COMBINED PHYSICAL AND BIOLOGICAL GEL-BASED HEALING OF CEMENTITIOUS MATERIALS

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Keywords: Self-healing cementitious materials, *Sporosarcina pasteurii*, Alginate

ABSTRACT

The outcomes of a preliminary experimental programme into a gel-based healing system that allows immediate healing of cementitious materials followed by longer-term development of robust healing through biological processes are reported. Alginate gels protect and maintain the viability of encapsulated microorganisms and have been used for protection of these and other cargoes in various situations. Soluble alginates form relatively strong, stable gels on contact with cations such as calcium, and can form gels on contact with cementitious materials. Calcium alginate gels were formed both in isolation and in contact with cementitious surfaces and assessed for their ability to protect encapsulated microorganisms (*Sporosarcina pasteurii*) from the harsh cementitious environment and their subsequent ability to generate calcium carbonate within the gel structure via urea biodegradation.

1. INTRODUCTION

Bacterial generation of calcite minerals can create or maintain construction materials such as soils and cementitious substances [1]. In the latter, e.g. mortar or concrete, microbial activity is significantly hindered due to the highly alkaline pH and difficulty in accessing the required nutrients and moisture. Efforts have been made to provide for microbial survival through immobilisation or encapsulation in a range of materials such as polyurethane [2, 3], silica gel [3] or expanded aggregates in conjunction with bacterial spores [4]. There has been some success in sealing (i.e. reducing permeability) materials through generation of calcite, but significant strength regain is often attributable to the immobilising matrix rather than the calcite itself [3].

Alginate gels have found use in protection and delivery of biological payloads such as bacteria [5]. They are formed simply through addition of liquid sodium alginate to a solution of calcium ions. Their ability to support biological payloads in relatively harsh environments has identified them as a potential support matrix in cementitious materials as part of an autonomic system for crack healing and repair. The presence of calcium ions in cementitious materials means alginate could form gels, and so heal or seal flaws, on contact, giving an immediate chemical effect upon crack formation and healing initiation with potential longer term effects through biological action. The potential for alginate to act in this way has been explored in this preliminary study.

2. MATERIALS AND METHODS

Sporosarcina pasteurii (NCIMB, UK; strain NCIMB8221), an aerobic, ureolytic bacterium, was grown in nutrient broth (Oxoid CM001, 13 g/L) amended with urea (20 g/L) for 48 hours at 30ºC. Four experiments were performed:

(i) Optimisation of S. pasteurii survival in alginate beads. Bacteria were mixed with sodium alginate (2.4, 2.8 and 3.2% w/w) and growth medium (Oxoid CM001 nutrient broth – 3 g/L; urea – 20 g/L; $NaHCO_3$ – 2.1 g/L; NH_4Cl – 10 g/L). Alginate beads were formed by pipetting 10 μl aliquots into calcium chloride ($CaCl_2$) solutions in deionised water (1, 3 and 5% w/w). Sets of beads were prepared in triplicate for each combination of alginate and $CaCl_2$ concentrations. After incubation for 7 days at 30ºC, active bacteria were identified by dehydrogenase assay (briefly, incubation for 4 hours at 30ºC with 5 mM 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) [6] then imaging on a Nikon LV100D epifluorescence microscope). Average cell counts were taken from ten images.

(ii) Bacteria survival on mortar surfaces. Portland cement mortar specimens (27 in total; surface dimensions 44x32 mm; water:cement ratio 0.45, sand:cement ratio 3.0) were cured for 28 days in water and split into 3 equal groups with further curing for 7 days in either air, water or $CaCl_2$ solution. Each group was sub-divided into 3 triplets (A, B and C) with the following solutions applied to the surface: A - alginate/growth medium; B - alginate/growth medium followed by $CaCl_2$ solution; C – alginate/growth medium and $CaCl_2$ solution mixed manually on surface. Optimal concentrations of alginate and $CaCl_2$ determined in *(i)* were used, both containing *S. pasteurii*. Following incubation at 30ºC for 10 days surfaces were examined using CTC, as above. Cell counts were determined manually using ImageJ software [7]. Surface material was removed by abrasion and examined by x-ray diffraction (XRD).

(iii) Bacterial strength development in artificial flaws. 9 mortar beams (mix as above; 25x25x140 mm) with a notch (1mm wide, 10 mm deep) at the mid-point were cured under water for 7 days and divided into 3 equal groups (X, Y and Z) with the following added to the notch: X – alginate/growth medium solution only; Y – alginate/growth medium solution with *S. pasteurii*; Z - alginate/growth medium solution and $CaCl_2$, both with *S. pasteurii*, placed in alternate layers (0.5 ml aliquots). Again, optimal alginate and $CaCl_2$ concentrations were used. After incubation at 30ºC for 21 days, 3-point bending tests were performed on the beams and surfaces examined microscopically as above.

(iv) Physical healing from alginate. 3 mortar beams (75x75x225 mm, mix as above) were fractured in a 3-point bending test, rejoined with sodium alginate solution (1, 2 and 3% w/w) and stored for 24 hours before repeating the bending test.

3. RESULTS AND DISCUSSION

(i) High levels of $CaCl_2$ (5% or 0.45 M) inhibited cell activity (Table 1), likely due to high salinity, with maximum activity at the median value of 3%. It is unclear why growth was hindered at 1% $CaCl_2$, though alginate beads did not form in such conditions and so continued bioavailability of $CaCl_2$ may be an issue. The optimal combination of alginate and $CaCl_2$ was 2.8% and 3% respectively.

Table 1: Active cell counts in alginate beads (cells per ml sample) – effect of alginate and $CaCl_2$ content. Presented as average (n = 3, standard deviation in brackets).

		Calcium chloride		
		1%	3%	5%
Alginate	2.4%	0 (0)	$8.9x10^4$ ($1.2x10^5$)	0 (0)
	2.8%	0 (0)	$4.5x10^5$ ($3.0x10^5$)	0 (0)
	3.2%	$1.4x10^5$ ($2.9x10^5$)	$2.4x10^4$ ($5.5x10^4$)	0 (0)

(ii) Activity of bacteria on mortar surfaces was determined by presence or absence of moisture. The applied alginate solution was, in some cases, found to have flowed off the surface leaving a dry environment. Specimens where it remained had observably moist areas (liquid or gel form). These areas contained amorphous gel layers in which large numbers of cells were embedded (

Figure **1**). Counts presented in Table **2** have significant variability due to loss of gel/moisture on certain specimens, which had no detectable active cells present. Little can be inferred as to the effectiveness of the application methods used, but it is clear that alginate gel can support significant numbers of active bacteria on a fresh mortar surface.

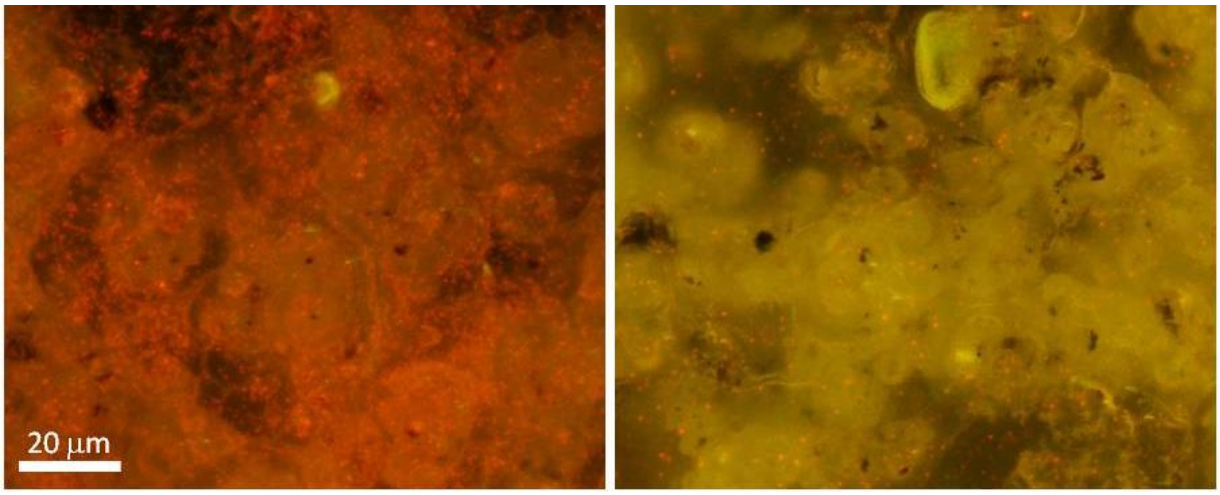


Figure 1: Images of mortar specimen surfaces showing gel and active cells (red).

Table 2: Active cell counts from gel-covered mortar surfaces (cells per mm^2). Data presented as average (n = 3) with standard deviation in brackets. Data are lower-bound values due to obscuration by gel and counting of large numbers.

		Alginate application method		
		A	B	C
Final curing conditions	Air	$7.0x10^3$ ($1.2x10^4$)	0 (0)	0 (0)
	Water	$5.2x10^4$ ($5.5x10^4$)	$3.5x10^3$ ($3.0x10^4$)	0 (0)
	$CaCl_2$	$2.7x10^4$ ($3.6x10^4$)	$2.1x10^4$ ($2.2x10^4$)	0 (0)

(iii) As in *(ii)* retention of alginate solutions in mortar beam notches was difficult and so no variation in flexural strength was demonstrated (Table 3). Apparently crystalline precipitation was observed on areas of the surface of a number of beams where gel was retained, and only in beams where *S. pasteurii* were present (Figure 2). XRD analysis of this precipitate indicated that it was calcite. Active bacteria were detected infrequently, possibly due to lack of moisture by the time of testing.

Table 3. Flexural strength of beams (kPa) from 3-point bending test.

Alginate application method	Average	Standard deviation
X	2460	80
Y	2389	279
Z	2303	97

(iv) Alginate gels formed a stable bond in fractured mortar beams, but had a relatively weak effect on mechanical performance (Table 4). Only up to 3% of the initial flexural strength was regained with 3% alginate, although the gel was able to support the self-weight of the cracked mortar beam during testing. Although this is relatively insignificant in the case of a fully fractured beam, its behaviour in smaller macroscopic and microscopic cracks may be different.

Table 4: Flexural strength (kPa) of mortar beams and after healing with alginate.

% alginate	Mortar	Alginate-healed
1	1648.4	0
2	1708.8	19.2
3	1651.2	49.8

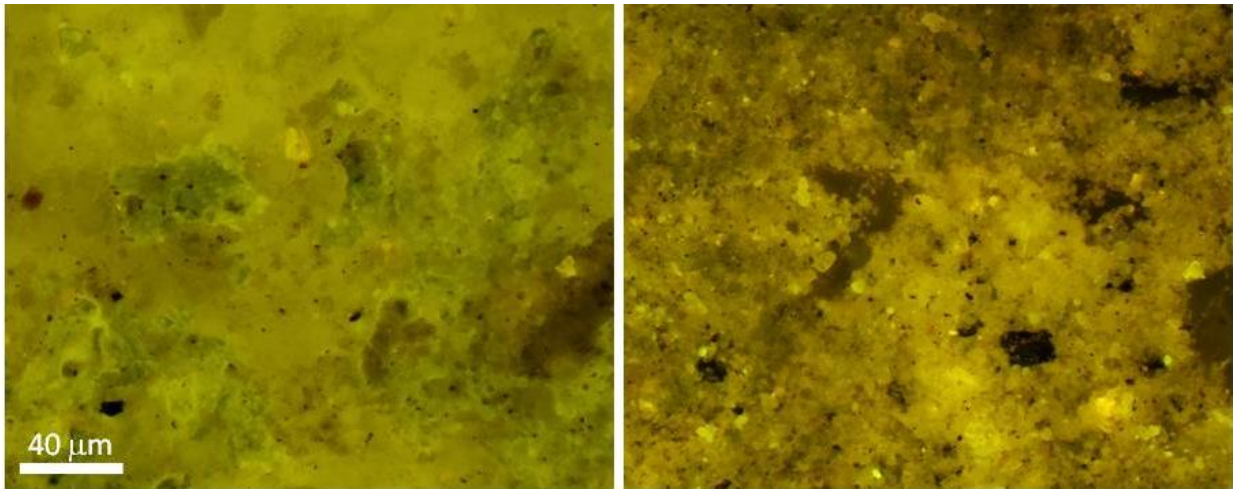


Figure 2: Surface layer development adjacent to notch on mortar beams (group Y, with bacteria, left) and lack of layer development (group X, without bacteria, right).

4. CONCLUSION

This paper reports the preliminary outcomes of an ongoing study, demonstrating that alginate gels optimised for survival of *S. pasteurii* are capable of supporting active cells over several weeks on highly alkaline mortar surfaces. There are indications that calcite precipitation occurs, although strength regain is yet to be demonstrated.

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