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Susceptibility of biocalcite-modified fiber cement to biodeterioration

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ABSTRACT

Fiber cement panels were treated with urea and various calcium solutions with and without live or dead cells of *Bacillus sphaericus* LMG 222 57, to produce a surface layer of biocalcite; they were then exposed to the environment in São Paulo, Brazil, for 22 months. The calcifying treatment that produced the most colonisation-resistant surface was living bacteria + medium B4 + urea. The resistance of these biocalcified panels was related to their low water absorption, porosity and surface hydrophilicity, linked to the smaller size of the crystals compared to other treatments. Carbonation of the fiber cement before calcification visually increased biofilm formation, but the same calcifying treatment produced highest fouling resistance in this pre-carbonated group. Control samples, without calcification, allowed the development of considerable fouling, sometimes including the filamentous cyanobacterial genus, *Scytonema*, indicative of mature sub-aerial biofilms. There was no significant visual degradation of the calcite crystals associated with the colonising fungi and phototrophs after 22 months' exposure. Biocalcification may safely be used to reduce the fouling-associated darkening of fiber cement and for protection and repair of cementitious building materials.

Introduction

Biocalcification has been used in a wide range of applications (De Muynck et al., 2010a, and references therein). Various biocalcifying microorganisms have been used for protection of cementitious building materials (Chunxiang et al., 2009; Zamarreño et al., 2009) and for remediation of concrete cracks (Ramachandran et al., 2001; Van Tittelboom et al., 2010). Another use for biocalcite could be to lighten and hence improve the reflectivity of darker-coloured materials. For example, fiber cement is much used in Brazil for roofing of low-income housing, and a layer of calcite over the base material could improve reflectivity and hence reduce temperatures within these buildings. However, microbial soiling of the surface would rapidly reduce this effect (Shirakawa et al., 2014) and so it is important to choose a calcification procedure that produces a fouling-resistant material. In literature, the application of water repellents and biocides is described to have a delaying effect on algal fouling of cementitious building materials (De Muynck et al., 2009). But the effect of microbially induced calcite on fouling has not yet been scientifically tested, although there is some anecdotal evidence that biocalcite-repaired buildings do not develop deteriogenic biofilms rapidly (Orial, 2000, cited in De Muynck et al., 2010b). As discussed in De Muynck et al. (2010a), some researchers have tried to develop methods based on the reintroduction of calcite into the pores of limestone. The lime-water technique, i.e. application of a saturated solution of calcium hydroxide, has been tried both for wall painting mortars and for some deteriorated calcareous stones, in order to impart a slight water repellent and consolidating effect (Tiano et al., 1999). As of yet, little success has been achieved in consolidating stone with inorganic materials. Some of the reasons for the poor performance of inorganic consolidants are their tendencies to produce shallow and hard crusts because of their poor penetration abilities, the formation of soluble salts as reaction by-products, growth of precipitated crystals and the questionable ability of some of them to bind stone particles together (Clifton and Frohnsdorff, 1982). In the case of the calcite reintroduction methods, the latter is attributable to the production of many small crystallites,

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Table 1
Treatment of fiber cement for formation of surface calcite layer.

Fiber cement treatment							
	Uncarbonated						None (control)
Code	**A**	**B**	**C**	**D**	**E**	**F**	**O**
Medium for calcite precipitation	B4 + U	$CaCl_2$ + U	B4 + U	$CaCl_2$ + U	B4 + U	$CaCl_2$ + U	–
Bacterial cells added	No	No	Live	Live	Dead	Dead	–
	Carbonated						None (control)
Code	**H**	**I**	**J**	**K**	**L**	**M**	**P**
Medium for calcite precipitation	B4 + U	$CaCl_2$ + U	B4 + U	$CaCl_2$ + U	B4 + U	$CaCl_2$ + U	–
Bacterial cells added	No	No	Live	Live	Dead	Dead	–

B4 = bacterial growth medium (Zamarreño et al., 2009); U = urea; Bacterial isolate = *Bacillus sphaericus* LMG 222 57.

which are not chemically bound to the internal surface of the pore and which are not able to bridge the pores (Tiano et al., 2006).

We exposed fiber cement that had been subjected to various biocalcification treatments to the aggressive environment of São Paulo for 22 months to assess its susceptibility to biofilm formation and biodeterioration.

Materials and methods

Fiber cement panels

Panels were provided by Infibra, São Paulo, Brazil. They were cut into 84 squares 4 × 4 cm and half of them were carbonated in 5% CO_2 at 75% RH for 30 days. 42 small squares (2 × 2 cm) were also used for X-ray analysis without carbonation. The squares were sanitised by immersion for 30 min in 70% ethanol, drying at room temperature in a laminar air flow cabinet for 6 h and exposing each face to UV irradiation for 30 min.

Biocalcification

Bacteria

Bacillus sphaericus (strain LMG 22257, Belgian Culture Collection, BCCM/LMG, Gent) was used for calcification. It was grown in liquid YE + U medium, composed of yeast extract (20 g/L) and urea (20 g/L) in deionized water. Where dead bacteria were required (see Table 1), bacteria were killed by autoclaving for 20 min.

Biocalcification process

Fiber cement specimens were immersed for 24 h in YE without bacteria (control) or in an overnight culture of living or dead bacteria (10^7 CFU/mL). Specimens were then incubated for 4 days in precipitation medium containing urea (20 g/L) and CaCl2.2H2O (50 g/L) (referred to as $CaCl_2$ + U), or in modified B4 medium (Zamarreño et al., 2009), containing calcium acetate (5 g/L), glucose (1 g/L), yeast extract (1 g/L), modified by the addition of urea (10 g/L) (referred to as B4 + U). After the 4 days of incubation, specimens were dried in an incubator at 30 °C for 2 days and a new cycle of biocalcification initiated. Two such cycles were carried out with each face of the square facing upwards. Table 1 shows the various regimes of treatment of the fiber cement squares.

The experiment was carried out in sextupulate, six 2 × 2 cm or six 4 × 4 cm squares being incubated in each large Petri dish with the appropriate medium. All incubations were carried out at 28 °C for 8 days.

Exposure of panels

The fiber cement squares were exposed at 45° facing South in a wooded area within the campus of the University of São Paulo, Brazil. They were placed approximately 1 m above the ground and left for 22 months.

Analyses

Macroscopic analysis

After 16 and 22 months' exposure, samples were examined with the naked eye and photographed. At the end of the exposure period (22 months) they were scanned and the digitalised images stored for analysis.

Microscope analysis

A confocal microscope (DCM-3D) was used to examine 3 areas in the dark centre of each of the 42 samples. Scanning electron microscopy with low vacuum (ESEM) was used to image the biofilms, using a Quanta 600 FEG (FEI) microscope with a pressure of 400 Pa and back scattering GAD detector. No pre-treatment of the samples was necessary.

Colour difference

Five cm^2 in the centre of each panel were measured using the L*a*b* system (Shirakawa et al., 2011). The dark/light value (L*) was determined and change in colour expressed as ΔE.

Water absorption

Samples were dried in a ventilated oven at 105 °C until they reached constant weight (±0.05%) and then immersed in 125 ml of distilled water in a Petri dish for 60 s. Free surface water was removed with moist gauze and the samples reweighed. The immersion process was repeated generating cumulative water immersion for 300, 600, 1200 and 1800 s. Water absorption kinetics was determined as the slope of the linear regression of the cumulative water absorption (g/100 g) against square-root of time. This figure gave the sorptivity of the sample (Wilson et al., 1999).

X ray diffraction

This was used to check the calcite crystals formed on the fiber cement surface. The analyses were carried out on non-pre-carbonated 2 × 2 cm samples, fixed to a 30 mm support. Powder diffraction analysis (XRD) was carried out in a Bragg-Brentano diffractometer (Panalytical X'Pert Pro) with a fine long focus CuKα tube anode, applying 45 KV/40 mA. The detector used was the X'Celerator, a multiple strip position sensitive detector that allows measurement in a shorter time than a point detector. The scans were obtained from 10 to 70°2θ with a step size of 0.017°2θ and 58 s

Table 2
Percentage of calcite and vaterite present in the crystals for each treatment, determined by XRD.

Treatment	A	B	C	D	E	F	O
Calcite	90	75	89	100	84	67	80
Vaterite	–	15	8	–	–	23	–