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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 catalyzed by microbial urease. Although most MICP studies are limited to stabilizing sandy soils, 42 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 investigates the effect of humic acid (HA), one fraction of humic substances, on MICP. For this 46 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 solubility: (i) humin, insoluble; (ii) humic acid (HA), only soluble under alkaline conditions; (iii) 63 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 numerous applications in various fields, such as fertilizers in agriculture, pollutant removal in the 66 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 exponential growth of related research in recent years. As an essential part of biocementation, 77 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, but the effectiveness was low even with 20% of CaCO₃ content in the treated samples. Sato et al. 86 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 combing 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 the obstacles when using MICP to stabilize organic soils, contributing to a broader applicationrange 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

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

Three sets of precipitation tests with HA addition of 0-8% were designed, as shown in Table 2. The concentration of Ca^{2+} and pH changes were monitored during the test (after 1-h, 18-h, 24-h, and 48-h). For each sampling, 100 µL was taken and diluted before measurement. The calcium meter used in this measurement is a compact Ca^{2+} meter B-751 (HORIBA, Ltd., Kyoto, Japan), with a measurement range from 4 to 9900 ppm. The pH meter (pH5S, needle-type sensor) is a product from CEM CORPORATION, Tokyo, Japan, with a wide measurable range from pH -2 to 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 measured for each case. In order to figure out the effect of HA on the morphology of CaCO₃ 155 156 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 OD₆₀₀ were examined. After 24 hours, the concentration of calcium ions was measured for all cases to confirm the percentage of Ca^{2+} precipitated. 161

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

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

169 Where *c* is the concentration of calcium ions after 24 hours; c_o 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 CaCO₃. 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 acidic condition, reducing the pH (Adusei-Gyamfi et al., 2019). Possibly, the out-layer 183 184 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 CaCO₃ precipitation rate of three strains

Figure 4 illustrates the monitoring of calcium ion concentration and pH changes in HA-added precipitation tests using three strains. It can be seen from this figure that these three strains showed completely different behaviors during the test. In Fig. 4a, the calcium ions consumed percentage by e-4 shows that approximately 25% of precipitation occurred after 1 hour and almost 100% after 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 CaCO₃ 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 CaCO₃ 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 strain to strain in responding to HA, and this response does not necessarily have to be negative. 215

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 comparing e-4 with PS-1, additional studies are needed before concluding. Considering long-term 236 effects, it is necessary to assess the viability of these two strains under the HA-rich condition. 237

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 were observed in HA-added cases. For a comprehensive picture of it, additional studies are needed. 272

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

The main goal of the current study was to contribute to a deeper understanding of organic soil stabilization using biocementation. Therefore, it was designed to investigate the influence of HA on microbial-induced calcium carbonate precipitation using three strains, two isolated from organic soil and one from sandy soil. The results show that bacterial response to HA differs for each species. In general, the following conclusions could be drawn. i) Native species generally have advantages over exogenous species. With HA addition
up to 8%, total precipitation obtained from two native species showed a slight decrease
(less than 15%) and a doubled increase separately, while the precipitation by exogenous
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.
- These findings contribute to a better prospect of organic soil improvement using biocementation, which confirmed that selecting suitable ureolytic bacteria and the chemical environment are two key factors that should be considered. Although it seems that applying MICP to organic soils is of great difficulty due to the complicated interactions involved, this report should help figure out potential solutions. There is, therefore, a need for further research that focuses on exploring how the microbes could counteract the effect of humic acid.
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329 **Competing Interests**

330 The authors declare no competing interests.

331 Author Contributions

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

338 Availability of data and materials

339 Data available on request from the authors.

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Tables

Table 1 Specification of practical grade HA

	Property	Description				
	Appearance	Blackish brown powder				
		HCl-insoluble: min.70%				
	Solubilities	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 s	slurry was measured in at 25°C				
463						

		Microbes		
Test ID	Ct	Optical density	Optimal	Measurement
	Strain	(OD600)	temp., °C	
Control		N/A	25	
A-1	e-4	5.56	25	$nH \& Ca^{2+}$ conc. monitoring
A-2	Ly.xy	6.6	25	SEM analysis
A-3	PS-1	8.74	30	SEM analysis
B-1	e-4	3.83-6.06	25	
B-2	Ly.xy	5.6-6.6	25	Conc. of Ca ²⁺ after 24 h
B-3	PS-1	6.71-8.74	30	

T. 4	Mechanisms						
Interactions	Activation	Inhibition					
	• Improvement of urease stability by	• Complexation of HA and					
Urease-HA	reducing collisions of proteins when	positively charged urease,					
	urease is negative-charged	blocking the active site					
	• HA as a carbon source for bacteria	• Acidification of environment					
Bacteria-HA	• Increase the yield of urease from	• Adsorption of HA to cells,					
	bacteria cells	leading to less nucleation site					

Table 3 Possible mechanisms from a microbiological perspective

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
- **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)

487

488

490 Figure 1. Urease activity of three strains (incubation temperature: e-4 and Ly. xy at 25°C, PS-1 at

491 30°C)



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



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



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,

and 48-hour incubation)



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





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



534 Figure 8. Illustration of potential effects of HA on microbial induced carbonate precipitation:

535 from microbes and HA-X complexation (hypothetical structure of HA modified from Stevenson,

1994)

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