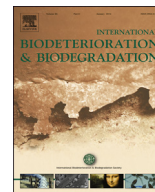




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Susceptibility of biocalcite-modified fiber cement to biodeterioration

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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.

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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 deteriorogenic biofilms rapidly (Oriol, 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% CO₂ 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 (10⁷ 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 CaCl₂ + 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 sextuplicate, 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 cm² 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	–

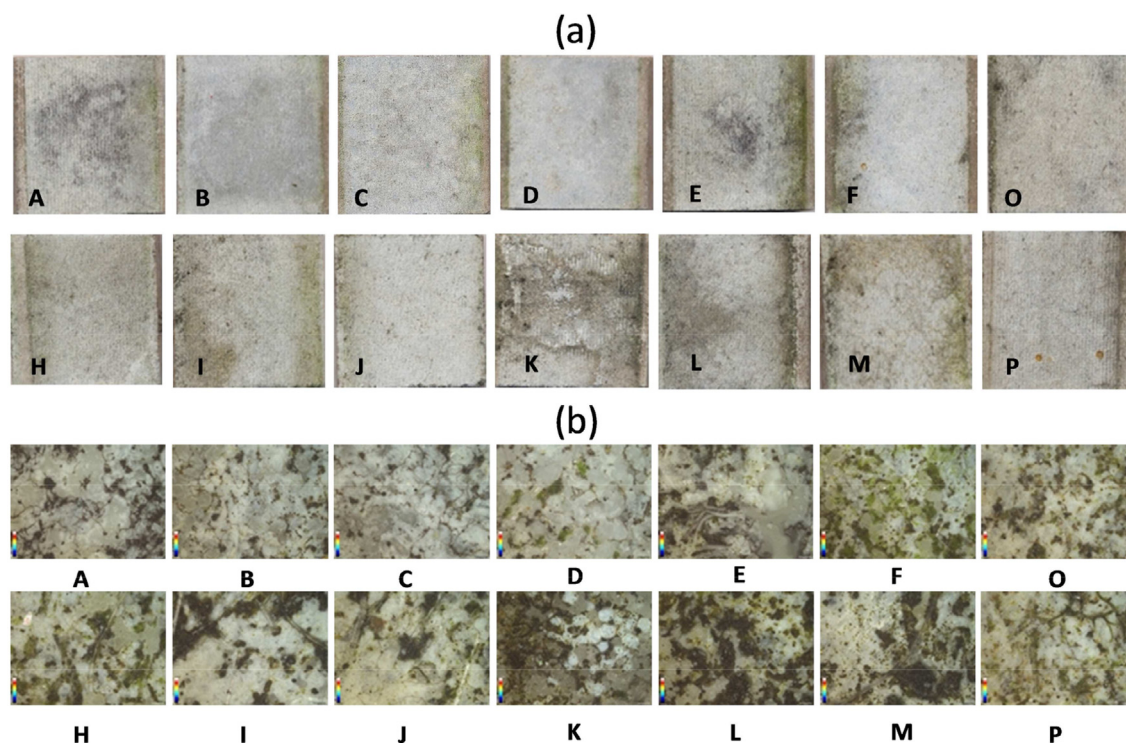


Fig. 1. Soiled fiber cement panels after 22 months exposure in São Paulo. Upper rows of each pair show uncarbonated and lower rows pre-carbonated samples. (a) macroscopic appearance; (b) compound confocal microscope images at low magnification.

time/step, under $\frac{1}{2}$ divergent slit configuration. Each measurement took 18 min. The Reference Intensity Ratio (RIR) analyses were performed using HighScore Plus (Panalytical, version 2.2c). RIR is an internal standard value obtained by relating the most intense peak of a phase to that of corundum (Al_2O_3). The database of known powder diffraction patterns supplied by the ICDD (International Centre for Diffraction Data) contains the RIR calibration factor for many phases (Snyder, 1992). This method has been used successfully in mineralogical semi-quantitative analyses of carbonates (Al-Jaroudi et al., 2007).

Porosity and contact angle

Porosity was measured by the vacuum immersion system described in Safiuddin and Hearn (2005). The samples were weighed dry (m_1), wet (m_2) and in hydrostatic conditions (m_3). Porosity (P) is given by

$$P = \frac{m_2 - m_1}{m_2 - m_3} \cdot 100$$

For measurement of hydrophobicity/hydrophilicity, contact angle of a water drop with the surfaces was measured. After treatments all samples were washed with deionized water and dried at 40 °C to constant weight. The contact angle sessile drop method was carried out with the EasyDrop device (Krüss) using 1 μL deionized water. Contact angle was measured repeatedly at various times from 4 to 240 s and the average calculated.

Statistical analysis

Colour change (ΔE) and L^* data was analysed by the two-factor factorial ANOVA test, using Statistica 12. The two factors considered important were: 1) Pre-carbonation (Uncarbonated vs Carbonated),

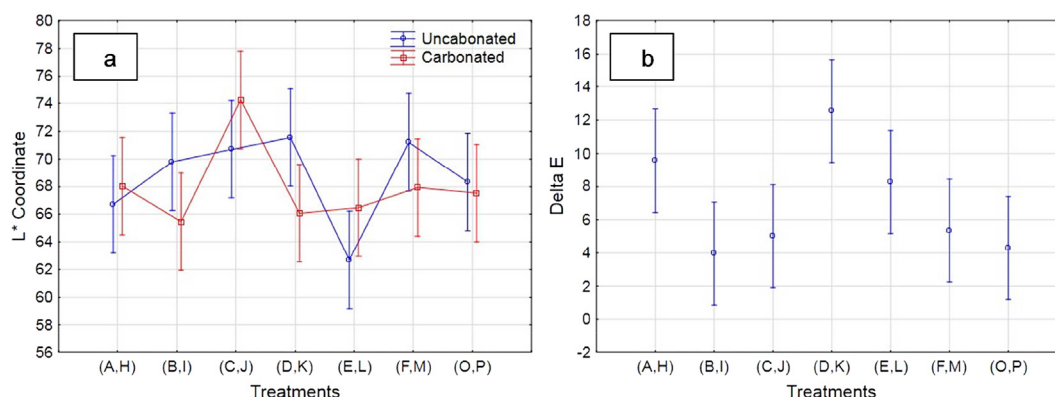


Fig. 2. a) Darkening of fiber cement panels measured as L^* ; there was interaction between pre-carbonation and treatment in the statistical analysis ($p = 0.02304$) b) Colour difference (ΔE) as a dependent predictor showed significant difference only within the 7 treatments ($p = 0.00163$) and not in the interaction between carbonation and treatments ($p = 0.10028$).

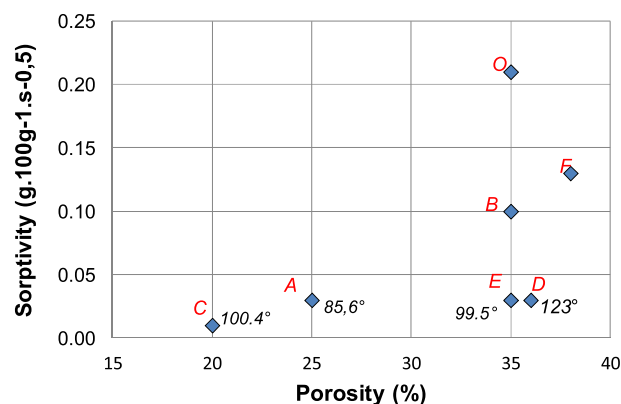


Fig. 3. Water sorptivity, porosity and water contact angle of biocalcite-modified fiber cements before exposure. Treatment shown as red letters and contact angle shown as numbers, next to the points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2) Treatments (culture medium and bacteria). As all factors were fixed, hypotheses about the main effects and factor interactions were easily formulated.

Results and discussion

All treatments resulted in calcium carbonate precipitation on the surface of the fiber cement samples, shown by ESEM (See Figs. 4 and 7) and by X-ray diffraction (Table 2). Treatments B, C and F produced, in addition to calcite, vaterite, which is an unstable form of calcium carbonate and is rapidly converted to calcite. The control (O) contained 80% calcite, doubtless used as a filler, and treatments

B, E and F gave values below, or little different from this. These were the treatments that did not contain living bacteria.

The macroscopic and microscopic appearances of the panels after 22 months' exposure are shown in Fig. 1(a and b). See complement of Figure 1 in supplementary material.

Samples that were carbonated before calcification appeared to become more intensely fouled with fungi and phototrophs than uncarbonated samples (Fig. 1), although the statistical difference in ΔE between carbonated and uncarbonated samples was not significant (Fig. 2), probably because of the heterogeneous nature of the biofilm. Carbonation reduced the average pH levels of the samples from 11 to 8. This reduction of superficial pH that occurs with carbonation is well known to encourage microbial colonisation and growth (see, for example, Shirakawa et al., 2003) and so the increased fouling seen visually was not unexpected. Fungal colonisation, seen on microscope examination of samples at 16 months (results not shown), preceded the appearance of phototrophs, a succession that was also found on freshly painted surfaces exposed in São Paulo (Shirakawa et al., 2002).

The samples treated with B4 + U and live *B. sphaericus* LMG 222 57 (treatments C and J) produced the modified fiber cement that best resisted biodeterioration, as shown by their higher L^* (less darkening) and visual inspection, after 22 months of exposure at the test site (Figs. 1 and 2a). This is related to the low water absorption and porosity and hydrophobicity showed by contact angle of treatment C (Fig. 3); for treatments B, F and control (O) the contact angle was not measured due to rapid absorption of the water drop; high absorption rates interfere with the measurement of water contact angle (Duncan et al., 2005).

Treatment C was also the one that produced the smallest crystals of calcite (compare C and D in Fig. 4), influencing its physical

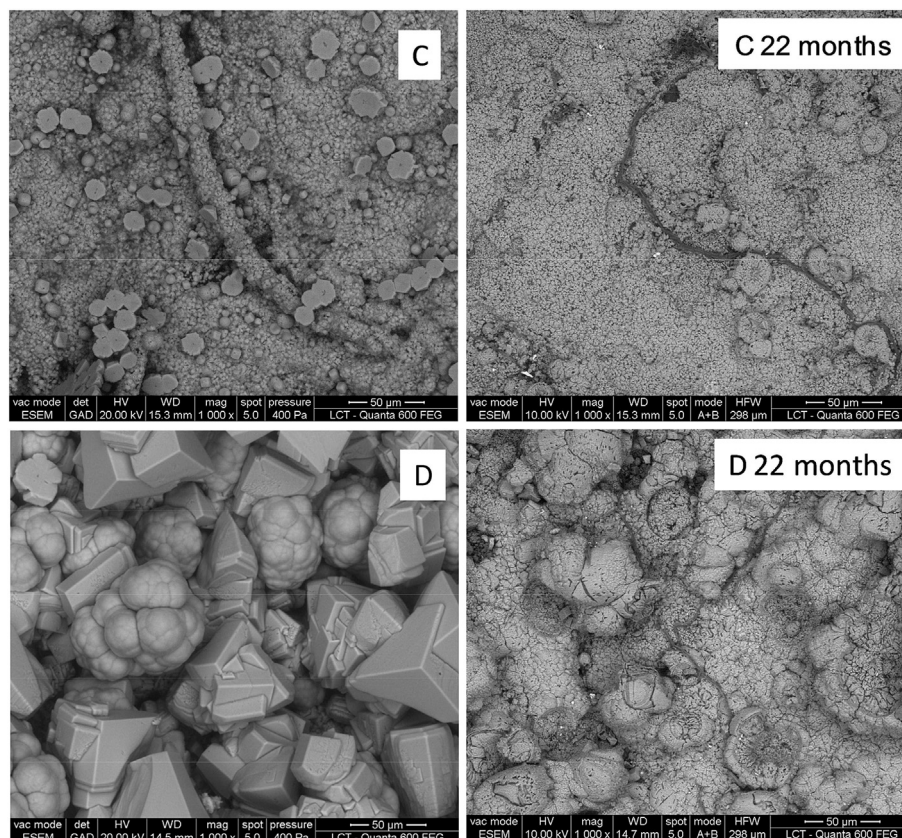


Fig. 4. ESEM micrographs of uncarbonated treatments C and D before exposure (left), showing difference in crystal size, and (right) after 22 months exposure. Bar markers 50 µm.

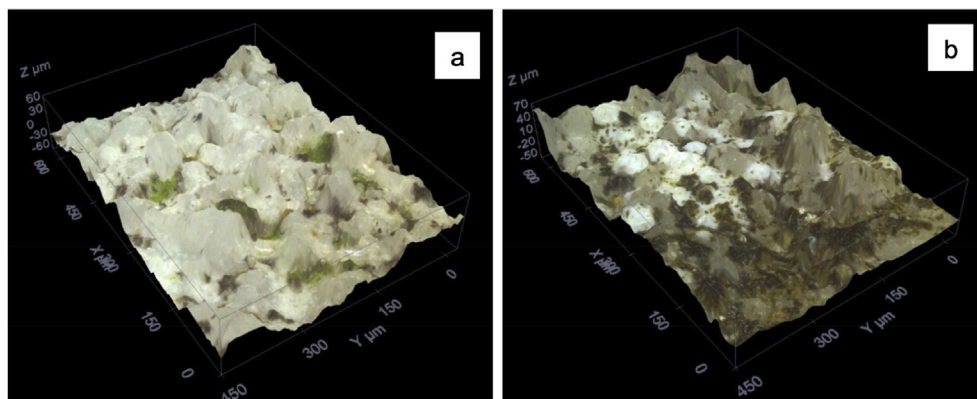


Fig. 5. Confocal microscope images of samples D (a) and its pre-carbonated form, K (b), showing phototrophic and fungal biofilms after 22 months' exposure. Axes (μm): x, 0 to 600; y, 450 to 0; z, –60 to 60 in (a) and –50 to 70 in (b).

properties. Sample J, the equivalent pre-carbonated cement, showed the best resistance to biodeterioration among this group.

The ESEM photo of sample C (Fig. 4) shows that the biocalcite layers are composed of smaller, more adherent crystals that formed a more homogeneous layer on the fiber cement surface, covering the previously exposed fibres, as well as the cement surface. This small-crystal structure is associated with low water absorption (Fig. 3) and these facts help to explain why this particular biocalcite is more resistant to microbial fouling than the others. Treatment D, which produced 100% calcite on the fiber cement surface (Table 2), also gave good results in the biodeterioration test, but its resistance was considerably reduced by pre-carbonation in treatment K (Figs. 1, 2b and 5). ESEM showed that this calcification treatment produced larger crystals, with larger inter-crystalline spaces (Fig. 4), corresponding to a higher water absorption (Fig. 3). These two samples (D and K) showed high phototroph colonisation (Figs. 1b and 5), which might be expected in more hygroscopic materials. Control, uncalcified, panels were amongst the worst biofouled. Indeed, biofilms on the carbonated control samples even included the filamentous cyanobacterial genus *Scytonema* (Fig. 6, probably *Scytonema myochrous*, Büdel et al., 2002), which is found only in well-developed subaerial biofilms (Gaylarde et al., 2012).

The biodeterioration determined in these tests was aesthetic, showing as discoloration of the panels. There was no apparent

degradation of the crystals close to the microbial cells (Fig. 7). This suggests that any acids produced by the cells were insufficient to corrode the calcite in this time. In addition, little exopolymer, a cell product that could be involved in degradation of calcite (Wanly and Seal, 2004) was seen in the biofilms. Some deterioration of the crystals was seen after 22 months (compare treatment D before and after exposure in Fig. 4), but this was not localised to colonised areas and was abiotic.

Conclusions

Fiber cement treated with living bacteria + medium B4 + urea produced a calcified layer that was more resistant to microbial fouling over 22 months of exposure to the environment in São Paulo, Brazil, than other biocalcified panels, whether the fiber cement was precarbonated, or not. The high resistance was related to low water absorption, porosity and surface hydrophilicity, which were linked to the smaller size of the crystals compared to those produced by other biocalcifying treatments. There was no significant visual degradation of the calcite crystals associated with the colonising fungi and phototrophs after 22 months' exposure,

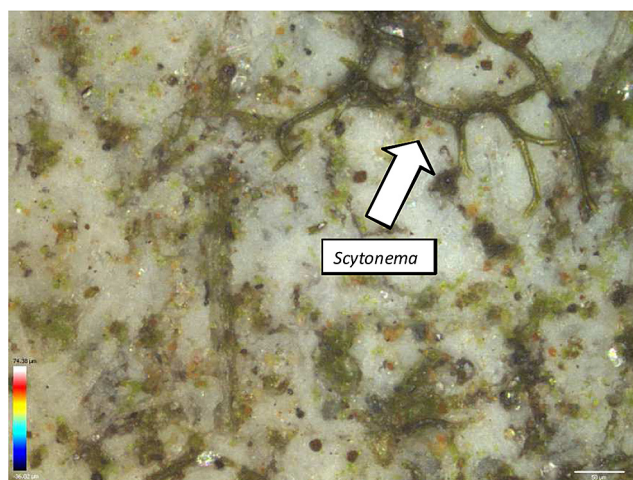


Fig. 6. Filaments of the cyanobacterium *Scytonema*, seen growing across the surface of non-calcified carbonated fiber cement after 22 months' exposure in São Paulo. Bar marker 50 μm.

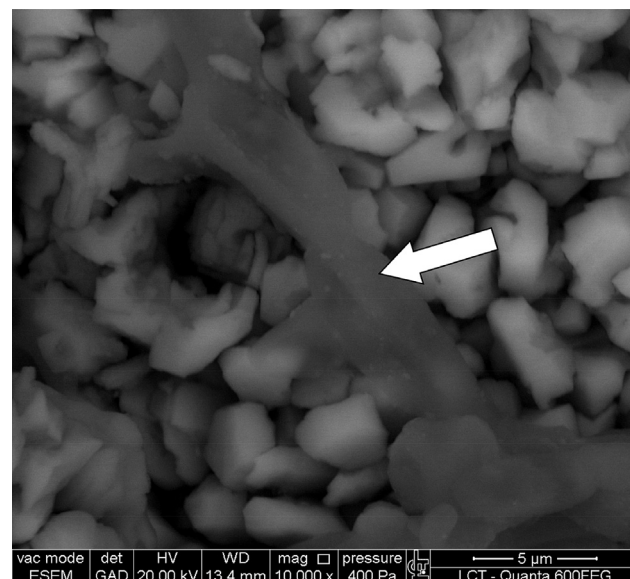


Fig. 7. ESEM micrograph of sample D after exposure, showing microbial filaments (arrow) on the biocalcite layer.

suggesting that biocalcification by this method can be safely used to avoid the fouling-associated darkening of fiber cement tiles and to protect or repair cementitious building materials without increasing biodeterioration potential. This treatment would be an improvement over the traditional limewater wash that has been used in the past in terms of longevity. Its future market acceptance will depend on the life cycle cost of a solution, influenced by business model and scale, which cannot be assessed in laboratory investigation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ibiod.2015.04.003>.

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