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39 **Abstract**

40 Microbial Induced Carbonate Precipitation (MICP) is one of the most commonly researched topics
41 on biocementation, which achieves cementation of soil particles by carbonate from urea hydrolysis
42 catalyzed by microbial urease. Although most MICP studies are limited to stabilizing sandy soils,
43 more researchers are now turning their interest to other weak soils, particularly organic soils. To
44 stabilize organic soils, the influence of humic substances should be investigated since it has been
45 reported to inhibit urease activity and disrupt the formation of calcium carbonate. This study
46 investigates the effect of humic acid (HA), one fraction of humic substances, on MICP. For this
47 purpose, the effects of HA content on CaCO₃ precipitation using three strains and on CaCO₃
48 morphology were examined. The results showed that native species in organic soils were less
49 adversely affected by HA addition than the exogenous one. Another interesting finding is that
50 bacteria seem to have strategies to cope with harsh conditions with HA. Observation of CaCO₃
51 morphology revealed that the crystallization process was hindered by HA to some extent,
52 producing lots of fine amorphous precipitates and large aggregated CaCO₃. Overall, this study
53 could provide an insightful understanding of possible obstacles when using MICP to stabilize
54 organic soils.

55 **Keywords:** Humic acid (HA); microbial induced carbonate precipitation (MICP); urease activity;
56 CaCO₃ morphology; soil improvement

57 **Introduction**

58 Humic substance, as one of the world's most widely distributed organic deposits, is still a mystery
59 to soil scientists even though the first attempt to figure out what it is could be dated back to the
60 18th century. The definition and formation of humic substances have been a controversial and

61 widely disputed research topic in soil science for a long time (Gerke, 2018; Baveye and Wander,
62 2019). From the traditional view, humic substances could be divided into three fractions based on
63 solubility: (i) humin, insoluble; (ii) humic acid (HA), only soluble under alkaline conditions; (iii)
64 fulvic acid, soluble (Stevenson, 1994). Among these fractions, HA, which functionally acts as an
65 acid due to the carboxylic and phenolic groups, is the one most researched and has profited
66 numerous applications in various fields, such as fertilizers in agriculture, pollutant removal in the
67 environment, and so on (Wen et al., 2019; De Melo et al., 2016; Tang et al., 2014). In natural soils,
68 Humic substances compose up to 85% of soil organic matter, and the proportion of each fraction
69 varies from different soil types (Ukalska-Jaruga et al., 2021). For instances, in Hokkaido, the
70 organic soils usually contains HA ranging from 7-40% (Odajima et al., 1990). As a ubiquitous
71 deposit, it becomes a challenging task for geoengineers to stabilize soils with high organic content
72 (up to 50%) due to their unique chemical characteristics that hinder the hydration of cement (Toda
73 et al., 2020; Beddaa et al., 2019). Since standard criteria for usual mineral soils might not be
74 applicable for organic soils, some researchers have turned their attention to some novel soil
75 improvement techniques.

76 In geoengineering, a new branch called biocementation is developing dramatically, with the
77 exponential growth of related research in recent years. As an essential part of biocementation,
78 MICP cements particles by carbonate from urea hydrolysis catalyzed by microbial urease,
79 precipitating with calcium ions (Mujah et al., 2017). Nowadays, many MICP studies have targeted
80 sandy soil improvement from several perspectives: (i) factors affecting MICP, (ii) characteristics
81 of bio-cemented soils (Naveed et al., 2020; Yu and Jiang, 2018; Montoya and DeJong, 2015); (iii)
82 durability evaluations under environmental erosions (Liu et al., 2019a; Gowthaman et al., 2020;
83 Gowthaman et al., 2021). Although these are still limited in stabilizing sandy soils, more

84 researchers are turning their attention to other problematic soils, such as organic ones. Canakci et
85 al. (2015) first tried to stabilize peat soil (one kind of typical organic soil) using MICP technique,
86 but the effectiveness was low even with 20% of CaCO₃ content in the treated samples. Sato et al.
87 (2016) have reported similar weak improvement after long term curing. Recently, Safdar et al.
88 (2021) tried two different methods to apply MICP: pressure-flow column and electrokinetic in
89 chambers, and reported the most remarkable improvement by MICP with a CaCO₃ content of less
90 than 2 %. The previous stage of this study also made some efforts to stabilize high water content
91 peat and amorphous peat combining MICP with fiber materials (bamboo fiber and wastepaper fiber),
92 but the effectiveness was somewhat limited (Chen et al., 2021a, b; Gowthaman et al., 2021a).
93 When it comes to applying MICP to organic soils, the existence of HA in organic soils should be
94 of particular concern. HA is well-known as one type of soil fertilizer due to its excellent cation
95 exchange capacity and ability to inhibit soil urease activity. When it is involved in MICP process,
96 on the one hand, it might disrupt the formation of CaCO₃ by interacting with calcium ions (Falini
97 et al., 2009; Kloster et al., 2013). On the other hand, HA inhibits urease activity by blocking the
98 enzyme's active site, which affects urea hydrolysis efficiency (Liu et al., 2019b).

99 Therefore, this study sought to investigate HA's influence on microbial-induced carbonate
100 precipitation. For this purpose, the following aspects were examined: i) effect of HA addition on
101 precipitation rate; ii) effect of HA on the response of three strains in precipitation tests; iii) effect
102 of HA on CaCO₃ morphology. Three strains used in this study were selected considering their
103 origins and urease activity. The well-designed carbonate precipitation tests quantitatively
104 evaluated the calcium ions consumption rate. This study tries to figure out how HA affects
105 microbial-induced calcium carbonate precipitation, the mechanism behind it, and how to overcome

106 the obstacles when using MICP to stabilize organic soils, contributing to a broader application
107 range of biocementation for the future.

108 **Materials and methodology**

109 **Chemical reagents**

110 The HA is a commercial product from FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan.
111 The specification is given in Table 1 below. According to the information provided by the
112 manufacturer, this product has 50-60% of carbon content, 4-6% of hydrogen content, and the rest
113 is mainly oxygen content. It should be mentioned that commercial HA products usually contain a
114 certain amount of impurities, including some clay minerals. Kobayashi et al. (2018) analyzed three
115 commercial HAs and compared them with standard soil HA, showing some differences in their
116 chemical characteristics. Although the commercial HA is not a matched representative of soil HA,
117 it was adopted in this experimental study considering the difficulty of extracting a sufficient
118 amount of HA from natural soils. Calcium chloride (FUJIFILM Wako) with a chemical purity
119 above 95%, pH (50g/L, 25 °C) in the range of 8.0-10.0, was the calcium source, and urea
120 (FUJIFILM Wako) with a chemical purity above 99% was the carbonate source.

121 **Tested strains**

122 Three strains, seen in Table 2, were tested to examine the effect of HA on bacterial response and
123 CaCO₃ precipitation. *Sporosarcina* sp. (e-4) and *Staphylococcus edaphicus* (PS-1) were isolated
124 from high water content peat (around 800%) that contains approximately 5% (wet mass basis) of
125 HA. *Lysinibacillus xylanilyticus* (Ly. xy) was previously isolated from Onuma sandy soil which
126 has much less organic matter and has been characterized in a previous study (Gowthaman et al.,
127 2019). Urease activities of three strains are compared in Fig. 1. Ly. xy was chosen to compare with
128 e-4 because it possesses a similar urease activity and preference for weak alkaline conditions. On

129 the other side, it has been expected that the strains isolated from peat soils might have more
130 tolerance towards HA, while strains from sandy soil might show relatively weaker resistance.

131 **Experimental design**

132 Figure 2 illustrates the processes in this experimental work. The preparation of bacteria is shown
133 in Fig. 2a. The culture medium used is NH₄-YE (ATCC 1376), with 15.75 g of Tris-aminomethane,
134 10 g of ammonium sulfate, 20 g of yeast extract, and 20 g of agar (for plate only) in 1 L of culture
135 medium. Bacterial colonies were inoculated to a 5 mL-preculture test tube from an NH₄-YE agar
136 plate. After 24 hours, 1 mL of preculture was added to 100 mL of the main culture and placed in
137 a shaker at 160 rpm under the optimal temperature for bacteria. After 48-72 hours of culture, the
138 bacteria population was confirmed by measuring optical density at the wavelength of 600 nm using
139 a spectrophotometer. Previous studies found that the metabolites produced by bacterial cells have
140 some influence on the morphology of CaCO₃ (Li et al., 2010; Azulay et al., 2018). Therefore, the
141 main culture was centrifuged at 8000 rpm for 5 min to separate bacterial cells and culture medium,
142 followed by replacing the medium with sterile distilled water. For each case, in total, 10 mL of
143 cementation solution (0.5 mol/L) and bacteria (2 mL of the main culture) are included in a 15 mL
144 test tube.

145 Three sets of precipitation tests with HA addition of 0-8% were designed, as shown in Table 2.
146 The concentration of Ca²⁺ and pH changes were monitored during the test (after 1-h, 18-h, 24-h,
147 and 48-h). For each sampling, 100 µL was taken and diluted before measurement. The calcium
148 meter used in this measurement is a compact Ca²⁺ meter B-751 (HORIBA, Ltd., Kyoto, Japan),
149 with a measurement range from 4 to 9900 ppm. The pH meter (pH5S, needle-type sensor) is a
150 product from CEM CORPORATION, Tokyo, Japan, with a wide measurable range from pH -2 to
151 16. Therefore, to minimize the effect of living bacteria cells, a sterile PES syringe filter with a 0.22

152 μm pore size (Hawach Scientific Co., Ltd., Xi'an, China) was used to remove bacteria in the sample
153 before measurements.

154 After precipitation tests, precipitates were oven-dried at 60° for 48 hours, and the dry weight was
155 measured for each case. In order to figure out the effect of HA on the morphology of CaCO_3
156 precipitation, the precipitates were observed using Miniscope TM 3000 (HITACHI, Tokyo, Japan).
157 The bacterial population is a crucial factor affecting the survival rate when living in harsh
158 conditions. Bacteria could generally survive a harsh environment and adapt to it more easily when
159 the initial population reaches a corresponding threshold. Therefore, precipitation tests using three
160 species with varying OD_{600} were examined. After 24 hours, the concentration of calcium ions was
161 measured for all cases to confirm the percentage of Ca^{2+} precipitated.

162 **Calculations**

163 The calcium ions consumption rate is a critical evaluation during the precipitation tests. The
164 percentage of calcium ion consumption is defined in Eq. (1). Comparing HA-added cases with
165 control cases (without HA), the percent decrease/increase of calcium consumption is calculated as
166 Eq. (2).

$$167 \quad \text{Percent of } \text{Ca}^{2+} \text{ consumed} = \frac{c - c_0}{c_0} \quad \text{Eq. (1)}$$

$$168 \quad \% \text{ Decrease} = \frac{c - c_{\text{ctrl}}}{c_0 - c_{\text{ctrl}}}, \text{ or } \% \text{ Increase} = \frac{c_{\text{ctrl}} - c}{c_0 - c_{\text{ctrl}}} \quad \text{Eq. (2)}$$

169 Where c is the concentration of calcium ions after 24 hours; c_0 is the initial concentration of calcium
170 ions; c_{ctrl} is the concentration of calcium ions in the control case.

171 **Results and discussion**

172 **Effect of HA addition on cementation solution**

173 As pointed out in the introduction part, HA has a wide range of applications, one of which is for
174 heavy metal removal in contaminated water due to its complexation with metal ions. Many
175 previous studies have stated that the cations, functional groups involved, and pH condition are the
176 key factors affecting the affinity between cations and HA (Kloster et al., 2013; Ai et al., 2020). In
177 this research, HA-Ca complexation and acidification of cementation solution are of great concern,
178 which might adversely influence the precipitation of CaCO_3 . Therefore, one set of control samples
179 was examined in advance by measuring the pH and calcium ions concentration changes. It can be
180 seen in Fig. 3a that 1-8% of HA showed a pH ranging from 5 to 6.5 when suspended in distilled
181 water. However, the pH dropped dramatically after adding cementation reagents. Based on some
182 reported studies, common metal ions could quickly form weak aggregation with HA under a weak
183 acidic condition, reducing the pH (Adusei-Gyamfi et al., 2019). Possibly, the out-layer
184 complexation between nanoparticles of HA and calcium ions is too weak, so calcium ions could
185 still be detected by the calcium meter. In the aspect of concentration of calcium ions, theoretically,
186 with 0.5 mol/L of calcium chloride, the concentration should be 20,000 ppm. As seen in Fig. 3b,
187 the concentrations were higher than the theoretical value but within an acceptable variation.

188 **Effect of HA on CaCO_3 precipitation rate of three strains**

189 Figure 4 illustrates the monitoring of calcium ion concentration and pH changes in HA-added
190 precipitation tests using three strains. It can be seen from this figure that these three strains showed
191 completely different behaviors during the test. In Fig. 4a, the calcium ions consumed percentage
192 by e-4 shows that approximately 25% of precipitation occurred after 1 hour and almost 100% after
193 48 hours. Interestingly, the pH of all HA-added cases increased from pH 3-4 to pH 7 after 1 hour

194 (seen in Fig. 4b), whereas a decrease was observed later on. This drop might be explained by the
195 bacterial response to modify the surroundings, which is a vital strategy for microbes to survive
196 when they are in harsh conditions (Ratzke and Gore, 2018), but the buffering capacity might
197 change over time. With the hydrolysis of urea going on, the final trend of pH was increasing
198 steadily. A comparison of all cases confirms that HA addition delayed the formation of CaCO_3 to
199 some extent. Turning to the evidence from the cases of *Ly. xy*, about 15% of calcium ions
200 precipitated after 1 hour in the control case, and no consumption was observed in other cases.
201 Nevertheless, the pH of all cases had increased to varying degrees. There is likely a connection
202 between the induced precipitation and the bacterial ability to modify the environment. During the
203 first hour of incubation, *Ly. xy* failed to manipulate the pH in cases with a relatively high HA
204 addition to favor the precipitation, even if it has relatively high activity. Therefore, the formation
205 of CaCO_3 was significantly inhibited by the acidic environment. As for PS-1, since it has a much
206 lower urease activity than the other two strains, no precipitation was observed after one hour.
207 Contrary to expectations, the pH of all cases increased significantly even though not much
208 hydrolysis of urea happened. These results verified the explanation discussed above, indicating
209 that the tested strains have responded to the harsh environment and modified the pH of the solution.
210 Furthermore, about 60% of calcium ions were consumed in cases with HA, while the control case
211 precipitated only 30% after 48-hour incubation. That is to say, HA addition might improve the
212 urease activity of PS-1 instead of inhibition. Generally, it is acknowledged that HA inhibits
213 enzyme activity, as one of the essential characteristics contributing to its wide application in the
214 agricultural field. The findings above confirmed that the microbial urease activity could differ from
215 strain to strain in responding to HA, and this response does not necessarily have to be negative.

216 **Percentage of calcium ions consumption**

217 As explained earlier, quite distinct differences among the three strains in terms of performance
218 were observed in monitoring measurements during precipitation tests. Figure 5a depicts the
219 calcium ions consumption rate in tests using three strains with varying bacterial populations. It can
220 be seen that e-4 was less affected by increasing HA addition rate and *Ly. xy* seems deactivated by
221 more than 4% of HA, whereas PS-1 was activated, indicating a distinguished tolerance towards
222 HA. The percentage decrease in e-4 and *Ly. xy* strains and percent increase in PS-1 are presented
223 separately in Fig. 5b and 5c. Overall, the decrease rate in e-4 cases was less than 20%, without
224 much difference between cases, while cases of *Ly. xy* showed a sharp decrease with HA addition
225 increasing. Regarding PS-1, the percentage increase in all cases was 150% on average, with a
226 relatively high deviation due to its relatively low initial activity. It should be mentioned that a
227 correlation between bacterial optical density and percent of calcium ions consumed was expected,
228 while no tendency was evident from the data. Theoretically, more bacterial cells could reduce the
229 HA/urease ratio in the cementation solution, contributing to a higher consumption rate. Since no
230 significant differences were observed, it is probably because of the narrow optical density range
231 tested in this experiment. Despite that, the obtained data are pretty revealing in several ways. First,
232 urease activity, usually quantified by some standard methods, is one of the critical factors when
233 selecting ureolytic bacteria for MICP, but values obtained from the standard condition may not
234 apply to some circumstances. For instance, PS-1 would be a more appropriate option than *Ly. xy*
235 when they were being applied to soils rich in HA, though it has a low urease activity. When
236 comparing e-4 with PS-1, additional studies are needed before concluding. Considering long-term
237 effects, it is necessary to assess the viability of these two strains under the HA-rich condition.

238 Turning to the inhibition mechanism, there is no comprehensive understanding of it, but most
239 researchers agree with the idea that the complexation between HA and enzymes hinders the access
240 of substrate to the enzyme by blocking the route to the active site of enzymes. For example, Liu et
241 al. (2019b) investigated the influences of HA on plant-derived urease and found that some
242 functional groups might contribute to the inhibition of urease activity. In terms of microbial urease,
243 a small number of reports are available to provide supporting information for this study. It should
244 be mentioned that urease from plants and microbes can exhibit significant differences in structure,
245 molecular size, and characteristics. On the other hand, HA varies from their origins (Kobayashi et
246 al., 2018), and microbial urease also differs from species to species (Mobley et al., 1995).
247 Therefore, it is difficult to explain this result, but it may be related to interactions between urease
248 and HA. Overall, these findings further support the previous hypotheses that native species possess
249 higher tolerance toward HA than exogenous species.

250 **Effect of HA on morphology of CaCO₃**

251 In terms of CaCO₃ precipitates, it should be noted that the weight of each case was confirmed to
252 be consistent with the percentage of calcium ions precipitated (seen in Fig. 6), which eliminated
253 the previous concern about the accuracy of calcium ions measurements. The morphology of
254 CaCO₃ precipitation induced by three species is compared in the figure below. Fig. 7a1-a3 presents
255 precipitations of e-4 cases. In the control case (0% of HA), CaCO₃ crystals were clear and found
256 in a similar shape and size. With HA addition increasing, precipitates became less ordered, and
257 many of them are fine amorphous particles. This might partly be explained by a lower precipitation
258 rate retarded by HA. In terms of morphology, it might be affected by the interactions between
259 calcium ions, urease, and HA particles. From the data of the previous section, *Ly. xy* was
260 significantly affected by a relatively high HA addition. Similarly, some differences were found

261 between cases with HA addition and control cases, as seen in Fig. 7b1-b3. More fine precipitates
262 and less large precipitates appeared in cases with 1 -2% HA addition. Only fine precipitates were
263 found in the 4% HA addition case. In the cases of PS-1, although the total precipitates were less
264 than the formers, the crystals were larger and easier to identify (seen in Fig. 7c1-c3). What is
265 interesting in these cases is that crystals precipitated around the HA particles, forming larger
266 aggregates, which might be partially explained by the aggregation of HA and calcium ions. The
267 reason for such formation is unclear, but it might be related to the interaction between urease and
268 HA particles. It has been reported that urease's charge state strongly affects the electrostatic
269 repulsion/attraction between urease and HA (Li et al., 2022). When the pH changes during the
270 precipitation tests, the interactions described above might also undergo many changes, which
271 could explain why some fine amorphous precipitations and some coarse aggregated precipitations
272 were observed in HA-added cases. For a comprehensive picture of it, additional studies are needed.

273 **Discussion on inhibition/ improvement mechanism**

274 There are still many unanswered questions about humic substances remained. With microbes
275 involved, this multidisciplinary research became even more exclusive. Fig. 8 illustrates some
276 factors and interactions likely involved in microbial-induced carbonate precipitation influenced by
277 HA addition. From the perspective of HA-X complexation, pH is a crucial contributing factor.
278 Urease undergoes conformational changes when influenced by HA under a low pH, while this
279 negative effect becomes less as pH increases to a certain level and alkaline conditions improve the
280 stability of urease (Li et al., 2022). In terms of complexation between metal ions and HA, it has
281 been reported in previous studies that there is a less available binding site at low pH, and the
282 interactions are dominated by aggregation of HA particles (Ai et al., 2020). Although more binding
283 sites are there under a high pH condition, the complexation of HA with common metal ions like

284 calcium ions is much weaker (Tan et al., 2019). From the perspective of microbial response, as
285 indicated previously, some bacteria have survival strategies to deal with harsh conditions. For
286 instance, *H. pylori*, a well-known species that causes infection of the gastrointestinal tracts,
287 survives stomach acid by producing a large amount of urease as part of their strategies (Lund et
288 al., 2014). Therefore, PS-1 may have a similar response toward the acidity condition created by
289 HA, showing an increased urease activity in HA-added precipitation tests. Moreover, some soil-
290 borne bacteria could utilize the HA as a carbon source (Tikhonov et al., 2010). In this way, HA
291 addition may enhance the survivability of bacteria in cementation solution without many nutrients.
292 On the other hand, adsorption of HA to bacteria cells might have some effects on bacterial
293 membrane transport (Feifičová et al., 2005). Usually, bacteria cells serve as nucleation sites for
294 precipitation during the MICP process. HA nanoparticles covering the bacteria cells could reduce
295 nucleation sites significantly. Table 3 below presents some possible activation and inhibition
296 mechanisms analyzed from a microbiological perspective. To avoid confusion, this study did not
297 go deeply into microbiological analysis, but there are many factors not discussed herein, such as
298 metabolites from bacterial cells and bacterial size that might also affect the final formation of
299 precipitates.

300 **Conclusions**

301 The main goal of the current study was to contribute to a deeper understanding of organic soil
302 stabilization using biocementation. Therefore, it was designed to investigate the influence of HA
303 on microbial-induced calcium carbonate precipitation using three strains, two isolated from
304 organic soil and one from sandy soil. The results show that bacterial response to HA differs for
305 each species. In general, the following conclusions could be drawn.

- 306 i) Native species generally have advantages over exogenous species. With HA addition
307 up to 8%, total precipitation obtained from two native species showed a slight decrease
308 (less than 15%) and a doubled increase separately, while the precipitation by exogenous
309 species was completely inhibited.
- 310 ii) Further examination of CaCO₃ morphology revealed that the crystallization of CaCO₃
311 was disrupted by HA, yielding lots of fine amorphous and large aggregated precipitates.
312 Fewer nucleation sites caused by HA adsorption on bacterial cells might contribute to
313 the morphology change.
- 314 iii) Mechanisms are discussed from perspectives of HA-complexation and bacterial
315 survival strategy. pH condition is one of the most contributing factors that control the
316 formation of HA-X complexation and the survivability of bacteria.

317 These findings contribute to a better prospect of organic soil improvement using
318 biocementation, which confirmed that selecting suitable ureolytic bacteria and the chemical
319 environment are two key factors that should be considered. Although it seems that applying
320 MICP to organic soils is of great difficulty due to the complicated interactions involved, this
321 report should help figure out potential solutions. There is, therefore, a need for further research
322 that focuses on exploring how the microbes could counteract the effect of humic acid.

323 **Ethical Approval and Consent to Participate**

324 Not applicable.

325 **Consent to Publish**

326 Not applicable.

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329 **Competing Interests**

330 The authors declare no competing interests.

331 **Author Contributions**

332 All authors contributed to the study conception and design. Meiqi Chen: laboratory
333 experimentations, analysis, interpretation of data, and drafting the manuscript. Dr. Sivakumar
334 Gowthaman: laboratory experimentations, analysis, critical reviewing. Prof. Kazunori Nakashima:
335 critical reviewing and technical support. Prof. Satoru Kawasaki: primary supervision, critical
336 reviewing, and final approval of the version to be submitted. All authors read and approved the
337 final manuscript.

338 **Availability of data and materials**

339 Data available on request from the authors.

340 **Reference**

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460 **Tables**461 **Table 1** Specification of practical grade HA

Property	Description
Appearance	Blackish brown powder
Solubilities	HCl-insoluble: min.70% NaOH-insoluble: max.20%
Loss on drying at 105°C	max.20%
Residue after ignition	max.20%
Melting point	> 300°C
pH*	5.0-6.5

462 * pH of 10 g/L aqueous slurry was measured in at 25°C

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Table 2 Experimental cases

Microbes				
Test ID	Strain	Optical density (OD ₆₀₀)	Optimal temp., °C	Measurement
Control		N/A	25	
A-1	e-4	5.56	25	pH & Ca ²⁺ conc. monitoring SEM analysis
A-2	Ly.xy	6.6	25	
A-3	PS-1	8.74	30	
B-1	e-4	3.83-6.06	25	Conc. of Ca ²⁺ after 24 h
B-2	Ly.xy	5.6-6.6	25	
B-3	PS-1	6.71-8.74	30	

Table 3 Possible mechanisms from a microbiological perspective

Interactions	Mechanisms	
	Activation	Inhibition
Urease-HA	<ul style="list-style-type: none"> • Improvement of urease stability by reducing collisions of proteins when urease is negative-charged 	<ul style="list-style-type: none"> • Complexation of HA and positively charged urease, blocking the active site
Bacteria-HA	<ul style="list-style-type: none"> • HA as a carbon source for bacteria • Increase the yield of urease from bacteria cells 	<ul style="list-style-type: none"> • Acidification of environment • Adsorption of HA to cells, leading to less nucleation site

470 **Figure captions**

471 **Fig. 1** Urease activity of three strains (incubation temperature: e-4 and Ly. xy at 25°C, PS-1 at
472 30°C)

473 **Fig. 2** Experiment process: (a) Preparation of bacteria culture; (b) CaCO₃ precipitation tests

474 **Fig. 3** Effect of HA on distilled water solution and on 0.5 mol/ L of CaCl₂ and urea (cementation
475 solution): (a) pH changes and (b) calcium ion concentration changes with 0-8% HA

476 **Fig. 4** Monitoring during the precipitation tests: (a) concentration of calcium ions and (b) pH
477 changes with 0, 1%, 2%, 4%, 8% of HA addition (measurement after 1-hour, 18-hour, 24-hour,
478 and 48-hour incubation)

479 **Fig. 5** (a) Percent of calcium ions consumed after 24 hours; (b) Percent decrease based on the
480 consumption rate of control cases: e-4 & Ly. xy (c) Percent increase based on the consumption
481 rate of control cases: PS-1

482 **Fig. 6** Dry mass of precipitates calculated based on weight changes in each case

483 **Fig. 7** CaCO₃ induced by three strains in precipitation tests with 0, 1%, and 4% HA addition. e-4:
484 (a1-a3); Ly.xy: (b1-b3); PS-1: (c1-c3)

485 **Fig. 8** Illustration of potential effects of HA on microbial induced carbonate precipitation: from
486 microbes and HA-X complexation (hypothetical structure of HA modified from Stevenson, 1994)

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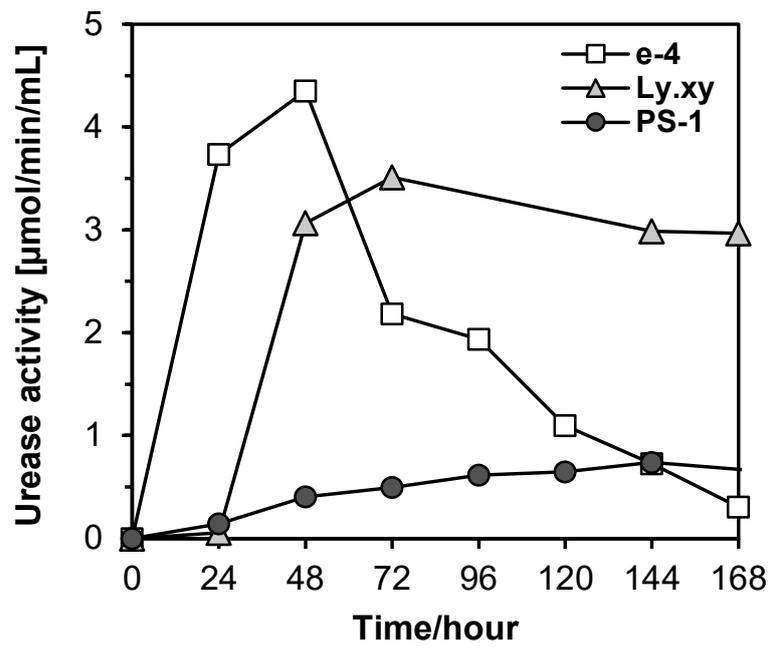
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491 30°C)

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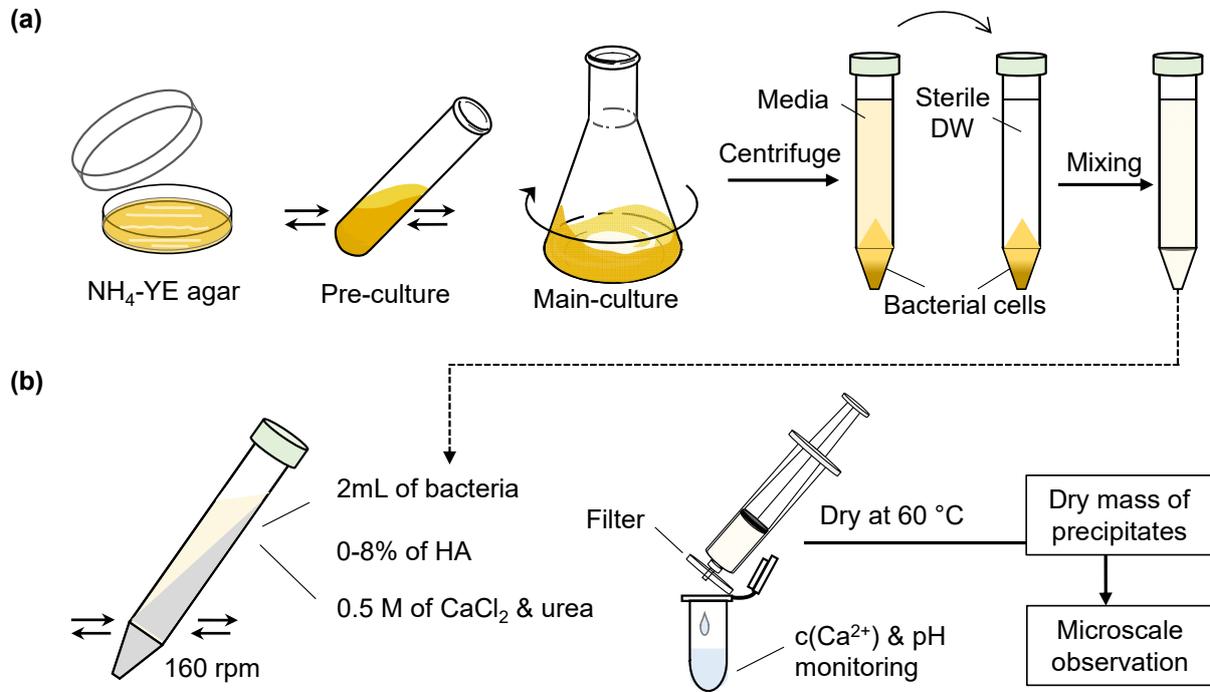


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496 Figure 2. Experiment process: (a) Preparation of bacteria culture; (b) CaCO₃ precipitation tests

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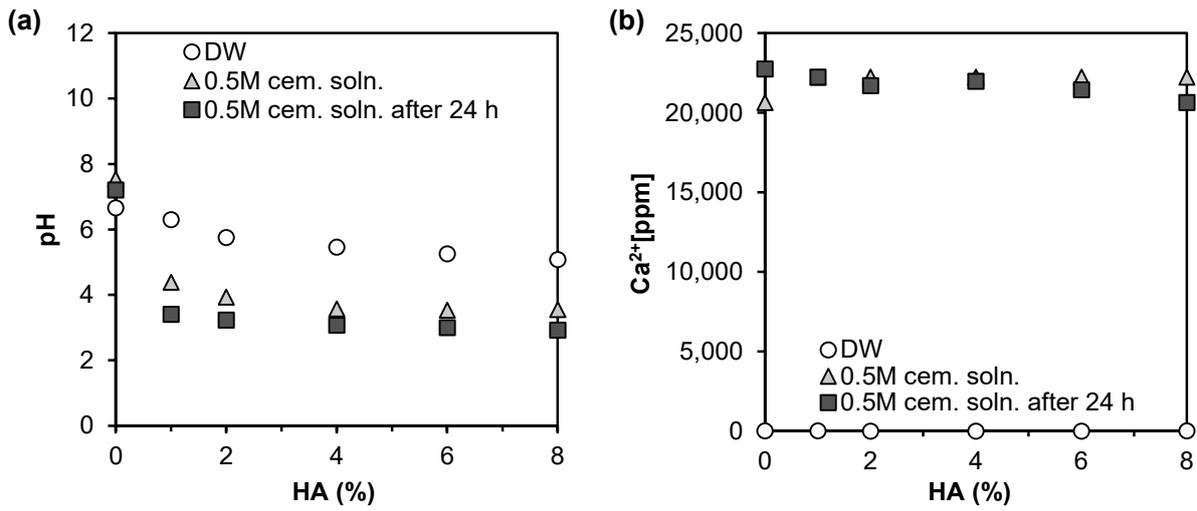


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501 Figure 3. Effect of HA on distilled water solution and on 0.5 mol/ L of CaCl₂ and urea
502 (cementation solution): (a) pH changes and (b) calcium ion concentration changes with 0-8% HA
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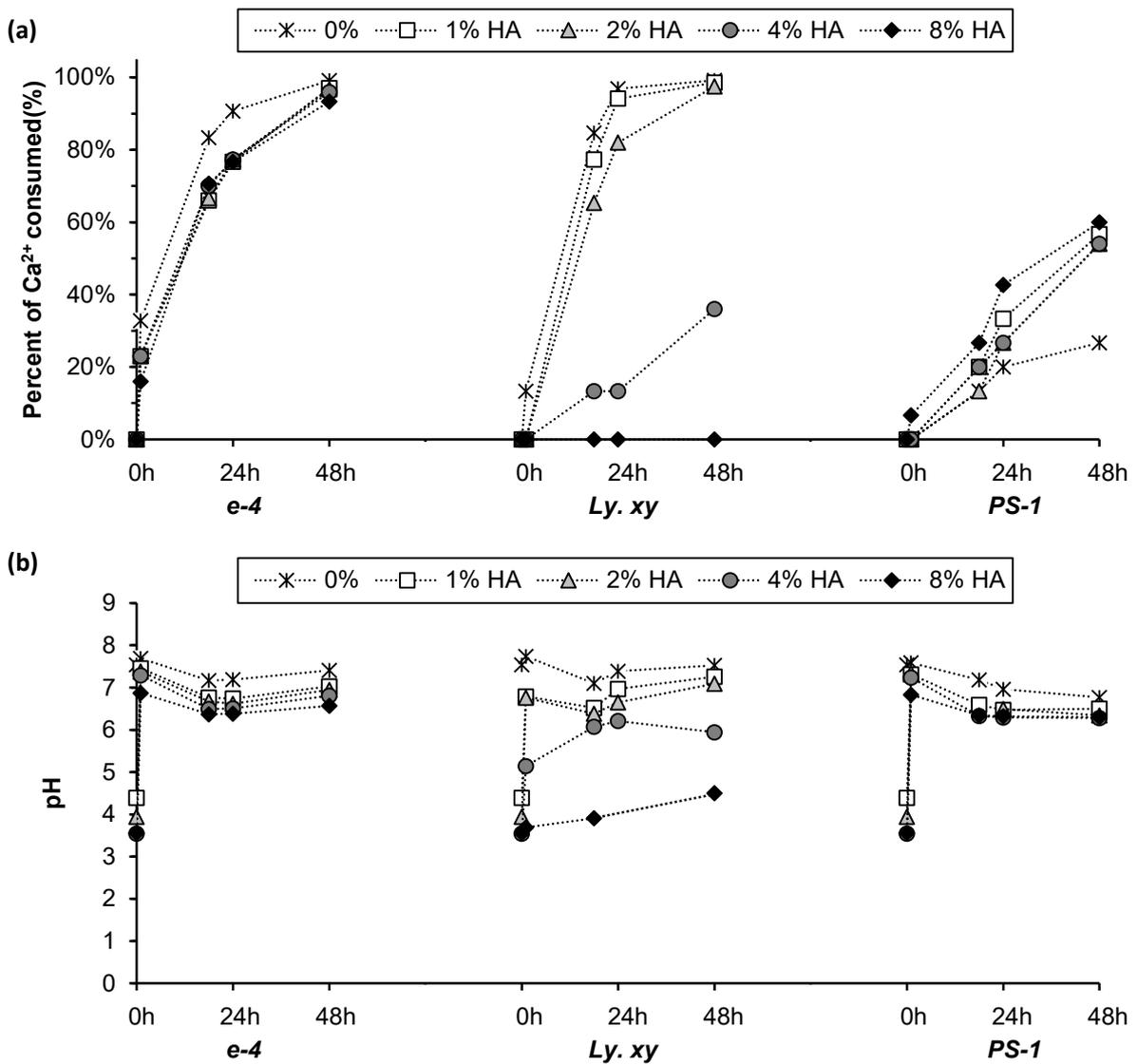
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511 Figure 4. Monitoring during the precipitation tests: (a) concentration of calcium ions and (b) pH
 512 changes with 0, 1%, 2%, 4%, 8% of HA addition (measurement after 1-hour, 18-hour, 24-hour,
 513 and 48-hour incubation)

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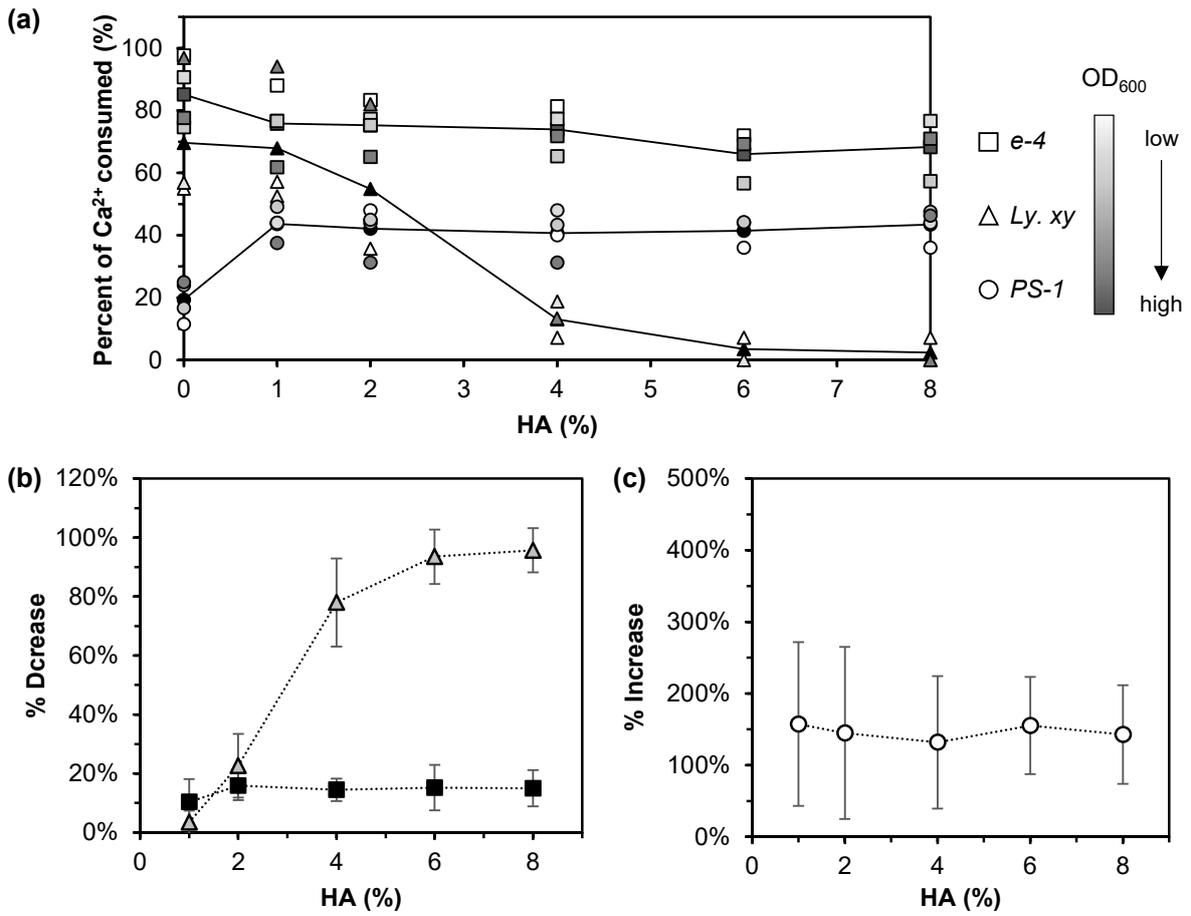


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518 Figure 5. (a) Percent of calcium ions consumed after 24 hours; (b) Percent decrease based on the
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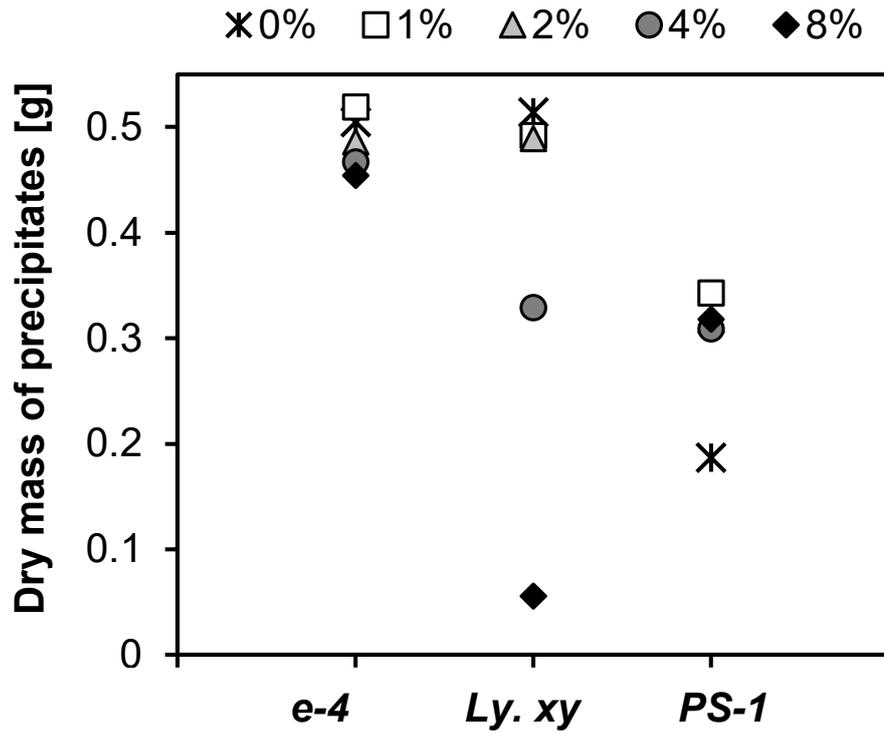
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524 Figure 6. Dry mass of precipitates calculated based on weight changes in each case

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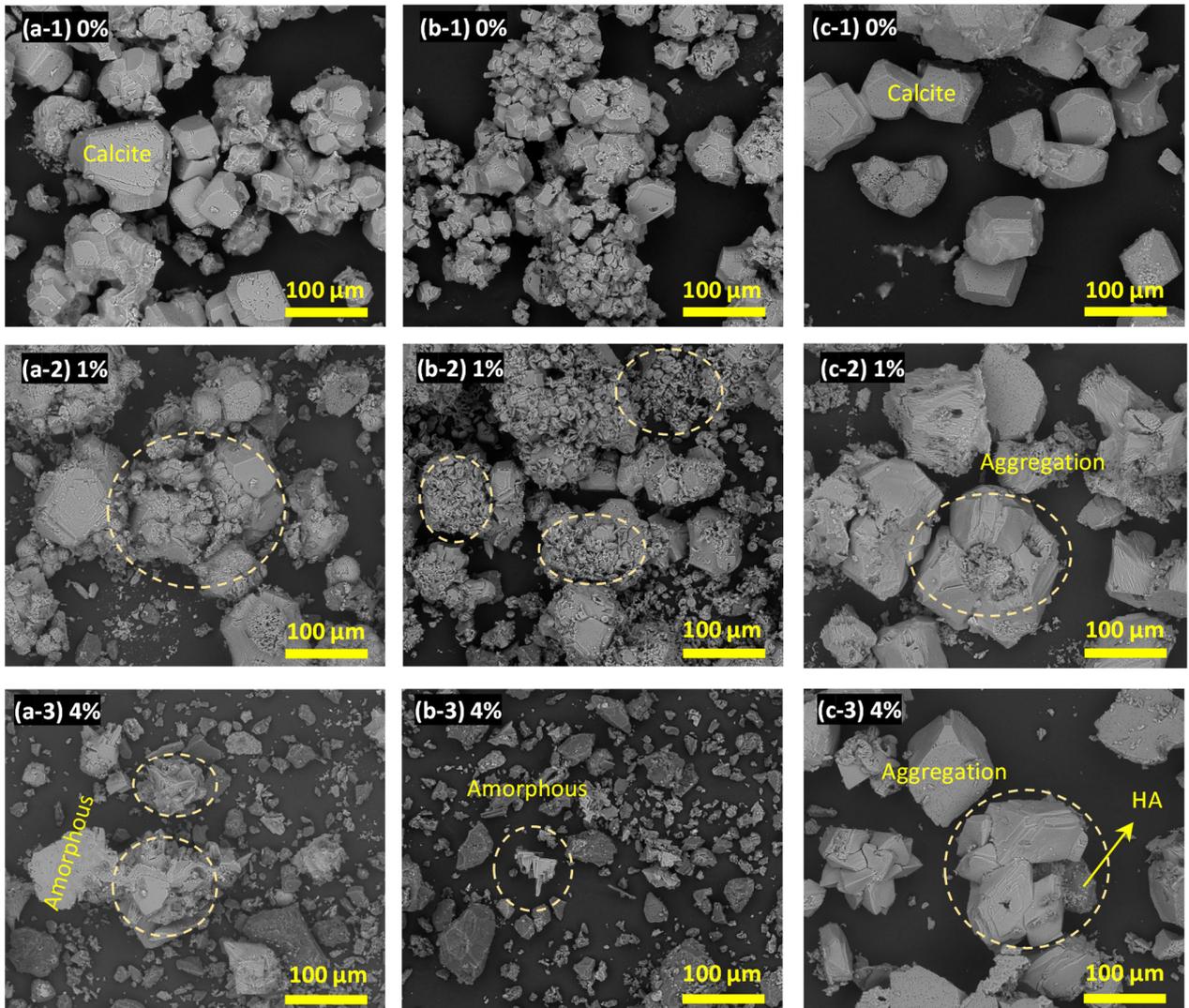
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529 Figure 7. CaCO₃ induced by three strains in precipitation tests with 0, 1%, and 4% HA addition.

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