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Biodeterioration of mortars exposed to sewers in relation to microbial diversity of biofilms formed on the mortars surface

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ABSTRACT

Strong deterioration of concrete in sewer systems is mainly due to microorganisms and especially to sulfur-oxidizing bacteria. Mortars made either with ordinary Portland cement (OPC) or calcium aluminate cement (CAC) were exposed in a waste water collector for five years. Mortar microstructure observed by microscopy reported a larger thickness of the degraded zone for OPC mortar. Taxonomic identification of bacterial communities performed on biofilms collected at the mortar surface reported similar bacterial diversities, but strong differences of relative abundance. A greater neutrophilic sulfur-oxidizing bacterial (NSOB) activity was observed for OPC mortar certainly in conjunction with its larger acid neutralization capacity. Thus, CAC mortar was less biodeteriorated than OPC mortar as less NSOB were able to settle on its surface in relation with its specific microstructure. The results of the reported field experiments have been compared with bioleaching laboratory experiments performed on identical mortars in the presence of *Halothiobacillus neapolitanus* as NSOB. As the deterioration mechanisms involved were similar, an acceleration factor with respect to the rate of *in situ* biodeterioration was determined for laboratory experiment.

1. Introduction

Concrete deterioration is one of the most serious problems affecting sewer infrastructure which has an enormous economic impact when the replacement or rehabilitation of sewer system is required. The deterioration rate of the concrete can reach up to several millimeters per year for sewer pipes (De Belie et al., 2004).

In sewer systems, concrete deterioration is mainly due to sulfuric acid generated by microbial sulfur oxidation because high concentrations of hydrogen sulfide (H_2S), oxygen (O_2) and moisture are present in the confined atmosphere of sewer network. Hydrogen sulfide (H_2S) is produced under anaerobic conditions by sulfate-reducing bacteria (SRB) from sulfate and other oxidized sulfur compounds present in water and sediments. H_2S volatilizes and dissolves in the moist concrete surface. Thus, it is mainly chemically oxidized to thiosulfate ($S_2O_3^{2-}$) and elemental sulfur (S^0). In addition of H_2S, CO_2 and other gases abiotically decrease the pH at the surface of concrete passing from a pH value of 12 to 9 (Jiang et al., 2015), enabling the colonization by microorganisms, such as neutrophilic sulfur-oxidizing bacteria (NSOB) (Mori et al., 1992; Jiang et al., 2014). These bacterial populations, such as *Thiobacillus* sp., *Thiomonas* sp., *Halothiobacillus* sp., oxidize H_2S and other reduced sulfur compounds to sulfuric acid (H_2SO_4) and polythionic acids (H_2SxO_6) that induce the dissolution of some minerals at the surface of the concrete leading to a drop of pH more or less rapidly depending on their acid neutralization capacity and the secondary minerals that may precipitate (Bielefeldt et al., 2009). At pH around 4.5, acidophilic sulfur-oxidizing bacteria (ASOB), such as *Acidithiobacillus thiooxidans* and *Thiomonas intermedia*, start to grow using sulfur compounds as donor electrons. ASOB can survive at pH as low as 1 and produce a high amount of sulfuric acid (Soleimani et al., 2013). As a consequence, the surface pH of concrete decrease to pH values between 1 and 3 depending on concrete composition (Parker, 1945). This promotes the further dissolution of minerals contained in concrete increasing the porosity of concrete and consequently reducing the mechanical strength of concrete that may fail. Moreover, these acidic conditions eventually inhibit neutrophilic sulfur-oxidizing populations in favor of more acid-tolerant sulfur-oxidizing populations (Islander et al., 1991). Additionally to the dissolution of the minerals that are not stable under acidic conditions, some secondary minerals can be precipitated and thus may participate to the biodeterioration of concrete.

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For example, in the case of concrete made with ordinary Portland cement (OPC), gypsum ($CaSO_4$, 2 H_2O) can be formed by the reaction between H_2SO_4 and Portlandite or calcium carbonate and lead to expansion and cracking (O'Connell et al., 2010; Montery et al., 2000). Segments of ductile cast iron coated with cementitious linings made with blast furnace slag cement (BFSC) reported abundant cracking caused by precipitation of secondary ettringite while no cracking was observed in the calcium aluminate cement (CAC) lining (Peyre Lavigne et al., 2016). Thus, the composition of concrete and especially of its cement paste is crucial to design concretes having a good resistance to biodeterioration. Amongst the different possibilities, concrete made with calcium aluminate cement is often reported to be more resistant than similar concrete made with OPC (Alexander and Fourie, 2011; Herisson et al., 2013). The different mineralogy and microstructure of the cement paste in CAC concrete are certainly some major parameters to explain the observed better performance. However, the possible interaction between sulfur-oxidizing bacteria (SOB) and the concrete especially at its surface on which biofilm is formed cannot be ruled out (Peyre Lavigne et al., 2015). Thus, it is necessary to finely characterize the microbial diversity of sulfur-oxidizing bacteria to better understand the concrete biodeterioration process. This can assessed using molecular based techniques, such as PCR-DGGE (Huber al., 2016), fluorescence hybridization (FISH) (Hernandez et al., 2002) and 16S rRNA sequencing (Dong et al., 2017). These latter techniques allow the identification of uncultured microbial communities contrarily to conventional culture dependent techniques which detect only a fraction of the total microbial population (Harisson, 1984; Islander et al., 1991; Keller and Zengler, 2004). Molecular microbiological methods (MMMs) are used to improve our understanding of the effect of microorganisms in biodeterioration. But, a multidisciplinary approach is required to establish the significance of molecular microbiological data in respect to biodeterioration mechanisms involved (Eckert and Skovhus, 2018).

The main objective of this study was to further explain the better performance of CAC concrete relative to OPC concrete by linking the biodiversity of the bacterial communities, especially SOB, developed on biofilm at the concrete surface and the intensity of the biodeterioration. Mortars, that are smaller and easier to manipulate than concrete samples, were placed during 5 years in a waste water collector. Then, bacterial communities were characterized by using 16S rRNA sequencing while the characterization of the microstructure of mortar was performed in order to estimate the intensity of biodeterioration. A second objective was to assess the relevance of laboratory bioleaching experiments using SOB, in order to estimate the performance of mortars. This was possible as identical mortars had already been studied after being subjected to bioleaching experiments in the presence of *Halothiobacillus neapolitanus* (Lors et al., 2017).

2. Materials and methods

2.1. Mortar specimens

Mortars were prepared by mixing cement, sand and water at a weight ratio of 2:6:1, according to NF EN-196-1 standard (2006). Two different cements were used to prepare the mortars named OPC and CAC mortars respectively: ordinary Portland cement (CEM I 52.5) supplied by HOLCIM (France) and calcium aluminate cement supplied by KERNEOS (France). The chemical composition of both cements was determined by X-ray fluorescence spectroscopy (Bruker, Pioneer S4, USA) (Table 1). The sand used was standard siliceous sand that is expected to be inert in the presence of sulfuric acid. The mortar was casted into PVC cylindrical moulds, in order to obtain cylindrical mortar samples (diameter 2.9 cm, height 6.3 cm, surface 70.6 cm^2 and volume 41.6 cm^3). Before the setting of the cement, a steel hook was inserted in the upper face of the mortar sample. The mortars were demoulded after 1 day and were cured in pure water at 20 °C during 28 days. After curing, the surface pH measured with pH surface electrode (SentixSur, WTW) was around 13. A specific device, named mortar collar, was designed, in order to suspend several mortar samples and to prevent the interactions between the samples. A mortar collar was made of four mortar samples that were attached by their steel hook to a steel cable protected by a Teflon sheath. Additionally, pieces of larger Teflon tube were used between the mortar samples to avoid any contact and cross contamination between them. Two mortar collars were made, each one with either mortars OPC or mortars CAC and then installed in the emerged part of the collector of waste waters of Urban Community of Lille (France) in august 2008. This collector was chosen due to easy access and because it was subjected to H_2S contents with average of 7 mg m^{-3}. It has to be noticed that sometimes the collector may be almost completely filled by waste water and consequently that the samples may be sometimes in direct contact with the waste water.

2.2. Mortars sampling

After 5 years of exposure (August 2014), one sample of each type of mortar was removed from mortar collar in order to be analyzed.

The biofilm was collected from the entire mortar surface of each mortar sample by scraping with a clean metal spatula, and then transferred in sterile 50 mL centrifuge tube. Moreover, a biofilm sample was collected on the concrete wall of the collector of waste waters (sample named C). For total bacterial counts, biofilm samples were directly used by mixing 0.5 g of biofilm with 10 mL of sterile Ringer solution (Ramsay, 1984). For molecular analysis, the biofilm samples were stored at −80 °C.

2.3. Microstructural and mineralogical analysis of mortars

Mortars were dried at 40 °C during 7 days. The mortar cylinder was impregnated in epoxy resin to avoid any damage during further preparation especially at the surface of the sample. The sample was then cut perpendicularly to the spherical sections in two equivalent pieces. The cut section enables us to observe the degraded areas located on its edges. Then, one of these two pieces was polished to produce a flat section. After hardening of the epoxy resin, the surface was pre-polished under water using several diamond-covered polishing discs having a decreasing particle size: 151, 75, 46 and 16 µm. To obtain a flat surface prior to fine polishing steps, samples were polished using a silicon carbide grinding wheel (particle size: 8 µm). The fine polishing steps were performed using diamond pastes of decreasing particle size (9, 6, 3, 1, ½, and ¼ µm). Observation corresponded to the bottom of the mortar cylinder and the two first cm of the sample.

Samples were gold coated before observation under a scanning electron microscopy (SEM) (Hitachi S-4300SE/N, Japan). Backscattered electron (BSE) imaging was performed (20 KeV and 2 KA) and complemented by energy-dispersive X-ray spectroscopy (EDS) analyses with a Thermoscientific Ultradry EDX detector running at 15 kV. BSE imaging gave some indications on the intensity of the deterioration of the

Table 1
Content (as atom %) of chemical elements containing in ordinary Portland cement (OPC) and calcium aluminate cement (CAC).

	O	Ca	Si	Fe	Al	S	K	Na	Mg	P	Ti	C	Sr
OPC	41.1	41.9	7.5	2.9	2.6	1.8	0.6	0.4	0.4	0.2	0.2	0.13	0.1
CAC	42.7	26	25.9	2.1	1.2	1.1	0.3	0.3	42.7	26	25.9	2.1	1.2