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ORIGINAL ARTICLE

Alteration of a cement matrix subjected to biolixiviation test

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Abstract This paper reports on the assessment of durability and long-term performance of a cement matrix subjected to heterotrophic microbial mediated degradation. In near surface disposal facilities for hazardous and radioactive wastes, microbial activities may likely, in a long-term perspective threaten the integrity of cement-solidified wastes. To investigate the detrimental impact of heterotrophic microorganisms on cement matrices, *Aspergillus niger* reputed as versatile and prevalent fungus in soil flora was selected as candidate. It was shown that this fungus has the potential of severely degrading ordinary Portland cement pastes through organic acids production. Cement pastes experienced chemical alterations such as substantial leaching of calcium, and mechanical degradation was evident as highlighted by the drastic decline in Young's modulus. Their poor behaviour with respect to heterotrophic biodeterioration and susceptibility to failure were therefore demonstrated. Consequently, biolixiviation scenario should be seriously considered in order to ensure safe long-term disposal for cement-solidified wastes.

Résumé *L'article concerne l'étude du comportement et des performances à long-terme d'une pâte de ciment vis-à-vis de la biodégradation associée aux microorganismes hétérotrophes. Dans les installations d'entreposage et de stockage en surface des déchets radioactifs, les activités biologiques recensées peuvent* in fine *compromettre l'intégrité des déchets solidifiés par liants hydrauliques. Afin d'évaluer l'impact de ces activités sur les matrices cimentaires, un champignon ubiquiste dans les sols,* Aspergillus niger*, a été sélectionné et mis en œuvre dans un essai de biolixiviation. Cette étude montre clairement que l'attaque d'une pâte de ciment Portland par* Aspergillus niger *est particulièrement sévère et due essentiellement aux acides organiques. L'attaque se traduisant essentiellement par une lixiviation substantielle en calcium et par la chute du module d'Young. Le scénario de biolixiviation devrait donc être sérieusement considéré dans l'optique d'un stockage sûr des déchets radioactifs stabilisés par liants hydrauliques.*

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Mots-Clés Biolixiviation · Ciment Portland · Champignons · Acides organiques · Durabilité des bétons.

1 Introduction

Concrete is an inherently durable material that will last for a long time if it is correctly mixed according to specific guidelines and properly designed for its intended environment and use. However, it is potentially vulnerable to a variety of deterioration mechanisms and, in particular, it may be corroded by biodeterioration processes promoted by microbial activities. It is believed that the high pH, almost 13.5, imposed by concrete will effectively curtail microbial growth; however there is some evidence that several microorganisms can readily colonize cement-based materials despite their alkalinity [1–3].

Concrete is commonly used as an engineered barrier material in near-surface disposal facilities for the solidification and stabilization of hazardous and radioactive waste. The ubiquitous occurrence of many types of microorganisms isolated from low-level radioactive waste environments has raised concerns that microbial activities could jeopardize the integrity and long-term performance of disposed waste. Moreover, concrete is fundamentally unstable in water and its properties change over time. It is therefore important to understand and assess the long-term interaction between the cement materials, groundwater and microorganisms likely to be present in the repository in order to ensure safe long-term disposal.

Cement-based materials are all prone to microbial attack. Most investigations in the literature related to concrete biodeterioration chiefly incriminate bacteria, whose activities are deemed to be the primary cause in the deterioration of concrete, especially in sewerage networks [4–7]. Fungi and heterotrophic bacteria have, to a certain extent, been underestimated in the biodeterioration process. Cement degradation by fungi isolated from soil and tolerant of alkaline conditions was demonstrated by Perfettini et al. [2]. Their results indicate a potential threat to cement materials if the associated microorganisms are supplied with substrate. Fungal induced degradation of concrete occurred more quickly than bacteria-influenced degradation with complexolysis suggested as the main mechanism of calcium dissolution [3]. Gaylarde et al. [8] reported that fungi isolated from building façades include many genera representative of air and soil flora and pointed out the susceptibility of concrete to degradation by heterotrophic microorganisms. Fungi are also particularly blamed for staining and spoiling the esthetic appearance of building façades [9].

Fungi are liable to colonize cement matrices and are able to impair their integrity *in fine* by excreting organic acid metabolites, which react with the hydrates and lead to substantial leaching of calcium [10]. Due to their filamentous growth habit and ability to produce and exude organic acids, protons and other metabolites, fungi are efficient biological weathering agents of building materials [11]. The biodeterioration phenomenon may be regarded either as a direct (or physical) attack by the biofilm coating the material, which results in hyphae penetrating through the accessible pores, and/or an indirect (or chemical) attack by their metabolites, mainly organic acids. Chemical attack, however, seems to be predominant [2, 10, 12]. In the agricultural environment, severe damage has been diagnosed in concrete floors and structures intended to store silage effluents or liquid manure, which is suspected to arise through chemical attack mainly induced by acetic and lactic acids [13–15]. These acids can severely deteriorate concrete especially at high concentrations. Bertron et al. [16] investigated the effect of organic acids on cement pastes. They reported decalcification of the altered zone, progressive dissolution of all crystallized phases and the probable formation of a silica gel enriched with silicon, aluminum and iron in the superficial layer. In their conclusion, they stated that attack by organic acids may be compared with that by strong acids.

This paper assesses the durability performance of cement matrices subjected to a bioleaching process (biodeterioration enhanced by leaching) by heterotrophic microorganisms, considered as a plausible scenario for concrete deterioration in near-surface disposal sites for hazardous and radioactive waste storage. Preliminary results on exposing an ordinary Portland cement paste to *Aspergillus niger* fungus for

Table 1 Composition of the fungal culture medium

Components	mg/l	Components	μg/l
KH_2PO_4	136	$CuSO_4 \cdot 5H_2O$	0.5
Na_2HPO_4	60	BH_3O_3	1
$MgSO_4 \cdot 7H_2O$	70	$MnSO_4 \cdot H_2O$	1
NH_4NO_3	100	$ZnSO_4 \cdot 7H_2O$	1
$CaCl_2$	10	$Mo_7(NH_4)_6O_{24} \cdot 4H_2O$	10
$FeSO_4 \cdot 7H_2O$	0.2	$Co(NO_3)_2 \cdot 6H_2O$	1
Glucose	10,000		

almost 15 months are discussed, with an emphasis on understanding the mechanisms of biodeterioration.

2 Materials and methods

2.1 Microorganisms and culture medium

The microorganism selected for investigating the detrimental impact of heterotrophic biodeterioration on the long-term performance of cement-based materials was the fungus *Aspergillus niger* van Tieghem (DSMZ[1] 823). This acidophilic fungus is ubiquitous and commonly found in soil environments. *Aspergillus niger* is known to tolerate relatively high pH, and is consequently a suitable candidate for assessing the durability of cement materials with regard to bioleaching phenomena. The composition of the fungal growth medium used in this study is given in Table 1. Ohshima et al. [17] demonstrated the ability of *Aspergillus niger* to colonize mortar and found that the fungus was highly dependent on a source of organic carbon for optimum growth. The *Aspergillus niger* strain was therefore supplied with glucose as a carbon source (substrate), in order to ensure optimum favorable conditions for fungal growth.

2.2 Cement paste specimens

This study was conducted on pastes made with an ordinary Portland cement designated in European Standard EN 197–1 as CEM I 52.5 N CP2. Their compressive strength and flexural strength measured on $4 \times 4 \times 16$ cm^3 prisms at 28 days age were 57.3 and 7.4 MPa, respectively. The chemical composition of the cement is given in Table 2. The water/cement mass ratio of the pastes was 0.5. The hardened cement paste specimens were cylindrical in form, 220 mm high and 110 mm in diameter. The specimens were demolded 24 h after pouring and stored in a basic solution (NaOH = 3 g/l, KOH = 10 g/l) reconstituting the interstitial phase of a hardened cement paste, in order to limit all preliminary leaching, at 20°C for 27 days. Afterwards, the specimens were cut into discs 30 mm thick and 110 mm in diameter. The density of the hardened cement paste determined after 28 days in accordance with AFREM-AFPC protocol [18] was 1.42 and the water-opened porosity was 46%. The porosity of the cement paste determined by mercury intrusion porosimetry, after drying at 50°C until constant mass, was 22.6%.

2.3 Experimental approach

The cement pastes were subjected to an accelerated biodeterioration test using the protocol described in detail in a previous paper [19]. The accelerated test consisted in putting 12 cement paste specimens in contact with the microbial growth medium (called the lixiviating solution) containing mainly glucose (substrate) and the candidate *Aspergillus niger*. The *L/S* mass ratio (mass of the leaching solution divided by the exchange surface of the solid) was 10 g/cm^2. The leaching solution was renewed at a ratio of 20% every 2 weeks. In parallel, *Aspergillus niger* inoculum was added regularly to the leaching solution also at the same frequency, i.e. every 2 weeks with a

Table 2 Chemical composition of ordinary Portland cement

Oxide	Wt. %	Oxide	Wt. %
CaO	65.82	K_2O	0.11
SiO_2	21.84	P_2O_5	0.07
Al_2O_3	4.13	Na_2O	0.04
Fe_2O_3	0.28	Loss on ignition	4.18
MgO	0.61	Total	99.84
SO_3	2.56	CaO_{free}	0.42
MnO	0.001		
TiO_2	0.2	Blaine (cm^2/g)	3970

[1] German Collection of Microorganisms and Cell Cultures

volume of 0.5 l. The age of the inoculum was 2 weeks. The ratio *L/S* was always maintained constant and equal to 10 g/cm^2. This procedure provided an accelerated simulation of fungal attack on cement pastes. The analyses carried out on the leaching solution included the investigation of electrical conductivity, pH, calcium (by inductively coupled plasma ICP), and biogenic organic acids (by high performance liquid chromatography HPLC) [19]. Degradation of cement pastes was assessed by analyzing the solid phase using various techniques, including optical and SEM observation, SEM-EDX micro-analysis, X-ray diffraction and indentation tests. Two cement paste samples were extracted every 12 weeks for analysis and readjustment of the leaching solution. This was done to maintain a constant *L/S* ratio over time. The biodeterioration test was performed under aerobic condition in order to enhance fungal growth and consequently the biodeterioration process. Two control tests were carried out in order to investigate the effect of the deionized water and the fungal culture medium (without glucose). The second test is more relevant to measure the deleterious action of heterotrophic microorganisms. Consequently, only the second control test will be presented in this paper.

3 Results and discussion

3.1 Naturalistic approach

SEM examination of the degrading cement paste specimens showed cracks over the surface, as well as dissolution–precipitation features such as precipitated calcite $CaCO_3$ and calcium oxalate (weddellite $CaC_2O_4 \cdot 2H_2O$ and whewellite $CaC_2O_4 \cdot H_2O$) which are characteristic of concrete chemical attack induced by carbonic and oxalic acids. Fungi can induce chemical weathering of cement matrices through the excretion of organic acid metabolites; moreover CO_2 released during respiration can lead to carbonic acid attack. Extensive hyphae in situ were clearly distinguished (Fig. 1e). However, it was not possible to identify *Aspergillus niger* in the biofilm structure coating the altered cement paste specimens; a point that may be explained by the fact that this fungus is completely aerobic.

3.2 Leaching phase analyses

3.2.1 Conductivity-pH

The conductivity gives a qualitative indication of the amount of ions leached by cement pastes during exposure to microbial (fungal) attack and therefore provides information concerning the mineralization of the leaching solution over time. Basically a cement matrix contains a porefluid (interstitial phase) with a very high pH (pH $>$ 13) which ensures and maintains the stability of the hydrated cement phases. Contact between this matrix and deionized water firstly leads to rapid lixiviation of K^+ and Na^+ alkalis and also of Ca^{2+} ions. This salting out of ions tends towards the establishment of an equilibrium between the interstitial phase and the surrounding environment, the equilibrium pH being around 12–13. This pH is relatively high and can therefore slow or even inhibit the development of microorganisms, including fungi. In order to accelerate the fungal colonization process, a buffered medium was used to moderate the pH to make it more favorable for fungal growth. The fungal culture medium (without glucose) provided the essential nutritional elements required by the microorganisms for growth and (due to the phosphates) buffered the lixiviating solution in contact with the cement paste to a pH of approximately 8 (Fig. 2).

In the presence of fungi, the electrical conductivity of the lixiviating solution rapidly evolved towards a value close to 10 mS/cm after 2 months trial time (transitional regime), then tended to stabilize at that value (stationary regime). This phase of increasing conductivity was associated with the dissolving of the calcium in the cement matrix and accompanied by a fall in pH to approximately 5. This drop in pH correlated to the production of metabolites (organic acids) by the fungi. We also observed, once the stationary regime had been established, a tendency for the pH to stabilize between 4 and 5, with occasional increases in pH probably due to a lack of glucose (in the fungal growth medium). When there is a shortage of glucose, the organic acids produced by the fungi may, in their turn, be assimilated and degraded, which could explain these increases in pH. The evolution of pH over time thus indicates that the biolixiviation of cement paste essentially corresponds to a chemical attack due to organic acids.

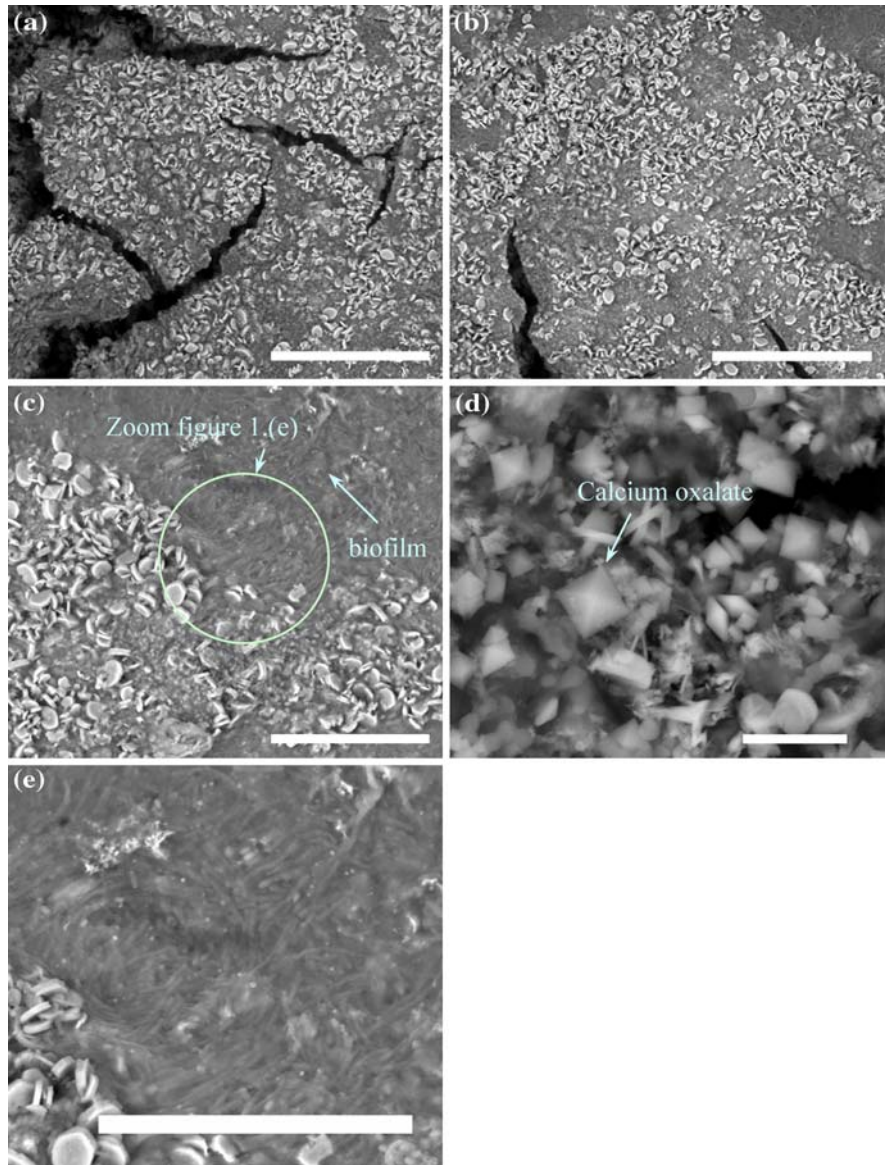

Fig. 1 SEM images of (**a**, **b**) deterioration of a cement paste exposed to microbial weathering for 6 months, cracking is evident, as well as the presence in situ of precipitated calcite and calcium oxalate (scale bar, 100 μm); (**c**) an extensive hyphal network over the surface, cf. (**e**) for more details (scale bar, 50 μm); (**d**) biogenic calcium oxalate dihydrate in situ (bipyramidal form) (scale bar, 10 μm). (**e**) Hyphal network observed over the cement paste surface, (scale bar, 50 μm)

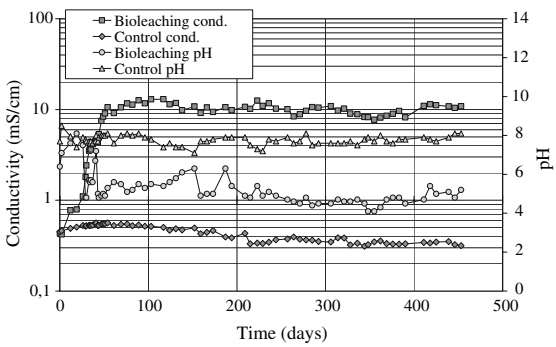

Fig. 2 Conductivity-pH versus exposure time

3.2.2 Leached calcium and organic acids

Once established in the microbial growth medium, fungi acidify their immediate environment by excreting organic acids and carbonic acid. These acids lead to a chemical attack of the cement paste. During the transitional regime, the total quantity of organic acids progressively increased to reach a value between 300 and 400 mmol/l (Fig. 3). A priori, this growth phase was the cause of the drop in pH. In parallel, the concentration of dissolved calcium also showed an increasing trend and reached a value of about 5 g/l.

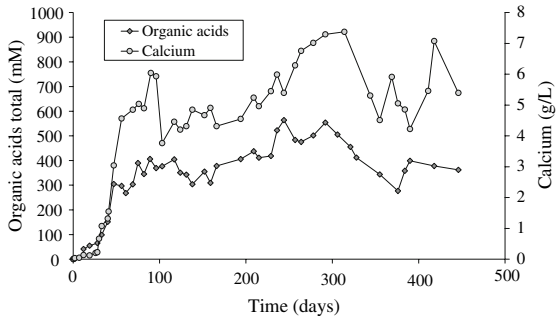

Fig. 3 Calcium-sum organic acids versus time

This significant salting out of calcium can be explained by the dissolving of portlandite $Ca(OH)_2$ and the decalcification of C–S–H hydrates and sulfoaluminates of calcium phases (aluminum–iron–mono AFm and aluminum–iron–tri AFt). After 2 months of biolixiviation, a stationary regime became established in the lixiviating solution, with the calcium stabilizing at around 5 g/l on average and the total organic acid content at around 400 mmol/l. Between 244 and 321 days (Fig. 3), we can also observe an above average increase in the calcium concentration, which was probably due to the increase in biomass accompanied by the production of more organic acids. However, it is not easy to corroborate this hypothesis, because the biomass present in the lixiviating solution was not quantified during the biolixiviation trial. The predominant organic acids (metabolites) secreted by fungi are oxalic, acetic, butyric and lactic acid. These acids are involved in the process of chemical attack of the cement matrix. Figure 4 shows the evolution over time of the concentrations of these acids as %. It can be seen from this figure that percentages of oxalic and butyric acids were relatively constant as from the 2nd month of the biolixiviation trial. The proportion of lactic acid was below 10% of total acids for the first 8 months and then increased to reach 30% of total acids. In parallel, acetic acid followed an inverse trend compared with lactic acid. The concentration of acetic acid was up to 30% of total acids during the first 8 months and then dropped below 10% of total acids. This phenomenon could be explained by a change in the metabolism of the microorganisms present in the lixiviating solution as from the 8th month, which favored the production of lactic acid to the detriment of acetic acid.

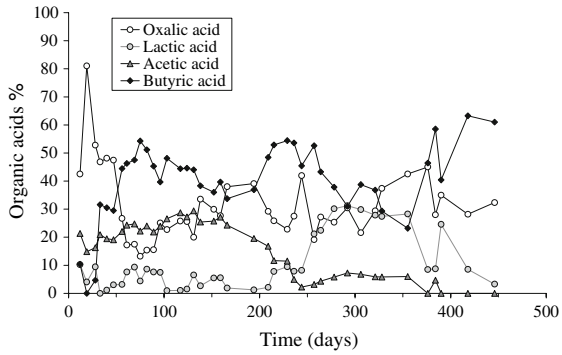

Fig. 4 Distribution of organic acids versus time

A priori, the chemical attack of Portland cement paste by organic acids takes place in three main stages [20]: decomposition of hydrates, mainly portlandite $Ca(OH)_2$ (1) and C–S–H (2), formation of calcium salts, and possibly dissolving these salts depending on their solubility in water (Table 3). In the formulas (1) and (2), the organic acid is represented by the generic formulae AH, and the calcium salt is represented by the formulae CaA_2. Table 3 shows that acetic and lactic acids are more corrosive than oxalic and butyric acids. The chemical attack due to oxalic acid (the predominant acid in the lixiviating solution, Fig. 4) could be considered as negligible or even beneficial, since it leads to the formation of an insoluble calcium salt. These oxalate crystals, which accumulate in the pores of the cement paste, can block the pores and act as a diffusion barrier, thus limiting the lixiviation of calcium.

$$Ca(OH)_2 + 2AH \rightarrow CaA_2 + 2H_2O \quad (1)$$

$$xCaO.ySiO_2.zH_2O + 2xAH \rightarrow xCaA_2 + ySi(OH)_4 + (x - 2y + z)H_2O \quad (2)$$

Bayoux et al. [20] carried out a study on the alteration of high alumina cements, which have a better behavior in comparison with ordinary Portland cements, in various acidic environments. In their conclusions, they stated that the main parameters governing acidic corrosion by organic acids are inter alia:

- The thermodynamic stability of the cement paste hydrates
- The acid concentration, which is directly related to the intensity of corrosion

Table 3 pK values of organic acids excreted by *Aspergillus niger* fungus and solubilities of associated calcium salts[a]

Acid	pK values			Calcium salt	Solubility, in g/l[b]	
	pK_1	pK_2	pK_3		Cold water	Hot water
Acetic	4.75	–	–	$Ca(C_2H_3O_2)_2 \cdot H_2O$	43.6^{0}	34.3^{100}
				$Ca(C_2H_3O_2)_2 \cdot 2H_2O$	34.7^{20}	33.5^{50}
Lactic	3.86	–	–	$Ca(C_3H_5O_3)_2 \cdot 5H_2O$	3.1^{0}	7.9^{30}
Butyric	4.81	–	–	$Ca(C_4H_7O_2)_2 \cdot 3H_2O$	s	sl. s
Gluconic	3.86	–	–	$Ca(C_6H_{11}O_7)_2 \cdot H_2O$	3.3^{15}	–
Propionic	4.88	–	–	$Ca(C_3H_5O_2)_2 \cdot H_2O$	49^{0}	55.8^{100}
Oxalic	1.23	4.19	–	$CaC_2O_4 \cdot H_2O$	0.007^{20}	–
Citric	3.08	4.74	5.4	$Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$	0.085^{18}	0.096^{23}
Formic	3.75	–	–	$Ca(HCO_2)_2$	16.2^{0}	18.4^{100}

[a] s: soluble, sl. s: slightly soluble

[b] Handbook of Chemistry and Physics, David R. Lide Eds., CRC Press, 1993–94, 447–450

- The solubility of the calcium salts (and perhaps aluminum salts if the pH is below 4), which is the most important parameter

3.3 Biodeterioration pattern

The degraded thicknesses of cement pastes are determined using one of the two specimens extracted approximately every 3 months. At 14 weeks of biolixiviation test, experimental results obtained for both cement paste specimens (degraded thicknesses, EDX and XRD analyses, and Rochart test) were almost identical, and thus it was decided to carry out the analyses on one specimen for next times. The potential for degradation of cement pastes by biogenic organic acids is evident from the biodeterioration pattern (Fig. 5). Examination of sectioned cement pastes subjected to bioleaching for almost 15 months showed typical zones of differential corrosion. A distinct difference was observed between the outer altered zone and the inner unaffected zone (sound zone) divided by a fairly sharp boundary. The altered zone (Fig. 5) could be subdivided into four distinct zones: the outer corroded zone including zones 1 and 1bis (4,900 μm thick) were severely degraded and had undergone significant microstructural alteration. Theses two zones obviously had a porous structure. The inner degraded zones, 2 and 3, were distinguished by a dense structure and were often traversed with cracks, in particular at the interface boundary, which is typical

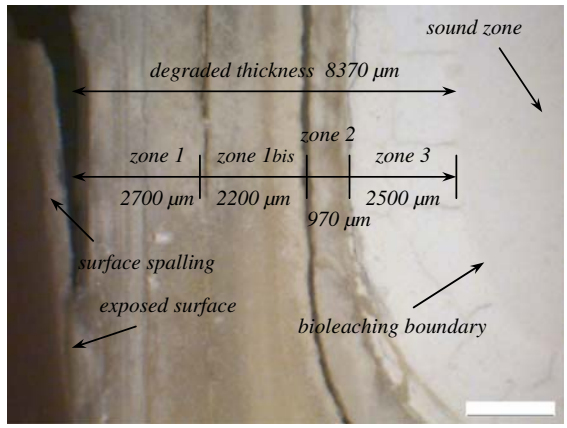

Fig. 5 Biodeterioration pattern of the cement paste (polished section) that was exposed to fungal biolixiviation for almost 15 months, exposed edge is on the left (scale bar, 2 mm)

of the expansive effects of sulfate attack but may simply have resulted from the drying of the sample. The kinetics of biodeterioration of the cement pastes could be investigated using the thickness of the altered zone and the degraded volume, which is determined by the formula (3).

$$V_d = \pi \cdot r^2 e \cdot \left[1 - \left(1 - \frac{\delta}{r}\right)^2 \cdot \left(1 - 2\frac{\delta}{e}\right)\right] \qquad (3)$$

where δ represents the thickness of the degraded zone and the couple ($r = 55$ mm, $e = 30$ mm) represent respectively, the ray and the thickness of the cylindrical test sample. Table 4 gives the thickness and

Table 4 Degraded thickness, variation in volume and calcium leached

Time (weeks)	14	27	39	51	63
Thickness (mm)	2.9	4.8	6.3	7.5	9.1
Volume (mm^3)	78,750	123,600	155,450	178,800	207,000
% Degraded vol.	27.6	43.4	54.5	62.7	72.6
% Calcium leached	10.5	16.4	27.8	33.4	37.0

volume of the altered zone after 14, 27, 39, 51 and 63 weeks of bioleaching. It should be noted that the thicknesses of the altered zones given in this table were based on those determined by optical microscopy (Fig. 5). The thickness of the altered zone after 15 months of bioleaching was about 8–9 mm, and five times higher than the thickness of the degraded zone for the control test (leaching test with deionized water). Figure 6 illustrates the evolution of the volume and thickness of the altered zone over time.

By way of comparison with other works on the alteration of cement pastes by organic acids, we can cite the study carried out by Bertron et al. [16] concerning the behavior of cement pastes in the presence of a mixture of organic acids designed to simulate manure slurries (aggressive solutions generally stored in concrete silos). This mixture, together with other components, included acetic and propionic acids at respective concentrations 12.6 and 2.8 g/l. The thickness of the degraded layer obtained after 18 weeks of immersion was estimated to be about 5.5 mm for a Portland cement paste with a water/cement ratio of 0.27 (pH maintained at 4, aggressive solution renewed every 6 weeks).

Braam et al. [13] carried out immersion tests on concrete prisms in a mixture of lactic and acetic acids at respective concentrations 50 and 17.5 g/l. They obtained a degraded thickness of 6.3 mm after 12 weeks of immersion (pH between 2.1 and 4, aggressive solution renewed when the pH rose above 4) for Portland cement concrete. De Belie et al. [14], who studied the effect of a mixture of lactic and acetic acids at 30 g/l on concrete prisms, estimated the degraded thickness after 32 days of immersion to be 1.4 mm (4 total renewal cycles, initial pH about 2.1 and pH maintained below 3 by addition of lactic and acetic acids).

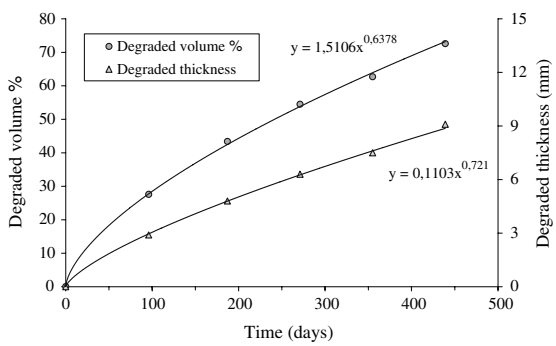

Fig. 6 Degraded volume % and degraded thickness versus time

3.4 EDX analyses

The degradation of the cement pastes was assessed by scanning electron microscopy (SEM) combined with energy dispersive X-ray (EDX) analysis of polished sections by investigating changes in elements along a transverse line on the specimen surface. The instrument used was a FEI QANTA 200 ESEM FEG equipped with an Oxford Inca Energy Dispersive X-ray system for chemical analysis. One of the two degraded cement paste specimens extracted almost every 3 months was analyzed.

Figure 7 shows quantitative oxide compositions along a transverse from the sample edge for a cement paste exposed to bioleaching for almost 15 months. Despite some noise resulting from the heterogeneity of hydrated cement paste, the distribution of oxides (Fig. 7) shows four zones of different chemical composition with regard to oxides. The thicknesses of the altered zones (4.8, 0.9 and 2.7 mm, respectively for the zones 1 + 1bis, 2 and 3) are very close to the thickness visually identified by optical microscopy. The sound zone is characterized by a relatively stable oxide composition. Calcium content gradually decreases in zone 3, and then shows a rather steep gradient pattern in zone 2. Outer zones 1 and 1bis exhibit substantial leaching of calcium and are mainly composed of SiO_2 and Al_2O_3, and therefore correspond to a silica–aluminum gel. Due to the drastic depletion of calcium in zones 1 and 1bis, the

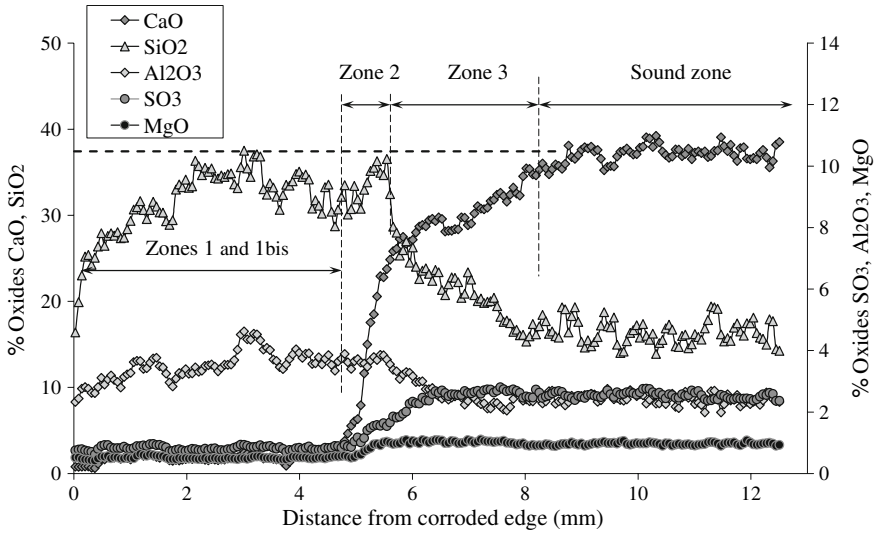

Fig. 7 Distribution of chemical elements along a transverse from the edge of the cement paste subjected to biolixiviation for almost 15 months

silica and aluminum contents of these zones are significantly higher than in the sound zone. This increase was also observed in the work of Bertron et al. [16], who studied the effect of a mixture of organic acids on cement pastes, and in the study carried out by Knight et al. [21] on the alteration of a Portland cement paste due to *Acidithiobacillus thiooxidans* bacteria. Zones 1 and 1bis also showed a significant decrease in sulfate, and magnesium was slightly reduced in comparison with the stable content of MgO in both zone 3 and the sound zone.

EDX analysis clearly revealed that calcium leaching is the main biodeterioration mechanism induced by microbial population, inter alia by *Aspergillus niger* fungi established in the leaching solution. The amount of calcium leached from the cement paste (qualitative percentage) can be determined using Fig. 7. The calcium leached is approximately calculated by the surface delimited by the curve of calcium and the plateau corresponding to the sound zone (trapeze method, $\delta \in [0–15\ \text{mm}]$ where δ is the distance from the edge). The qualitative amounts of calcium leached from the altered cement paste specimens are given in Table 4.

Figure 8 illustrates the variation of the CaO/SiO_2 ratio in function of the depth of the altered cement paste. The sound zone is characterized by a relatively stable ratio below 2.5 and corresponds to the CaO/SiO_2 ratio of C–S–H hydrates increased by the calcium from portlandite $Ca(OH)_2$ and hydrated sulfoaluminates corresponding to the AFm and AFt phases. In zone 3, where Portlandite is completely dissolved (Fig. 9), the CaO/SiO_2 ratio specifically associated to C–S–H hydrates, AFm and AFt phases, gradually decreases and reaches a minimum value of almost 1 at the interface between zones 2 and 3. This result highlights the decalcification of C–S–H hydrates in this zone. In zone 2, the CaO/SiO_2 ratio drops rapidly to 0.2. The most altered zones, 1 and 1bis, have a ratio below 0.1, suggesting a substantial depletion of calcium in these zones.

3.5 X-ray diffraction analyses

In addition to the EDX, a mineralogical analysis of powdered samples of the degraded cement pastes was carried out. The apparatus used was a Bruker AXS D8 ADVANCE diffractometer, fitted with a copper anticathode and a nickel filter, voltage 40 kV, current 40 mA. X-ray diffraction analyses were performed, using one degraded cement paste specimen, on three samples for each zone. Except a slight difference in the height of the peaks, the experimental results were the same for the three samples. Figure 9 shows X-ray diagrams of the sound zone and the various altered zones associated to one sample for each zone.

In the sound zone we can observe the presence of various peaks corresponding to the hydrates portlandite and ettringite. The gypsum peak also appears quite clearly, as does a halo centered on the reticular distance 3.04 Å ($2\theta_{Cu} = 29.36°$) due to C–S–H hydrates. The anhydrous C_2S and C_3S are absent, suggesting almost complete hydration of the cement paste.

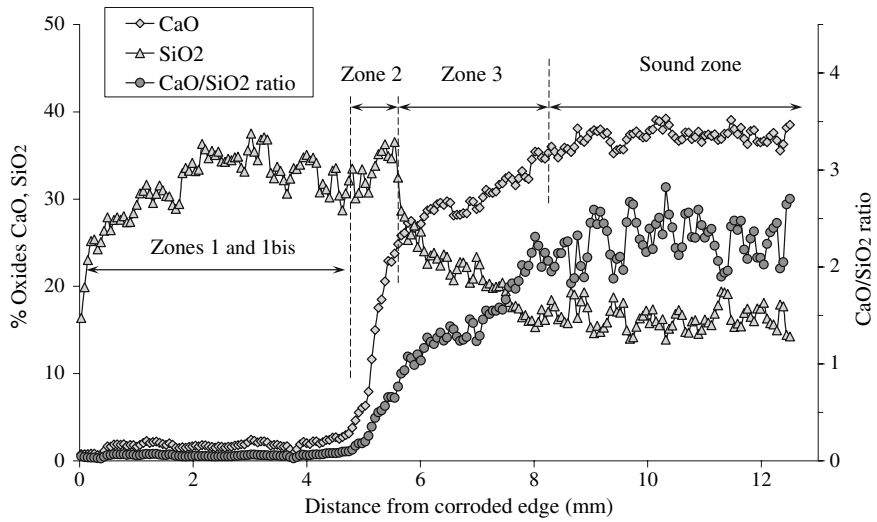

Fig. 8 CaO/SiO_2 ratio in function of degraded thickness

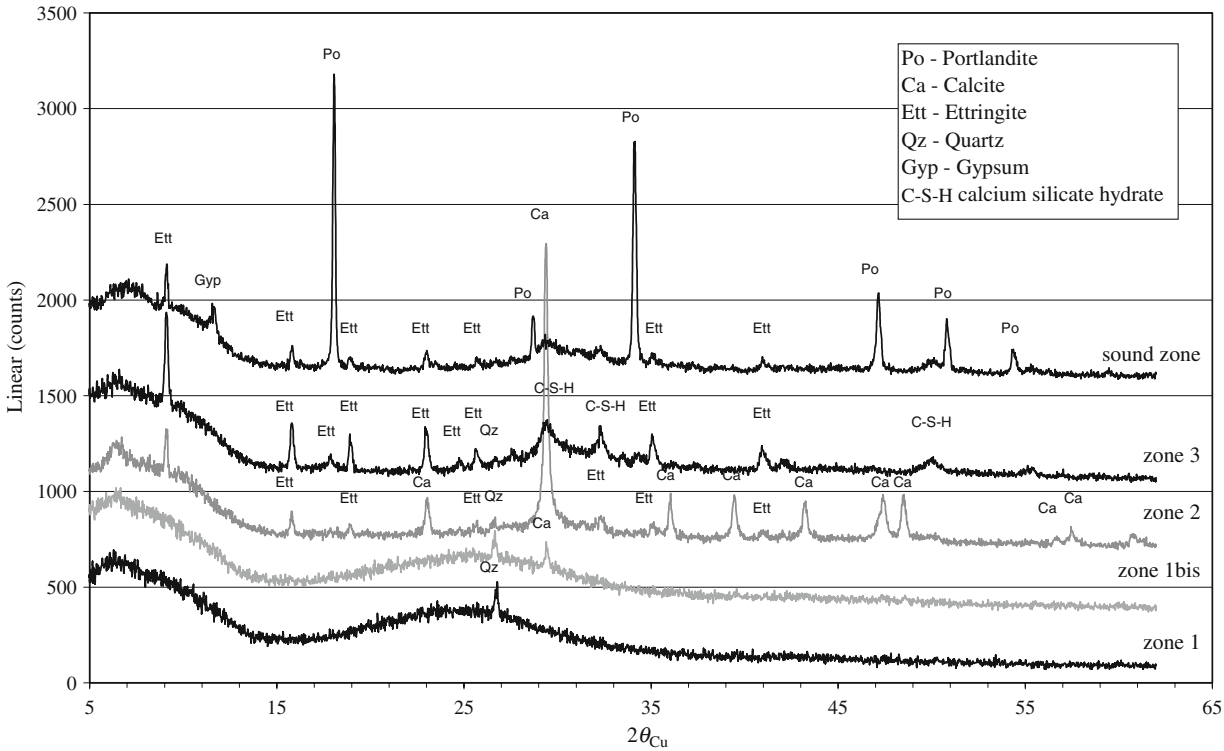

Fig. 9 X-ray analysis of degrading cement paste that was subjected to fungal biolixiviation for almost 15 months

In zone 3 the portlandite is completely dissolved, and the gypsum peak disappears, the ettringite peaks are accentuated (suggesting inward diffusion of sulfate) and the C–S–H hydrates appear more clearly at reticular distances 3.04 Å, 2.79 Å and 1.82 Å ($2\theta_{Cu} = 29.36°$, 32.04° and 50.08°, respectively). In zone 2, the ettringite peaks are attenuated by comparison with zone 3. Additional peaks at reticular distances 3.85 Å, 3.04 Å, 2.49 Å, 2.28 Å, 2.09 Å, 1.91 Å and 1.87 Å ($2\theta_{Cu} = 23.08°$, 29.40°, 35.99°, 39.43°, 43.18°, 47.51° and 48.52°, respectively) suggest considerable precipitation of calcite in this zone. In zone 1bis the only peak that subsists is that of calcite at reticular distance 3.04 Å ($2\theta_{Cu} = 29.40°$), although

much attenuated in height, and a quartz peak (reticular distance = 26.75 Å, $2\theta_{Cu} = 26.75°$) becomes more apparent compared to zone 2. One can also observe the presence of a halo whose center is slightly shifted with respect to the quartz, indicating an almost amorphous structure. Zone 1 is very similar to zone 1bis apart from the absence of calcite. The quartz peak remains but is slightly accentuated.

The results of the X-ray diffraction analysis show that the portlandite is completely dissolved in the degraded zones and that zones 1 and 1bis are the most severely affected by the biodeterioration. It also shows the occurrence of sulfate attack in zone 3, as indicated by the increased ettringite peaks. This attack may explain the fissures observed in this zone (Fig. 5), particularly at the interface with zone 2. It should also be noted that the X-ray diffraction analysis did not indicate the neoformation of calcium oxalates or other calcium complexes in zone 1 (Fig. 1), apparently because these crystals were not sufficiently abundant or are in an amorphous form.

3.6 Rochart indentation tests

3.6.1 Principle and experimental procedure

The mechanical properties of the altered cement matrices were investigated using a specific indentation test called the Rochart test, which characterizes the behavior of rocks and other materials, in particular the elastic Young's modulus in an interval ranging between 15 and 45 GPa. The Young's modulus $E_{rochart}$ obtained is between 0.5 and 1.5E, where E is the exact Young's modulus determined by a compression test on a specimen with embedded strain gages [22].

The Rochart test works according to the following principle: an indenter, normal to the sample surface, with high rigidity and known geometry is driven into the sample by applying an increasing load up to a preset value. The load is then gradually decreased after a partial relaxation phase. The load applied to the cement paste and the depth penetration displacement are continuously recorded throughout this process. The curve representing the load versus depth penetration obtained (Fig. 10) is then used to determine the Young's modulus through analysis of the unloading slope.

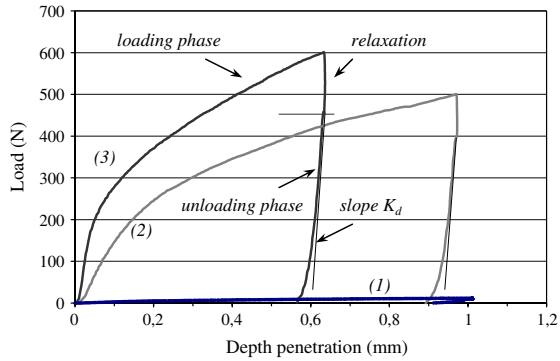

Fig. 10 Example of a load–depth penetration displacement curve for a degraded cement paste subjected to fungal biolixiviation for almost 15 months, (1) zone 1, (2) zone 3 and (3) sound zone

The indentation test was calibrated using an aluminum standard with known Young's modulus $E_{alu} = 71{,}400$ MPa and Poisson coefficient $v = 0.34$. The internal rigidity K_i of the indenter used (circular section with a diameter $\varnothing_{ind} = 1$ mm) was then calculated using formulae (4), where the rigidity K_d represents the unloading slope of the load–displacement curve (Fig. 10), v Poisson's ratio, D the diameter of the indenter and E the Young's modulus. The internal rigidity of the indenter (average calculated by means of five indentation tests) was $K_i = 65{,}637$ MPa. Rochart indentation tests were carried out on the altered and sound zones.

The compression test with embedded strain gages was performed on a cylindrical sound cement paste specimen (140 mm high and 70 mm in diameter). The compression test was performed according to the methodology developed by Torrenti et al. [23]. The Young's modulus E obtained was 15,600 MPa.

$$\frac{1}{K_d} = \frac{1 - v^2}{ED} + \frac{1}{K_i} \tag{4}$$

3.6.2 Results

Indentation tests (Fig. 5) were carried out on zone 1, zone 3, and the sound zone in order to provide a qualitative estimate of the mechanical deterioration occurring in the altered cement pastes. Zone 2 was not tested because of its narrowness. It should be noted that Rochart test results were associated to one of the two cement paste specimens extracted every almost 3 months (mean value of five tests for each

deteriorated zone analyzed). The Young's modulus E was calculated according to formula (4) for 14, 27, 39, 51 and 63 weeks of bioleaching and the results are given in Table 5. The measurements carried out on zone 1 should be treated with caution because the modulus determined is too low with respect to the confidence zone of the apparatus. The results given in Table 5 indicate a drastic drop in elasticity in zone 1, on average by 95.5% compared to the sound zone.

The Young's modulus calculated for each zone and time w ($w = 14$, 27, 39, 51, 63 weeks) can be associated with the average CaO/SiO_2 ratio calculated by EDX analysis for each zone (Fig. 7). The results are given in Fig. 11, which shows a linear correlation between Young's modulus E and the CaO/SiO_2 ratio. This is a very interesting finding because it can provide a rapid estimation of the Young's modulus in the degraded zone based on the profile of the CaO/SiO_2 ratio in function of thickness and the Young's modulus of the sound material.

4 Conclusions

Portland cement paste is particularly severely attacked by biolixiviation. The biolixiviation phenomenon appears to be due to attack by organic acids. The principal organic acids involved are oxalic, butyric, acetic and lactic acid. These acids react with hydrates, notably portlandite and C–S–H, leading to the lixiviation of calcium by complexation. The concentrations of the organic acids secreted by the microorganisms in the lixiviating solution are very high, comparable to the concentrations of organic acids encountered in aggressive agricultural environments (silage, slurry, etc.). These environments cause the deterioration of concrete storage containers, which explains the considerable thickness of the degraded zone of the Portland cement pastes, about 8 or 9 mm after 15 months.

Table 5 Young's modulus E (MPa) in function of time and zone i

Time (weeks)	14	27	39	51	63	Mean value
Zone 1	600	700	700	700	600	660
Zone 3	13,600	13,500	13,000	12,900	12,500	13,100
Sound zone	16,000	16,000	16,000	15,500	15,800	15,860

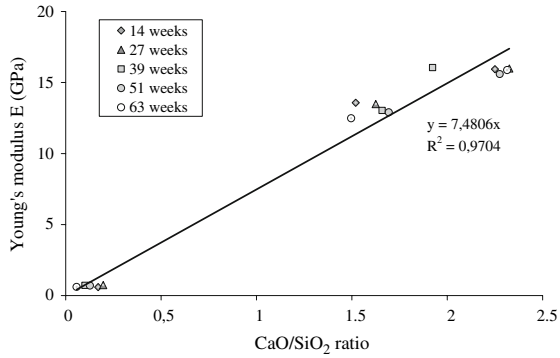

Fig. 11 Young's modulus in function of the CaO/SiO_2 ratio

Textural examination of the cement pastes after 15 months of biolixiviation showed an essentially chemical degradation with several altered zones and a sound zone. The outermost zones, 1 and 1bis, resembled a silica and alumina gel. These zones were decalcified, with a very porous structure. Zone 2 was very dense, with considerable precipitation of calcite. Zone 3 was characterized by complete dissolution of portlandite. The degradation profile also showed fissures indicating sulfate attack. This attack was also shown by X-ray diffraction analysis and can be explained by the diffusion of sulfate ions toward the sound core of the sample in the opposite direction to the lixiviation of calcium in order to balance the electrochemical equilibrium.

The deterioration of the matrix during the biolixiviation test was progressive, culminating in complete elimination of portlandite (sound core), as indicated by the propagation of the biolixiviation interface (between the altered zone and the sound core) toward the interior. Nevertheless, the kinetics of this propagation are slowed down by the gradual thickening of the silica and alumina layer (outermost degraded zone), which in a certain sense acts as a diffusion barrier. There is obvious mechanical degradation of the cement pastes due to biolixiviation, as indicated by the results of the mechanical analyses and also by the highly porous texture and friable appearance of zones 1 and 1bis.

In the light of these results, it therefore appears essential that biological attack should be taken into serious consideration when evaluating the stability of industrial and radioactive waste.

A certain number of points still need to be clarified, in particular the effect of regular feeding of the fungi with glucose, which tends to dissolve the portlandite and other calcium phases together with the organic acids, the role of the biofilm in the degradation, the identification and quantification of the contaminant microorganisms present in the lixiviating solution, and finally the influence of the type of cement. Once the various mechanisms have been identified it will be possible to begin modeling the process.

In this study we investigated biolixiviation on ordinary Portland cement paste, since it is relatively easy to understand its structure, in order to clarify the deleterious actions of heterotrophic microorganisms on the durability of the hydrates such as $Ca(OH)_2$, C–S–H, AFm and AFt phases. Once the mechanisms of biodeterioration have been elucidated, the biolixiviation test will be applied to much more complex construction materials such as mortar and concrete.

References

1. Ribas Silva M, Deloye FX (1991) Dégradation biologique des bétons. Bulletin de Liaison des Laboratoires des Ponts et Chaussées 3629(176):87–91
2. Perfettini JV, Revertegat E, Langomazino N (1991) Evaluation of cement degradation induced by the metabolic products of two fungal strains. Experientia 47(6):527–533
3. Gu JD, Ford TE, Berke NS, Mitchell R (1998) Biodeterioration of concrete by the fungus *Fusarium*. Int Biodeterior Biodegradation 41(2):101–109
4. Diercks M, Sand W, Bock E (1991) Microbial corrosion of concrete. Experientia 47(6):514–516
5. Tazawa EI, Morinaga T, Kawai K (1994) Deterioration of concrete derived from metabolites of microorganisms. In: Malhotra VM (ed) Durability of concrete. Proceedings of the 3rd CANMET-ACI international conference, Nice, France, 22–28 May, American Concrete Institute, pp 1087–1097
6. Kawai K, Morinaga T, Tazawa EI (2000) The mechanism of concrete deterioration caused by aerobic microorganisms. In: Ribas Silva (ed) Microbial impacts on building materials. Proceedings of 1st international RILEM workshop, São Paulo, Brazil, 6–7 July, RILEM Publications, pp 58–63
7. Scrivener K (2003) Effect of cement type on bacteria induced acid attack in sewage networks. In: Ribas Silva (ed) Microbial impacts on building materials. Proceedings of the international RILEM conference, Lisbon, Portugal, 8–9 September, RILEM Publications, pp 58–64
8. Gaylarde CC, Glyn Morton LH (1999) Deteriogenic biofilm on buildings and their control: a review. Biofouling 14(1):59–74
9. Moraes Pinheiro SM, Ribas Silva M (2004) Microorganisms and aesthetic biodeterioration of concrete and mortar. In: Ribas Silva (ed) Microbial impacts on building materials. Proceedings of 2nd international RILEM workshop, Vitória, Brazil, 7–9 July, RILEM Publications, pp 47–54
10. Jestin A, Libert M, Thouvenot P, Sellier R, Bournazel JP (2004) Biodégradation de matrices cimentaires. Revue Européenne de Génie Civil 8(9–10):1087–1104
11. Gadd GM (2004) Mycotransformation of organic and inorganic substrates. Mycologist 18:60–70
12. Gaylarde C, Ribas Silva M, Warscheid Th (2003) Microbial impact on building materials: an overview. Mater Struct 36(5):342–352
13. Braam RC, Kramer WA, Frénay JW (1998) Concrete with rapid-hardening blastfurnace slag cement: the rate of deterioration by lactic and acetic acid. In: Stoelhorst von D, Den Boer GPL (eds) Challenges for concrete in the next millennium. Proceedings of the 13th FIP Congress, Amsterdam, Netherlands, 23–29 May, A A Balkema, Rotterdam, Netherlands, pp 77–81
14. De Belie N, Verselder HJ, de Blaere B, Nieuwenburg DV, Verschoore R (1996) Influence of the cement type on the resistance of concrete to feed acids. Cement Concrete Res 26(11):1717–1725
15. De Belie N, Debruyckere M, Van Nieuwenburgs D, de Blaere B (1997) Concrete attack by feed acids: accelerated tests to compare different concrete compositions and technologies. ACI Mater J 94(6):546–554
16. Bertron A, Escadeillas G, Duchesne J (2004) Cement pastes alteration by liquid manure organic acids: chemical and mineralogical characterization. Cement Concrete Res 34:1823–1835
17. Ohshima A, Matsui I, Yuasa N, Henmi Y (1999) A study of growth of fungus and algae on mortar. Trans Japan Concrete Inst 21:173–178
18. AFPC-AFREM (1997) Durabilité des bétons, Méthodes recommandées pour la mesure des grandeurs associées à la durabilité. Compte-Rendue des Journées Techniques, Institut National des Sciences Appliquées, Université Paul Sabatier, Toulouse, France
19. Lajili H, Degorce-Dumas J -R, Roussy J, Grambin-Lapeyre C, Devillers P, Bournazel J -P (2006) Methodology of study of the bioleaching of a cement matrix. In: Fort R et al (eds) Heritage, weathering and conservation. Proceedings of the international conference, Madrid, Spain, 21–24 June, Taylor & Francis, vol 1, pp 373–379
20. Bayoux JP, Letourneux JP, Marcdargent S (1990) Acidic corrosion of high alumina cement. In: Mangabhai RJ (ed) Calcium aluminate cements. Proceedings of the international symposium held at Queen Mary and Westfield College, University of London, United Kingdom, 9–11 July 1990, E. & F.N. Spon, New York, pp 30–240
21. Knight J, Cheeseman C, Rogers R (2002) Microbial influenced degradation of solidified waste binder. Waste Manage 22(2):187–193
22. Wang W (2005) Identification du comportement mécanique de géomatériaux par micropénétration. Thèse de doctorat, Université de Montpellier II
23. Torrenti JM, Dantec P, Boulay C, Semblat JF (1999) Projet de processus d'essai pour la determination du module de deformation longitudinale du béton. Bulletin de Liaison des Laboratoires des Ponts et Chaussées 4263(220):79–81