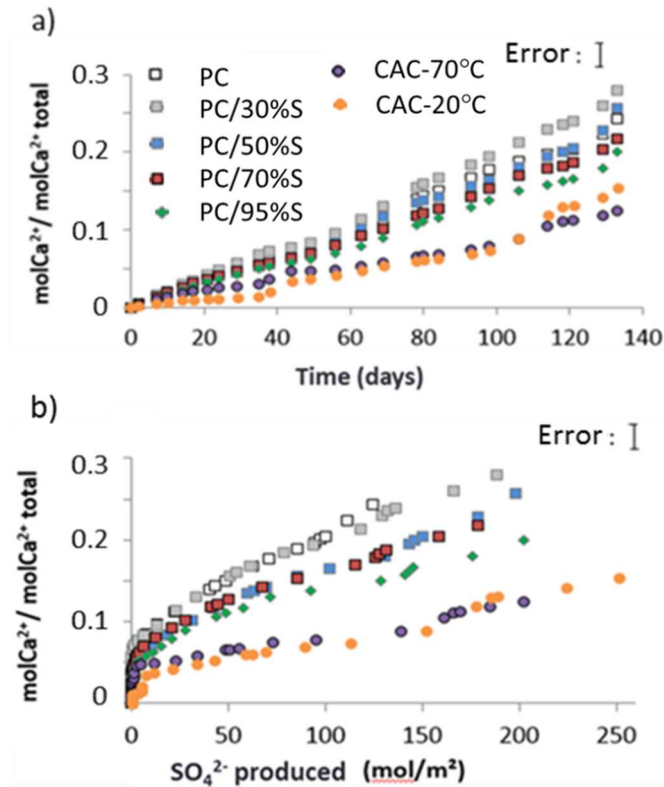
512

513 **3.3.1 Calcium and aluminium concentration in leaching solution during biodeterioration**

514

515 During the exposure of the tested materials to the BAC test, the sulfuric acid produced by SOB  
516 induced leaching and dissolution/precipitation of phases in the cement matrix. Calcium and aluminium  
517 concentrations in the leaching solution were monitored to evaluate the kinetics of biodeterioration of  
518 the different materials. To enable comparison of leaching among the different materials, the leached  
519 concentrations were standardised, i.e. divided by the initial concentration of the element in the  
520 material. Figure 10a shows the evolution of standardised calcium concentration in the leaching  
521 solutions as a function of time. To evaluate the dependence between the leaching of Ca and the acid  
522 production of acidophilic SOB, the leaching of Ca is also represented as a function of sulfate produced  
523 in Figure 10 b (as a reminder, there is a stoichiometric relationship between acid and sulfate  
524 produced).

525



526

527 **Figure 10. Evolution of calcium concentration in the leaching solution divided by the initial calcium concentration**  
 528 **in the material as a function of a) time b) sulfate concentration in the leaching solution.**

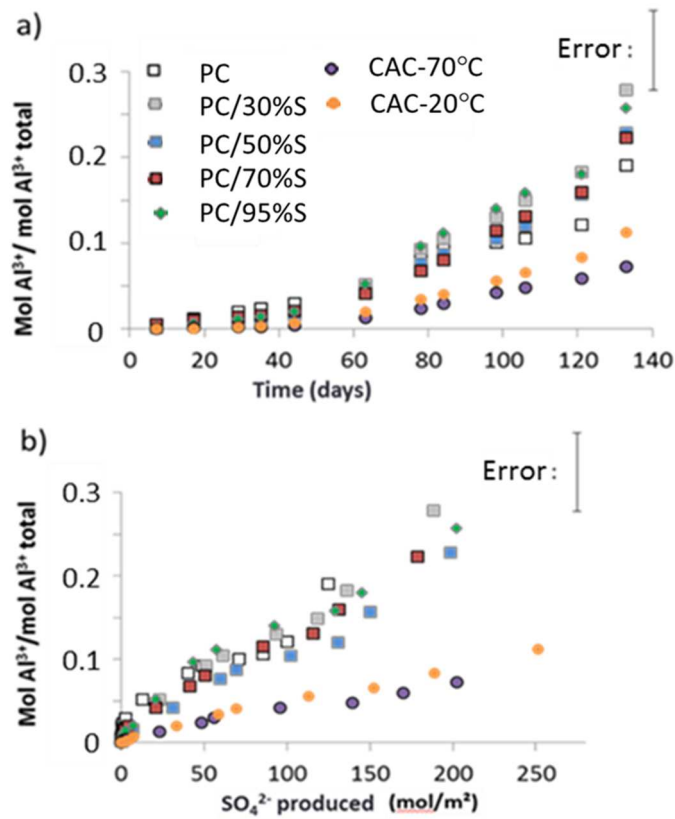
529

530 Figure 10a shows that CAC binders released less Ca<sup>2+</sup> than PC binders (relatively to their initial  
 531 composition of calcium). The difference between CAC and PC-based binders increased significantly  
 532 from 60 days, corresponding to the end of the pH fall to around 3.5 for all materials.

533 For all materials, the evolution of the calcium concentration in the leaching solution was a linear  
 534 function of time on the whole, even before the pH drop. Therefore diffusion limitation phenomena  
 535 may not have occurred over the entire test duration. Figure 11b shows that the normalised calcium  
 536 concentration represented as a function of the sulfate concentration was also linear, i.e. the calcium  
 537 concentration released by the material was linearly dependant on the amount of acid produced by SOB  
 538 (except for very low concentrations of sulfate, i.e. < 10 mol/m<sup>2</sup>). With increasing GGBS content, the  
 539 normalised calcium concentration in the leaching solution decreased. For a similar acid attack strength,  
 540 there was no significant difference between the leaching of CAC-70°C and CAC-20°C, i.e. no  
 541 influence of conversion phenomena, in the conditions of the test.

542 Figure 11 shows the evolution of standardised aluminium concentration (i.e. divided by the initial  
 543 aluminium concentration in the material) in the leaching solution as a function of time (a) and sulfate  
 544 concentration (b), in order to compare leaching for a similar attack strength.

545



546

547 **Figure 11. Evolution of aluminium concentration in the leaching solution divided by the initial aluminium**  
 548 **concentration in the material as a function of a) time b) sulfate concentration in the leaching solution.**

549

550 CAC-based material released relatively less aluminium than PC-based materials. Whatever the  
 551 material composition, aluminium leaching increased significantly after 60 days, corresponding to low  
 552 pH conditions. Figure 11 a shows that standardised aluminium leaching was higher for PC-based  
 553 materials than for CAC systems. Figure 11 b shows that, for a similar attack, nearly 4 times less  
 554 aluminium was leached from CAC-based materials than PC-based materials. Part of the aluminium  
 555 contained in CAC phases was not released, probably because of the precipitation of  $\text{Al(OH)}_3$  as a  
 556 deterioration product. There was no significant difference between CAC-70°C and CAC-20°C, i.e.,

557 again, no influence of the conversion was detected in the conditions of the test. In addition, no  
558 significant difference can be observed whatever the substitution of PC-based materials (with higher  
559 relative errors compared to the calcium measured previously, due to lower concentrations).

560

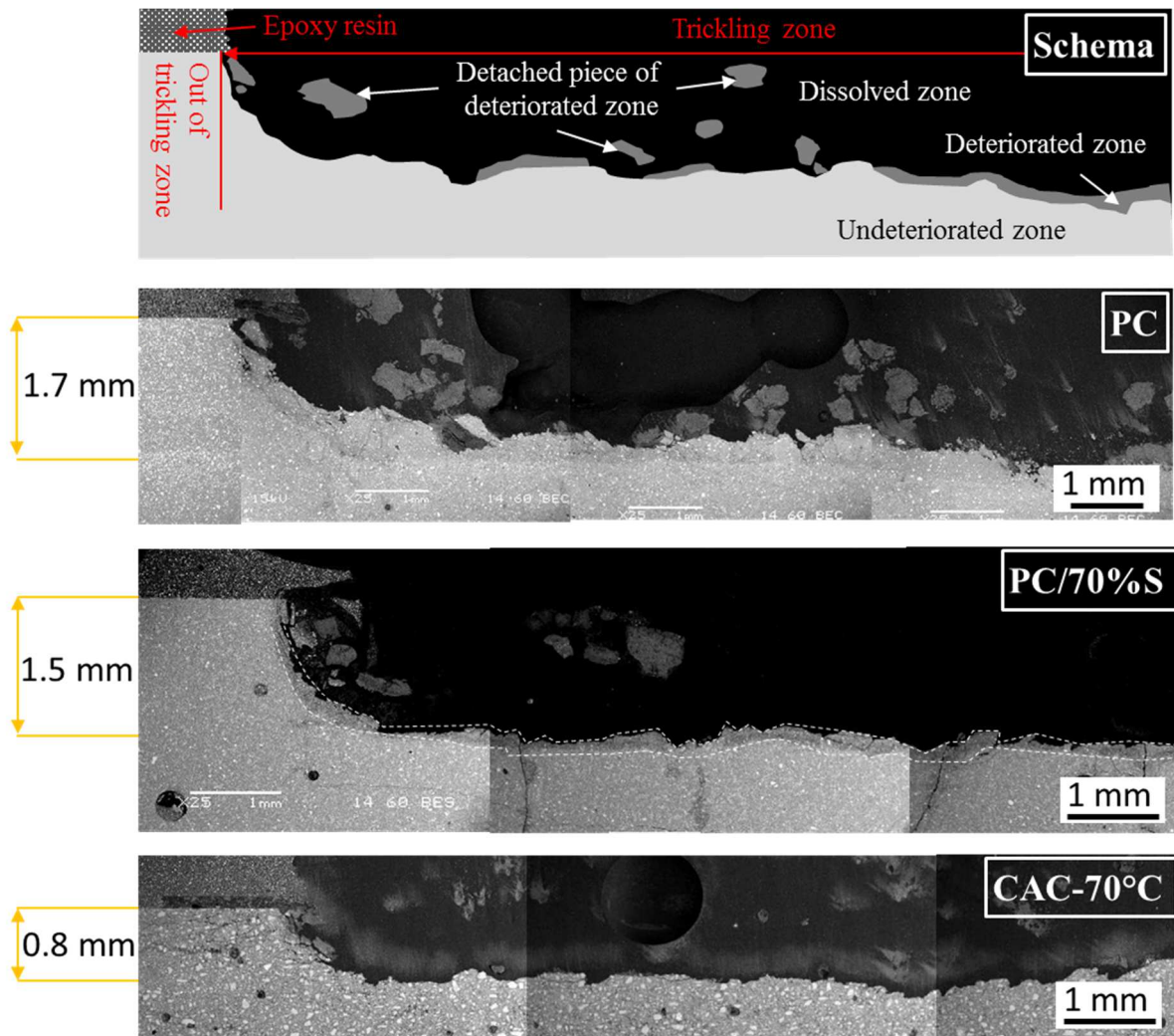
### 561 **3.3.2 Characterisation of cement pastes exposed to the BAC test**

562

563 The SEM images of polished cross sections of PC, PC/70%S and CAC-70°C are presented, with a  
564 scheme of the image observations, in Figure 12. The inoculated surface is facing upward.

565 The deterioration zone started only at around 1 mm from the left hand side of the image due to the  
566 presence of a protective epoxy resin, which was initially deposited in order to have a reference for  
567 measuring depth of deterioration. The deteriorated area corresponded to the trickling zone where the  
568 leaching solution flowed. It can be seen that the deteriorated surfaces of the samples were relatively  
569 smooth. Because of the preparation protocol for SEM analyses, some pieces of the non-cohesive  
570 deteriorated zone were detached and are visible above the specimen in the theoretical dissolved zone  
571 (in black).

572



573

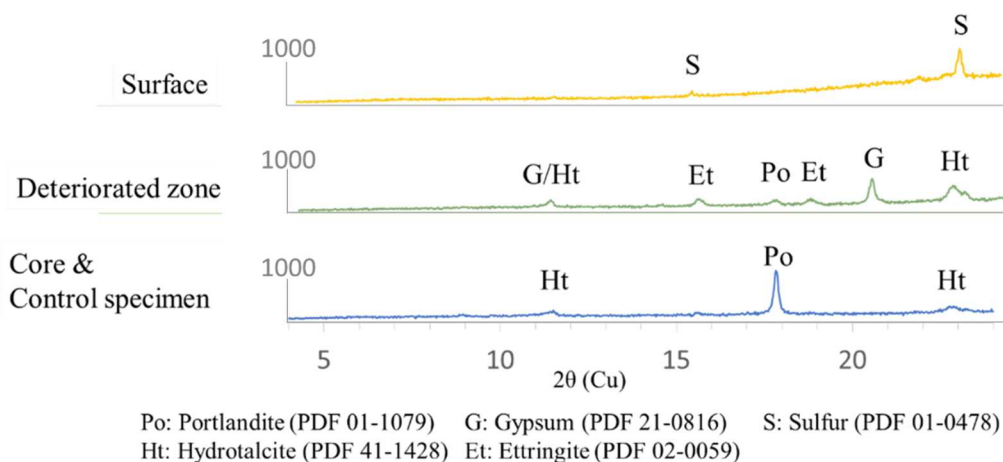
574 **Figure 12. Scheme and SEM-BSE image of cross section of deteriorated PC, PC/70%S and CAC-70°C cement**  
 575 **paste after 133 days in the BAC test.**

576

577 Figure 12 shows that a part of the cement pastes was dissolved. The upper surface in contact with the  
 578 biofilm appeared with dark grey contrast, meaning a lower density. EDS analyses on this zone showed  
 579 that it was almost totally decalcified and mainly composed of Al and Si for PC systems, and of Al for  
 580 CAC matrices (data not shown). This zone was thin, probably because (i) it was almost totally  
 581 dissolved by the acid, and (ii) it was fragile and thus removed during sample preparation. The  
 582 deteriorated layer thickness of CAC-based material (0.8 mm) was about half that of pure PC-based  
 583 materials (1.7 mm). The addition of 70% of GGBS led to a slightly smaller depth of deterioration (1.5

584 mm) than pure PC matrix. Some cracks perpendicular to the surface could be observed for the  
585 PC/70%S sample, and for other high GGBS content systems (data not shown).

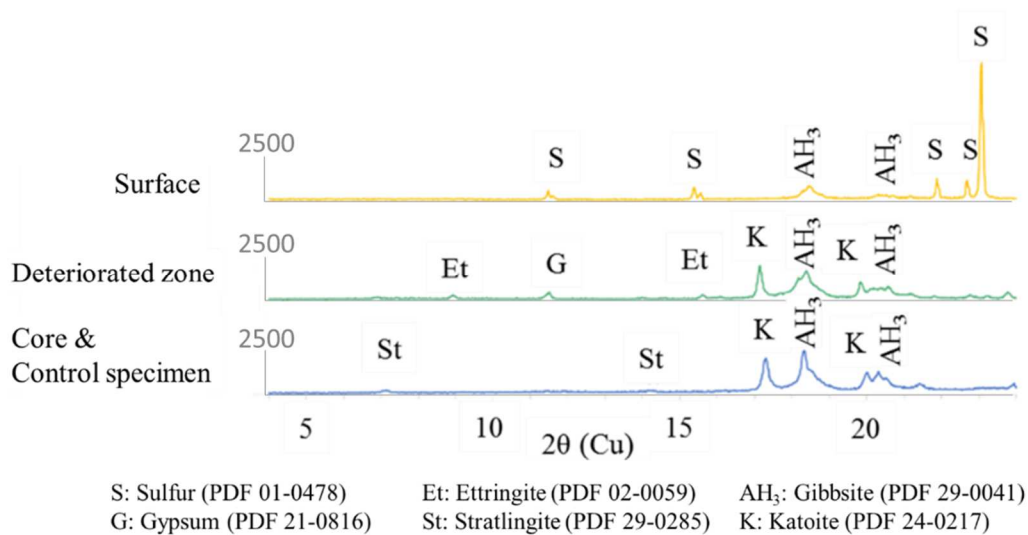
586 XRD analyses were also carried out for the different samples: 1) on the top of the deteriorated surface,  
587 2) a few hundred micrometres below the top surface and 3) in the sound area of the sample (1.5 cm  
588 deep). Reference specimens were also analysed. Figure 13 shows an illustration of XRD analysis for  
589 the PC/30%S specimen. The core and the reference specimens showed the same diffractograms. CH,  
590 hydrotalcite, and ettringite phases were logically identified in the core of the samples on the 0-24°  
591 range. In the intermediary zone, located a few hundred microns below the surface, ettringite and  
592 gypsum were detected, as were small quantities of CH. The diffractogram of the surface showed  
593 several halos, indicating an essentially amorphous nature of the microstructure, i.e. a gel (made of Si  
594 and Al, if we consider the results of EDS analyses), and some peaks of elemental sulfur, which is a  
595 product of the microbial reduction of  $S_4O_6^{2-}$  in anaerobic conditions in the biofilm.



596  
597 **Figure 13. XRD analysis on massive sample of PC/30%S cement paste on the surface, in an intermediate zone (a**  
598 **few hundred microns below the surface) and in the core of the sample, as well as the control specimen.**

599  
600 Figure 14 shows XRD analysis of CAC-70°C in the same three different areas.

601



602

603

**Figure 14. XRD analysis on massive sample of CAC-70°C on the surface, in the deteriorated zone, in the core of the sample, as well as the control specimen.**

604

605

The core and the reference specimens showed the same diffractograms, comprising typical phases of a converted hydrated CAC: stratlingite, katoite and AH<sub>3</sub>. In the intermediary deteriorated zone, gypsum and ettringite were found. The surface was composed of elemental sulfur and AH<sub>3</sub>. The presence of elemental sulfur was due to the anaerobic biological and/or chemical reduction of tetrathionate in the biofilm zone.

610

## 611 4. Discussion

612

### 613 4.1 Biological resistance

614

#### 615 4.1.1 Influence of soluble aluminium on SOB

616

Investigation of the inhibitory effect of Al<sup>3+</sup> on SOB biofilms carried out in a reactor showed a temporary inhibition of the maximal growth rate  $\mu_{max}$  coupled with an increase of the oxidation yield with the first injection of AlCl<sub>3</sub> up to 50 mM. The oxidation yield highlights the energy needed for bacteria to survive in their environment (it increases when bacteria need to move, balance their inner

620

621 pressure, etc.). The modification of the environment of the bacteria implied that they had to adapt to  
622 new conditions. The addition of  $\text{AlCl}_3$  changed the ionic strength of the liquid, and thus induced an  
623 osmotic stress which temporally decreased the bacterial activity [43]. The acclimation of SOB to the  
624 new environment required energy and time. During the acclimation period ( $t_1+24$  hours) the bacterial  
625 yield increased, revealing the overconsumption of oxygen. More energy was temporarily used for the  
626 maintenance processes, i.e. to survive. But, at the end of this period, the maximal specific growth rate  
627 was in the same range as before the addition of soluble aluminium; i.e. SOB bacteria developed a  
628 resistance to the  $\text{AlCl}_3$  environment. For the second addition up to  $\text{Al}^{3+} \approx 100$  mM, the SOB growth  
629 was not significantly impacted. The oxidation yield increased temporarily. A complementary study  
630 showed that  $\text{NaCl}$  also induced a decrease in the bacterial growth of SOB. The effect on the maximal  
631 specific growth rate recorded just after the addition may be attributed to the sudden modification of the  
632 ionic pressure, inducing osmotic stress. The effect of aluminium could be a secondary parameter to  
633 explain the temporary modification of  $\mu_{\max}$  after the first addition of  $\text{AlCl}_3$ .

634

635 Bacteria developed at pH 2 were less affected by the addition of  $\text{AlCl}_3$ ; they were probably already  
636 adapted to a concentrated ion environment due to the low pH during the selection of microorganisms.  
637 Similar phenomena explained the lower influence of the aluminium injection on SOB developed on  
638 CAC material, which was aluminium rich and released a high ion concentration when exposed to the  
639 BAC test (Figure 6). Microorganisms in biofilms developed in inert conditions at pH 4 were more  
640 impacted by the 50 mM  $\text{AlCl}_3$  addition in the very short term, but their behaviour was equivalent to  
641 that of microorganisms grown in other conditions 24h after the injection and then for the 100 mM  
642 injection.

643

644 In a sewer network, pipes have a lifespan of 50 years, which gives bacteria plenty of time to adapt to  
645 their environment. In such an environment, it is likely that, after their acclimation, they are little  
646 affected by aluminium. In the light of these results, it may be concluded that the better resistance of



647 CAC mortar in aggressive conditions comparable to those implemented in this study may not be due to  
648 the inhibition of the SOB activity caused by the release of soluble aluminium in the aqueous phase.

649

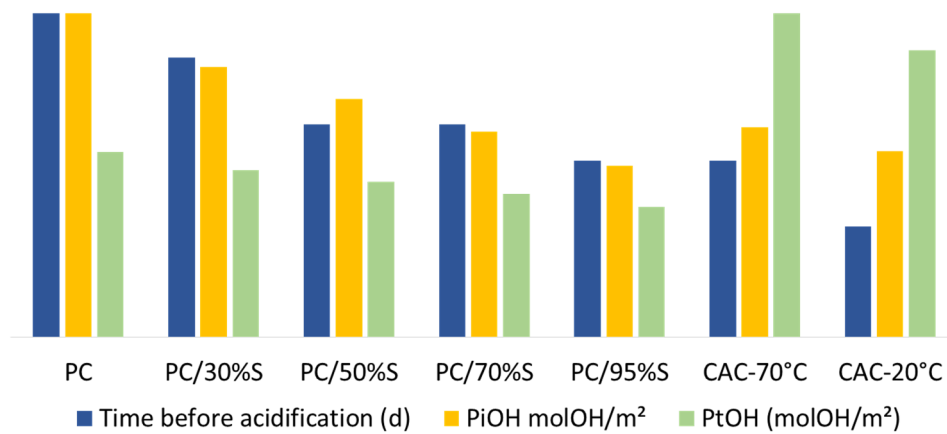
#### 650 **4.1.2 Influence of material composition on SOB development**

651

652 Even if free  $Al^{3+}$  was not responsible for the SOB inhibition, the solid material itself may have an  
653 influence on the sulfur-oxidising activity development or the biofilm composition. The better  
654 resistance of CAC might be due to a bacteriostatic effect related to the physicochemical parameters of  
655 the material [12,26]. In this study, the role of the chemistry and the mineralogy of the material were  
656 mainly considered but other parameters may have an influence on the colonisation of the material by  
657 microorganisms, such as the surface roughness, the surface tension, and the type of porosity  
658 (modifying the attachment of the microorganisms to the material).

659 The biogenic acid attack of the cementitious material induced a decrease of the pH in the solution  
660 trickling onto the surface. The pH evolution of the leaching solution showed a delayed acidification  
661 which depended on the material composition (Figure 7). One of the first reactions induced by the  
662 presence of acid on the surface of the material was the decalcification of calcic hydrates (CH, C-A-S-  
663 H,  $C_3AH_6$  and  $CAH_{10}$  and anhydrous), and their release of  $OH^-$ . Figure 15 gives a comparison between  
664 the delay before biogenic acidification and  $PiOH$ , the intermediate hydroxide potential, as defined in  
665 2.2.2.

666



667  
668 **Figure 15. PiOH, PtOH and the delay before biogenic acidification for each material tested**

669

670 Figure 15 shows the correlation between PiOH, PtOH and the time before biogenic acidification for  
 671 each material. The lower PiOH of CAC explains the earlier decrease of pH for this material compared  
 672 to PC. The increasing GGBS substitution level with PC also lowers the PiOH and the time before  
 673 acidification. There is no clear correlation between PtOH, the total potential in OH<sup>-</sup> and the steps of  
 674 acidification.

675 At the early stage of acidification, production of sulfuric acid was low because of the small amount of  
 676 NSOB in the inoculum, the unfavourable growth conditions for ASOB and the low production of  
 677 sulfuric acid by NSOB compared to ASOB [2]. So, when an inoculation introduced few neutrophilic  
 678 bacteria, the buffer potential of the material had a significant influence on microbial development.  
 679 Elsewhere, the delay before acidification induced a delay before the ASOB development and therefore  
 680 in sulfuric acid production. CAC-based materials underwent earlier acidification, so the biogenic  
 681 sulfuric acid flow seen by these systems was higher. The conditions of aggressiveness were then  
 682 higher during the beginning of the experiment for CAC materials than for PC systems.

683 The analyses of the bacterial population revealed that there was a selection of similar populations on  
 684 each material tested, with predominance of ASOB and a minor proportion of NSOB. The composition  
 685 of the material did not induce the selection of a specific population depending on the nature of the

686 binder. The predominant bacterium at the end of the experiment was *Acidithiobacillus thiooxidans*.  
687 The bacteria observed have already been mentioned in different studies of the literature as the  
688 predominant species responsible for sulfuric acid production in the range of pH from ~4 to ~1 in sewer  
689 networks [2,3,13]. It can also be mentioned that no *Acidithiobacillus ferrooxidans* has been observed.  
690 This may be explained by bacterial growth conditions which were not suitable to such species,  
691 especially with the use of white PC with low iron content. *Ac. ferrooxidans* has been reported in the  
692 depth of concrete in an iron-rich front [44]. The biofilms analysed developed on the surface of the  
693 material. It should be underlined that the bacterial population analyses were not quantitatively  
694 absolute, and just informed on the relative proportions of microorganisms. However, the relatively  
695 similar production of sulfate on each material (delayed or not) showed that the sulfur-oxidising  
696 activity was not affected by the nature of the material.

697 Microscopic observation of the biofilms confirmed the development of microorganisms on the surface  
698 of each material whatever its composition. Bacillus was observed on the surface of the materials but  
699 was probably distributed throughout the volume of the biofilm before its dehydration. The sulfur-  
700 oxidising species reported on cementitious material in sewer networks with such morphology were  
701 generally *Acidithiobacillus* or *Thiobacillus* [13,45,46]. Filaments of ~1 µm diameter may correspond  
702 to filamentous bacteria or exopolysaccharide (biopolymer produced by the bacteria for the  
703 structuration of the biofilm). Filaments of diameter >3µm indicated probable presence of fungi. There  
704 was no evidence of the material composition having a bacteriostatic effect on the development of  
705 biofilms. However, it is notably that the experimental setup was operated in optimal conditions for  
706 culture of SOB (continuously water, nutrients and sulfur substrate).

707 In this study, the only detected effect of the material on the biology was the influence of the PiOH on  
708 the delay before acidification. This effect was responsible for a lower impact of CAC-based materials  
709 (and higher PC-GGBS substitution) on the sulfate and thus acid production by SOB. CAC materials  
710 were exposed to highly acid conditions earlier but exhibited more resistance to deterioration. The  
711 study of the biological activity also showed that there was no significant effect of materials rich in  
712 aluminium on the SOB settlement activity or SOB selection using the BAC test, in contrast with the

713 literature hypothesis put forward in [17,26,29]. Moreover, the neutralisation capacity, corresponding to  
714 the PtOH in this study, did not induce a lower sulfuric acid production by microorganisms on CAC  
715 materials, as may have been previously assumed [12].

716

## 717 **4.2 Chemical resistance**

718

719 The better resistance of CAC binders could be mainly related to intrinsic physicochemical  
720 characteristics (nature of the phases, reactivity, porosity, etc.) of the materials rather than a  
721 bacteriostatic effect.

722 The biodeterioration phenomenology observed in cement matrices using the BAC test was globally in  
723 accordance with in situ and laboratory data from the literature. The use of tetrathionate as reduced  
724 sulfur substrate in the feeding solution allowed the selection of sulfur-oxidising activity [23,24].  
725 Neutrophilic and then acidophilic SOBs were successively selected. The microbial activity induced the  
726 production of sulfuric acid, which decreased the surface pH to about 2.5. The presence of elemental  
727 sulfur was an indicator of the succession of oxidising reactions occurring from the tetrathionate  
728 consumed by SOBs. The biogenic acid attack induced the decalcification and dissolution of the main  
729 cementitious phases and the formation of a deteriorated zone that was rich in silicon and mainly  
730 amorphous for PC systems, and rich in aluminium (AH<sub>3</sub> type) for CAC materials.

731 In the current study, sulfur based phases were precipitated in the deteriorated areas. Literature data  
732 confirmed the presence of gypsum and ettringite in concrete deteriorated layers [15,25]. Calcium ions  
733 released from dissolved calcic phases (C-A-S-H, CH, carbonates, C<sub>3</sub>AH<sub>6</sub>, etc.) diffused and reacted  
734 with sulfate ions from sulfuric acid to form gypsum in oversaturation conditions relative to this phase.  
735 A thick layer of calcium sulfate phases (gypsum, bassanite and anhydrite) was sometimes observed  
736 during experimental campaigns on site [17,23,47]. Only a small quantity of gypsum and no bassanite  
737 nor anhydrite was observed in the present study. The aggressive nature of the BAC test, which was  
738 designed for accelerated testing, may explain the lack of massive precipitation of calcium sulfate, due

739 to the very low pH conditions. Moreover, the wet-dry cycles, temperature, humidity, or hydraulicity  
740 present in sewer conditions may play an important role in the precipitation of calcium sulfate phases  
741 [3]. Wet-dry cycles induce a higher precipitation of such phases but also cause regular interruption of  
742 the biological activity.

743 Different hydrates (AFm, C-A-S-H, ettringite, hydrotalcite) may be a source of aluminium for the  
744 precipitation of ettringite. Reaction between gypsum and aluminate phases present in the system  
745 induced the formation of ettringite in oversaturated solutions (rich in Al, SO<sub>4</sub> and Ca). Additionally,  
746 some anhydrous phases (residual GGBS grains, C<sub>2</sub>AS, etc.) could release aluminium even though their  
747 contribution is not well identified in the literature. Ettringite was not always detected in experimental  
748 campaigns of the literature and was generally localised near the sound area, due to its high solubility at  
749 pH lower than 10.7 [22,24,26,47]. The formation of this expansive phase may lead to microcracking  
750 phenomena with high crystallisation pressure. Some positive effects on porosity filling are not  
751 documented in the field of biodeterioration.

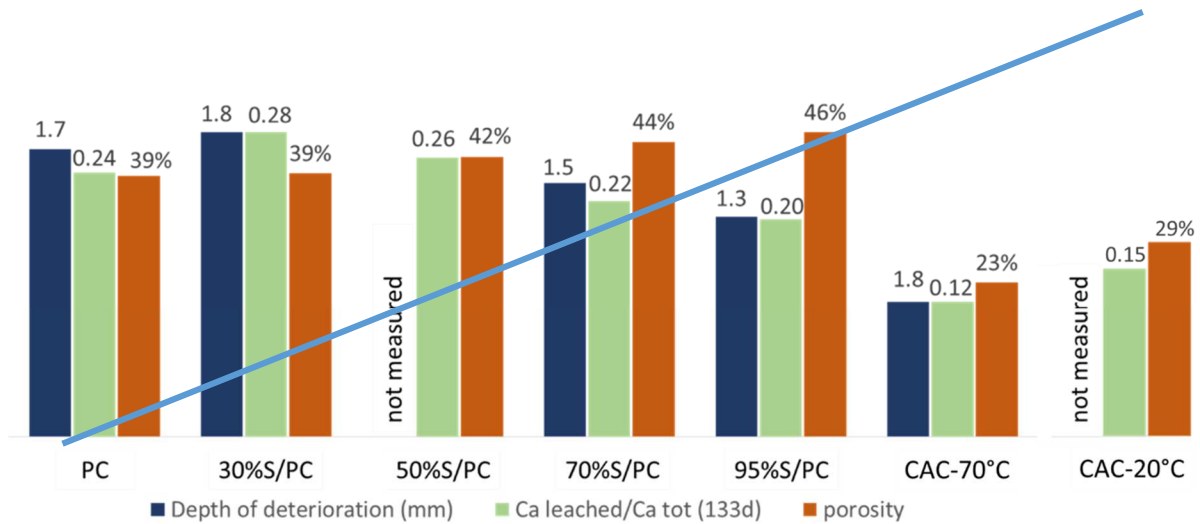
752 The deterioration zone seemed to be more porous than the sound one, with darker shades on SEM  
753 observations. The thickness of the deteriorated area was low and may be related to the high  
754 aggressiveness of the BAC test caused by the low pH and the constant rinsing of the material surface,  
755 inhibiting the oversaturation of the pore fluids. The biodeterioration mechanisms have been simulated  
756 by comparing ion equilibrium. They explain the precipitation of the different phases and will be  
757 presented in a further publication.

758 The total depth of deterioration was smaller for CAC-based materials than for PC-based materials.  
759 [15,17,25]. This is in accordance with data obtained in laboratory and in situ conditions [17], [26].  
760 Figure 16 shows that the depth of deterioration was strongly correlated to the standardised  
761 concentration of calcium in leaching solution (i.e. divided by the initial concentration in the material).  
762 This comparison showed the ability of leaching solution analyses to identify the intensity of the  
763 biodeterioration and thus their possible use as a tool to compare different types of binders.

764 A hypothesis to explain the better resistance of CAC-based material is the lower reactivity of the  
765 initial and decalcified phases [27,28]. With increasing GGBS content in PC-based material, the  
766 standardised calcium leaching decreased. CAC-based materials underwent significantly lower  
767 aluminium and calcium leaching than PC-based materials (Figure 10 and Figure 11). The theoretical  
768 dissolution constants of the hydrated phases of the PC-based materials are lower than those of CAC-  
769 based ones [48]. So, the dissolution of katoite ( $C_3AH_6$ ) would be slower than that of common phases  
770 in PC systems like CH. The  $AH_3$  phase seemed to be intermixed with  $C_3AH_6$  in the degraded area as  
771 shown in Figure 12. The lower total content of calcic hydrates in PC-GGBS systems (due to the high  
772 proportion of residual anhydrous GGBS grains) influenced this resistance. Anhydrous phases may be  
773 less reactive to acid attack than hydrated phases [49,50]. In biodeteriorated zones of mortar pipe  
774 sections exposed to the BAC test, Peyre Lavigne et al. observed an area where the hydrated paste was  
775 dissolved but the anhydrous phases were preserved [25]. CAC-based materials presented a lower  
776 standardised  $Al^{3+}$  leaching than PC-based systems, which meant that aluminium was more stable in  
777 CAC systems.  $AH_3$  phases are known to be stable over a wide pH range (pH ~ 4 to ~10 depending on  
778 the aluminium concentration) [26,51]. In addition, no difference related to thermal curing of CAC-  
779 based materials was observed: CAC-20°C and CAC-70°C showed equivalent Ca and Al leaching. The  
780 presence of katoite ( $C_3AH_6$ ) or  $CAH_{10}$  was not a determining parameter for the resistance of CAC  
781 binders in the very aggressive conditions developed in the BAC test.

782 In the literature, the lower diffusion properties of CAC phases are assumed to account for the better  
783 resistance of these binders [15,26–28]. Figure 16 compares the deterioration parameters (depth of  
784 deterioration, standardised calcium leached) and initial water intrusion porosity for the different  
785 systems.

786



787

788 **Figure 16. Comparison between depth of deterioration, calcium leaching and initial water porosity for PC,**  
789 **PC/30%S, PC/50%S, PC/70%S, PC/95%S and CAC-70°C.**

790

791 With increasing GGBS content in PC-based material, the initial porosity increased slightly [52]. This  
792 effect has often been reported in the literature but was generally combined with a refinement of  
793 porosity limiting the diffusion process. CAC-based material had the lowest initial porosity. This was  
794 partly due to the initial lower water/solid ratio (0.3 against 0.4 for PC-based materials). No correlation  
795 between the initial total water porosity and the leaching of the cementitious matrix can be evidenced  
796 on the series of OPC-slag based materials (Ca leaching and depth of deterioration tend to decrease  
797 while the initial matrix porosity increases in higher slag content mixes), which would indicate that  
798 initial porosity was not a critical characteristic in the biodeterioration of materials in such aggressive  
799 conditions. For CAC-based materials, the difference in phase composition and in porosity may  
800 influence the calcium leaching. Moreover, as it can be seen in Figure 8, CAC 20°C specimens were  
801 exposed to a higher sulfate flow due to the earlier biogenic acidification (Figure 7). The biogenic acid  
802 attack induced the formation of deteriorated areas, possibly with different diffusion properties. These  
803 deteriorated areas may have had a positive effect by limiting both the diffusion of aggressive ions  
804 inside the matrix and leaching of calcium outside the cementitious binder. To understand the influence  
805 of the porosity in such conditions, the biodeterioration of a theoretical CAC material with initial

806 porosity similar to that of PC but having the same composition as CAC-70°C has been simulated and  
807 will be the subject of a further publication.

808

809 In conclusion, the reactivity of the phases is likely to control the dissolution of the cement matrix and  
810 thus the resistance of the materials submitted to the BAC test in aggressive conditions. Diffusion  
811 properties were probably a secondary parameter accounting for the deterioration process kinetics in the  
812 aggressive conditions of this study. The difference of chemical resistance between the cementitious  
813 phases and their associated diffusion properties has been simulated using a biodeterioration model  
814 combining biological activity and dissolution/precipitation of solid phases and will be presented in a  
815 further publication.

816

## 817 **5. Conclusion**

818

819 The present paper studied the reliability of the main hypotheses found in the literature to explain the  
820 better resistance of CAC-based material than PC systems in a sewer network. The absence of a long-  
821 term bacteriostatic effect of soluble aluminium up to 100 mM (from  $\text{AlCl}_3$ ) on SOB activity was  
822 demonstrated using controlled batch reactors bacterial consortia selected in different conditions.

823 Matrices with a wide range of aluminium contents and different phase assemblages were considered.

824 They were based on PC with different GGBS substitution levels (from 0 to 95 wt.%) and CAC. The  
825 cement pastes were exposed to an accelerated laboratory biodeterioration test (BAC test) for 133 days  
826 with final pH of 2.5 to simulate sewer conditions. It was shown that the intermediate hydroxide  
827 potential (i.e. the quantity of hydroxide ions released by the dissolution of calcic phases) was  
828 correlated with the beginning of strong acidification and so the start of the activity of ASOBs. More  
829 aggressive conditions (due to an earlier acidification) were obtained for CAC systems and, to a lower  
830 extent, for PC-GGBS systems with high slag replacement levels. However, these materials were more  
831 resistant to biodeterioration than PC systems. Moreover, no difference in population selection or



832 characteristics of the biofilm was evidenced between the different materials. There was also no  
833 evidence of a bacteriostatic effect of aluminium rich materials on the SOB activity.

834 The better resistance of CAC-based materials compared to PC-based was verified by SEM  
835 observations. The leaching of sulfate, calcium and aluminium produced during the biodeterioration test  
836 were consistent with the SEM observations, which demonstrated the ability of leaching analysis to  
837 monitor the kinetics of biodeterioration. The phenomenology of deterioration was consistent with the  
838 literature. Calcic phases were dissolved whereas deteriorated areas rich in silica, for PC-based  
839 systems, and rich in aluminium, for CAC systems, were formed. The thicknesses of these areas were  
840 reduced due to the high aggressiveness of the deterioration test, which led to dissolution of the surface  
841 material. Moreover sulfur based products were precipitated in the upper part of the sample in contact  
842 with the biofilm. The resistance to biodeterioration of CAC systems was linked to physicochemical  
843 characteristics of the material when faced with biogenic attack. The transport phenomena limiting the  
844 dissolution of the cement matrix seemed to constitute a secondary process in the conditions of the  
845 BAC test. The phase stability of initial and secondary precipitated phases was probably the key  
846 parameter explaining the better resistance of CAC-based material and PC-GGBS compared to PC  
847 binders in sewer network conditions. To confirm and improve the understanding of deterioration  
848 mechanisms, a simulation has been performed using a model taking account of the biological activity  
849 (only influenced by the  $PiOH$ ) of the different populations of SOBs and the physicochemical  
850 evolutions of the material (leaching solution composition, dissolution/precipitation of phases,  
851 evolution of porosity and diffusion properties) and will be presented in a further publication.

852

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854

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861

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