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Fungal Deterioration of Barrier Concrete used in Nuclear Waste Disposal

M. Fomina, V. S. Podgorsky, and S. V. Olishevska
Zabolotny Institute of Microbiology and Virology of National Academy of Sciences of Ukraine, Kyiv, Ukraine

V. M. Kadoshnikov and I. R. Pisanska
Institute of Geochemistry of the Environment of National Academy of Sciences of Ukraine, Kyiv, Ukraine

S. Hillier
Macaulay Institute, Craigiebuckler, Aberdeen, AB15 8QH, Scotland, UK

G. M. Gadd
Division of Molecular and Environmental Microbiology, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, Scotland, UK

Fungal biogeochemical activity over a long-term scale may have negative environmental consequences for the management of barrier materials used in nuclear waste disposal. Fungal deterioration of barrier concrete was studied in microcosms simulating a heterogeneous environment with an external source of nutrients for the fungi. Fungi successfully colonized barrier concrete, generally avoiding granite aggregates, and biochemically (by excretion of protons and ligands) and biomechanically deteriorated the concrete. Fungi dissolved the cement matrix leaching structural elements and accumulating them within the fungal biofilm and associated microenvironment. Oxalate-excreting *Aspergillus niger* formed abundant calcium oxalate crystals on the concrete and encrusting fungal hyphae.

Keywords fungi, concrete, biodeterioration, calcium, silicon, oxalate

INTRODUCTION

The safe long-term storage of both existing and future nuclear wastes is of vital importance in protecting the environment. Cement and concrete are used as barriers in all kinds of nuclear waste repositories. Despite the theoretical service life of concrete reaching up to one million years, microbially induced corrosion is one of the most important factors to take into account. All types of building and ceramic materials, including concrete and cement, are deteriorated by microorganisms (Diercks et al. 1991; Gaylarde and Morton 1999; Kikuchi and Sreekumari 2002; Roberts et al. 2002). Although most laboratory and field microbially induced corrosion studies have concentrated on bacterial involvement, other microorganisms, especially fungi, can also influence corrosion processes.

In some environments, e.g., the fuel-water interface and soil, fungi may dominate the microbiota and be a significant cause of corrosion. The potential for mycocorrosion of metal containers selected for storage of nuclear waste in terrestrial environments has been stressed (Geesey 1993). Fungi can be very radiation-resistant and can survive and colonize concrete barriers under severe radioactive contamination. In 1997–1998, extensive fungal growth was observed on the walls and other building structures in the inner part of the "Shelter" built over the fourth Unit of the Chernobyl nuclear power plant damaged in 1986 (Zhdanova et al. 2000). The high radiation selection pressure inside Reactor No.4 of Chernobyl Nuclear Power Plant (radiation α = 500 Bq/cm^2, β = 20,000 Bq/cm^2, γ = 700 mR/h) led to some genetic alteration of fungal strains inhabiting the concrete surfaces (Mironenko et al. 2000). The most frequently isolated microfungi in the first years after the accident and later in the habitats with severe radiation were predominantly melanized species from the genera *Alternaria*, *Cladosporium*, and *Aureobasidium* (Zhdanova et al. 2000).

Fungi can be very desiccation-resistant and many survive on only traces of nutrients (oligotrophy). It was found that *Fusarium* sp., *Penicillium* sp. and *Hormoconis* sp. could degrade hydrocarbon-based lubricants *in situ* and produce organic acids that caused localized corrosion of post-tension structures

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Address correspondence to G. M. Gadd, Division of Molecular and Environmental Microbiology, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, Scotland, UK. E-mail: g.m.gadd@dundee.ac.uk.

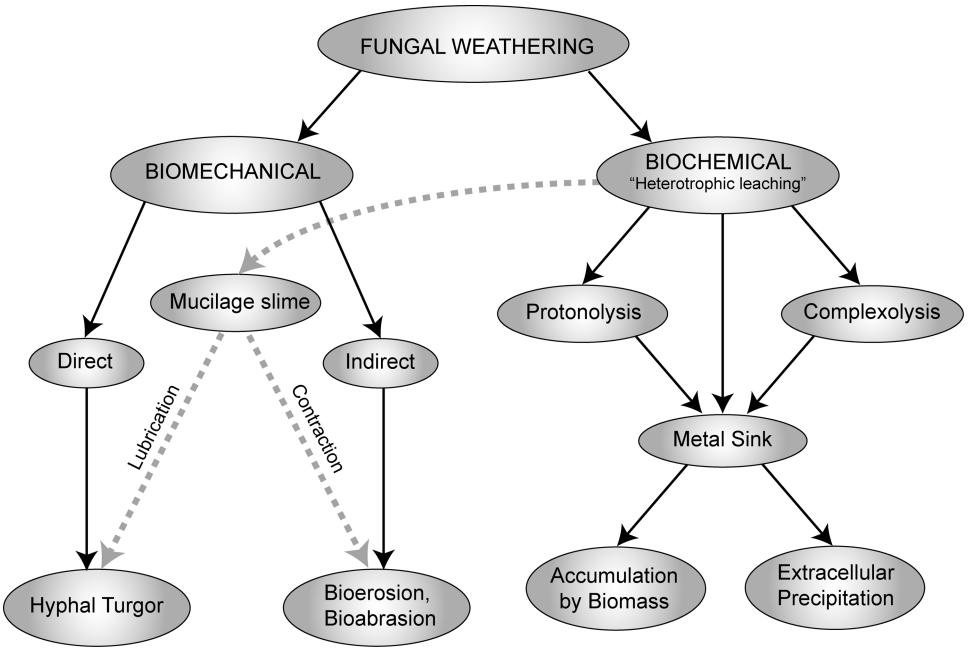

FIG. 1. Mechanisms of fungal bioweathering of mineral surfaces applicable to myco-deterioration of concrete.

used in buildings, bridges and nuclear power plants (Little and Staehle 2001). Other studies have also indicated that fungi play an important role in the deterioration of concrete (Perfettini et al. 1991; Gu et al. 1998; Nica et al. 2000).

The deterioration of concrete by fungi is effected by mechanisms common to fungal weathering of rocks and minerals (Gadd 2007) (Figure 1). Two synergistic actions by which fungi can degrade mineral substrates are biomechanical and biochemical (Burgstaller and Schinner 1993; Banfield et al. 1999; Burford et al. 2003; Fomina et al. 2005a, 2006; Gadd 2007). Biomechanical weathering of minerals by fungi can be direct or indirect. Direct biomechanical degradation of minerals can occur through penetration by fungal hyphae into decayed rocks and by tunnelling into otherwise intact mineral matter which can occur along crystal planes, cleavage, cracks and grain boundaries in, e.g., sandstone, calcitic and dolomitic rocks (Banfield et al. 1999; Kumar and Kumar 1999; Sterflinger 2000; Burford et al. 2003; Money 2004).

Fungal hyphae can exert considerable mechanical force which derives from the osmotically generated turgor pressure within hyphae (Money 2004). Biomechanical penetration into mineral matter is facilitated by thigmotropic reactions (Bowen et al. 2007a) and lubrication with mucilaginous slime produced by fungi that may contain acidic and metal-chelating metabolites (Burford et al. 2003). Thigmotropism or contact guidance is a directed mode of fungal growth towards grooves, ridges and pores in solid material (Watts et al. 1998; Bowen et al. 2007a, 2007b) and may explain how fungal hyphae explore and exploit weakened sites in mineral surfaces. Indirect biomechanical weathering can also occur because of shrinking or swelling effects of the hydrated mucilage produced by many fungi (Warscheid and Krumbein 1994).

All of the processes involved in biomechanical weathering of rocks by fungi are strongly connected with biochemical processes which are believed to be much more important than mechanical degradation (Kumar and Kumar 1999; Burford et al. 2003; Gadd 2007). The two main mechanisms of solubilization of rocks and minerals by fungi are acidolysis and complexolysis, which may be enhanced by metal accumulation in and/or around the fungal biomass (Sand and Bock 1991a, 1991b; Burgstaller and Schinner 1993; Burford et al. 2003; Gadd et al. 2005).

Acidolysis (or proton-promoted dissolution) occurs when fungi acidify their microenvironment as a result of the excretion of protons, organic acids, and the formation of carbonic acid resulting from respiratory CO_2 (Burgstaller and Schinner 1993). Many fungi are able to excrete metal-complexing metabolites which are associated with complexolysis or ligand-promoted dissolution (Burford et al. 2003). These include carboxylic acids, amino acids, siderophores and phenolic compounds (Manley and Evans 1986; Muller et al. 1995; Gadd 1999). Carboxylic acids provide a source of protons for solubilization and chelating anions which can complex metal cations (Devevre et al. 1996). Fungal-derived carboxylic acids with strong chelating properties (e.g., oxalic and citric acid) perform an aggressive attack on mineral surfaces (Gadd 1999; Fomina et al. 2005a). Fungal (*Fusarium* sp.) degradation of concrete proceeded more rapidly than bacterium-mediated (*Acidithiobacillus* sp.) degradation with complexolysis suggested as the main mechanism of calcium mobilization (Gu et al. 1998). Fungi can be very efficient bioaccumulators of soluble and particulate forms of

metals (Gadd and Mowll 1985; Gadd et al. 1984; Gadd and White 1985; White et al. 1995).

Metal immobilization reduces the external free metal activity and may shift the equilibrium to release more metal into aqueous solution (Gadd 1993; Sterflinger 2000). Mobile metal species can be bound, accumulated or precipitated by fungal biomass via biosorption to biomass (cell walls, pigments and extracellular polysaccharides), transport and intracellular accumulation, and extracellular precipitation and formation of secondary mycogenic minerals (Gadd and Mowll 1985; Gadd and White 1985; Fomina et al. 2005a, 2006).

The aim of the present work was to experimentally study the long-term mycodeterioration of barrier concrete and elucidate the fungal role in alterations of concrete geochemistry.

MATERIALS AND METHODS

Fungal cultures selected for this study were strains isolated from toxic metal or radionuclide-polluted soils in Ukraine: *Aspergillus niger* van Tieghem strain 42, *A. versicolor* Veillemin Tiraboschi strain 26, *Fennelia flavipes* (=*Aspergillus flavipes*) Wiley et Simmons strain 38, *Eurotium herbariorum* (=*Aspergillus repens*) (Wiggers : Fries) Link strain 28, *Paecilomyces lilacinus* (Thom) Samson strain 20, *Cladosporium cladosporioides* Fresenius de Vries strain 4, and *Alternaria alternata* (Fries : Fries) von Keissler strain 37 (Zhdanova et al. 2001; Fomina et al. 2005b). These species of fungi have been commonly reported as airborne microflora in dwellings and occupational premises, frequently detected on and potentially causing deterioration of building materials (Koval et al. 1991; Zhdanova et al. 2000; Lugauskas et al. 2003; Shirakawa et al. 2003; Do et al. 2005). Fungal strains were maintained at 25°C on modified Czapek-Dox agar medium comprising: $NaNO_3$ (3.0 $g \cdot l^{-1}$), K_2HPO_4 (1.0 $g \cdot l^{-1}$), $MgSO_4 \cdot 7H_2O$ (0.5 $g \cdot l^{-1}$), KCl (0.5 $g \cdot l^{-1}$), sucrose (30 $g \cdot l^{-1}$) and agar No. 2 (20 $g \cdot l^{-1}$): 14-day-old cultures were used as fungal inoculum.

The concrete samples consisted of chips of barrier concrete manufactured for the disposal of low and intermediate level radioactive waste within reinforced concrete containers ("PivdenAtomSpecBud" Trust Factory, Vyshgorod, Ukraine). Mineralogical analysis of the concrete showed quartz, feldspar, calcium silicates, calcite, calcium aluminate and aluminoferrite. Concrete chips were sterilized by autoclaving 3 times (121°C, 60 min) and were then oven-dried at 100°C at least overnight.

Concrete samples were inserted into Petri dish microcosms (Figure 2). The top external surface area of the chips varied from

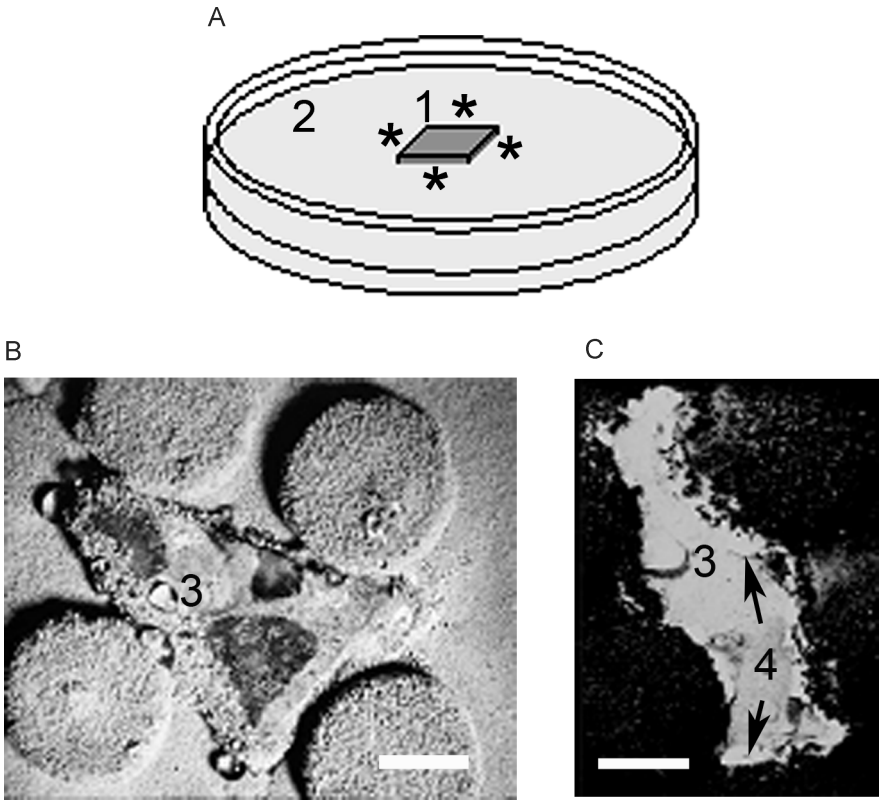

FIG. 2. Design of the concrete-containing microcosm simulating a heterogeneous environment with an external source of nutrients for the fungi. (A) Petri dish microcosms with concrete chip [1] inserted into a hole cut out of the agar medium [2] leaving an approx. 2 mm gap between the concrete chip and agar; discs of fungal inoculum [*] were placed around the concrete chip also leaving a 2 mm gap; (B, C) fungal colonization of the concrete chip and formation of exudates [3] and crystalline precipitates [4]; (B) *F. flavipes* after 1 month exposure and (C) *A. niger* after 1-year exposure. Scale bars are 5 mm.

50 to 600 mm^2 (see Figure 2). The depth (height) of the chips was approximately 10 mm. The microcosm design in this study was intended to provide a nutritionally and mineralogically heterogeneous environment for filamentous fungi in an approach similar to that for previous studies of fungal responses to toxic metals in tessellated agar tile microcosms (Figure 2) (Fomina et al. 2003). A concrete chip was inserted into a hole of corresponding size that had been cut out from the centre of the Czapek-Dox agar in the Petri dish, leaving a 2 mm gap between the concrete and the agar (Figure 2). Four 10 mm diameter disks of fungal inoculum cut from the edge of fungal colonies were placed around the concrete chip, also leaving a 2 mm gap. Plates were sealed with Parafilm and incubated for one month at 25°C and then over one year at ambient temperature. Our concrete chips at the start of the experiments were devoid of significant organic matter, unlike the chips of nutrient broth-saturated mortar used by Shirakawa et al. (2003) in 1-month mortar bioreceptivity tests. The agar medium was the external source of nutrients for the fungi invading the surface of the concrete.

Petrographic and mineralogical observations of concrete surfaces were carried out using a Polmi-A microscope (Carl Zeiss, Germany).

Fungal colonization of the surface of concrete samples was estimated as a percentage (%) of the area covered by the fungal biofilm to the total surface area of the concrete sample. The surface area was calculated using conventional mathematical formulae for surface area measurements of rectangles, parallelograms, triangles, trapezoids and circles, depending on the shape of the concrete chip and the pattern of fungal colonization.

Following light microscopic observations of fungal colonization and deterioration of concrete samples in microcosms, scanning electron microscopy (ESEM, high vacuum mode) was used to analyze concrete transformations by fungi. Air-dried samples of concrete were coated with 30 nm Au/Pd using a Cressington 208 HR sputter coater and examined using a Philips XL30 environmental scanning electron microscope (ESEM) field emission gun (FEG) operating at an accelerating voltage of 15 or 25 kV.

Mineralogical and elemental analyses of concrete transformations were carried out using X-ray powder diffraction (XRD) and energy dispersive X-ray analysis (EDXA) coupled with ESEM. For XRD, samples were mounted onto single crystal silicon substrates and examined using a Panalytical X-pert Pro diffractometer equipped with a position sensitive "X-celerator" detector. Diffraction patterns were identified by reference to patterns in the International Centre for Diffraction Data (ICDD) Powder Diffraction File (PDF) set 51 (2001), using Bruker AXS Diffrac Plus Eva software.

The concentration of calcium, silicon, aluminium and iron leached from concrete and accumulated within the agar was evaluated by Emission Spectrochemical Analysis (CTE-1, USSR) in discs cut out of the agar (dia. 10 mm) at 10 mm from the concrete samples with four discs removed from each microcosm.

The chemical groups within the fungal biomass harvested from the surface of concrete and associated with biofilm exudates sampled from microcosms after 5 months of growth were analyzed using a Carl Zeiss infrared spectrophotometer UR-20 (Germany). Biofilm exudates sampled from microcosms after 5 months of growth were also analyzed for metal content using an atomic absorption spectrophotometer AAS–8500 F (Japan) with reference to appropriate standard solutions.

RESULTS

The fungi in our tests were able to colonize the surface of concrete chips (Figures 2B, C). Colonization depended on the concrete chip dimensions with the percentage of colonized area negatively correlating with total surface area of the concrete (Figure 3). It was observed that colonizing fungi avoided areas of granite aggregates on the concrete surface. Fungi produced drops of exudate on the surface of colonized concrete which were retained for at least 6 months of exposure (Figures 2B, C).

After 1 year of exposure of the concrete chips to the fungi, deterioration symptoms such as expansion and discolouration of the cement matrix, discolouration of clinker grains, crystallization and formation of cracks were observed by light and petrographic microscopy (petrographic data not shown) (Table 1). However, the appearance of the first crystals visible using light microscopy occurred much earlier, after six months of exposure. Other authors have reported observation of crystals on the surface of broth-saturated mortar after three months exposure to *Cladosporium sphaerospermum* (Shirakawa et al. 2003).

Among the fungal cultures tested, *A. niger* demonstrated the highest capacity for concrete deterioration (Table 1). Electron microscopic studies of concrete exposed to *A. niger* over one year showed spalling layers covered with a mycelial net, cracking, and the formation of abundant crystals on the concrete surface and encrusting fungal hyphae (Figure 4). EDXA spectra of

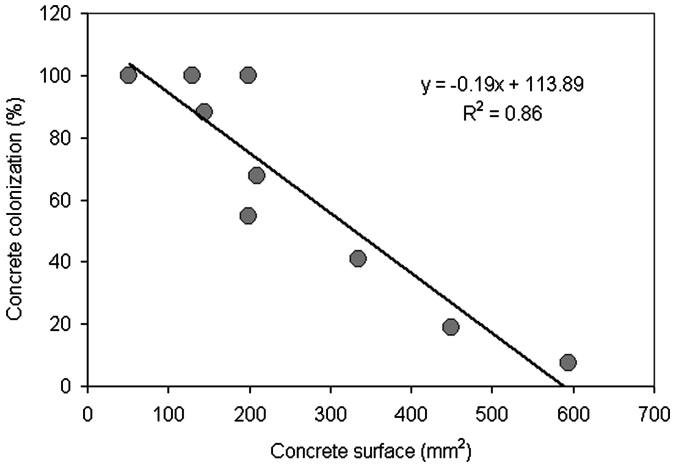

FIG. 3. Correlation between percentage of concrete surface colonized by fungi and total exposed surface area of concrete.

TABLE 1
Deterioration symptoms of concrete exposed to fungi after 1 year observed using light microscopy

Fungi	Concrete deterioration symptoms			
	Cement matrix	Clinker grain discolouration	Cracking	Crystals
Alternaria alternata	Discolouration	−	+	+
Aspergillus niger	Expansion, Discolouration	+	+	+
Aspergillus versicolor	Expansion	−	−	+
Cladosporium cladosporioides	Expansion, Discolouration	−	−	+
Eurotium herbariorum	Discolouration	−	−	−
Fennelia flavipes	Expansion, Discolouration	−	−	
Paecilomyces lilacinus	Discolouration	−	−	−

crystals formed by *A. niger* showed the presence of calcium (Figure 5A). XRD analysis identified crystalline precipitates within the biomass and on the concrete surface as calcium oxalate dihydrate (weddellite) and calcium oxalate monohydrate (whewellite) (Figure 5B). XRD also showed the presence of some quartz in some samples (data not shown).

Infrared spectroscopy of the *A. niger* biofilm and exudates collected from the concrete after five months of exposure indicated the presence of calcium and silicon both coordinated with oxygen (Ca─O, Si─O), and absorption frequency bands typical for carboxylate groups (C─C, C─H, C─O, C═O, O─H) (Table 2). Carboxylate ligands are therefore the most likely to be involved in coordination of cations leached from concrete.

Evaluation of metal and silicon concentrations within exudates drops showed a substantial presence of calcium and silicon compared to aluminium and iron (Table 3). The highest metal concentration was found for calcium being at least 30-times greater than for silicon.

The migration of structural elements of the concrete into the agar was also revealed (Table 3). The highest accumulation within the agar was observed for calcium followed by silicon (Table 3).

TABLE 2
Chemical groups detected by infrared spectroscopy within biomass and exudate drops after 5 months growth of *A. niger* in a concrete-containing microcosm

Absorption frequencies, ν (cm^{-1})		Chemical Groups
Biomass	Exudate	
465	465	Si─O, Ca─O
517	517	Si─O, Ca─O
785	785	Si─O
1090	1080	Si─O
1320	1320	C─O
1630	1650	C─C, C═O
1370, 3260, 3080	2930	C─H
3480	3400	O─H

DISCUSSION

All tested fungal cultures were able to colonize the surface of concrete. Fungi are successful colonizers of rock, mineral surfaces and building materials, and have been reported from a wide range of rock types (Staley et al. 1982; Gorbushina et al. 1993; Sterflinger 2000; Verrecchia 2000; Burford et al. 2003a, 2003b; De Los Rios et al. 2004; Gadd 2007). Despite the apparent inhospitality of the rock environment, the presence of organic and inorganic residues on mineral surfaces or within cracks is thought to encourage proliferation of fungi and other microbes. Waste products of algae and bacteria, dead cells, decaying plant material, dust particles, aerosols and animal faeces can all act as nutrient sources for fungi (Sterflinger 2000; Gaylarde et al. 2001; Gadd 2007). Mineral grains within the host-rock may also serve as a source of metals essential for microbial growth.

TABLE 3
Concentration of structural elements of the concrete within exudate drops [analyzed by AAS after 5 months] and agar [analyzed by emission spectroscopy after 1 year] for *A. niger* concrete-containing microcosms (n = 4, standard error of the mean did not exceed 10%)

Element	Exudates ($mg \cdot l^{-1}$)	Agar (% w/w)
Ca	15.10	2.00
Si	0.50	0.45
Al	<0.10	0.20
Fe	<0.05	0.20

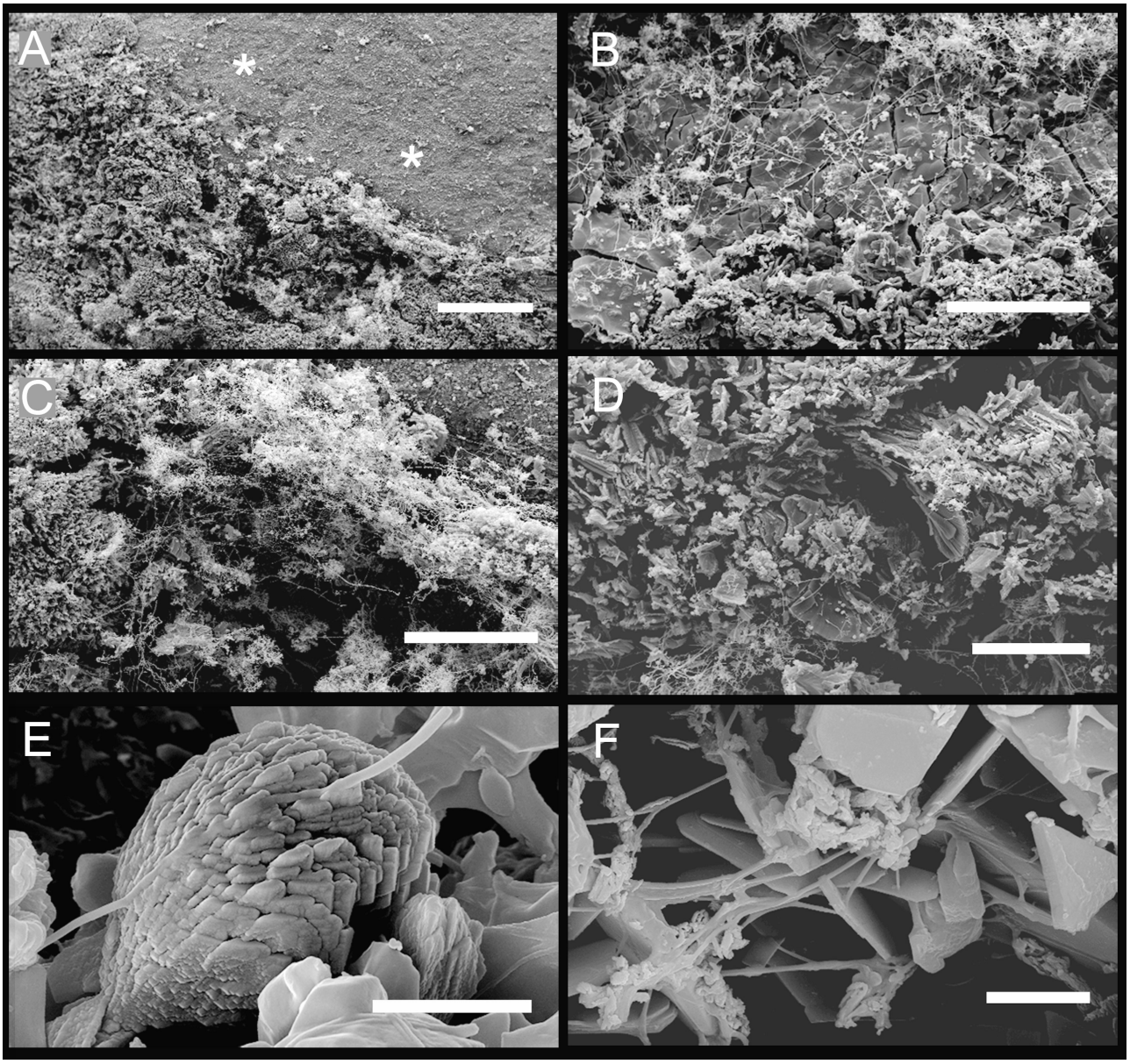

FIG. 4. (A–F) High-vacuum mode ESEM images of air-dried Au/Pd-coated samples showing deterioration and transformation of concrete by *A. niger* after 1 year of exposure in a microcosm: spalling, cracking, and massive crystallization associated with fungal mycelium. In (A), it can be seen that the areas of granite [*] aggregates were much less susceptible to fungal attack. In (E and F), calcium-containing crystals can be seen encrusting the fungal hyphae. Scale bars are (A) 500 μm, (B, C) 200 μm, (D) 100 μm, (E, F) 5 μm.

The transfer of biological material (e.g., fungal spores and other reproductive structures) from external sources including the ability of filamentous fungi to translocate nutrients within the mycelial net may also play a role in the colonization of sub-aerial environments by fungi. The negative correlation between fungal colonization of the concrete surface and the total surface area of the concrete sample may be a consequence of translocation phenomena (Boswell et al. 2002; Jacobs et al. 2002). Mycelial colonization of the borders of the concrete chips perhaps reflects efficient nutrient translocation by the mycelial network from adjacent parts of colonies growing on the agar. However, the limited colonization of the concrete surface at distance may reflect spatial limitations in the ability of the mycelial system to translocate nutrients and also tolerate the extreme alkalinity of the concrete.

Colonisation of rock and mineral substrates by microbes and the development of a microbial consortium is likely to be influenced by physical and chemical properties and interactions based on environmental (e.g., macro/micro-climate) and biological factors resulting in and influencing ecological succession at

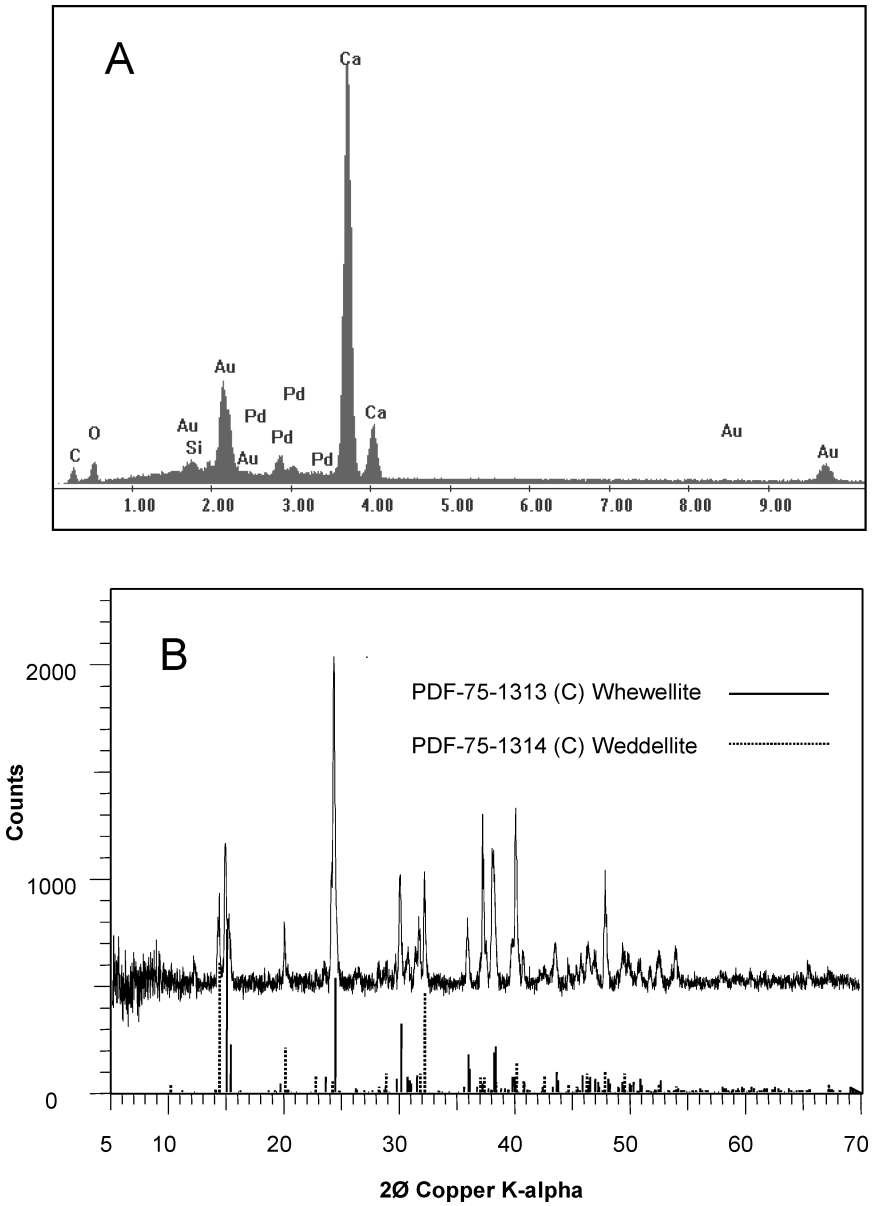

FIG. 5. Typical patterns of elemental (EDXA/ESEM) and mineralogical (XRD) analyses of fungal precipitates on concrete formed by *A. niger* after 1 year of exposure in a microcosm. (A) EDXA spectra of Au/Pd coated samples showing that crystals associated with hyphae contained calcium, (B) XRD identifying fungal crystalline precipitates as calcium oxalate dihydrate (weddellite) and calcium oxalate monohydrate (whewellite).

the micro-scale level (Gleeson et al. 2005, 2006). The succession of a complex microbial population consisting of heterotrophic and autotrophic bacteria, and fungi was thought to be involved in the corrosion of concrete sewage collection pipes (Nica et al. 2000). It has also been suggested that concrete corrosion was accelerated by a mutualistic relationship between *Acidithiobacillus thiooxidans* (formerly *Thiobacillus thiooxidans*) and fungi inhabiting severely corroded sewer pipes (Kyeoung-Suk and Mori 1995).

Microbial attack on minerals may be specific and may depend on the groups of microorganisms involved, e.g., some lichen hyphae overgrew augite and mica but avoided quartz (Aristovskaya 1980). We observed some specificity for the tested fungi colonizing the surface of concrete since the areas with granite aggregates were avoided. Alkaline (basic) rocks are generally more susceptible to fungal attack than acidic rocks (Eckhardt 1985; Kumar and Kumar 1999). Nevertheless, along with other organisms, fungi are believed to contribute to the weathering of silicate-bearing rocks, e.g. mica and orthoclase, and iron- and manganese-bearing minerals, e.g., biotite, olivine, and pyroxene (Kumar and Kumar 1999). Fungal degradation of aluminosilicates and silicates is believed to occur as a result of the

production of organic acids, inorganic acids, alkalis and complexing agents (Rossi and Ehrlich 1990). It is also likely that respiratory CO_2 can enhance silicate degradation by carbonic acid attack (Sterflinger 2000). *Aspergillus niger* has been reported to degrade olivine, dunite, serpentine, muscovite, feldspar, spodumene, kaolin and nepheline, *Penicillium expansum* to degrade basalt, and *Penicillium simplicissimum* and *Scopulariopsis brevicaulis* to release aluminium from aluminosilicates (Mehta et al. 1979; Rossi 1979; Sterflinger 2000). Fungal weathering of limestone, sandstone and marble, is also known to occur (Kumar and Kumar 1999; Ehrlich 2002).

The tested concrete was made of cement, water and aggregates (granite gravel and quartz sand). Calcium silicates (Ca_3SiO_5 and β-Ca_2SiO_4) are the main constituents of the cement prior to hydration (75% of cement by weight) and major contributors to cement strength. Calcium aluminate ($Ca_3Al_2O_6$) and calcium aluminoferrite ($Ca_4Al_2Fe_2O_{10}$) each comprise only 10% of the cement weight. Hydration of calcium silicates leads to the formation of amorphous calcium silicate hydrate as the main product, with a Ca/Si molar ratio of around 1.8 and representing approximately 60% (by volume) of all hydration products and, proportionately, calcium hydroxide occupying a further 20% (by volume) (Double 1983). Although water is essential for concrete hydration and hardening, long-term contact of concrete with water may be detrimental to concrete stability leading to a progressive leaching of the cement phases and removal of calcium. Moisture is also the major factor determining microbial growth on construction materials. The formation of hydrophilic slimes and biofilms by microorganisms colonizing the surface of concrete contributes to moisture retention, and to biophysical/biomechanical deterioration (Gaylarde and Gaylarde 2004). It has been shown that microbial polysaccharides and polyols may bind to the siloxane layers within layered silicaceous minerals, such as micas and soapstone, causing expansion of the crystalline layer, and allowing entry of chelating agents which mobilize the ions stabilising the crystal structure (Gaylarde and Gaylarde 2004).

Environmental scanning electron microscopic studies have previously shown that fungal mycelium is covered with a hydrated mucilaginous sheath which provides a physico-chemical microenvironment for diffusion of mobilized metals and fungal metabolites and re-precipitation of secondary mineral phases (Fomina et al. 2005a, 2006). In the microcosm experiments, tested fungi formed a hydrated biofilm and drops of exudate on the surface of colonized concrete. *A. niger*, which can be commonly isolated from building materials (Shirakawa et al. 2003; Biyik et al. 2005; Do et al. 2005), showed a very high ability to deteriorate concrete that can be attributed to the excretion of strong chelators (e.g., oxalate, citrate). Our previous studies have shown that *A. niger* grown in the presence of calcium–bearing minerals and various nitrogen sources was able to excrete a variety of low molecular weight carboxylic acids into liquid media: oxalic (32–63% of total excreted organic acids), citric (4–12%), gluconic (23–37%), and succinic (10–19%) acid, with the oxalate concentration reaching up to 9 mM. Oxalic acid is a strong organic acid with dissociation constants of $pK_1 = 1.46$ and $pK_2 = 4.40$, which can complex with calcium to form highly insoluble calcium oxalate crystals (solubility product, K_{sp}, at 25°C of 2.32×10^{-9}) (see Gadd 1999, 2007). In this study, oxalic acid produced by *A. niger* reacted with calcium from the cement matrix of the concrete to form the secondary mycogenic mineral phases, whewellite and weddellite.

Organic ligands accelerated the leaching of calcium, silicon, iron and aluminium from concrete. We hypothesise that mobile substances leached from the concrete migrated into the agar through hydrated fungal biofilms via diffusion and translocation (Boswell et al. 2002; Jacobs et al. 2002). Leaching of major constituents of the cement matrix, especially calcium, indicated structural changes, dissolution and ultimately failure of the cement matrix due to fungal action. Calcium leaching was significantly higher than that of silicon, which was similar to previously reported results on cement degradation by sulphur-oxidizing bacteria (Aviam et al. 2004). This can be attributed to the rapid dissolution of calcium hydroxide $Ca(OH)_2$ followed by the incongruent dissolution of calcium silicate hydrate, releasing significantly more calcium than silicon, based on the comparatively low solubility of the major mobile form of silicon "silicic acid," H_4SiO_4, and its oligomeric condensation products "amorphous silica," $SiO_n(OH)_{4-2n}$ (Kaim and Schwederski 1994; Aviam et al. 2004). In water between pH 1 and 9, silicon solubility is about 100–140 mgl^{-1}, but in the presence of cations such as calcium, aluminium or iron, solubility decreases markedly (e.g., down to 5 mgl^{-1} in sea water). Suppression of silicon solubility is even more pronounced in a typical cement environment with a pH range of 12–13. The mobilization of some silicon as the anion SiO_4^{4-} occurring under such very alkaline conditions cannot be completely ruled out.

Scanning electron microscopy of the deteriorated concrete samples revealed spalling layers and deep cracks explored by the fungal mycelium, and precipitation of abundant crystals of calcium oxalate, often associated with and encrusting fungal hyphae. Such massive crystallization of mycogenic minerals may be regarded as expansion attack on concrete inducing more cracking and a dramatic fall in concrete mechanical resistance. The presence of a quartz phase, particularly resistant to weathering, in the fungal precipitates may be explained by fungal interactions with the spalling layers resulting in mechanical entrapment of quartz grains within the fungal biofilm.

In conclusion, our results have demonstrated that fungi can successfully colonize barrier concrete, change their physicochemical microenvironment, deteriorate concrete and mediate redistribution and cycling of calcium, silicon, aluminium and iron (Figure 6). Such phenomena have obvious negative environmental consequences and should be taken into account in the use of concrete in safe nuclear waste disposal, as well as in other building contexts.

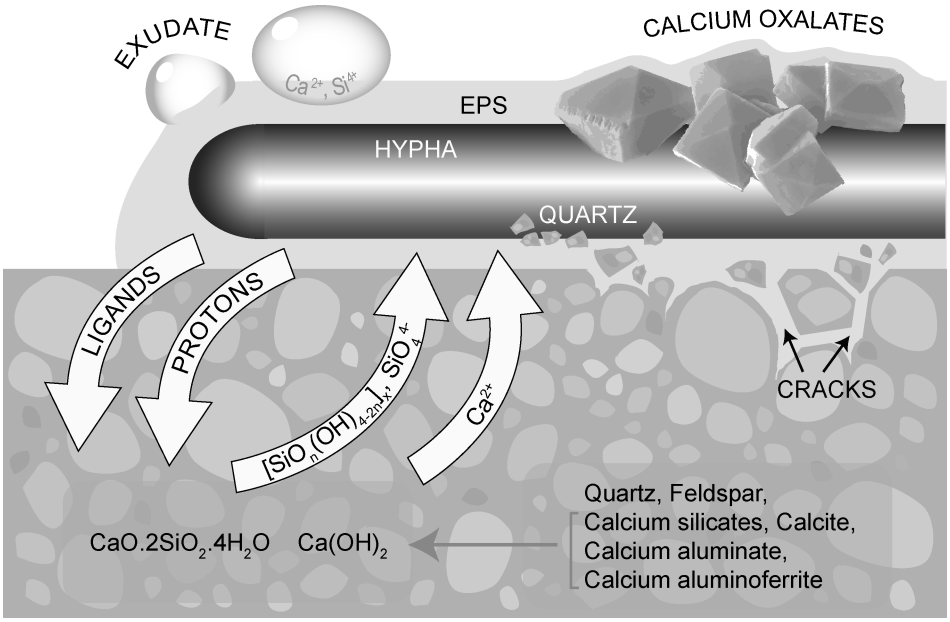

FIG. 6. Simplified diagram of geochemical transformations of concrete by fungi based on our observations. On the surface of colonized concrete, fungi formed a hydrated biofilm consisting of hyphae and extracellular polymeric substances (EPS) retaining moisture, and drops of exudate containing fungal metabolites. Hydrated slime served as a matrix for physicochemical processes, including dissolution-precipitation reactions and diffusion of mobile metal species and organic anions. Mineral composites of concrete are quartz, feldspar, calcium silicates, calcite, calcium aluminate and aluminoferrite (listed in the right bottom corner) with quartz and feldspar derived from added aggregates being largely unreactive. Fungi biochemically attack the concrete surface, excreting protons and ligands leading to the dissolution of concrete, by first reacting with cement paste components, $Ca(OH)_2$ and the backbone of cement paste calcium-silicate hydrate. The mobilized major elements of cement calcium (Ca^{2+}) and silicon (mainly as silicic acids $[SiO_n(OH)_{4-2n}]_x$) were leached from the concrete and accumulated within the biomass and exudates, tending to form complexes with fungal metabolites (e.g., organic acids), and re-precipitated. Oxalate-excreting fungi could form secondary mycogenic mineral phases of calcium oxalates (whewellite and weddellite) on both fungal hyphae and concrete surfaces. Some mechanical interaction between the fungal biofilm and the quartz released from dissolved concrete may also occur. As a result of fungal biochemical and biomechanical deterioration the concrete shows signs of expansion, cracking and spalling.

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