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1 **Use of Colloidal Thickeners to Inject and Retain Bacterial**
2 **Growth Media to Repair Cracked Concrete**

3
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10

11 **Abstract** – This study aimed at developing a bacteria-based method to
12 repair cracks in concrete (crack opening < 1 mm) that would be suitable for full-
13 scale operations and economically viable. CaCO₃-producing bacteria were
14 injected into calibrated cracks with bioavailable calcium and nutrients in a
15 suspension thickened with a combination of Welan gum and Attagel® 50. These
16 thickeners have synergistic effects, enhancing viscosity and shear-thinning
17 behavior. These rheology characteristics made injection without drainage possible
18 for crack widths ranging from 150 to 800 µm. Bacterial growth and the associated
19 CaCO₃ biomineralization were enhanced in the presence of thickeners.

20

21 **Keywords:** *bacteria, cracks, concrete, thixotropy, thickeners, CaCO₃*

22 1. Introduction

23 Cementitious materials are prone to developing cracks. Cracks narrower
24 than 100 μm can be closed by natural self-healing, mainly due to the formation of
25 calcium carbonate (CaCO_3) [1]. Larger cracks, however, can dramatically reduce
26 the lifespan of a structure by allowing aggressive compounds to penetrate deeper
27 layers of materials and accelerate deterioration. Fine cracks (<1 mm) are currently
28 mostly repaired with synthetic resins, which are easy to use, inexpensive, and
29 watertight. These compounds are, however, pollutants. In addition, the treatment
30 has to be repeated every 10 to 20 years due to chemical degradation of the
31 polymers, resulting in additional costs and emissions [2]–[4]. Supplemental
32 CaCO_3 (mostly calcite) formed through bacterial activity [5] could provide an
33 environmentally friendly alternative to synthetic resins: calcite is stable and long-
34 lived in most concrete service environments. Consequently, a growing number of
35 studies are focusing on producing self-healing concrete or repairing cementitious
36 structures through biomineralization [6]–[9].

37 When used as repair agents, calcite-producing bacteria have to be injected
38 into cracks. Maintaining the bacteria alive and able to produce enough calcium
39 carbonate remains a challenge, as cracks are hostile environments. Moreover,
40 conventional liquid bacterial cultures cannot keep bacteria, nutrients, and water
41 inside the cracks. Therefore, a specific growth medium has to be developed. The
42 literature has already reported on several bacteria immobilization techniques
43 already in use, such as polyurethane foam [10], glass beads [11], sand [12], [13],

44 silica gel [14]–[16], and agar gel [17]. Natural colloidal thickeners, however,
45 seem to have the most potential for designing an injectable medium to repair
46 cracked concrete. Indeed, these compounds are nonpolluting and inexpensive as
47 well as being well suited for bacterial immobilization [18]. Besides, most
48 colloidal thickeners have highly shear-thinning and/or thixotropic behavior [19],
49 which enhances injection efficiency. Clays, in particular, can entangle bacteria in
50 biofilm-like networks providing protection against protozoa predation,
51 desiccation, and washout [20]–[23]. Used in combination, clays and
52 polysaccharides often show synergistic effects, increasing the stability of the
53 suspension and the effect of both thickeners, making highly shear-thinning and
54 thixotropic suspensions used for various applications [24]–[29].

55 The aim of this study was to use the synergistic effect of two colloidal
56 thickeners—Welan gum and Attagel® 50 palygorskite clay—to create a thickened
57 medium suitable for injection into concrete cracks from 150 to 800 μm in width.
58 This study focuses first on the rheological characterization of the thickened
59 growth medium using different Welan and Attagel concentrations to determine the
60 most promising composition. Second, the behavior during injection and
61 subsequent drying of the selected growth medium was tested on calibrated cracks
62 in mortar samples. Lastly, the bacterial activity and the associated amounts of
63 bioprecipitated calcite were assessed.

64

65 2. Materials and Methods

66 *2.1. Suspension Preparation*

67 All assays were performed in a solution containing 75 g.L⁻¹ calcium L-
68 lactate hydrate (Sigma-Aldrich), 47 g.L⁻¹ calcium nitrate tetrahydrate (Fisher
69 Scientific), 20 g.L⁻¹ calcium gluconate (Sigma-Aldrich), and 3 g.L⁻¹ yeast extract
70 (Difco), dissolved in ultrapure water (referred to below as LNG medium).

71 Welan gum (Kelco) and Attagel® 50 (BASF) powders were sterilized
72 separately at 120°C in an autoclave for 15 minutes. Welan or Attagel suspensions
73 were prepared by dispersing the powder at 22°C in the LNG medium, followed by
74 homogenization with a magnetic stirrer at 1000 rpm for 24 hours. To prepare
75 suspensions containing both thickeners, each thickener was separately dispersed
76 in the LNG medium at 22°C at twice the concentration (ensuring better
77 dispersion), then mixed together, and homogenized with a magnetic stirrer at
78 1000 rpm for 24 hours. The concentration of Welan and Attagel is expressed as a
79 weight percent of the total weight of the suspension (w:w).

80

81 *2.2. Rheological Assays*

82 The suspensions were prepared 24 hours before the rheological
83 measurements in order to allow the suspensions to rest after homogenization.
84 Rheological characterizations were carried out with a rheometer with coaxial

85 cylinders (MCR302, Anton Paar) equipped with a CC27/P6 (grooved) inner
86 cylinder (Anton Paar). After being poured into the cylinder, the suspensions were
87 allowed to rest for 30 minutes with the inner cylinder in lower position.

88 The first measurement program applied an increasing, then decreasing
89 rotational shear to the fluid. The measuring program set shear rates at 0,1, 1, 10,
90 50, 100, 250, 500, 250, 100, 50, 10, 1, and 0,1 s⁻¹. Each stage lasted 20 seconds
91 and included 50 measurements of shear stress. The second program, starting
92 immediately after the first, was divided in three steps: first, negligible oscillatory
93 shear (oscillation; amplitude: 0,001%; angular frequency: 10 s⁻¹) for 100 seconds,
94 second, high rotational shear at 500 s⁻¹ for 60 seconds; and lastly, negligible
95 oscillatory shear (oscillation; amplitude: 0,001%; angular frequency: 10 s⁻¹) for 10
96 minutes. The negligible oscillatory shear simulates rest in order to characterize the
97 thixotropic behavior of the suspension.

98 The rheological characterization of the LNG medium thickened with
99 Welan and Attagel followed a two-step experimental design. A first step was to
100 determine the boundaries of the rheological characterization with a Hadamard
101 matrix (Table 1).

102 *Table 1. Hadamard matrix for the rheological characterization of the thickened medium*

N	Welan (% w:w)	Attagel (% w:w)
1	0,2	4
2	0,2	8
3	0,4	4
4	0,4	8

103

104 Based on the experimental results, the response of the medium to changes
 105 in thickener concentration was determined according to a composite experimental
 106 design. This design had two factors—Welan and Attagel concentrations—and
 107 three variables for each. As a result, 9 experiments had to be performed (Table 2).
 108 Two additional suspensions were characterized to complete the surface graphs:
 109 WA34, containing 0,3% Welan and 4% Attagel, and WA38, containing 0,3% and
 110 8% Attagel.

111 *Table 2. Extended experimental design for the rheological characterization of the thickened*
 112 *suspensions*

N	Welan		Attagel	
	Factor value	% (w:w)	Factor value	% (w:w)
1	-1	0,2	-1	5
2	0	0,3	-1	6
3	+1	0,4	-1	7
4	-1	0,2	0	5
5	0	0,3	0	6
6	+1	0,4	0	7
7	-1	0,2	+1	5
8	0	0,3	+1	6
9	+1	0,4	+1	7

113

114 **2.3. Study of Pseudoplastic Behavior**

115 The pseudoplastic behavior of each suspension was quantified with the
 116 Ostwald-deWaele law for viscoelastic fluids, also referred to as the power law:

117
$$\eta = K\dot{\gamma}^{n-1} \quad (1)$$

118 where η = viscosity, K = consistency index, $\dot{\gamma}$ = shear rate, and n = power-law
119 index.

120 The power-law index is defined by the shear-thinning behavior of the suspension.
121 It is equal to 1 for Newtonian fluids and decreases as the shear-thinning behavior
122 increases. The consistency index K defines the overall viscosity and is equal to the
123 viscosity of the fluid at 1 s^{-1} . In this study, a custom indicator—the “viscosity drop
124 factor”—was used to illustrate the power-law index. This factor is equal to the
125 viscosity at $0,1 \text{ s}^{-1}$ divided by the viscosity at 500 s^{-1} . It represents the loss of
126 viscosity of the suspension under shear.

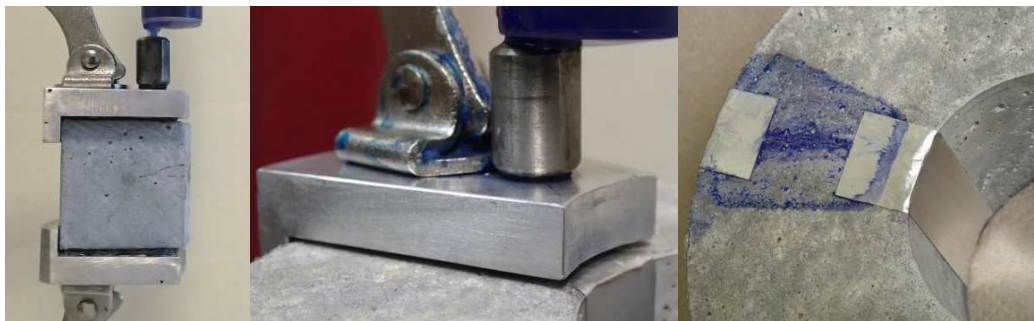
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128 ***2.4. Injection Assays***

129 Injection assays were performed on annular cracked mortar samples made
130 as described by Ducasse-Lapeyrusse [30] (height: 50 mm, outer diameter:
131 150 mm, inner diameter: 55 mm, reinforced with a 95 mm steel ring). Mortars
132 were cast according to ASTM C109M [31] with a water/cement ratio of 0,485 and
133 a sand/cement ratio of 2,75 with standard pure silica Ottawa sand and ordinary
134 Canadian Portland cement (GU, Blaine fineness $381 \text{ m}^2.\text{kg}^{-1}$), Curing lasted for
135 28 days at 23°C and 100% RH. Crack openings between 150 and $500 \mu\text{m}$ were
136 made by forcing a steel expansive core inside the mortar, as described by Gagné
137 and Argouges [1]. Samples were then carbonated for one month at 4% CO_2 , 20°C ,
138 40% RH, allowing crack self-healing. Crack openings were measured with a
139 videomicroscope on 24 points evenly distributed on both sides of the mortar.

140 Crack width was determined with air permeability measurements according to the
141 method described by Gagné and Argouges [1]. The samples were dried at ambient
142 temperature for 48 hours before the air permeability measurements.

143 For the injection tests, 10 mL of suspension were injected inside the cracks
144 at low pressure with a custom injection device (Fig. 1). The suspension was
145 slowly injected inside the crack, then the injection device was removed and the
146 excess levelled off. If the crack had not been completely filled, the suspension
147 was considered to be too thick. Conversely, the suspension was too fluid if
148 drainage was observed after injection. In total, 52 cracks were tested for injection
149 of thickened suspensions, including 18 with the WA36 suspension to take crack
150 diversity into account. Six cracks were selected to test repeated injections of
151 WA36.



152

153 *Fig. 1. From left to right – cross-sectional view of the injection device setup on a mortar sample;*
154 *upper side view; cracked mortar sample after injection of a colored solution*

155

156 After injection, the sample was dried at ambient temperature for 48 hours,
157 and then sawn to observe the cross section of the crack under scanning electron

158 microscopy (SEM) and energy dispersive spectroscopy (EDS) analysis. A small
159 part of the inner surface of each crack was directly observed, and referred to as the
160 “raw surface.” All samples were metallized with carbon thread prior to
161 observation.

162 **2.5. Bacterial Growth Assays**

163 In order to assess the effect of the thickeners on the production of calcium
164 carbonate by the bacteria, the bacterial strain *Bacillus pseudofirmus* (DMS 2516,
165 issued by DSMZ) was cultivated at 20°C in LNG medium versus in thickened
166 LNG (referred to as LNGW) medium (Table 3).

167

168 **Table 3. Composition of the different media used for bacterial growth**

Name	Composition
NBN medium	11,8 g/L calcium nitrate (Sigma-Aldrich) 8 g/L nutrient broth (Difco)
LNG medium	75 g/L calcium lactate (Sigma-Aldrich) 20 g/L calcium gluconate (Sigma-Aldrich) 47 g/L calcium nitrate (Fisher) 3 g/L yeast extract (Difco)
LNGW medium	LNG 0,3% Welan (w/w) 6% Attagel (w/w)

169

170 Stock bacteria were stored at -85°C in cryotubes containing 1 mL of
171 bacteria in NBN medium (Table 3) and 0,5 mL glycerol (30%) at a bacterial
172 concentration of $5 \cdot 10^9$ to $1 \cdot 10^{10}$ bacteria/mL. During the pre-culture, the bacteria
173 were cultivated for 30 hours in NBN medium at 30°C on an orbital shaker
174 (150 rpm). After 30 hours, the pre-culture was centrifuged at 4000 rpm for
175 20 minutes at 20°C . One pellet from 100 mL of pre-culture was used to inoculate
176 each 100 mL batch of the growth assay.

177 The growth assays were performed in 250 mL Erlenmeyer flasks
178 containing 100 mL LNG or LNGW medium (Table 3). Each condition was
179 performed in triplicate. The bacteria were cultivated for 35 days at 20°C on an
180 orbital shaker (150 rpm). Three milliliters of bacterial suspension were
181 periodically sampled in order to monitor pH (Sensitix 41 probe, WTW), bacterial
182 concentration, and lactate concentration during the assay. After 35 days, the assay
183 was stopped and the cultures were centrifuged at 4000 g for 20 minutes at 20°C ,
184 rinsed twice with 40 mL ultrapure water, then dried at 40°C and weighed to
185 determine the concentration of solids. A fraction of the dry pellet was used to
186 determine the calcium carbonate concentration by TGA (Jupiter STA 449 F3,
187 Netzch). To monitor bacterial concentration, the samples were diluted in saline
188 suspension ($c = 9 \text{ g}\cdot\text{L}^{-1}$), stained with acridine orange (Sigma-Aldrich), and fixed
189 on nuclepore filters (Whatman). The bacterial cells were then counted under an
190 epifluorescence microscope (Leica, France) at 490 nm. Lactate concentration was
191 measured by ion chromatography (ICS-3000, Dionex).

192

193 3. Results and Discussion

194 *3.1. Rheological Assays*

195 3.1.1. Characterization of Shear-Thinning Behavior 196 and Viscosity

197 First, suspensions defined by the Hadamard matrix were studied (Table 4).
198 LNG medium containing 4% of Attagel did not show a strong viscosity recovery
199 at rest. These suspensions were stable and presented no segregation or loss of
200 consistency after being stored for three days at ambient temperature. The LNG
201 medium containing 8% Attagel, however, was prone to segregation. The optimal
202 range of concentration of Attagel was higher than 4% to ensure a significant effect
203 of the thickener, but lower than 8% to prevent its destabilization.

204 Welan did not have a strong impact on the stability or on the thixotropic
205 behavior of the suspension, but did affect viscosity. Indeed, suspensions
206 containing 0,2% Welan had low viscosity and might therefore be prone to
207 drainage after injection into cracks. Increasing the Welan concentration to 0,4%
208 dramatically increased the viscosity of the studied suspensions. This concentration
209 was sufficient to avoid post-injection drainage at any Attagel concentration.
210 Indeed, suspension WA48, containing 0,4% Welan and 8% Attagel, was paste-
211 like under high shear and formed a weak gel after 4 hours at rest.

212 *Table 4. Hadamard matrix and rheological characterization of the thickened suspensions*

	0,2% Welan	0,4% Welan
4% Attagel	Too fluid Slightly thixotropic Viscosity at 1 s ⁻¹ : 2,3 Pa.s	Correct viscosity Slightly thixotropic Viscosity at 1 s ⁻¹ : 4,9 Pa.s
8% Attagel	Correct viscosity Slight tendency to segregate Viscosity at 1 s ⁻¹ : 12,1 Pa.s	Excessive viscosity Slight tendency to segregate Viscosity at 1 s ⁻¹ : 15,3 Pa.s

213

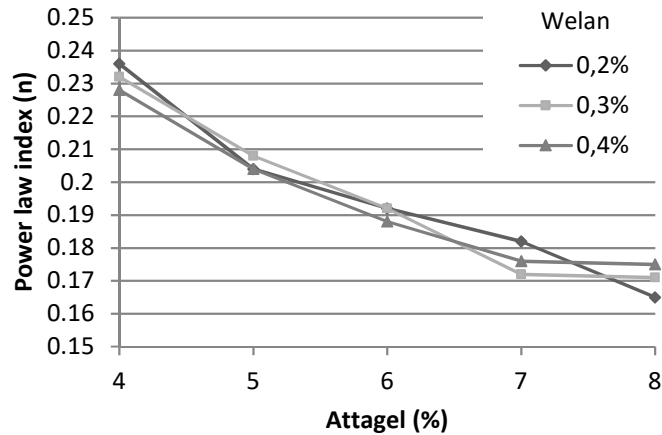
214 The nine suspensions in the experimental design described in Table 2
 215 yielded flow curves accurately fitting the Ostwald law ($R^2 > 0,99$) and had strong
 216 shear-thinning behavior, with power-law indexes lower than three (Table 5).

217 *Table 5. Fitting of suspensions containing Welan gum and Attagel with Ostwald law for*
 218 *viscoelastic fluids*

Sample	Welan (%)	Attagel (%)	K	n	Viscosity Drop Factor	R ²
WA24	0,2	4	2,33	0,235	633	0,997
WA25	0,2	5	3,93	0,204	829	0,998
WA26	0,2	6	5,82	0,185	933	0,998
WA27	0,2	7	8,57	0,173	1038	0,999
WA28	0,2	8	11,9	0,164	1175	0,999
WA34	0,3	4	3,22	0,239	651	0,999
WA35	0,3	5	4,94	0,211	810	0,999
WA36	0,3	6	7,31	0,191	958	0,999
WA37	0,3	7	10,9	0,175	1093	0,999
WA38	0,3	8	14,8	0,164	1134	0,999
WA44	0,4	4	4,18	0,247	664	0,999
WA45	0,4	5	6,34	0,214	833	0,999
WA46	0,4	6	8,99	0,194	979	0,999
WA47	0,4	7	12,5	0,177	1105	0,999
WA48	0,4	8	16,7	0,171	1147	0,999

219

220 Figure 2 provides the effect of each thickener on the consistency index K.
221 Both thickeners increased the value of K and, therefore, the viscosity of the
222 suspensions proportionally to their concentrations.



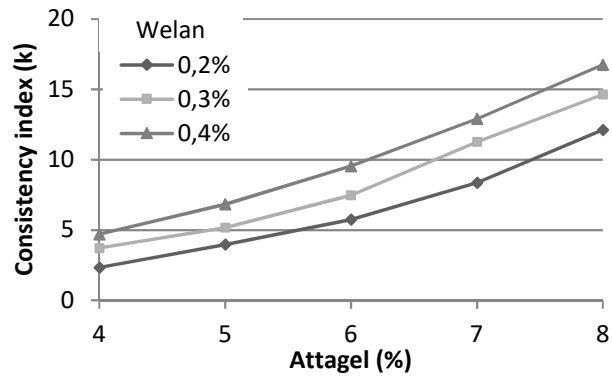
223

224 *Fig. 2. Evolution of the consistency index of the studied suspensions as a function of Welan and*
225 *Attagel contents*

226

227 Figure 3 displays the power-law index “n” as a function of Welan and
228 Attagel concentrations. The value of “n” decreased as Attagel concentration
229 increased, but it was not affected by the Welan concentration. Therefore, in this
230 range of concentrations, only the Attagel concentration played a role in reducing
231 the value of “n” and increasing the shear-thinning behavior of the suspensions.

232



233

234 *Fig. 3. Evolution of the power law index of the studied suspensions as function of Welan and*

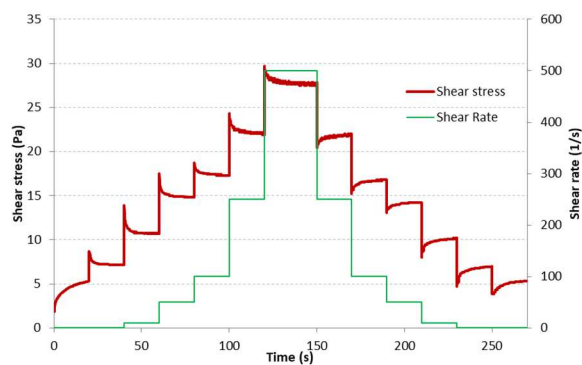
235

Attagel contents

236

237 In all samples, when shear stress is displayed as a function of time, the
 238 modification of shear rate was followed by a progressive response of shear stress
 239 (Fig. 4: for clarity, only one sample (WA36) is displayed as an example). The
 240 suspensions therefore have short-term thixotropic behavior. The time needed for
 241 viscosity stabilization after a change in shear is approximately of 15 seconds,
 242 which is similar for destructureation (following an increase in shear rate) and
 243 recovery (following a drop in shear rate).

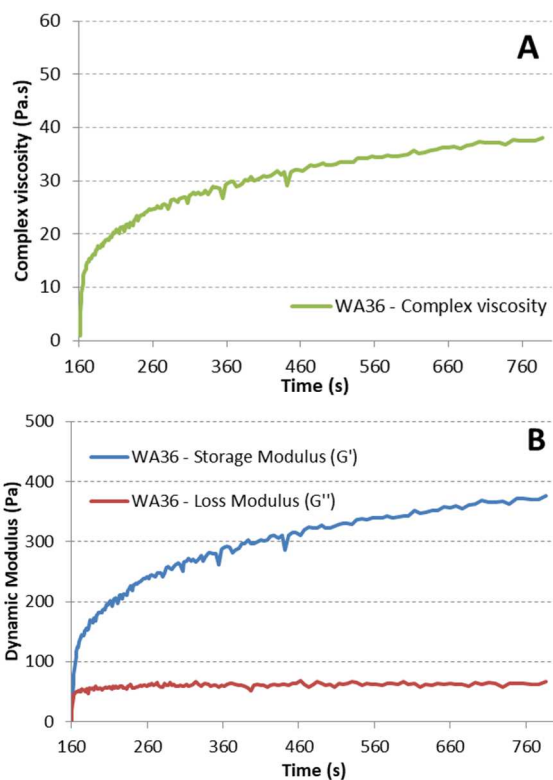
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245

246 *Fig. 4. Flow curve of suspension WA36 containing 0,3% Welan and 6% Attigel, displayed as*
 247 *shear stress / shear rate as a function of time*

248 Moreover, the complex viscosity and storage modulus of all the samples
 249 progressively increased when subjected to 10 minutes of oscillatory
 250 measurements, following destructuration at 500 s⁻¹ (Fig. 5). The Welan–Attigel
 251 suspensions therefore had long-term thixotropic behavior. After 10 minutes, the
 252 complex viscosity of the suspension was still increasing, suggesting a greater
 253 buildup in viscosity. The most concentrated suspensions (WA46, WA37, WA38,
 254 and WA48; see Table 5) were able to form weak gels after 6 hours at rest, which
 255 confirms that viscosity recovery continued to greatly increase viscosity.



256

257

258 *Fig. 5 (A) complex viscosity and (B) dynamic modulus of the suspension WA36 containing 0,3%*
 259 *Welan and 6% Attagel under oscillatory shear after 30 s., stirring at 500 s⁻¹*

260

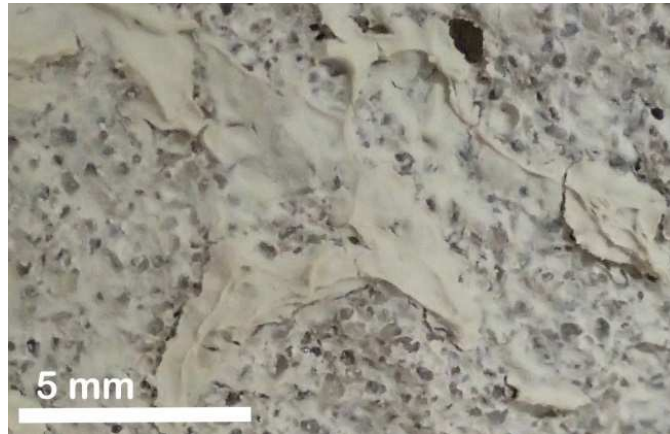
261 Combining Welan and Attagel yields shear-thinning, thixotropic, and
 262 settling-resistant suspensions for injection into cracked mortar. The rest of the
 263 experiments using thickened solutions were performed with suspension WA36,
 264 which contained 0,3% Welan and 6% Attagel dispersed in the LNG medium
 265 (referred to hereafter as LNGW).

266 **3.2. Injection Assays with the LNGW Suspension**

267 The LNGW suspension was successfully injected into all the cracks (150
 268 to 500 μm) and filled at least 80% of the cracks with no post-injection drainage.

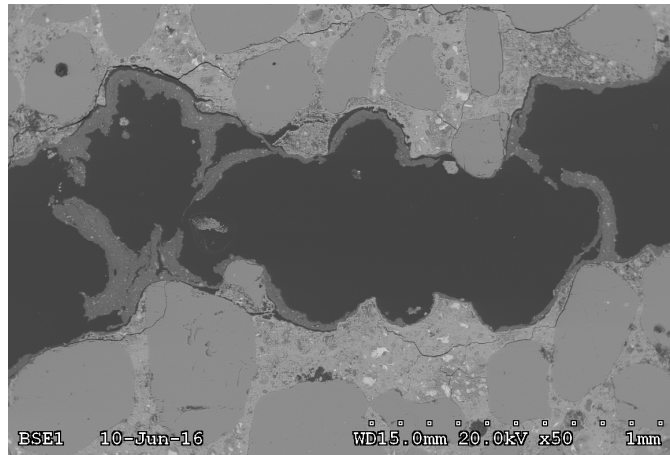
269 Additional injections into cracks up to 800 μm filled cracks to 100% without post-
270 injection drainage, which suggests a wider injection range. This versatility is due
271 to the shear-thinning and thixotropic behavior of the suspension: viscosity can be
272 strongly lowered by shaking to facilitate injection, while recovery prevents post-
273 injection drainage.

274 Upon drying, the thickened suspension formed a thin film covering most
275 of the inner surface of the crack, with a network of crests almost joining both
276 sides of the crack (Figs. 6 and 7). The film deposited on the inner surface of the
277 crack was irregular and less than 50 μm thick on most of the surface.



278

279 *Fig. 6. Film formed inside a 800 μm crack by the thickened suspension (LNGW) after injection*
280 *and drying*



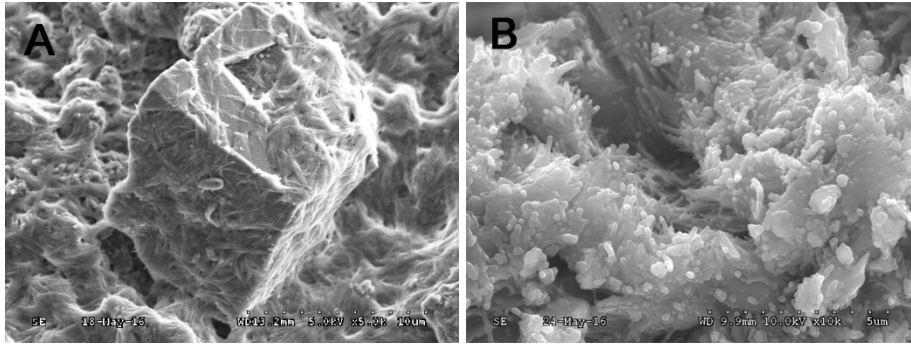
281

282 *Fig. 7 Cross-sectional SEM observation of a 580 μm crack after injection of the thickened*
283 *suspension (LNGW)*

284

285 The film was made of interlaced 5 μm long needles with some particles,
286 such as dolomite cubes, mortar debris, and precipitates (Fig. 8). This observation
287 is similar to SEM observations of Attagel described in the literature [32], showing
288 that the film is mainly composed of clay. Indeed, the composition of the solid
289 deposit, as determined by EDS analysis (Table 6), was close to the composition of

290 the Attagel as provided by the supplier [32], although SiO₂ concentrations varied
 291 in the sample.



292
 293 **Fig. 8. SEM observations of the inner surface of a crack after injection of thickened suspension.**
 294 **(A) dolomite cube covered with clay needles; (B) clay deposit**

295

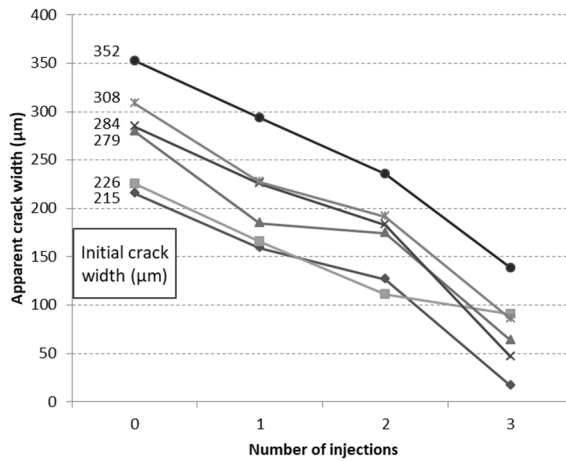
296 **Table 6: Average measured composition of the solid deposit and commercial Attagel**
 297 **composition [32]**

Mineral (as element)	Average Measured Value (mass%)	Attagel Concentration (mass%)
SiO ₂	77,7 (+/- 9,0)	65,2
Al ₂ O ₃	11,7 (+/- 1,2)	12,7
MgO	11,1 (+/- 0,9)	12,3
CaO	5,6 (+/- 3,2)	3,9
Fe ₂ O ₃	4,5 (+/- 1,0)	3,5

298

299 A comparison of six mortars with different crack widths (225–350 μm)
 300 shows that the deposit of clay polymer film in the crack significantly reduced the
 301 air permeability (Fig. 9). This led to a reduction in the apparent width of the crack
 302 by more than 50 μm after each injection of thickened suspension. The fact that the
 303 film itself was mostly thinner than 50 μm confirms the important role of the crest

304 network in air permeability reduction. Repeated injections led to an accumulation
305 of layers, producing a proportional reduction of the apparent crack width up to
306 200 μm .



307

308 *Fig. 9 Apparent width of cracks in six mortar samples, initially and after repeated injections of*
309 *the LNGW medium*

310

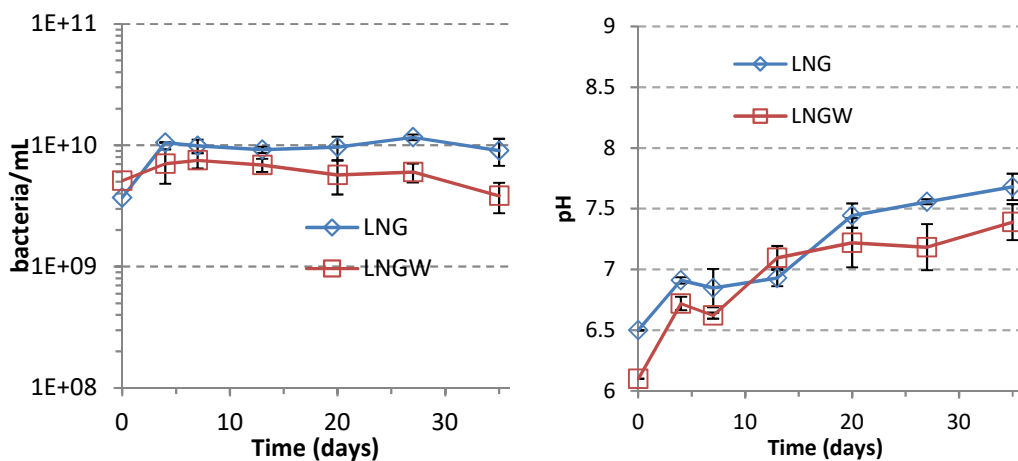
311 **3.3. Bacterial Growth Assays with the LNGW Suspension**

312 Figure 10 shows the evolution of the bacterial population during the
313 growth assay. As concentrated inoculum was used, the initial population already
314 ranged between $3 \cdot 10^9$ and $4 \cdot 10^9$ bacteria/mL at day 0. In the non-thickened
315 medium (LNG), the bacterial population rapidly increased and stabilized at
316 $1 \cdot 10^{10}$ bacteria/mL during the rest of the assay. The bacteria were in the form of
317 small cocci, mostly assembled in tetrads or small clusters (Fig. 11). In the
318 thickened medium (LNGW), the bacterial population also initially increased, but
319 to a lesser extent than in LNG. Then, the bacterial population decreased slightly

320 during the assay, but this might be attributed counting bias. Indeed, the cocci
321 tended to form massive clusters with time, hampering counting accuracy.

322 The pH progressively increased during the assay both in the LNG and in
323 LNGW media. After 35 days, the pH increased roughly 1 point, reaching a final
324 value of approximately 7,5 at the end of the assay, with or without thickeners.
325 Without thickeners, there was a steady decrease of lactate concentration in the
326 LNG medium (Fig. 12), stopping after 20 days and leading to the consumption of
327 80% of the lactate. Lactate consumption was quicker in the LNGW medium,
328 leading to complete consumption after 20 days. Thus, the presence of thickeners
329 did not hinder the bacterial activity and even seemed to be beneficial.

330



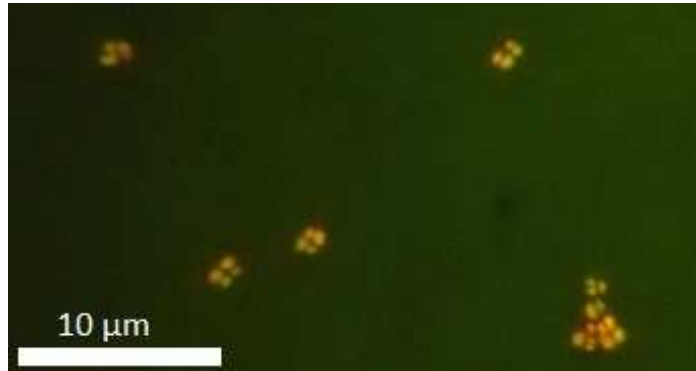
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332 *Fig. 10, Evolution of bacterial population (A) and pH (B) in the LNG and LNGW suspensions*

333

at 20°C. Experiment performed in triplicate

334



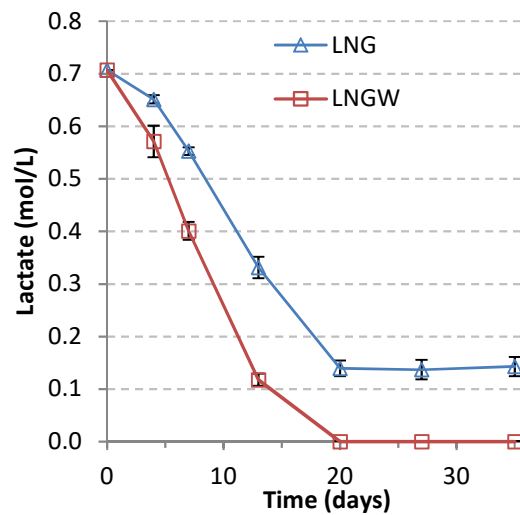
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336

Fig. 11. Bacterial cells after 10 days in the LNG bacterial suspension at 20°C, after acridine orange staining, observed by epifluorescence

337

338



339

Fig. 12. Evolution of lactate concentration in the LNG and LNGW bacterial suspensions.

340

Experiment performed in triplicate

341

342

343 In the LNG medium, the average CaCO_3 production (Table 7) was 17,0

344 g/L (+/- 0,9 g/L) after 35 days. In the LNGW medium, the average CaCO_3

345 production after 35 days was 25,1 g/L (+/- 3,0 g/L). The production of calcium

346 carbonate was therefore more important in the thickened medium, which is
 347 consistent with the greater lactate consumption. The presence of thickeners also
 348 contributed to increasing the total dry weight at the end of the assay, as Attagel
 349 added 60 g/L to the final solid composition in the samples.

350 Moreover, the protective effect of clays on bacteria have been largely
 351 documented [20–23], and might constitute a major advantage as maintaining
 352 bacterial survival and activity is a major challenge for outdoor bioprocesses. As
 353 for the scope of crack sealing, the combination of biomineralization and clay
 354 deposit is particularly interesting as both factors help reduce crack permeability.
 355 Moreover, the clay deposit might serve as a scaffold for calcium carbonate
 356 crystals, improving their durability inside the crack. Additionally, this growth
 357 medium shows interesting properties for a pilot-scale test:

- 358 - It is nonpolluting and nontoxic, and involves inexpensive materials.
- 359 - Its rheological properties make it suitable for various sizes of cracks.
- 360 - It can be prepared by simple dispersion of dry powders and does not
 361 require complex protocols or specific precautions.
- 362 - It forms a washout-resistant film inside the crack, contributing its
 363 sealing.

364

365 *Table 7. Dry weight and calcium carbonate production after 35 days in the LNG and LNGW*
 366 *suspensions*

Sample	LNG-	LNG-	LNG-	Average	LNGW-	LNGW-	LNGW-	Average
--------	------	------	------	---------	-------	-------	-------	---------

	1	2	3		1	2	3	
Dry weight (g/L)	19,7	21,2	20,3	20,4 (+/- 0,8)	94,3	88,3	88,5	90,3 (+/- 3,4)
CaCO₃ in dry residue (%)	83%	84%	85%	84 (+/- 1)	38%	34%	35%	36 (+/- 2)
CaCO₃ production (g/L)	16,4	17,7	17,2	17,1 (+/- 0,7)	28,5	23,2	23,6	25,1 (+/- 3,0)

367

368

369 4. Conclusion

370 Adding polysaccharide gum (Welan) and a palygorskite clay (Attagel) to
371 growth medium made it possible to design stable suspensions with strong shear-
372 thinning and thixotropic behavior. The thickened suspension was efficiently
373 injected into cracks ranging from 150 to 800 μm . Cultivated in a growth medium
374 containing 0,3% Welan and 6% Attagel, *Bacillus pseudofirmus* was able to grow
375 and precipitate larger amounts of calcium carbonate than in the non-thickened
376 growth medium. Future studies will aim at confirming the healing potential of the
377 thickened medium developed in this study by testing it on an outdoor pilot scale
378 or on real-scale concrete structures.

379

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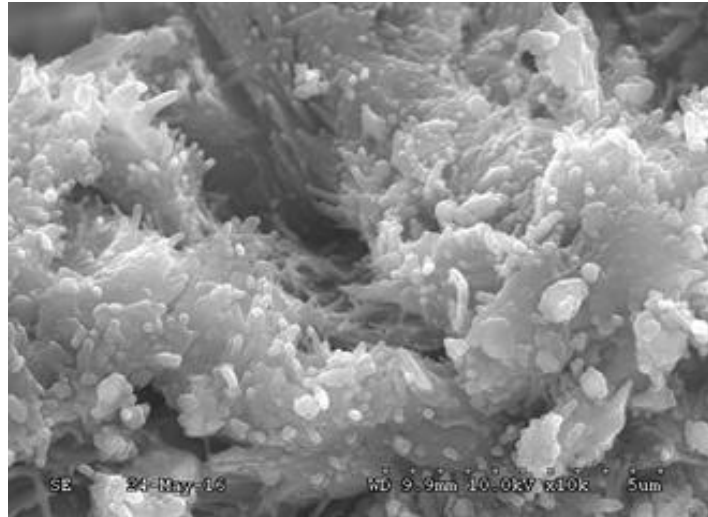
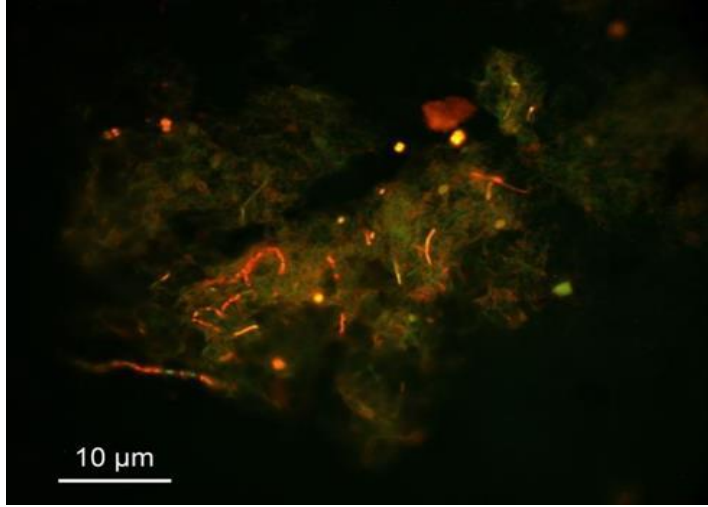
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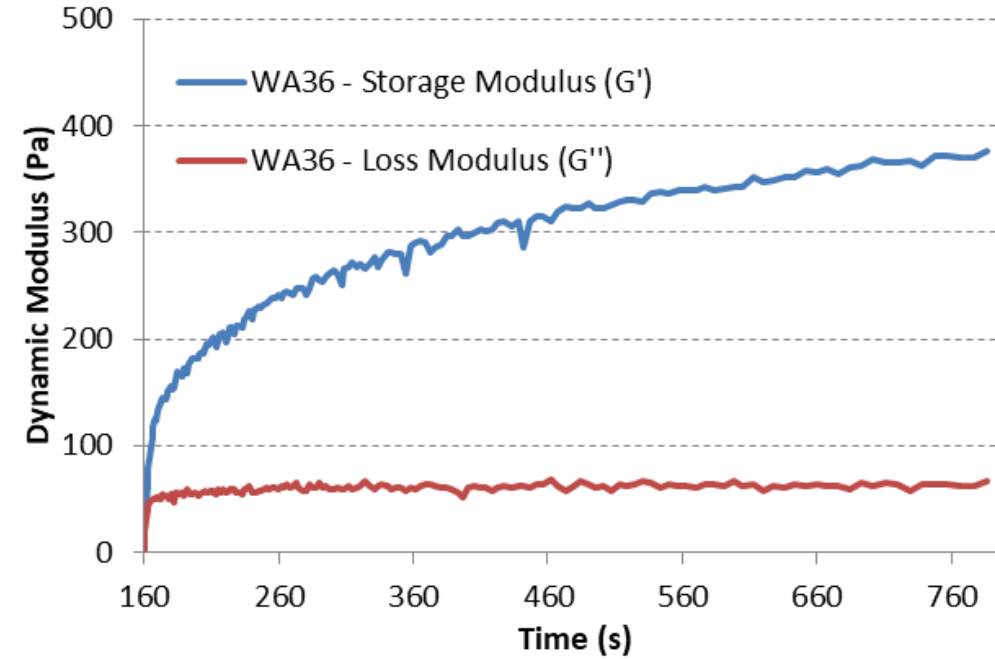
Bacillus pseudofirmus
calcite-forming bacteria



Attagel clay and Welan gum



Clay-biopolymer thixotropic
suspension



Injection in
concrete cracks

In-situ calcite
biomineralization



Composite (calcite – clay) clogging:
Long-lasting crack repair

