Cement and Concrete Research 41 (2011) 429–438

Contents lists available at ScienceDirect

Cement and Concrete Research

journal homepage: http://ees.elsevier.com/CEMCON/default.asp

A new test method to assess the bacterial deterioration of cementitious materials

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ARTICLE INFO

Article history:
Received 3 August 2010
Accepted 19 January 2011

Keywords:
Cement paste D
Durability C
Bacteria
Degradation C

ABSTRACT

The biodegradation of cement-based matrices by agro-industrial effluents is a very complex phenomenon. In this work, a test was developed – the *Build-Mat Bio-test* (*BMB test*) – to examine the degradation mechanisms caused by microbial activity on any type of building material. The main objective of this device was to analyze and distinguish between the effects caused by the bacteria and those caused by their metabolites in the deterioration. In this study, the BMB test was used to evaluate the impact on cementitious matrices of the bacterium *Escherichia coli*, found in liquid manure. The mechanisms and kinetics of deterioration resulting from exposure to the bacterial culture or to the metabolites were compared with those obtained with synthetic organic acids alone. It was notably observed that the bacterial suspension caused more intense deterioration and higher alteration kinetics as compared to the medium without microorganisms and to the synthetic acid solution.

1. Introduction

Agro-industrial and agricultural structures made of concrete (animal houses, treatment plants, dairy plants, storage silos, etc.) are severely damaged by agrofood and breeding waste waters, such as whey, silage effluents and liquid manure [1]. These effluents, the compositions of which are complex and very variable, contain compounds in solution or in suspension: organic substances, mineral compounds, and, in most cases, microorganisms. Organic matter is degraded by these microorganisms (Aerobacters sp., faecal *Streptococci, Lactobacillus*, etc.) and may then be transformed into biomass (microbial proliferation) and into various compounds, notably organic acids (volatile fatty acids, lactic, citric acids, etc.) [2–4]. Acidic and biological components in the effluents are aggressive agents for concrete since it is a strongly basic, mineral material.

The chemical degradation caused in cementitious matrices by organic acids found in effluents has been investigated extensively by considering organic acids alone [5–13]. However, the influence of microbial activity in the effluents on the degradation of concrete has not been well identified. The most detailed studies addressing the mechanisms of interaction between microorganisms and cementitious matrices concern sewage systems [14–18]. In this case, the organisms responsible for the deterioration belong to the sulphate reducing and sulphur oxidizing bacteria [17–19]. These bacteria interact with concrete through the H_2S cycle, which differs considerably from the situation occurring in agro-industrial environments (possible carbonation, consumption of some elements of the cement paste) [20–22], except, perhaps, for those occurring under certain conditions in manure (collecting systems, for example).

Moreover, progress in the understanding of the interactions between materials and media bearing microorganisms is mainly hindered by the difficulty of designing and implementing a test method allowing examination of each component of the system individually. Up to now, most experimental studies performed to analyze the interactions between microorganisms and concrete have used laboratory test benches based on quite a simple design: the immersion of specimens in a microbial culture medium [23–28] or sometimes a more elaborate one [29]. These studies have enabled interesting preliminary results to be obtained on the bio-receptivity of mortars and cement pastes [25,26] and some interesting mechanisms for degradation of the cementitious matrix to be highlighted [27]. However, in all cases, the specific effect of the action of the microorganisms could not be distinguished from the degradation induced by the metabolites produced by the microorganisms (dissolved CO_2, H_2S, organic acids, nitric acid, etc.) in the growth medium. Moreover, it is impossible to directly isolate the growing bacterial cells in order to analyze their specific effect on the matrix. During growth, bacteria cannot be separated from their metabolites since they are continuously produced by the microorganisms. A suitable approach for identifying the specific alterations of matrix caused by the microbial cells would be to subtract the effects caused by the metabolites alone from those caused by the growing bacterial cells plus their metabolites.

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doi:10.1016/j.cemconres.2011.01.014

The aim of this work was to design a new test method, the *Build-Mat Bio-test* or *BMB test*, for examining the physicochemical and biological phenomena occurring at the interface between building materials and bacteria-bearing liquid media, here agro-wastes. In this test, building material specimens are exposed to model environments (selected microorganisms) in controlled biological conditions. The device was designed to identify the effect of bacterial cells in the deterioration of the material and to distinguish this effect from that of their metabolites alone (such as organic acid and CO_2). This paper provides details about the architecture of the *BMB test* and reports the results of an exploratory study using a cementitious matrix. In this first series of applications, cement paste specimens were exposed to a growing culture of *Escherichia coli*, which is a bacterium found in liquid manure. The mechanisms of deterioration were examined by X-ray diffraction (XRD), electron microprobe (EPMA) and scanning electron microscope (SEM). The results were compared to those obtained with the synthetic organic acids alone in the same exposure conditions.

2. Materials and methods

2.1. Materials

The study was conducted on hardened cement pastes made with ordinary Portland cement CEM I 52.5 R [30] using a water/cement mass ratio of 0.27. The specimens were removed from their moulds (25-mm diameter, 75-mm-high cylinders) 24 h after pouring and stored in water at 20 °C for 27 days before any treatment.

2.2. Bacteria and cultivation medium

The model bacterium used in this study was an ampicillin-resistant strain of *E. coli*, which was cultivated in a Luria Bertani (LB) medium composed of tryptone (10 $g.l^{-1}$), sodium chloride (10 $g.l^{-1}$), glucose (10 $g.l^{-1}$), yeast autolytic extract (5 $g.l^{-1}$) and glycol polypropylene 2000 (antifoam, 0.5 ml l^{-1}).

The culture medium was heat sterilized and an antibiotic, ampicillin (100 $mg.l^{-1}$), was added in order to prevent contamination and to enable the inoculated *Escherichia coli* bacteria (ampicillin-resistant strain) to be correctly targeted in this first exploratory work.

2.3. Sterilization procedure for cementitious specimens

Cement paste cylindrical samples (diam = 25 mm, h = 10 mm, slices sawn from the initial cylinders) were sterilized before being used. Several methods were evaluated to sterilize the cement samples: (i) tyndallization, (ii) formalin bath, (iii) exposure to formalin aerosols and (iv) ethanol bath. The aim was to identify a method combining efficiency in terms of prevention of bacterial proliferation, rapidity and no or low chemical or mineralogical impact on the cementitious matrix. Tyndallization is a low-temperature sterilization method which consists of heating cycles at 70 °C separated by 24 h. Three to five cycles were tested on the samples with storage temperature of 20 °C between heating cycles. Immersions of the samples in a formalin solution for 2 h or in ethanol for 1 to 3 days were also tested. Some samples were exposed to a formalin aerosol (NP 30 TER One Shot®).

In order to evaluate the efficiency of the various processes, cement paste specimens, previously subjected to the sterilization methods, were immersed in a sterilized, fresh culture medium and the bacterial growth was monitored for 32 h by optical density (wavelength: 620 nm). In case of bacterial proliferation, the optical density of the solution increased whereas it remained constant when the sterilization of the paste was efficient. Afterwards, the samples were analyzed by electron probe microanalysis (EPMA) and X-ray diffraction (XRD) to evaluate the chemical and mineralogical impact of the treatment on the matrix. The preparation of the specimens prior to these tests is detailed in Section 2.6.

The formalin aerosol process was rejected because it caused the formation of a 100-μm-thick degraded zone in the outer part of the specimen. This zone was decalcified and had low sulphate content. Immersion in ethanol for 1 and 2 days and immersion in formalin did not prevent bacterial proliferation. Tyndallization and the 3-day immersion in ethanol were the most efficient methods.

Therefore, in all further experiments, the cement paste specimens were sterilized by tyndallization at 70 °C (3 cycles of 1 h of heating at 70 °C separated by 24 h at 24 °C).

2.4. Experiment with BMB test

The components of the *Build-Mat Bio-test*, or *BMB test*, are presented in Fig. 1. Bacteria cells (*E. coli* in this experiment) were grown in continuous culture in the reactor described below. The overflow from the reactor was divided into two portions to provide continuously circulating feeds to two columns (diam = 3.5 cm, h = 17 cm, liquid total volume = 164 ml) containing the building material samples. One column was fed with the raw culture medium coming from the reactor and containing the growth medium, the bacterial cells and their metabolites. The other portion of the reactor overflow was filtrated through a Sartolon 0.2 μm polyamide membrane (Sartorius) to remove the bacterial cells before it was introduced into the second column. The second column was thus fed with residual substrates and bacterial metabolites produced in the bioreactor. The cementitious samples (5 cement paste slices per column) were fully immersed in the circulating fluid. The cement paste samples were separated by small PVC cylinders in the columns to favour the contact of the circulating fluid with all the faces of the samples. The solid/liquid volume ratio in the columns was approximately 0.15. The flow of circulating fluid in the tubes was about 40 ml h^{-1} and the residence time of the liquid in each tube was about 4.1 h. Fig. 1 shows the differences in turbidity of the circulating fluid between the two columns, reflecting the presence or absence of bacterial cells. Downstream of each column, the liquid phase was collected into a bin to avoid any contamination.

The reactor (Inceltech®, max. vol.: 2 l) was continuously fed with fresh sterilized medium at a mean dilution rate of 20 ml h^{-1}. The pH in the reactor was maintained at 7 by adding NH_4^+ and the temperature was maintained at 37 °C. It should be noted that the NH_4^+ base was combined with OH^- and thus was not expected to be detrimental to the cementitious matrix as the exchange reaction between Ca^{2+} from the cement paste (in $Ca(OH)_2$ for example) and NH_4^+ would lead to the formation of $Ca(OH)_2$. The bacterial growth in the reactor was monitored by determination of the biomass by measuring the turbidity of the medium at 600 nm (Hitachi U-1100 spectrophotometer) and the dry mass of cells collected by filtration (0.2 μm pore size filter) and dried (24 h, 200 mm Hg, 60 °C). Temperature and pH were checked regularly downstream of the columns.

Oxygen was supplied in the reactor from ambient air through an air pump and a drilled cannula. The gas flow in reactor was controlled by an air flow rate regulator. Small holes in the cannula allowed small air bubbles to form, which increased the air–liquid exchange area and thus the oxygen transfer. The oxygen content, fixed at 20% of the saturation content, in the reactor was controlled by a pO_2 probe (Bronkhorst High-Tech). Gas from the reactor was evacuated through a filter (0.2 μm Sartorius filter) located in the upper part of the reactor and ensuring the sterility of the device.

Concentrations of the main organic acids produced by *E. coli* (acetic and lactic acids) during growth were measured in the reactor and at the outflow of the columns by HPLC (Aminex HPX-87 H column, PDA 994 Waters, 210 nm UV detector, RI 410, Waters refractometer).

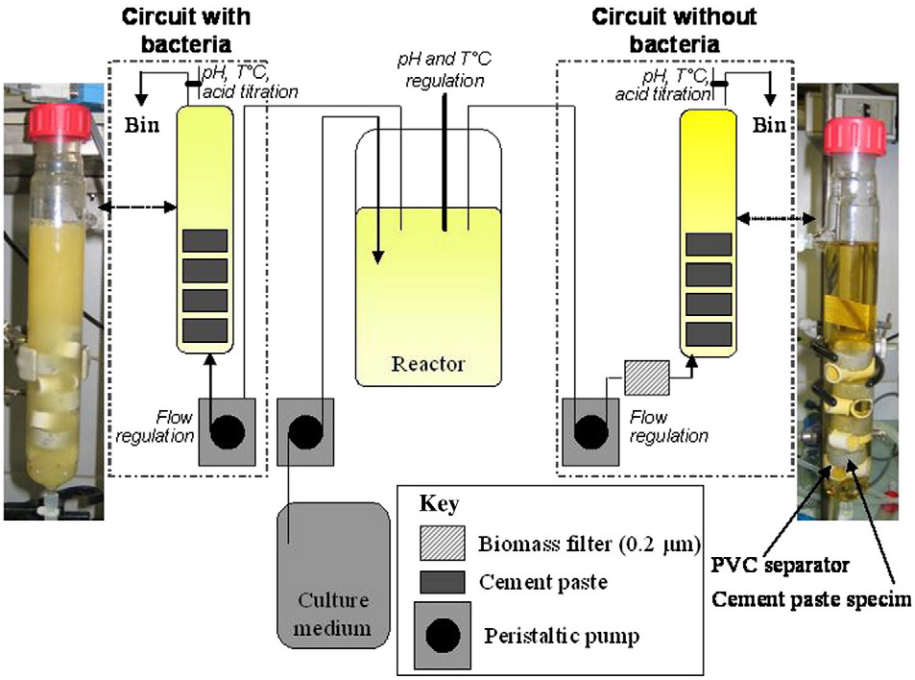

Fig. 1. Schematic diagram of the Build-Mat-Bio-test (BMB test).

After 4 weeks of exposure, the cementitious matrix samples were withdrawn from the device and investigated mineralogically and chemically.

2.5. Experiment with synthetic organic acid alone

In order to assess the mechanisms and kinetics of deterioration induced by the acidic part of the culture media, cement pastes were exposed to organic acid solutions in the same hydrodynamic conditions as in the columns of the *BMB test*. The aggressive solution was made with acetic acid alone since (i) acetic acid was the major acid titrated in the columns, and (ii) deterioration mechanisms of the cement matrix by acetic acid were shown to be equivalent to those of the various acids produced by *E. coli*, i.e., acetic, succinic, formic, lactic acids [6,9,31,32].

The molar concentration of acetic acid was taken to be equal to the total molar concentration of acids of the raw and filtered cultures of the BMB test (0.120 M). This solution was added with NaOH (0.113 M) in order to bring the initial pH up to 5.8. This pH is lower than that of *BMB test* media (pH 7, buffered with NH_4^+) but pH 7 is an unstable pH zone for the acid solution (equivalence) which should be avoided to ensure low amplitude of pH variations inside the columns and thus keep steady aggressive conditions for the cementitious matrices.

In this experiment, the column – containing 5 cement paste slices – was fed with the acetic acid solution, itself contained in a tank, through a peristaltic pump (Masterflex) with a flow rate of 40 $mL.h^{-1}$ (same flow rate as that of *BMB test* experiments). Downstream of the column, the solution was collected in a bin and its pH was measured daily (average pH = 5.94, standard deviation = 0.07).

After 4 weeks of exposure, the cementitious matrix samples were withdrawn from the column and investigated mineralogically and chemically according to the methods detailed in Section 2.6.

2.6. Chemical and mineralogical analysis of cementitious specimens

Chemical modifications were analyzed using an electron microprobe (Cameca SX 50; accelerating voltage: 15 kV; current: 10 nA; beam scanning area: $2 \times 2\ \mu m^2$).

After their exposure to the aggressive media, 2 slices per column were sawn according to a diametric plane to obtain two half-discs. One half-disc per slice was then embedded in epoxy resin (Mecaprex MA2 by Presi) in small moulds for ease of positioning and polishing. The flat sawn sides of the half discs were then polished using a series of three silicon carbide polishing disks (Presi). Finally, EMPA analyses were performed on the polished sections of the half-discs, perpendicularly to the surface in contact with the aggressive media, at different depths from the surface to the center of the specimens (about 12.5 mm depth). Chemical composition profiles of the cementitious specimens were thus obtained. More details about the preparation of the specimens and about the EPMA operating conditions are given in Ref. [33]. A control specimen was also analyzed 4 weeks after pouring.

Mineralogical analyses using X-ray diffraction were performed on the specimens (Siemens D5000; copper cathode; anode voltage: 40 kV; current strength: 30 mA). The measurements were performed on two specimens per column according to the distance to the surface in contact with the aggressive media. The first analysis was carried out on the plane external face of the slice, which was then abraded and submitted to the next analysis. The last analysis, which was on the core of the specimen, was carried out at a depth of 5 mm. The upper and lower specimens in each column were chosen for these analyses so that the whole plane surface was in contact with the aggressive media (any masking effect or other disturbance linked with the presence of the PVC separators was then avoided). A control specimen was also analyzed 5 weeks after pouring.

Microstructural observations and chemical analyses were performed using a scanning electron microscope (Jeol JSM-6380LV) fitted with an EDS detector (Rontec XFlash® 3001).

3. Results

3.1. Analyses of the liquid phases of the aggressive media

Fig. 2 gives the dry mass and turbidity in the reactor according to time of *E. coli* culture at the beginning of the experiment. First, batch culture was carried out in the reactor and biomass increased fast. After 28 h of growth, when the biomass reached a stable value, the

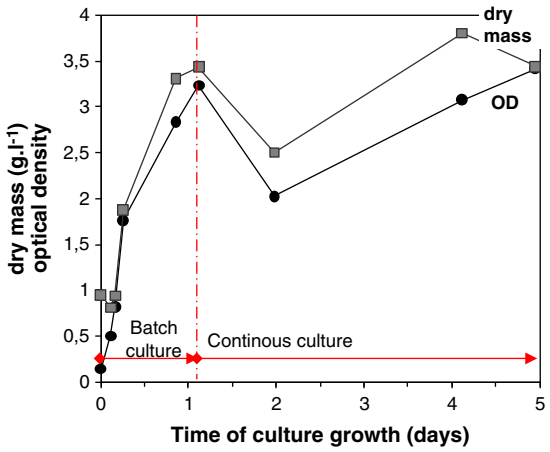

Fig. 2. Bacterial growth in reactor according to time at the beginning of the experiment.

continuous culture was commenced by connecting the reactor to the columns.

Fig. 3 shows the variations of pH and concentrations of acids in the raw and filtered culture media downstream of the columns containing the cementitious specimens. The pH of the solutions, initially 7, increased in contact with the cement paste specimens in the columns (residence time in the columns: 4.1 h).

In accordance with the metabolism of *E. coli* under the selected operating conditions, acetic acid was the major metabolite and it was detected in the two columns whereas the lactic acid concentration remained low in both columns.

Some slight differences in the concentrations of these acids were still observed between the two columns and the pH was slightly lower in the column fed with the raw culture (containing the bacteria cells). It is likely that these differences were due the microbial activity in the column containing bacterial cells since the bacteria could have continued to produce organic acids and/or could have consumed some of the previously produced compounds under substrate limitation or under anaerobic conditions as some bacteria can do [34].

It should be noted that the bacteria may also have produced other types of acids, e.g., formic or succinic acids (that were not titrated during the experiment) and that the sum of acetic and lactic acid concentrations did not necessarily represent the total concentration of acids in the solutions.

Despite this difference, the total concentrations of titrated acids were very similar between the two columns (0.12 M) providing the necessary basis for comparison between the two media. This concentration of acids was used to make the synthetic organic acid solution.

3.2. Observations of the cementitious specimens with video microscope

Fig. 4a, b and c shows cross-sections of cement paste specimens after 4 weeks of exposure to the *Build-Mat Bio-test* media and to the synthetic organic acid solution, respectively.

3.2.1. Specimens exposed to the BMB test media (raw and filtered cultures)

From the inside of the specimens to the surface, the following zones can be observed (Fig. 4a and b):

- The deeper parts of both samples, zone 1 (core) with a color identical to that of the control sample and zone 2, a light-colored paste layer of similar thickness (around 0.65 mm) for both samples. It should be noted that zones 1 and 2 caused phenolphthalein to change color (from transparent to violet), which means that the pH of these zones was higher than about 9.5.
- Three distinct zones visible on the samples in contact with the raw culture media (containing *E. coli* cells, Fig. 4a): from brownish nearest to the center to whitish layers towards the outside (zones 3 to 5). The total thickness of zones 3 to 5 was about 1.30 mm.
- In contrast, for the samples in contact with the culture filtrate (without *E. coli* cells, Fig. 4b), the outer part of the specimen showed a single brownish colored zone (zone 3). Its thickness was about 0.65 mm.

The thickness of these outer layers was twice as great in presence of bacteria as in their absence.

A deposit was observed on the surface of the specimens exposed to the solution containing *E. coli* cells. Observations with SEM made since then on the occasion of another experiment, performed with similar experimental conditions, showed that this was a deposit of bacteria. In a further study, the viability of the cells deposited on the cementitious specimens will be assessed using confocal microscopy associating markers of viability.

3.2.2. Specimen exposed to the synthetic organic acid solution

From the inside of the specimens to the surface, 3 zones were observed (Fig. 4c):

- The color of zone 1 (core) was identical to that of the control sample.

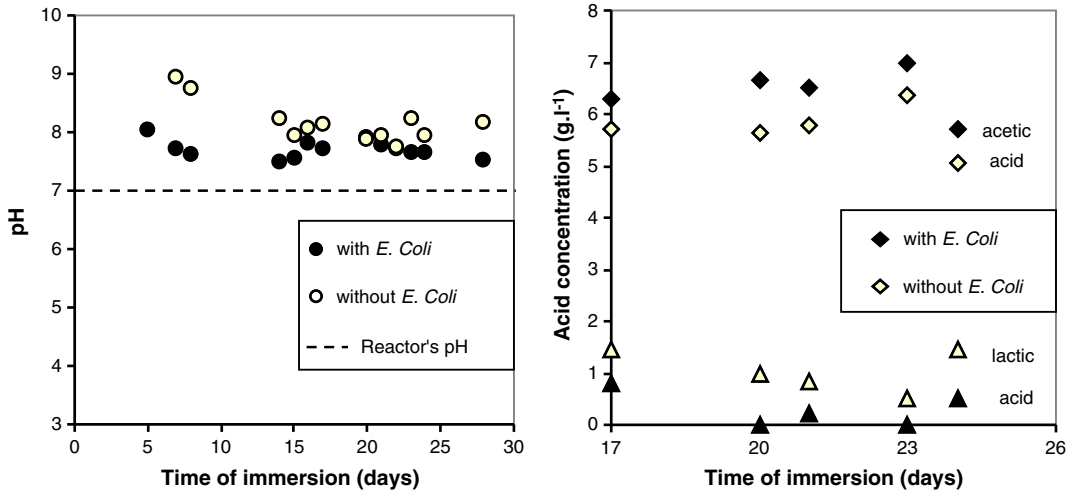

Fig. 3. pH and concentrations of acetic and lactic acids for raw solution (with *E. coli* cells) and filtered solution (without *E. coli* cells) according to the time of immersion.

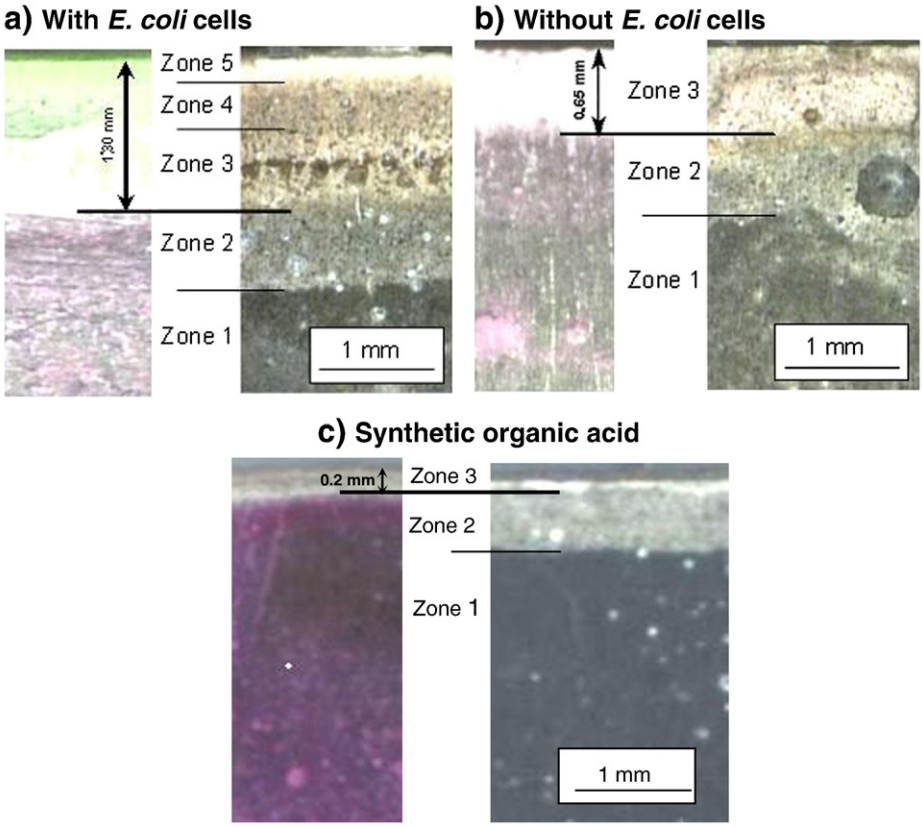

Fig. 4. Cross-sections of altered hardened cement paste after 4 weeks of exposure in the columns: (a) without *E. coli* cells; (b) with *E. coli* cells; (c) of synthetic organic acid.

— Zone 2 was a lighter grey transition zone (around 0.50 mm thick). As for the specimens exposed to BMB test media, zones 1 and 2 caused phenolphthalein to change color (pH>9.5).
— The outer part of the specimen showed a single yellowish colored zone (zone 3), 0.20 mm thick. The thickness of this zone was smaller than the equivalent zone of specimens exposed to the *BMB test*.

3.3. Chemical analysis with electron microprobe (EPMA)

The results of EPMA analysis of cement pastes exposed to the *BMB test* media and to synthetic organic acid, according to the distance to the surface, and of the control sample are shown in Fig. 5a, b and c. On each graph, zones 1 to 3 or 1 to 5, as defined in Fig. 4, are reported. For all the specimens, zone 1 (or the core) had the same chemical composition as the control specimen.

3.3.1. Specimen exposed to the raw culture (with E. coli cells)

Zone 2 presented regular, progressive decalcification and was enriched in SO_3. At the limit between zones 3 and 2, the CaO content was around 30%. In zone 3, the CaO profile was disrupted and fluctuated between 20% and 38%. In zones 4 and 5, (i) the CaO content continued to decrease, reaching zero at the surface of the specimen, (ii) the SO_3 content was almost zero and (iii) SiO_2, Al_2O_3 and Fe_2O_3 oxides were well preserved. Given the CaO content in zone 5 (almost zero), calcium silicate hydrates or C-S-H, the main hydrated phase of cement paste, must have been dissolved (Fig. 5a).

3.3.2. Specimen exposed to the culture filtrate (without E. coli cells)

Zone 2, or the transition zone between the core and the zones with pH<9.5, was progressively decalcified and was enriched in SO_3. At the limit between zone 3 and zone 2 (change in color of phenolphthalein), the CaO content was around 30%. In zone 3, the CaO mass content continued to decrease towards the surface, where it was around 10%. Fe_2O_3, SiO_2 and Al_2O_3 seemed to be well preserved in zone 3 (higher contents of these oxides than in zone 1, probably linked with the decalcification of zone 3). SO_3 content in zone 3 tended to be zero (Fig. 5b).

3.3.3. Specimen exposed to the synthetic organic acid solution

The total amount of oxides, i.e., the compactness of the paste, greatly decreased between zones 2 and 3. The limit between zones 2 and 3 matched a significant variation of the calcium amount. Zone 2 was slightly (i) decalcified and (ii) enriched with sulphur, as compared to zone 1. Zone 3 lost much of its calcium. It was made up of silicon, aluminum, and iron. Sulphur was totally absent from the zone. Given the calcium content in zone 3, it may be assumed that calcium silicate hydrates were dissolved (Fig. 5c).

3.4. Mineralogical analysis by X-ray diffraction

Fig. 6a, b and c shows the mineralogical characterization by XRD of specimens exposed to the *BMB test* media and to synthetic organic acid for each zone described previously. For each type of degradation, zone 1 showed essentially the same X-ray pattern as the control specimen. In zone 2, the $Ca(OH)_2$ peaks had disappeared, but the anhydrous residual phases were still present. The intensity of ettringite peaks was greater than in zone 1.

3.4.1. Specimen exposed to the raw culture (with E. coli cells)

In zone 3, some calcite had formed. Peaks of ettringite and anhydrous residual grains were largely reduced. In zone 4, calcite and C_4AF (a residual anhydrous grain of the cement paste) peaks were still present. In zone 5, a large halo had formed, centered on the main

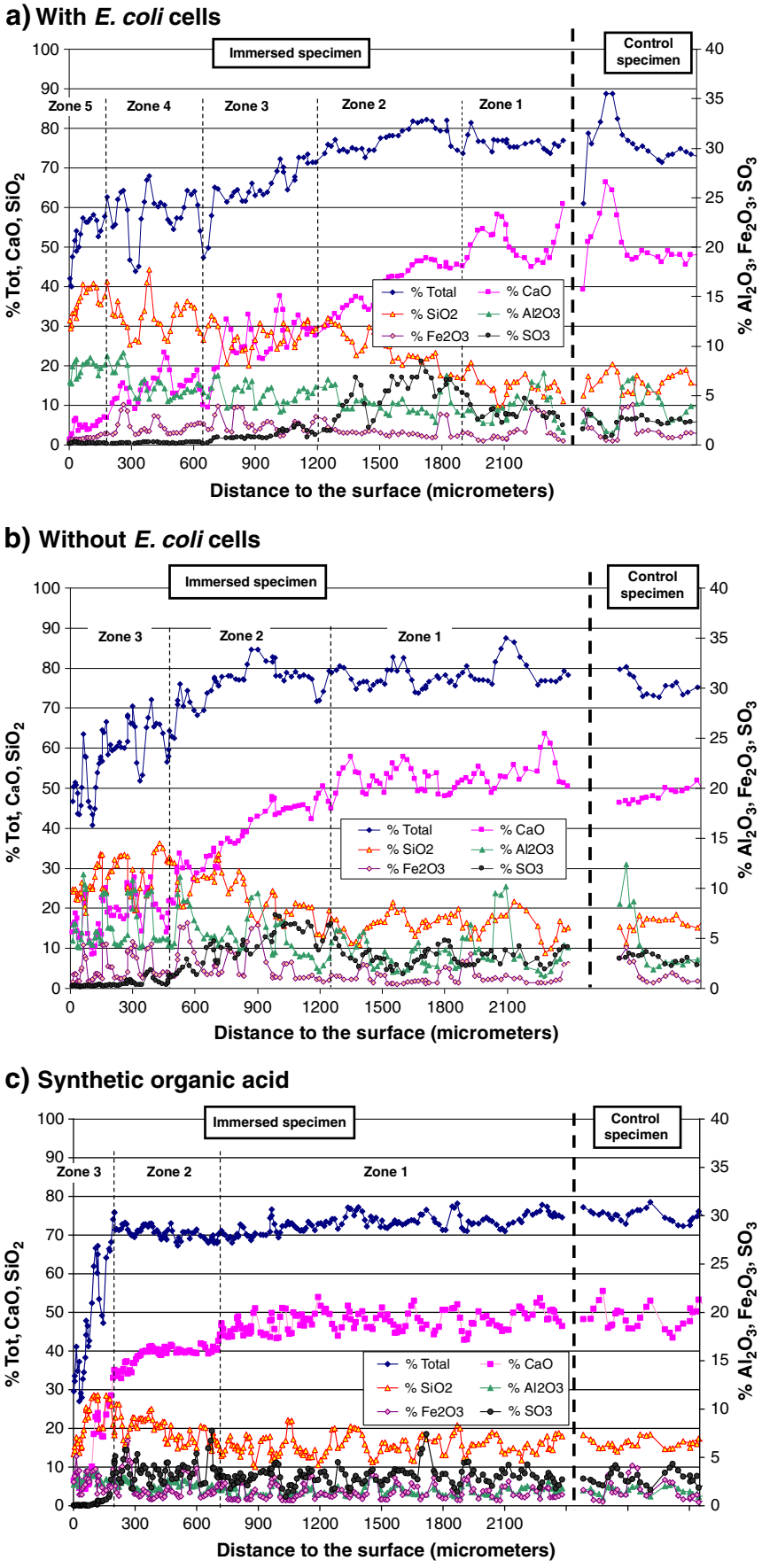

Fig. 5. Cement paste chemical analysis with EPMA according to the distance to the surface after 4 weeks of exposure in the columns: (a) without *E. coli* cells, (b) with *E. coli* cells, (c) of synthetic organic acid.

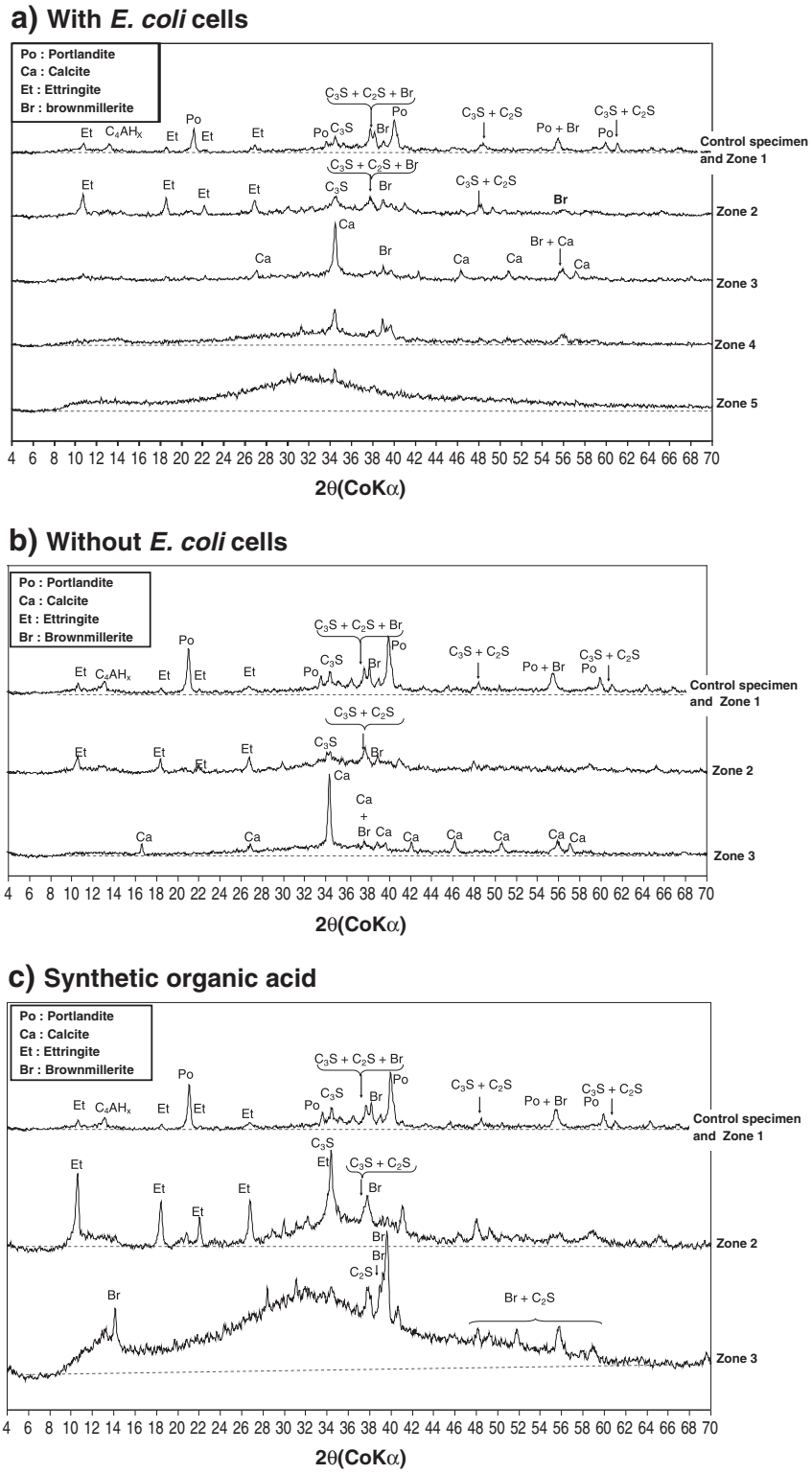

Fig. 6. Mineralogical analysis by XRD of the cement paste after 4 weeks of immersion in solution (a) with *E. coli* cells, (b) without *E. coli* cells, (c) of synthetic organic acid. X-ray patterns of the various zones of the immersed specimens and of the control specimen.

quartz peak: the structure was quasi-amorphous and may have been composed of silica gel containing aluminum and iron (see microprobe analysis) (Fig. 6a).

3.4.2. Specimen exposed to the culture filtrate (without E. coli cells)

In zone 3, the only crystallized phase observed was calcite (Fig. 6b).

3.4.3. Specimen exposed to the synthetic organic acid solution

In zone 3, the structure was mainly amorphous but some lines typical of C_4AF and C_2S anhydrous phases were present. Their intensity was higher than in the control specimen. This may have been linked with the rather amorphous nature of the structure, which tended to increase the intensity of the crystallized phase (Fig. 6c).

3.5. Microstructural observations with SEM

Fig. 7a, b and c shows the polished sections of specimens exposed to the *BMB test* media and to the synthetic organic acid solution, observed with a scanning electron microscope in backscattered electron mode.

Zones 3 to 5 for the specimen exposed to the raw culture (Fig. 7a) and zone 3 for the specimen exposed to the culture filtrate and to the organic acid solution (Fig. 7b and c) showed sensibly lower density than zones 1 and 2, indicating a more intense alteration of the matrix.

For all the specimens, large amounts of residual anhydrous grains were visible in zone 1. These grains were also observed in zones 2 to 5 although some of them were progressively dissolved.

In zones 3 and 4 of the sample exposed to *E. coli* cells, dense compounds were visible. These grains were assumed to be calcite grains since EDX analyses showed that they contained sensibly higher amounts of calcium than the surrounding paste (Fig. 8). In zone 4, the proportion of these grains was lower than in zone 3. For the specimen exposed to the filtrated medium, a thin Ca-bearing deposit, assumed to be calcite, was observed at the surface in contact with the aggressive medium. These results were in accordance with the XRD patterns of these zones.

4. Discussion

4.1. Mechanisms of deterioration by the raw culture (*E. coli* cells, metabolites: organic acids, CO_2)

The fluid circulating in the column with the raw culture contained the feeding medium, *E. coli* cells and their metabolites: notably organic acids (mainly acetic and lactic acid with a total concentration of 0.12 M) and CO_2. In the column, the bacterial activities may have modified the medium composition as suggested by the pH (7.8) of this circulating fluid, which was lower than that of the filtered one.

Regarding chemical and mineralogical modifications, the attack resulted in a well-marked zonation of the cement paste specimens. Five zones were observed. Zone 1 had the same characteristics as the control specimen, zone 2, or the transition zone, showed (i) a slight decrease of calcium content linked with the dissolution of calcium hydroxide and (ii) an enrichment in sulphur, probably coming from the outer part of the specimen (zones 3 to 5), where it was dissolved, and then precipitated in zone 2 to form ettringite as shown through XRD analyses.

Zones 3 to 5, more intensely degraded (pH<9.5) than zone 2, showed a progressive and almost total decalcification. In the inner

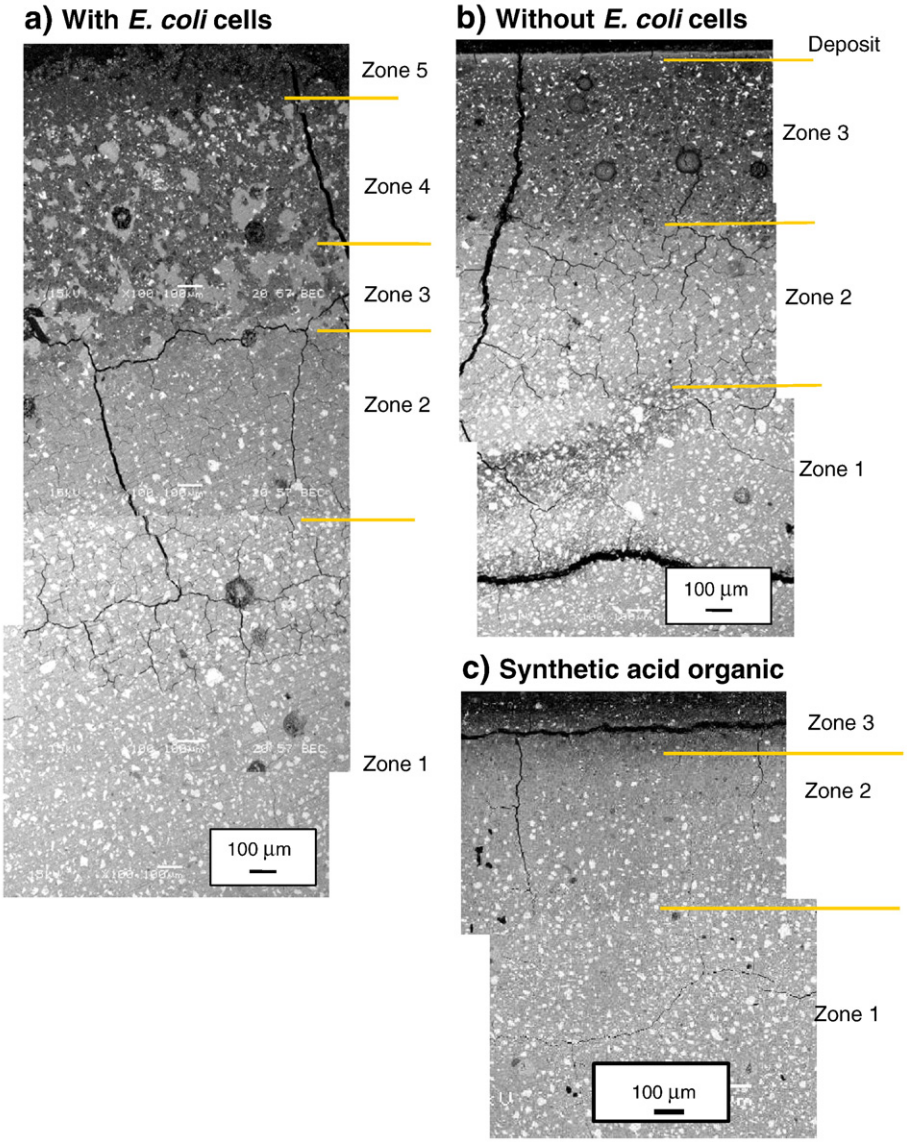

Fig. 7. SEM observation in BSE mode of the cement pastes after 4 weeks of immersion in solutions (a) without *E. coli* cells, (b) with *E. coli* cells, and (c) of synthetic organic acid.

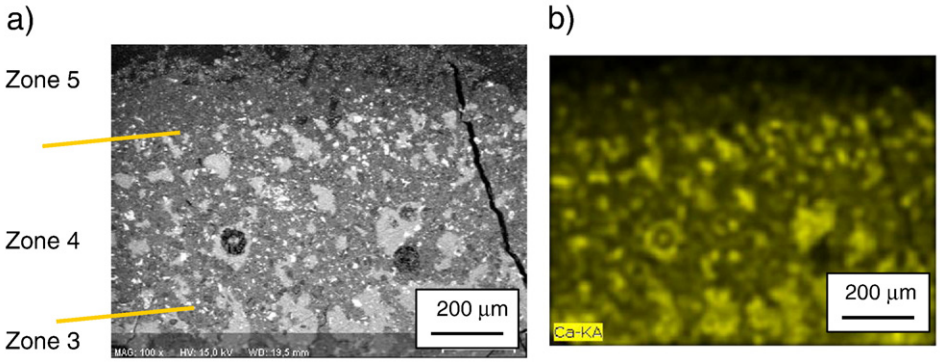

Fig. 8. Observation with SEM of the outer part of the cement paste of Fig. 7 exposed to the solution with *E. coli* cells. (a) BSE mode picture and (b) calcium mapping with EDX.

part of this zone (in zone 3), a carbonation front was found. This precipitation of calcite, probably linked with the presence in the solution of carbon dioxide resulting from the bacterial activity (which unfortunately could not be measured in the medium), has already been observed by Lajili et al. [27] in a study of alteration of cement matrix by fungi (*Aspergillus niger*) at a pH of 5. These authors also detected calcite in the vicinity of the transition zone. As modeled by De Windt et al. [11,12] on the same study, the precipitation of calcite is caused by the diffusion of dissolved CO_2 from the aggressive solution through the degraded zone of the specimen.

The outer part of the altered zone (zone 5) was completely decalcified. It was amorphous and was made up of a silica gel containing aluminum and iron. The CaO content, almost zero, suggests that C-S-H were dissolved in this zone.

The thickness of zones 3 to 5 was about 1.3 mm after 4 weeks of immersion.

4.2. Mechanisms of deterioration by the culture filtrate (metabolites: organic acids, CO_2...)

The fluid circulating in the column with the culture filtrate (without *E. coli* cells) contained the feeding medium and the bacteria's metabolites: notably organic acids (mainly acetic and lactic acid with a total concentration of 0.12 M) and CO_2.

Zones 1 and 2 exhibited features similar to those of the samples immersed in the raw culture.

However, in contrast to the specimen in the raw culture,

- the outer part of the specimen (here, zone 3) was not completely decalcified (CaO content about 15%);
- the precipitation of calcite, probably linked with the bacterial activity in the reactor, was observed at the surface of the specimen, and not deeper in the matrix as was the case for the specimen in the raw culture;
- the thickness of the outer part (with pH<9.5—here, zone 3) of the specimen after 4 weeks of experiment was sensibly lower than that of the specimen immersed in the raw culture (with E. coli cells) (500 µm vs. 1300 µm).

These differences in chemical features of the specimens are linked with the presence/absence of bacterial cells. But they may not be entirely explained by the slight difference of organic acid concentrations between the two media. Two supplementary reasons can be considered: (i) the bacteria in the raw medium continued to produce CO_2 and so changed the medium equilibrium and, above all, (ii) the deposit of bacteria at the surface of the specimen induced locally higher concentrations of acids (the measured concentration of acid downstream of the column is not representative of the concentration at the surface of the specimen) and lower pH.

These local conditions of acid concentrations and pH might explain the difference of calcite precipitation depth between the two aggressive media. In the column without bacteria, calcite could precipitate at the surface of the specimens where the pH was 8, as in the whole medium. In contrast, in the column with bacteria, the production of acids at the surface inhibited the precipitation of calcite, which occurred deeper in the matrix where pH conditions were suitable.

4.3. Mechanisms of deterioration by synthetic organic acids alone

The attack by the organic acid solution – with same concentration of acids as the *BMB test* media – resulted in a well-marked mineralogical and chemical zonation in which three zones were observed.

Zones 1 and 2 had the same characteristics as those of the specimens exposed to the *BMB test.*

Now, no carbonation was observed in the specimen, as was to be expected since the medium contained no CO_2. Zone 3 (with pH<9.5) was almost totally decalcified and was amorphous. It was made of a silica gel containing aluminum and iron. C-S-H was dissolved. The thickness of zone 3 was about 200 µm after 4 weeks of immersion.

4.4. Comparison of the various attacks and specific effect of each aggressive agent

The attack by the acidic part of the medium is thus less aggressive than the *BMB test* media with and without bacteria. Comparing the organic acid solution and the filtrate one (OA + metabolites + residual nutrients), the presence of CO_2 dissolved in the medium might induce more severe degradation of the specimens than the acids alone. This may be linked with the presence of bicarbonate anion HCO_3^-, the major carbonic species at the pH in question (7.8 to 8) which induces additional acid attack on the matrix. Moreover, NH_4^+ might have a specific effect; this should be analyzed in a further study.

The attack by the raw culture (bacteria + residual nutrients + metabolites) is the most aggressive of the three media tested in terms of kinetics of degradation. This cannot be explained only by the slight difference in organic acid concentrations observed between the two columns of the *BMB test* (linked with the continuation of metabolic activities). It seems to be a real, specific effect of the presence of the bacteria that deposit on the specimen surface and produce acids directly in contact with the surface. The concentrations of acids and the pH of the circulating fluid are not representative of what really occurs at the surface of the specimen. The presence of bacteria here generated sensibly more severe degradation of the matrix.

5. Conclusions

A new experimental device was developed for a test method to evaluate the mechanisms of the degradation of building materials caused by the microbial activity: the *Build-Mat Bio-test* (*BMB test*). In

this exploratory study, cementitious matrices were exposed to a culture of *E. coli* (which included the bacterial cells, the products of their metabolism and the residual substrates) or to the same culture after filtration to remove the bacterial cells.

Regarding the degradation mechanisms, both conditions (with and without cells) resulted in a well-marked mineralogical and chemical zonation, causing a progressive and more or less intense decalcification of the matrix. In both columns, the respiration of bacteria caused carbonation of the matrix. In the absence of *E. coli* cells, the carbonation occurred in the outer part of the specimen but, in the presence of the *E. coli* cells, the precipitation of calcite took place deeper in the matrix. In the outer part of the samples, the conditions of pH and of acid concentration seem to have been more severe, probably because of the production of high concentrations of acids at the surface, where the bacteria formed a deposit. The results enabled specific effects of the bacteria to be identified in the degradation: the presence of microorganisms generated higher degradation kinetics and more intense degradation of the matrix, notably in terms of decalcification.

In future studies, the test method will be used in particular to investigate (i) the effect of other types of bacteria and (ii) the behavior of various cementitious matrices (several binders and water/binder ratios) in order to analyze their relative performance toward biodegradation in terms of kinetics and intensity of degradation.

Acknowledgments

The authors thank the Institut National des Sciences Appliquées of Toulouse, France, (http://www.insa-toulouse.fr/en/index.html) for its financial support.

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