

# Understanding interactions between cementitious materials and microorganisms: a key to sustainable and safe concrete structures in various contexts

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**Abstract** Building materials can be exposed to microorganisms (mainly bacteria, fungi and algae) in almost every aqueous medium or damp environment, water being the indispensable condition for life development. The activity of microorganisms can be responsible for mineralogical, chemical and micro-structural damage to the material (biodeterioration). Deleterious effects can also concern the aesthetics of a building (proliferation of colored biological stains on façades and roofs) or the quality of indoor air (presence of microorganisms in damp buildings). However, microorganisms can also have positive effects (healing of materials) and their action is explored through the development of bio-based protective systems intended for building materials. In all cases, understanding interactions between building materials and microorganisms is an indispensable step toward the development of more sustainable, better quality, safer structures in many environments. This paper presents two examples where the action of microorganisms has—or is likely to have—strong impact on the durability and safety of concrete structures. The first example concerns the biodeterioration of concrete in agricultural and agro-food environments. The second example is that of the abiotic and biotic reactivity of

nitrate in repository of intermediate-level long-lived nuclear wastes. The paper presents the approaches used to explore and understand the phenomenology of bio-geo-chemical interactions in these complex environments. These studies notably comprise the development of test methods and experimental pilots to enable these explorations to be carried out. Current shortcomings in the scientific literature and in the standardization environment are also highlighted.

**Keywords** Microorganisms · Concrete · Biodeterioration · Organic acids · Nuclear waste repository

## 1 Introduction

Infrastructures and buildings are exposed to microorganisms in a variety of contexts. In every situation where building materials can be in contact with water or moisture, an indispensable condition for life to develop, they can be subject to the action of microorganisms. Interactions between life and materials may, in some cases, condition the service properties, the durability and the safety of building materials, products and structures [1–4]. There is growing awareness of these interactions, which are now the subject of more and more research.

### 1.1 General aspects about microorganisms

Microorganisms are microscopic, pioneer organisms in any development of life, and are ubiquitous in air,

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water and soil. They are characterized by their metabolic functions i.e. all the biochemical reactions through which the microbial cells can develop, grow, react and reproduce (proliferate). The creation of microbial biomass, resulting from a succession of redox reactions, necessitates an energy source (from light or from the oxidation of chemical, organic or inorganic compounds, i.e. electron donors) and a carbon source (organic or inorganic, i.e. CO<sub>2</sub>) [5]. The major mode of classification of microorganisms is based on metabolism, which defines the way microorganisms interact with their environment and notably with substrates (for example, building materials). It is also a resultant of the characteristics of the environment: the type of predominant microorganisms in a given medium is determined by the characteristics of the environment, which act as selection factor from an high initial diversity, e.g. in fully aerobic conditions, ammonium-rich environment, nitrifying microorganisms will become significant (case of sludges from wastewater treatment plant with high solid retention time) [6], whereas in sulfur-rich aerobic media, sulfur-oxidizing microorganisms (SOM) will grow (case of crown part of sewer pipes [7, 8]). Besides their rapid adaptation to the environment, one characteristic of microorganisms is their ability to act on the environment. At the surface of a substrate, populations can follow one another and, through their metabolism, cause the chemical conditions to progressively evolve toward more favorable ones (for example, decrease of surface pH of concrete in sewer environments because of the succession of neutrophilic and acidophilic SOM [9, 10]). Microorganisms can also organize themselves in the form of a biofilm (aggregates of organized microbial communities held together by the polymers they excrete), which makes the bacterial population more resistant to severe environmental conditions or to variations in these conditions [5, 11–13].

## 1.2 Main types of interactions between microorganisms and building materials

### 1.2.1 Biodeterioration

In many cases, microorganisms have detrimental effects on the structures and construction materials which compose them. In aggressive aqueous media such as waste waters, ground waters, sea waters,

agricultural or agro-industrial environments, and industrial effluents, structures—often made of concrete—can suffer deterioration linked to the activity of microorganisms [14–20]. The main microorganisms responsible for biodeterioration are bacteria and fungi. Bacteria are unicellular microorganisms that can exhibit all types of metabolisms. Fungi are uni- or multi-cellular heterotrophic microorganisms: they need organic matter as a carbon source and electron donor, which means that they generally develop in rich organic carbon environment or on a primary layer of autotrophic microorganisms in the case of environment with low organic matter content [21, 22]. Multicellular fungi produce thin filamentous structures called hyphae, which absorb nutrients in the environment [5]. These microorganisms act on the structures through their metabolism. They produce metabolites, many of which are chemically aggressive to building materials and especially concrete (organic and mineral acids, CO<sub>2</sub>, sulfur compounds, etc.) [23–26]. They can also degrade materials through some specific effects, such as the formation of biofilms on the surface, which locally generate high concentrations of aggressive metabolites [27], or the physical action of hyphae of fungi on the material [22, 28, 29]....The deterioration occurring in concrete materials in connection with mineralogical, microstructural and chemical changes is as varied as loss of alkalinity, erosion, spalling of the concrete skin, corrosion of rebars, loss of water- or airtightness, and collapse [18, 30, 31].... Such deterioration leads to a significant increase in the direct cost of repairing structures and indirect costs linked to loss of production income (e.g. during repair work inside damaged plants), and may also lead to pollution resulting, for example, from waste water leakage to the environment (case of damaged infrastructures intended for collecting or storage of effluents). A case in point is the risk of damage to repositories intended for nuclear waste storage, by microbial action on the geological and chemical conditions within the infrastructures [2, 4]; this will be developed further in this paper.

### 1.2.2 Microbial stains on building materials

Microorganisms can also act on structures through their own appearance. The facades of buildings or monuments can be subject to proliferation by colored microorganisms. These are mainly algae or



cyanobacteria, which are photoautotrophic microorganisms, i.e. they use light as their energy source and mineral compounds as carbon source ( $\text{CO}_2$ ) and electron donors (photosynthesis). They can thus grow on strictly mineral supports such as stone, roofing tiles and concrete or cementitious coatings (with lowered pH following carbonation or other chemical weathering), etc. The consequences are mainly aesthetic: green, black or red stains develop, sometimes very quickly, on infrastructures, depending on a variety of climate, environmental and architectural parameters [3, 32–36]. Fungi can also participate in this kind of attack. In this case, beside aesthetic alterations, building materials can suffer mineralogical and microstructural damage [29, 37]. The biological stains lead to significant cleaning costs and to image prejudice in the case of prestigious buildings. Although the microorganisms responsible for these alterations have been quite well identified [32, 38–41], research is now focusing on determining colonization mechanisms, notably the influential material-related factors [3, 35, 36, 42], and on developing preventive or curative solutions to protect external walls [43–47]. However, some research works seek to improve the bio-receptivity of materials in order to favor the growth of microorganisms such as algae or lichens and higher plants that can improve the appearance and the thermal performance of buildings [48].

### 1.2.3 Indoor microbial proliferation

Building materials are also exposed to microbial proliferation in indoor environments. Microorganisms thriving in damp indoor environments (molds, bacteria, fungi) are known to be one of the main causes of the degradation of indoor air quality and can be serious health hazards to occupants because of the production of airborne particles such as spores, allergens, toxins and other metabolites. Health troubles include irritations and toxic effects, superficial and systemic infections, allergies and other respiratory and skin diseases [49]. The resulting social and economic impact is very significant [50, 51]. The surfaces of building materials (plasterboard, mortar, etc.) are generally highly porous and rough. In damp environments, these materials can provide an support favorable to the proliferation and growth of microorganisms. Although the microorganisms that develop in building environments are quite well identified for conventional

building materials, studies linking the properties of these materials with the nature of the proliferation in terms of species and kinetics are still lacking in the literature [49]. Current research work notably aims to develop different solutions to inhibit, or at least, reduce microbial proliferation on building materials and substrates [52–57]. Moreover, the literature on the susceptibility of bio-based materials, incorporating plant aggregates and/or fibers, to microbial proliferation is scarce [58, 59]. This topic needs serious consideration, in particular with the increasing interest in these materials [60–63].

### 1.2.4 Positive effects of microorganisms

However, in some cases, microorganisms can have beneficial effects on cementitious materials, for example when they are used as a way to protect and/or repair concrete, in applications such as bacteria-based protective systems. New formulations of cementitious materials, incorporating selected bacteria and suitable chemical precursors, are being developed with the aim of filling micro-cracks in concrete and thus improving its durability properties [64, 65]. Surface treatments such as biodeposition are also being explored [66–68]. In other cases, the formation of microbial biofilm on the surface of cement-based materials can provide a protective layer against biological deterioration, e.g. either by the excretion of protective organic polymers (EPS = ExoPolymeric Substances), where beneficial precipitation of calcium carbonate can occur for example [69], or by the proliferation of non-aggressive microbes capable of competing with undesirable microorganisms [70, 71]. Also, the metabolites produced by some fungi or lichens, such as oxalic acid, can protect Ca-bearing materials by the precipitation of calcium oxalate at their surface [72, 73].

## 1.3 Understanding microorganisms-cementitious materials interactions

Since concrete is the most widely used building material, understanding the interactions between microorganisms and cementitious materials is crucial and constitutes a fundamental step toward more durable, safer, better quality structures in many contexts. Nevertheless, except for the case of

biodeterioration in sewer systems [1, 7–9, 74], these phenomena have only recently been considered by building material and product manufacturers, owners, civil engineers and contractors, and finally, research funders. In the scientific literature, it is also quite a recent topic, the coverage of which has been increasing, especially since the late 1990s. Current unanswered questions pertain to both scientific and technical aspects. Among other things, the specific impact of microorganisms on concrete structures in terms of biodeterioration mechanisms—apart from that of their metabolites—is not well understood. Moreover, the influence of the material properties (chemical and physical) on the activity of the microorganisms and their structuring into biofilm, for example, is not well apprehended, although it conditions the deleterious impact on the material [75]. Moreover, no standard test method exists to qualify concrete or building materials exposed to the action of microorganisms. So, current research is interested in developing relevant, rational and representative test methods in the various contexts concerned [27, 33, 34, 76–81]. The modeling of interactions between cementitious materials and the microorganism-bearing environment is also in its early stages. It is, however, a high-priority issue to complete the understanding of these interactions on the one hand and to predict the material/product/structure service life duration on the other. The double challenge is to model (i) the activity of microorganisms and biofilm at the surface of a highly reactive material such as concrete and (ii) the impact of specific metabolites ( $H_2SO_4$ , citric acid, etc.) that produce expanding secondary products by reaction with the cementitious matrix, which in turn creates cracking within the matrix and modifies its transfer and mechanical properties.

RILEM Technical Committee 253-MCI—microorganisms cementitious materials interactions, was created in 2013 and aims to address all these issues by bringing together specialists from the different domains (cementitious materials, microbiology, process engineering...). The activity of this TC, which should end in 2018, will include the writing of a State-of-the-Art report in which all the aspects mentioned above will be developed in much greater detail.

The present paper focuses on two types of interactions between microorganisms and cementitious materials that I have had the opportunity to work on.

The first concerns concrete in agricultural and agro-food environments. The aim of this research work was to improve the durability of cementitious materials under the action of both chemical and biological attacks induced by agro-industrial media (agro-food, agricultural and breeding effluents, mainly). Mechanisms of concrete alteration in these environments were investigated. In particular, chemical attack of cementitious materials by organic acids, which are quite common microbial metabolites, was thoroughly explored. The impact of microorganisms on the alteration mechanisms and kinetics was also investigated. The second type of interaction concerns a little known aspect of the safety of nuclear waste repository structures, at least from the civil engineering standpoint: the possible influence of microbial activity on the redox conditions within the cells intended for intermediate-level long-lived nuclear wastes. Both studies have notably focused on the development of laboratory pilots to rationally investigate the interactions among microorganisms, materials and chemical species in controlled microbial conditions. The paper also aims to point out current shortcomings in the scientific literature and in the standardization environment.

## 2 Biodeterioration of concrete structures in agricultural and agrofood environments

### 2.1 Context and problems

In their initial state, agricultural effluents, such as liquid manure, molasses, silage juices and effluents from biogas systems, contain, among other species, more or less complex organic matter (including organic acids), and microorganisms (bacteria, yeast). The activity of these microorganisms can degrade the organic compounds and, in turn, produce organic acids (acetic, lactic, succinic) together with  $CO_2$  and  $CH_4$ . The various acids found in these effluents are thus either produced by this bacterial activity or are contained in the raw materials (fruit or plants in the case of molasses and wine effluents), or they result from industrial treatments applied to the raw matter at the different steps of the industrial process. The concentration of acids can reach several millimoles per liter depending on the effluents, the pH of most effluents being between 4 and 8 [26].



The effluents lead to serious degradation of the structures intended for their production, collection, storage and processing, which are often made of concrete (cowsheds, housing structures, milking rooms, anaerobic digesters, etc.) [16, 18, 30, 82, 83]. Progressive erosion of the concrete has been observed, which may be accelerated by mechanical action (high pressure cleaning, animal and machine traffic...). In the long term, embedded reinforcement can become corroded and result in the destruction of facilities [30]. The financial and environmental consequences of the damage were mentioned in the introduction.

The aggressiveness of agro-food effluents toward concrete structures is linked to their chemical components (organic acid and CO<sub>2</sub> in particular), on the one hand, and to their microbiological components, on the other hand [16, 27, 83]. Developing concrete that perform well in such environments necessitate to understand the degradation mechanisms by the various aggressive compounds contained in the effluents. Our strategy was to separate chemical and biological phenomena and to use model media: synthetic organic acids [84–86] on the one hand, and model microbial environments [27] on the other. Studies were then validated using real effluents (liquid manure, silage juices, biogas system juices) [16]. The first part of this report will concern research work focusing on attack by organic acids and the second part will look into biological action in such media.

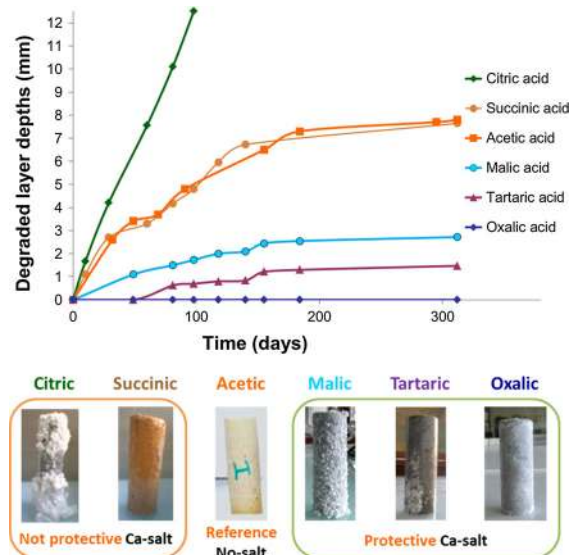
## 2.2 Attack by organic acids

Aggressive media consist of mixes of a wide range of organic acids where the chemical and physical properties of the acids and of their salts and organo-metallic complexes are many and varied [26, 87]. In order to move toward an approach predicting the aggressiveness of such or such medium according to its acid composition, the work aimed to identify the relationships between the aggressiveness of the acids, the physical and chemical properties of the acids and their salts, and the chemical and mineralogical properties of the cement matrix. Works were first carried out with acids taken separately [84–86, 88, 89] before mixes of acids were considered [90, 91]. One of the aims was also to identify the resistance of ordinary and special binders in such environments [92, 93]. The work was based on experiments and modeling. Mineralogical, chemical and microstructural changes

were studied using various analytical techniques for liquids and solids, the results of which were cross-compared to establish the alteration mechanisms [85, 94]. The influence of some parameters and/or phenomena such as polyacidity or complexation being difficult to highlight experimentally, equilibriums in solutions were also investigated using CHESS and HYTEC software on a wide range of pH and concentrations. Particular attention was paid to the formation of complexes in solution [87, 90].

### 2.2.1 Mechanisms and kinetics of degradation by the acids

The kinetics of cement paste alteration varies greatly from one acid to another (Fig. 1). Immersion of cement pastes in synthetic organic acid solutions with the same concentration of acids showed that: (i) the most aggressive of the acids tested was citric acid (pH4) which dissolved a 25-mm-diameter cement paste specimen in less than 4 months, (ii) the least aggressive of the acids tested was oxalic acid, despite the very low pH of the acid solution (0.85). This example showed that the pH parameter is not sufficient to characterize the aggressiveness of an acid medium.



**Fig. 1** Kinetics of alteration of various organic acids toward Portland cement paste [26] and aspects of the corresponding specimens. All the acid solutions were 0.28 M and with a pH of 4 (adjusted by addition of soda), except for the oxalic acid solution, the pH of which was 0.85



The composition of acids of the medium should be considered in any attempt to predict its aggressiveness. It should be noted that pH is, however, the main criterion to be considered in NF EN 206-1 (2004) and FD P 18-011 (2009) for the classification of aqueous acid environments. Finally, two groups of acids can be considered: (i) acids with soluble calcium salts (no formation of salt occurs during the attack in usual laboratory conditions, i.e. regular renewal of acid solution or addition of concentrated acid and periodic renewal of solution); (ii) acids with slightly soluble salts. Within the latter group, the formation of the salt in the conditions of the experiments is either protective or destructive for the matrix, depending on the properties of the acids and their salts (Fig. 1).

*Acids with soluble salts* (acetic acid, propionic, and butyric acids, etc.). These acids are present in most agricultural/agrofood effluents and in many microorganisms-bearing environments (sewer systems, etc.). The alteration mechanisms have been described by several authors [84, 85, 88, 95, 96]. This attack expresses itself by calcium leaching from the matrix and the formation of a Si–Al-skeleton gel with high porosity [85] and very weak mechanical properties. The altered zone is not dissolved during the attack. In this case, the aggressiveness of the acids is related to their pKa [88] and to the chemical and physical properties of the matrix [85] and the aggregates. The attack by acids with soluble Ca-salts is close to that induced by strong acids with soluble salts such as  $\text{HNO}_3$  or  $\text{HCl}$  [24]. These organic acids can advantageously replace strong acids in laboratory experiments as advantage can be taken of the acids' buffer zone (low pH changes for significant release of  $\text{OH}^-$  anion by the cement matrix in the pH interval  $[\text{pKa} - 1, \text{pKa} + 1]$ ) [97].

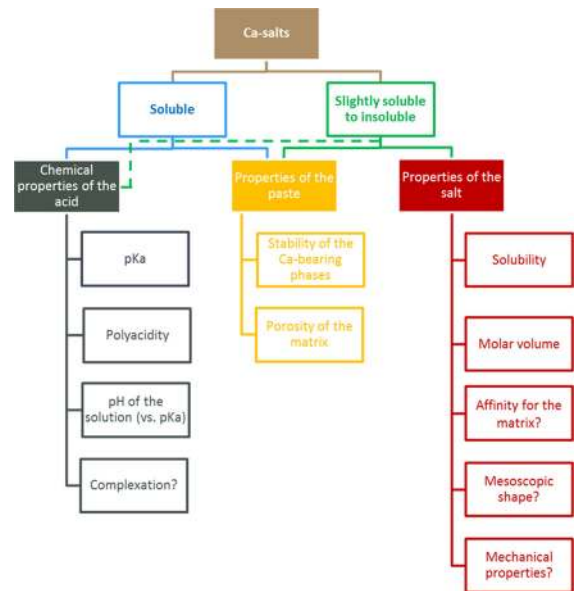
*Acids with slightly soluble to insoluble salts* (oxalic, citric, succinic, tartaric, and malic acids). These acids can be found in agro-food effluents (winery effluents, vinasses, dairy effluents) and in the plants or fruits themselves. Oxalic and citric acids can also be excreted by some fungi or lichens and deteriorate building materials [98–102]. The attack by these acids leads to the formation of Ca-salts. These salts are systematically formed by the reaction between Ca cations released by the cement paste and the most dissociated form of the acid (in the case of a poly-acid, such as succinic, tartaric or citric acid) [26, 86]. Although the properties of the salt largely determine

the intensity of the attack, the mineralogical composition of the matrix also has an influence. Two groups of acids can be distinguished: those having salts that are protective toward the matrix (i.e. the precipitation of the salt reduces the kinetics of the front progression inside the matrix compared to the reference acid: acetic acid) and those having salts that worsen the degradation kinetics. A correlation between the molar volume of the salt and the degradation occurring on the matrix has been observed [26, 86]. The volume of the paste porosity, and the phases at the expense of which the salt forms, are also influencing parameters. Citric acid, the most aggressive of the acids tested, forms Ca-citrate tetra-hydrate through the reactions with the cement matrix. The molar volume of this salt is very high ( $518 \text{ cm}^3/\text{mol}$ ) compared to the molar volume of the phases at the expense of which it precipitates, i.e.  $\text{C-S-H}$  and  $\text{Ca(OH)}_2$  ( $125$  and  $33 \text{ cm}^3/\text{mol}$ , respectively). The outer layer of the cement paste is severely damaged by the precipitation of Ca-citrate. The high aggressiveness of citric acid is also assumed to be linked to its tri-acidity: for a given concentration of acid, three moles of hydronium ions are released and participate in the hydrolysis of the cement matrix. The least aggressive acid for cementitious matrices made of ordinary binders is oxalic acid. In the case of oxalic acid, the precipitation of Ca-oxalate, which is hardly soluble, if at all, protects the matrix. In the case of OPC pastes,  $\text{Ca(OH)}_2$ , which dissolves when pH values are lower than 12.5, is likely to play a sacrificial role in the attack:  $\text{C-S-H}$  are preserved and the paste keeps its integrity even in the outer altered layer exposed to pH as low as 0.85 [86]. In contrast, CEM III pastes with high substitution rates are progressively attacked (but with slow degradation rates): in the absence of  $\text{Ca(OH)}_2$  in the hydrated matrix,  $\text{C-S-H}$  are attacked. The highly beneficial effect of Ca-oxalate is probably related to (i) the molar volume of the salt which enables it to fill the volume of  $\text{Ca(OH)}_2$  and the porosity of CEM I paste and probably a small part of hydrates porosity [86], and (ii) the high stability of this salt over a wide range of pH and concentrations of acids [87]. It should be noted that the protective effect of oxalic acid toward calcium-bearing stony materials is quite widely reported in the literature [100, 103].

*Synthesis of parameters influencing aggressiveness of the acids.* Finally, an attempt was made to classify the parameters influencing the aggressiveness of the acids toward cementitious materials (Fig. 2). The



phenomenology of organic acids' attack is primarily related to the acid salt solubility. When the salt is soluble, the chemical properties of the acids (pKa, polyacidity, as well as the pH of the solution versus the pKa [88]) and the properties of the paste, influence the acid's aggressiveness [92, 95]. The polyacidity of the acid may also influence its aggressiveness to the cement matrix [87], although no polyacid with soluble Ca-salt has been studied in the literature. Moreover, it is likely that complexation phenomena have an impact on the intensity of the hardened matrix alteration (through a cation-pump effect) but no direct evidence has been provided yet, either from experimental [26] or from modeling studies [104]. Finally, the composition of the cement matrix and notably the chemical stability of the Ca-bearing phases influence the resistance of the matrix to the attack. Ordinary binders with aluminum-rich supplementary cementitious materials (SCM) have been found to offer a good resistance to attack, especially with slag additions [95, 105, 106]. The stability of the anhydrous residual grains toward the acid attack may also influence the resistance of the matrix [84, 85, 88, 107]. In very aggressive conditions (low pH, high concentration of acid), the porosity of the matrix was found to be only a secondary parameter of the resistance of the matrix to the attack by acetic acid [95]. When the salts are slightly soluble to insoluble, as mentioned above, the properties of the salts greatly influence their aggressiveness to the cementitious matrix. A strong correlation was highlighted between the molar volume of the salt—in relation to the porosity of the matrix, and the acid's aggressiveness [26, 86]. The solubility and stability versus pH of the salt also influence the intensity of alteration: the very low solubility of Ca-oxalate salt and its wide stability domain (as a function of pH and concentration of acid) accounts for its protective effect on the matrix [87] in comparison with Ca-tartrate, for example [26, 108]. Although they are not well understood yet, other salt-related parameters could influence the acid's aggressiveness, such as the affinity of the salt for the matrix, its mesoscopic shape (which should condition its ability to cover the cement matrix and thus protect it against further degradation), or its mechanical properties. The properties of the paste are also major parameter of the acids' aggressiveness and, in the case of acids with little soluble salts, they can act adversely to the case of acids with soluble salts (Fig. 2). For example, the presence of the

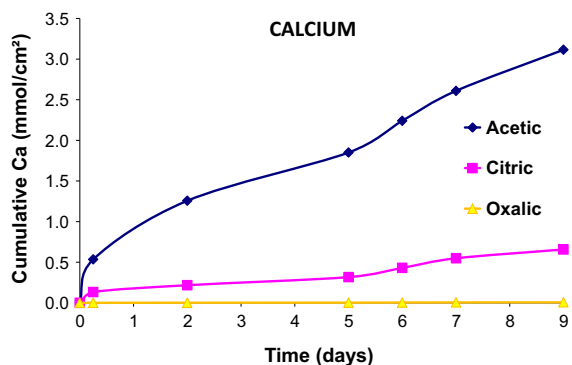


**Fig. 2** Organization chart of parameters influencing the aggressiveness of organic acid toward the cement matrix (from experimental and modeling works). The dotted line illustrates secondary influencing parameters (see text)

low stable  $\text{Ca}(\text{OH})_2$  in the matrix is favorable to the protective effect of oxalic acid (sacrificial role of portlandite which contributes to preserve C–S–H from dissolution) [86]. Also, higher porosity of the cement matrix seems to promote the protective effect of oxalic acid on the cement matrix [91]. Finally, the chemical properties of the acids (pKa, polyacidity...) seem to play a secondary role in the aggressiveness of acids with slightly soluble to insoluble Ca-salts.

### 2.2.2 Performance of cement based materials

The performance of cement based materials exposed to organic acids, estimated by the kinetics of alteration, can be measured through altered depths, mass losses/gains and variations of mechanical strength for example. Altered depth should be preferred as the prime indicator of resistance as it expresses the time taken for the alteration front to reach reinforcements in the case of reinforced concrete structures (which are commonly encountered in the types of media considered). Mass losses should be considered only as a secondary indicator as they depend, in the case of acids with soluble salts, on the initial Ca content of the cement matrix. From this point of view, considering



**Fig. 3** Kinetics of calcium leaching of Portland cement paste immersed in organic acids [108]. All the acid solutions were 0.28 M and with a pH of 4 (adjusted by addition of soda), except for oxalic acid, the pH of which was 0.85. Leaching of calcium in acetic acid was much higher than in citric acid, although citric acid is much more aggressive to the matrix than acetic acid in terms of altered depths (see Fig. 1). The lower calcium concentration in the citric acid solution made was due to the precipitation of Ca-citrate [86]

mass losses instead of degraded layer depths can lead to significantly different ranking of the resistance of binders because of the different Ca contents of the materials [92, 97]. The use of mechanical strength testing should also be considered with care: the continuation of hydration in the sound zone can lead to biased results in the estimation of the kinetics of alteration. Moreover, specimens subjected to mechanical tests should be prepared carefully (the zones of loading should be protected from the chemical attack or they should be removed prior to loading in case they have been altered). Finally, estimations of durability should not be made by considering the leaching of calcium only as, depending on the degradation mechanisms, the leaching behavior can give opposite results in terms of acid's aggressiveness to degraded layer depths (Figs. 1, 3). Ideally, a combination of several durability indicators should be used. Several recommendations regarding test methods for cementitious materials exposed to organic acids are made in [97].

Most studies carried out to evaluate the performances of cement pastes toward organic acids have used acetic acid. Not surprisingly, the best performance in such environments was observed with low W/C matrices that contained small amounts of Ca, and large amounts of Si and Al [84, 85]. Supplementary cementitious materials such as slag and metakaolin

were found to improve the resistance of the matrix [85, 93, 95, 106]. Long curing periods (>3 months) substantially enhanced the resistance of the hydrated phases (slag hydrates were found to preserve their calcium during leaching at pH as low as 4) [95]. Nevertheless, without this long curing time, in pH as low as 4 (pH at which experimental campaigns are generally conducted) the gain of resistance obtained using SCM was limited. CAC appeared to be the most resistant of the binders tested in these conditions [92, 93]. Few data exist regarding the durability of cement matrices exposed to organic acids with slightly soluble Ca-salts, such as citric or tartaric acids [93]. Alkali-activated materials could offer good resistance to organic acids [105, 109, 110] but more research data are necessary to identify the benefits of such binders [111].

### 2.2.3 Perspectives

Several aspects deserve attention in further studies on the durability of cementitious materials in organic acids. Regarding the attack by acids with slightly soluble salts, the influence of some properties of salts, not well understood yet, such as the affinity of the salt for the matrix, its mesoscopic shape and its mechanical properties still have to be investigated. The promising behavior of slag based binders should also be confirmed and explained. Moreover, oxalic acid could be explored as a way to protect the cementitious materials against acid environments [91]. Knowledge of the mechanisms of attack in mixes of acids has also to be completed [26, 91, 93]. Finally, geochemical modeling of interactions between organic acids and cementitious matrix should be continued and improved. In particular, attack by organic acids creating expansive salts which generate cracks in the matrix and in turn modify its transfer properties needs to be adequately modeled in order to better predict the service life duration of concrete products and structures subjected to acid environments. It was finally clearly highlighted that pH should not be considered as the only indicator of aggressiveness of acid environments: the composition of acids should also be considered (nature of acids and concentrations). In this view, standards defining classification of chemically aggressive environments to concrete should be improved.



### 2.3 Microorganisms-concrete interactions in biodeterioration processes of agro-industrial environments

Experiments with real media have shown that it is not sufficient to consider synthetic chemical compounds when the aim is to understand and express all the interactions between the materials and medium, in particular when microbial activity is involved [16, 17, 112]. As mentioned in the introduction, microorganisms may have specific effects on the material, for example by the formation of biofilm at the surface, which may worsen degradation compared to the purely chemical attack [16, 27]. Identifying the impact of microorganisms on substrates requires specific laboratory methods. At the moment, there is no standardized method to qualify concrete in microorganism-bearing media. Efforts are made by research groups worldwide to propose such methods. The methods have to be representative of the different environments to be simulated. They generally require specific microbial metabolisms and nutrient supplies, and they have to simulate specific environmental conditions (aerobic/anaerobic, mode of humidity/water supply: immersion, condensation, capillary ascent, run-off, etc.). Different environments have been considered in the literature so far: sewer systems (attack by biogenic sulfuric acid excreted by SOB) [1, 76–80], external concrete walls exposed to humidity (proliferation of algae or fungi at their surface) [32, [33, 36, 113, 114], etc. For agricultural environments, we proposed a test method aimed at mimicking hydraulic, microbial and chemical conditions occurring in storage silos of agricultural and agro-food effluents and identifying the effect of microorganisms in the degradation [27].

#### 2.3.1 Test device

The aim was to develop a test—the *Build-Mat Bio* test or *BMB* test—that enabled the specific effect of bacteria to be identified in the alteration of building materials by microorganism-bearing liquid media in immersion conditions. The purpose was to work either with a model environment (isolated or mixed pure strains) or with consortia, under controlled biological and environmental conditions (temperature, nutrients, flow rate, selected microorganisms, etc.). As it is impossible to isolate the bacteria from their

metabolites (such as organic acids, CO<sub>2</sub>, etc.) since they produce them continuously, the idea was to work by difference between a medium containing the bacteria and the metabolites and another medium identical in chemical composition but free of any bacteria thanks to a biomass filter (Fig. 4) [27]. The culture of microorganisms was separated from the exposure to the cement matrix by using a pre-culture in a bioreactor from which the aggressive media were extracted and passed through a column containing the cement matrices. The system was based on dynamic feeding that prevented any accumulation of the cement leachates in the media. In the study case, the test was intended to simulate the quasi static hydraulic conditions occurring in storage silos (liquid manure or silage silos) so very low flow rates were imposed in the system. However, other hydraulic and biological conditions can be simulated with the *BMB* test.

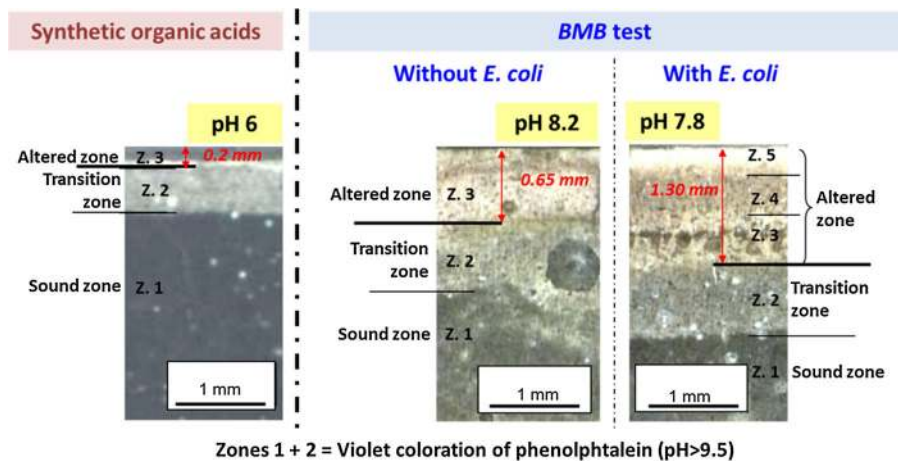
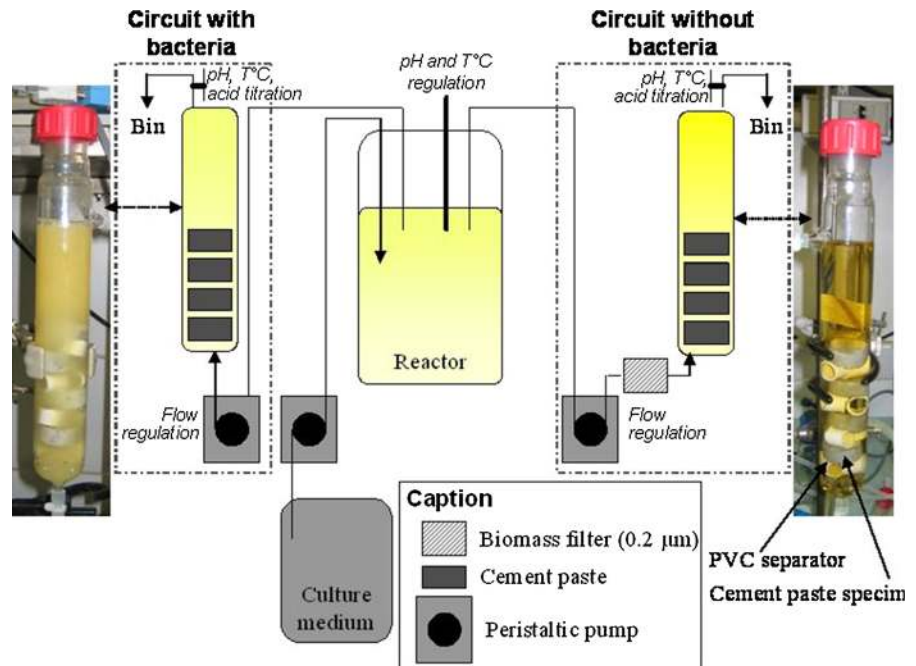
#### 2.3.2 Decoupling degradation mechanisms

The test device was used with the model bacterium *Escherichia coli*, a common, well-know and easy-to-grow bacterium found in many organic effluents. A specification for the device was that the chemical characteristics (pH and composition of acids) of the two liquid media circulating in the columns of the *BMB* test (with and without bacteria) were very close and representative of a liquid manure, the simulated medium in this case (pH 8, total concentration of lactic and acetic acids: 0.12 M). The mineralogical and chemical changes and the altered layer depths were investigated on the cement paste specimens in the two columns (with and without *E. coli*). For comparison, cement pastes were exposed to the organic acid compound of the medium using synthetic acetic and lactic acids with same concentrations as in the *BMB* test and by adjusting the pH to 6 (using NaOH) (Fig. 5).

It was found that the least severe attack came from the synthetic acids alone (with the lowest pH of the three media considered). The depth of the degraded layer of the paste was 0.2 mm after 4 weeks of exposure. In the *BMB* test, the degraded layer was twice as deep in the presence of bacteria (1.3 mm) as in their absence (0.65 mm). Moreover, EPMA and XRD analyses showed that the outer layer of specimens exposed to bacterial cells were completely decalcified and amorphous whereas quite intense



**Fig. 4** Schematic diagram of the *Build-Mat Bio-test* (BMB test) [27]



**Fig. 5** Cross sections of cement paste specimens exposed to the *BMB* test for 4 weeks (*column* with and without bacteria) and to synthetic organic acids in the same hydraulic conditions. The *upper zone* of the pictures matches the surface of the specimen

in contact with the aggressive media (corresponding pH given in the yellow rectangles). The *lower parts* of the pictures match the cores of the cement specimens (cylinders 25 mm in diameter). Adapted from [27]

carbonation and only partial decalcification was identified in the outer zones of cement pastes exposed to the metabolites only. This highlighted not only the supplementary effect of bacterial respiration (compared to the acid constituents alone) but also, and most importantly, the influence of the formation of biofilm at the surface of the specimens in the column with

bacteria, which markedly intensified degradation. These results show that considering only the chemical compounds of the bacteria-bearing media leads to an underestimation of the alteration in terms of its intensity and kinetics. The ability of bacteria to form biofilms at the surface can become really detrimental to structures and these aspects must be taken into

account when evaluating the durability of materials, products and structures in such environments.

### 2.3.3 Perspectives

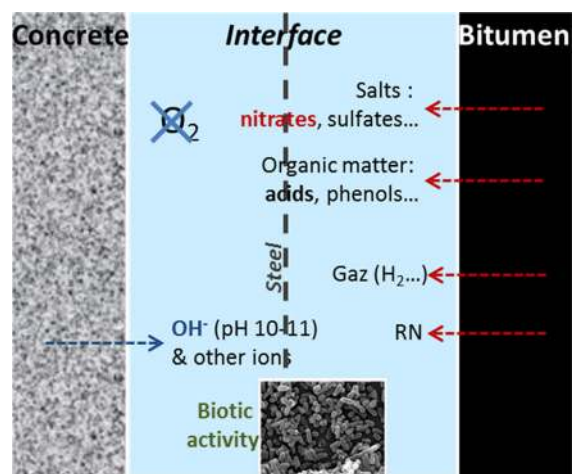
As mentioned above, there is currently a lack of standardized test methods for qualifying concrete in microorganism-bearing environments. Progress should be made in the coming years, notably on the basis of test methods proposed in the scientific literature. It should be kept in mind that the test methods have to be specific to the type of environment considered. Moreover, prescriptions for concrete formulation in such environments must also be adapted to take better account of the specificity of biodeterioration. Regarding interactions between microorganisms and cementitious materials, while the mechanisms of alteration of the cement matrix should be further completed in the different contexts where biodeterioration occurs, the impact of the cement paste properties (chemical, mineralogical, physical) on the biofilm structuration and activity also has to be investigated as, in some cases, it plays a significant role in the durability of the cement based materials and products [75, 76, 115, 116].

## 3 Bio-geo-chemical interactions and reactivity of nitrates in nuclear waste repository

### 3.1 Context and issue

Deep repository of nuclear wastes is an unexpected context where microbial activities and their interactions with materials deserve attention because these interactions can significantly influence the geo-chemical conditions—and thus the safety—of the storage. In France, a significant fraction of intermediate-level, long-lived radioactive wastes (IL-LLW) consist of a mixture of inorganic salts immobilized in a bitumen matrix. They are poured into steel containers grouped in reinforced concrete over-packs that will then be placed inside concrete waste cells built at a depth of 450–550 within a Callovo–Oxfordian clay host rock formation [117]. After closure of the cells, water re-saturation should enhance the release of chemical species into the interstitial aqueous medium. These include soluble salts (notably nitrates or sulfates) and organic matter such as organic acids, phenols, etc.

contained within the bituminous matrix [118], together with gas (mostly  $H_2$ ) produced via anaerobic corrosion of the steel and/or radiolysis of organic matter and water in the repository (Fig. 6). However, the presence of nitrates ( $NO_3^-$ ) and other oxidized species (such as  $SO_4^{2-}$ ,  $Fe^{3+}$ , and  $CO_2$ ) in the vicinity of waste packages may result in oxidizing conditions favorable to the mobility of a series of radionuclides (Se, U, Tc, Pu, Np, etc.) [2]. However, in the geochemical conditions prevailing in the cells (notably anoxic, after depletion of oxygen, and highly alkaline, imposed by concrete leaching) different redox reactions are likely to occur driving the system back to a reducing environment favorable to the safety of the repository. Among these reactions, nitrates reduction will play a key role in the cell since reactions at high pH necessitate a high energy contribution. Reduction of nitrate ( $NO_3^-$ ) may occur (i) from surface catalysis provided by the different types of steels in the waste cell, and/or (ii) from biological catalysis through bacterial activity [119–121]. Microbial investigations of candidate host rocks intended for deep nuclear waste repositories in France and Switzerland have highlighted small viable communities, these microorganisms being almost dormant (inactive) because of very limited space and water availability (low porosity of the unperturbed rock formations) [122–125]. Nevertheless, the repository excavation and construction works may allow further development of microbial communities in the cell, (i) either by



**Fig. 6** Schematic representation of the physico-chemical conditions at the concrete-bitumen interface in the disposal cells of IL-LLW (adapted from [129])

proliferation of the indigenous microbial population from the surrounding host rock after disturbances that will provide space, water and nutrients, likely to revive the dormant organisms and/or (ii) by contamination by non-indigenous microorganisms brought by human activities during the construction and exploitation of the cells before their closure. Moreover, studies on natural analogues (such as sediments from a lime working site or from a soda lake) have shown that microbial activities are possible in highly alkaline (pH up to 12–13) and anaerobic chemical environments similar to those which should develop in a nuclear waste deep repository [126–128].

The reactions involving nitrates—whether they be abiotic or biotic i.e. without or with bacteria—may lead to the formation of nitrite ( $\text{NO}_2^-$ ), nitrogen ( $\text{N}_2$ ) and/or ammonium ( $\text{NH}_4^+$ ), depending on a variety of parameters not well understood yet, particularly in the alkaline conditions imposed by the surrounding concrete. Both types of reduction reactions involve electron donors and numerous candidates are available in the waste cell or in the host rock (organic acids,  $\text{H}_2$ , zero-valent metals, ...) [2]. The overall study aims to investigate the reduction of nitrates within a system comparable to a “real” waste-cell where bacterial activity is likely to occur (denitrifying alkalophilic bacteria, in the conditions considered here) and notably to determine the phenomenology and kinetics of reactions and the role of each potential electron donor (i.e. organic acids released by bitumen, such as acetic or oxalic acids,  $\text{H}_2$ , the various metallic phases in the repository, etc.) in the reactions [129–131]. The environment under consideration is complex, multi-component and multi-phase. A step-wise approach was thus adopted, starting with simplified decoupled model systems (model media studied in batch conditions) [129, 130, 132], the complexity of which was progressively increased to move to close-to-real systems using an experimental pilot specifically developed for the study [131].

It should be noted that, apart from the problem considered here regarding the fate of oxyanions such as nitrates, there are other types of possible interactions between microorganisms and the various chemical compounds and materials (concrete, bitumen, metal, clay rock, etc.) within the nuclear waste repository that pertain to microbiologically induced corrosion (MIC) [4, 120, 133–137]. Moreover, microorganisms can modify the fate of radionuclides

themselves [138, 139]. All these aspects show that microbial activities should be taken into account when predicting the geochemical conditions in the repository cells and evaluating how safe the storage will be.

### 3.2 Physicochemical interactions under abiotic conditions

Physical and chemical interactions between the various compounds of interest in the systems (nitrates, bitumen leachates, concrete, and steel) were first studied in the absence of bacteria, in order to decouple abiotic and biotic phenomena likely to occur in the repository.

Among the organic matter released by bitumen of IL-LLW, short-chain organic acids (notably acetic, formic and oxalic acids [118, 140–143])—easily assimilable by bacteria in the repository—are likely to react with concrete of the repository). These reactions may, on the one hand, alter the durability of concrete cells [84, 88, 129, 132, 144], and, on the other hand, change the availability of organic matter usable by heterotrophic microorganisms for the reduction of oxyanions such as nitrates. It should also be noted that other sources of organic acids than bitumen can be found in nuclear waste storage: when cellulosic materials are immobilized with other low- and intermediate level radioactive wastes in cement matrices, cellulose may degrade, mainly to isosaccharinic acid, but also to formic and acetic acids following the reactions of polymers which break down under alkaline conditions [145, 146].

Beside acid-cement paste interactions, investigations have also focused on the reactivity of nitrates under abiotic conditions. The reduction of nitrates has been proved to be possible in the presence of different types of steels, in conditions similar to those occurring in a repository (i.e. alkaline pH and anoxic conditions), and it leads to the formation of  $\text{NH}_4^+$ . At pH up to about 11.5, nitrates can be reduced in the presence of C-steel, but the reaction rates strongly decrease when pH increases. In this case, carbon steel is likely to act as the electron donor [121, 131, 147], visible signs of corrosion being observed at the steel surface. Stainless steel, also present in the repository, could be involved in nitrate reduction, up to pH 12, in the presence of  $\text{H}_2$  gas as an electron donor. In this case, stainless steel act as a real surface catalyst, i.e. no corrosion of steel occur [148, 149].

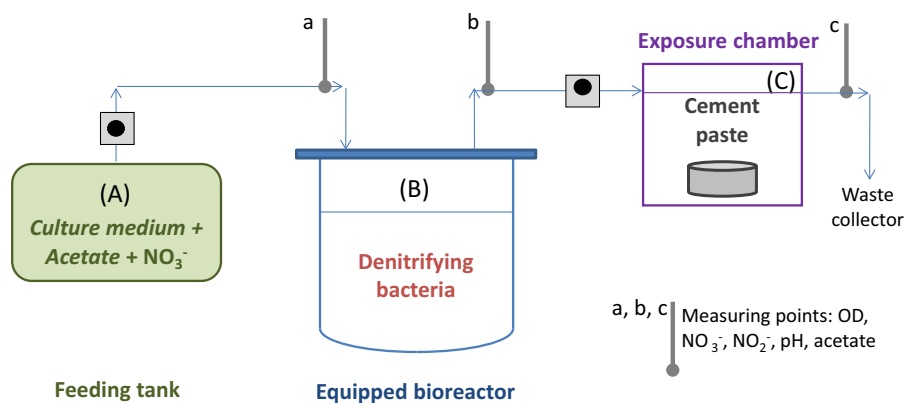


### 3.3 Interactions under biotic conditions

Microbial denitrification transforms nitrates to gaseous nitrogen via several intermediates, notably nitrites:  $\text{NO}_3^-_{(\text{aq.})} \rightarrow \text{NO}_2^-_{(\text{aq.})} \rightarrow \text{NO}_{(\text{enzyme complex})} \rightarrow \text{N}_2\text{O}_{(\text{gas})} \rightarrow \text{N}_{2(\text{gas})}$  [150]. Microbial denitrification, in the particular context of the disposal of radioactive waste as defined in Fig. 6, has so far been a matter of pure speculation. However, many microorganisms are known to lead to denitrification under conditions similar to those likely to occur in the repository, i.e. notably at high pH (in the range of pH 9–11) and anoxic conditions (conditions that will prevail in the storage in the long term). Works carried out to explore the biologically induced reduction of nitrates in such conditions have considered selected pure strains and wild consortia (using natural analogs) [120, 129, 131, 151–154]. The first step of such studies was therefore to identify microorganisms capable of (i) growing in alkaline conditions, (ii) using nitrate as electron acceptor and (iii) using one or more electron donors present in the repository. Two types of electron donors in the repository are explored in the literature: organic acids [129, 131, 152–154] and  $\text{H}_2$  [120, 153]. Bacteria using organic carbon (organic acids here) as substrates and energy source are called heterotrophic, whereas bacteria using  $\text{CO}_2$  as carbon source and chemical compounds such as  $\text{H}_2$  as electron donor are said to be autotrophic.

Among heterotrophic alkalophilic denitrifying bacteria, *Halomonas desiderata* (*Hd*) [155] has been identified as an interesting candidate as this bacteria

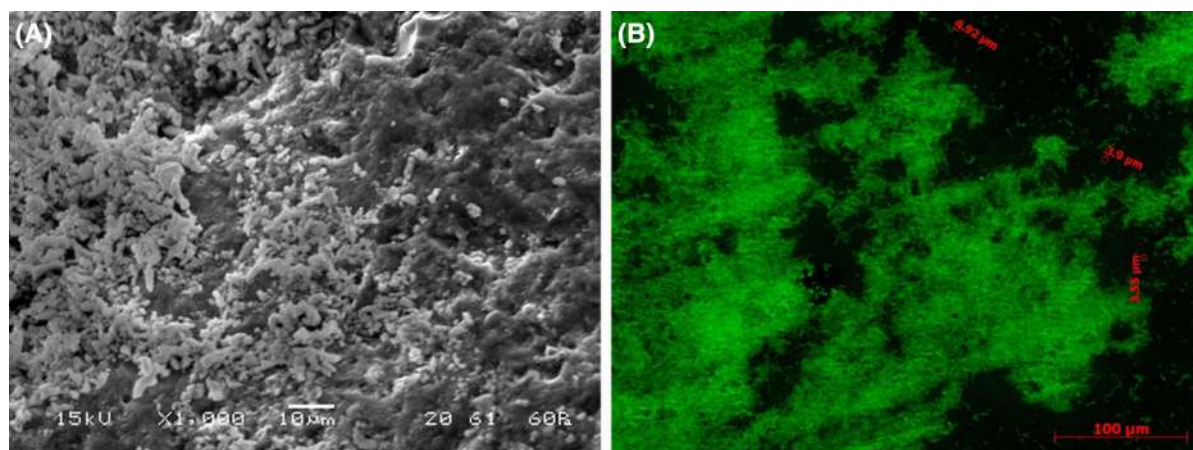
has been shown to reduce nitrates at pH up to 10 in batch and planktonic (bacterial cells in suspension) conditions [129, 130]. Moreover, the presence of cement matrices was shown to enhance denitrification kinetics at pH 10 (compared to experiments without solid cement matrices) and enabled denitrification reactions at pH between 10 and 12, which was taken to be linked to the ability of the bacteria to form a biofilm at the surface of solid CEM V cement paste matrices (Fig. 8a) [129]. In addition to these exploratory experiments carried out in batch conditions, a specific experimental device was implemented to simulate conditions close to those in the repository. The system consisted of a central bioreactor—where selected bacteria were grown—and separate exposure chambers containing cement paste matrices connected in series upstream and/or downstream of the bioreactor. The system was continuously fed with nitrate solution enriched or not with some culture medium. The system was intended to simulate various scenarios likely to occur in the repository [151] and to refine the understanding of microorganism denitrification strategies (calculation of microbial reaction rates in steady-state conditions). In particular, the configuration of the pilot device presented in Fig. 7 explored the impact of alkaline interstitial aqueous medium on the denitrification reaction by planktonic microorganisms (in the bioreactor) and then the impact of the presence of solid cement paste matrices and thus of adhered biomass (or biofilm) on the reactions (in the exposure chamber downstream). The experiments carried out either with enriched medium or with limited-nutrient medium



**Fig. 7** Schematic representation of the experimental device used for the study of interactions between microorganisms and solid materials during continuous microbial denitrification.

Arrows indicate the direction of the advective flow in the system. **b, c** temperature = 37 °C; stirring = 300 rpm [131]





**Fig. 8** Microscopic observation of the surface of solid matrices colonized by *Hd* (at pH 12, after 600 h of exposure): **a** SEM and **b** epifluorescence [131]

made of cement leachate and with acetate as electron donor showed denitrifying activity of *Hd* up to a feeding medium pH of 12 [131, 151]. The denitrifying reactions were improved in the exposure chamber compared to the those occurring in the bioreactor (complete reduction of nitrate to  $N_2$ , consumption of intermediate nitrites in the exposure chamber) presumably because of the formation of biofilms (Fig. 8b) at the surface of the cement paste specimens: the planktonic (in suspension) cells were inhibited at pH 12, whereas cells in biofilm at the surface of cement paste were still active. This organization of bacterial cells in an attached three-dimensional architecture allows them to avoid wash-out and possibly to resist difficult chemical conditions better [11–13].

Finally, the rates of biologically induced nitrate reduction recorded in biotic experiments were much higher than those measured under abiotic conditions (steel catalysis), although the two experimental campaigns were carried out in very similar chemical conditions [131]. These results highlighted the importance of biologically catalyzed reactions and the need to consider them in evaluating the fate of nitrates in radioactive waste repositories.

### 3.4 Perspectives

Further study will explore the possible synergy phenomena between steel surface catalysis and biological catalysis of nitrate reduction. It will also

evaluate  $H_2$  as an electron donor for microbial catalysis of nitrate reduction in much more details.

On the basis of these experimental results, microbial activity and its influence on the reactivity of several oxyanions and radionuclides in the cell is being modeled [156–158].

## 4 Concluding remarks

Interactions between building materials and microorganisms can occur in many contexts and these interactions can condition the durability, the safety or the aesthetics of numerous buildings and infrastructures. It has been shown, notably through the two examples presented in this paper, that the phenomena occurring in such environments cannot be assimilated to the purely chemical or geo-chemical reactions that they may comprise. One technological issue is the development of test methods that enable the mechanisms of the interactions to be identified and the performances of building materials to be qualified, under representative conditions. This is currently the subject of several research projects, focusing on different contexts of Construction. The test methods should be specific of the environmental conditions considered. Standards have now to evolve to take better account of the building material-microorganism reactions, both from the point of view of test methods and from that of formulation prescription (in

particular, regarding concrete). Among the main scientific issues to be tackled in the next few years, progress still has to be made in the understanding of biodeterioration mechanisms and the influence of biofilm in the intensity and kinetics of alteration. But the impact of the material properties on the activity of microorganisms and their structuration into biofilm for example is very important and very poorly understood as yet, despite the fact that it can condition the durability of the materials and products. These aspects are crucial steps in the development of building materials that perform well in such environments. This is the aim of the RILEM TC 253-MCI, recently created to deal with all these aspects and also with other topics such as bacteria-based protective systems or indoor microbial proliferation. Finally, one constant challenge inherent in research on microorganism-building material interactions stems from its need for multidisciplinary teams involving microbiologists, civil engineers, chemists, process engineers, etc. This constant dialog between researchers of different skills, which is absolutely necessary if we are to understand each other and if our research is to progress, makes it incomparably fascinating and rewarding.

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