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Abstract

Microbial Induced Carbonate Precipitation (MICP) is one of the most commonly researched topics 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, more researchers are now turning their interest to other weak soils, particularly organic soils. To stabilize organic soils, the influence of humic substances should be investigated since it has been 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 purpose, the effects of HA content on $CaCO_3$ precipitation using three strains and on $CaCO_3$ morphology were examined. The results showed that native species in organic soils were less adversely affected by HA addition than the exogenous one. Another interesting finding is that bacteria seem to have strategies to cope with harsh conditions with HA. Observation of $CaCO_3$ morphology revealed that the crystallization process was hindered by HA to some extent, producing lots of fine amorphous precipitates and large aggregated $CaCO_3$. Overall, this study could provide an insightful understanding of possible obstacles when using MICP to stabilize organic soils.

Keywords: Humic acid (HA); microbial induced carbonate precipitation (MICP); urease activity; $CaCO_3$ morphology; soil improvement

Introduction

Humic substance, as one of the world's most widely distributed organic deposits, is still a mystery to soil scientists even though the first attempt to figure out what it is could be dated back to the 18th century. The definition and formation of humic substances have been a controversial and widely disputed research topic in soil science for a long time (Gerke, 2018; Baveye and Wander, 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) fulvic acid, soluble (Stevenson, 1994). Among these fractions, HA, which functionally acts as an 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 environment, and so on (Wen et al., 2019; De Melo et al., 2016; Tang et al., 2014). In natural soils, Humic substances compose up to 85% of soil organic matter, and the proportion of each fraction varies from different soil types (Ukalska-Jaruga et al., 2021). For instances, in Hokkaido, the organic soils usually contains HA ranging from 7-40% (Odajima et al., 1990). As a ubiquitous deposit, it becomes a challenging task for geoengineers to stabilize soils with high organic content (up to 50%) due to their unique chemical characteristics that hinder the hydration of cement (Toda et al., 2020; Beddaa et al., 2019). Since standard criteria for usual mineral soils might not be applicable for organic soils, some researchers have turned their attention to some novel soil improvement techniques.

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, MICP cements particles by carbonate from urea hydrolysis catalyzed by microbial urease, precipitating with calcium ions (Mujah et al., 2017). Nowadays, many MICP studies have targeted sandy soil improvement from several perspectives: (i) factors affecting MICP, (ii) characteristics of bio-cemented soils (Naveed et al., 2020; Yu and Jiang, 2018; Montoya and DeJong, 2015); (iii) durability evaluations under environmental erosions (Liu et al., 2019a; Gowthaman et al., 2020; Gowthaman et al., 2021). Although these are still limited in stabilizing sandy soils, more researchers are turning their attention to other problematic soils, such as organic ones. Canakci et 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 $CaCO_3$ content in the treated samples. Sato et al. (2016) have reported similar weak improvement after long term curing. Recently, Safdar et al. (2021) tried two different methods to apply MICP: pressure-flow column and electrokinetic in chambers, and reported the most remarkable improvement by MICP with a $CaCO_3$ content of less than 2 %. The previous stage of this study also made some efforts to stabilize high water content peat and amorphous peat combing MICP with fiber materials (bamboo fiber and wastepaper fiber), but the effectiveness was somewhat limited (Chen et al., 2021a, b; Gowthaman et al., 2021a). When it comes to applying MICP to organic soils, the existence of HA in organic soils should be of particular concern. HA is well-known as one type of soil fertilizer due to its excellent cation exchange capacity and ability to inhibit soil urease activity. When it is involved in MICP process, on the one hand, it might disrupt the formation of $CaCO_3$ by interacting with calcium ions (Falini et al., 2009; Kloster et al., 2013). On the other hand, HA inhibits urease activity by blocking the enzyme's active site, which affects urea hydrolysis efficiency (Liu et al., 2019b).

Therefore, this study sought to investigate HA's influence on microbial-induced carbonate precipitation. For this purpose, the following aspects were examined: i) effect of HA addition on precipitation rate; ii) effect of HA on the response of three strains in precipitation tests; iii) effect of HA on $CaCO_3$ morphology. Three strains used in this study were selected considering their origins and urease activity. The well-designed carbonate precipitation tests quantitatively evaluated the calcium ions consumption rate. This study tries to figure out how HA affects 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 application range of biocementation for the future.

Materials and methodology

Chemical reagents

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

Tested strains

Three strains, seen in Table 2, were tested to examine the effect of HA on bacterial response and $CaCO_3$ 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 the other side, it has been expected that the strains isolated from peat soils might have more tolerance towards HA, while strains from sandy soil might show relatively weaker resistance.

Experimental design

Figure 2 illustrates the processes in this experimental work. The preparation of bacteria is shown in Fig. 2a. The culture medium used is NH_4-YE (ATCC 1376), with 15.75 g of Tris-aminomethane, 10 g of ammonium sulfate, 20 g of yeast extract, and 20 g of agar (for plate only) in 1 L of culture medium. Bacterial colonies were inoculated to a 5 mL-preculture test tube from an NH_4-YE agar plate. After 24 hours, 1 mL of preculture was added to 100 mL of the main culture and placed in a shaker at 160 rpm under the optimal temperature for bacteria. After 48-72 hours of culture, the bacteria population was confirmed by measuring optical density at the wavelength of 600 nm using a spectrophotometer. Previous studies found that the metabolites produced by bacterial cells have some influence on the morphology of $CaCO_3$ (Li et al., 2010; Azulay et al., 2018). Therefore, the main culture was centrifuged at 8000 rpm for 5 min to separate bacterial cells and culture medium, followed by replacing the medium with sterile distilled water. For each case, in total, 10 mL of cementation solution (0.5 mol/L) and bacteria (2 mL of the main culture) are included in a 15 mL 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

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

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 $CaCO_3$ precipitation, the precipitates were observed using Miniscope TM 3000 (HITACHI, Tokyo, Japan). The bacterial population is a crucial factor affecting the survival rate when living in harsh conditions. Bacteria could generally survive a harsh environment and adapt to it more easily when the initial population reaches a corresponding threshold. Therefore, precipitation tests using three species with varying OD_{600} were examined. After 24 hours, the concentration of calcium ions was measured for all cases to confirm the percentage of Ca^{2+} precipitated.

Calculations

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

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

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

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

Results and discussion

Effect of HA addition on cementation solution

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

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

Percentage of calcium ions consumption

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

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

Effect of HA on morphology of $CaCO_3$

In terms of $CaCO_3$ precipitates, it should be noted that the weight of each case was confirmed to be consistent with the percentage of calcium ions precipitated (seen in Fig. 6), which eliminated the previous concern about the accuracy of calcium ions measurements. The morphology of $CaCO_3$ precipitation induced by three species is compared in the figure below. Fig. 7a1-a3 presents precipitations of e-4 cases. In the control case (0% of HA), $CaCO_3$ crystals were clear and found in a similar shape and size. With HA addition increasing, precipitates became less ordered, and many of them are fine amorphous particles. This might partly be explained by a lower precipitation rate retarded by HA. In terms of morphology, it might be affected by the interactions between calcium ions, urease, and HA particles. From the data of the previous section, Ly. xy was significantly affected by a relatively high HA addition. Similarly, some differences were found between cases with HA addition and control cases, as seen in Fig. 7b1-b3. More fine precipitates and less large precipitates appeared in cases with 1 -2% HA addition. Only fine precipitates were found in the 4% HA addition case. In the cases of PS-1, although the total precipitates were less than the formers, the crystals were larger and easier to identify (seen in Fig. 7c1-c3). What is interesting in these cases is that crystals precipitated around the HA particles, forming larger aggregates, which might be partially explained by the aggregation of HA and calcium ions. The reason for such formation is unclear, but it might be related to the interaction between urease and HA particles. It has been reported that urease's charge state strongly affects the electrostatic repulsion/attraction between urease and HA (Li et al., 2022). When the pH changes during the precipitation tests, the interactions described above might also undergo many changes, which 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.

Discussion on inhibition/ improvement mechanism

There are still many unanswered questions about humic substances remained. With microbes involved, this multidisciplinary research became even more exclusive. Fig. 8 illustrates some factors and interactions likely involved in microbial-induced carbonate precipitation influenced by HA addition. From the perspective of HA-X complexation, pH is a crucial contributing factor. Urease undergoes conformational changes when influenced by HA under a low pH, while this negative effect becomes less as pH increases to a certain level and alkaline conditions improve the stability of urease (Li et al., 2022). In terms of complexation between metal ions and HA, it has been reported in previous studies that there is a less available binding site at low pH, and the interactions are dominated by aggregation of HA particles (Ai et al., 2020). Although more binding sites are there under a high pH condition, the complexation of HA with common metal ions like calcium ions is much weaker (Tan et al., 2019). From the perspective of microbial response, as indicated previously, some bacteria have survival strategies to deal with harsh conditions. For instance, *H. pylori*, a well-known species that causes infection of the gastrointestinal tracts, survives stomach acid by producing a large amount of urease as part of their strategies (Lund et al., 2014). Therefore, PS-1 may have a similar response toward the acidity condition created by HA, showing an increased urease activity in HA-added precipitation tests. Moreover, some soil-borne bacteria could utilize the HA as a carbon source (Tikhonov et al., 2010). In this way, HA addition may enhance the survivability of bacteria in cementation solution without many nutrients. On the other hand, adsorption of HA to bacteria cells might have some effects on bacterial membrane transport (Feifičová et al., 2005). Usually, bacteria cells serve as nucleation sites for precipitation during the MICP process. HA nanoparticles covering the bacteria cells could reduce nucleation sites significantly. Table 3 below presents some possible activation and inhibition mechanisms analyzed from a microbiological perspective. To avoid confusion, this study did not go deeply into microbiological analysis, but there are many factors not discussed herein, such as metabolites from bacterial cells and bacterial size that might also affect the final formation of precipitates.

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.

ii) Further examination of $CaCO_3$ morphology revealed that the crystallization of $CaCO_3$ was disrupted by HA, yielding lots of fine amorphous and large aggregated precipitates. Fewer nucleation sites caused by HA adsorption on bacterial cells might contribute to the morphology change.

iii) Mechanisms are discussed from perspectives of HA-complexation and bacterial survival strategy. pH condition is one of the most contributing factors that control the 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.

Ethical Approval and Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

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Competing Interests

The authors declare no competing interests.

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.

Availability of data and materials

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
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

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

Table 2 Experimental cases

Test ID	Microbes			Measurement
	Strain	Optical density (OD_{600})	Optimal temp., °C	
Control	N/A		25	pH & Ca^{2+} conc. monitoring SEM analysis
A-1	e-4	5.56	25	
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	

468 **Table 3** Possible mechanisms from a microbiological perspective

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

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Figure captions

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

Fig. 2 Experiment process: (a) Preparation of bacteria culture; (b) $CaCO_3$ precipitation tests

Fig. 3 Effect of HA on distilled water solution and on 0.5 mol/ L of $CaCl_2$ and urea (cementation solution): (a) pH changes and (b) calcium ion concentration changes with 0-8% HA

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

Fig. 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

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

Fig. 7 $CaCO_3$ induced by three strains in precipitation tests with 0, 1%, and 4% HA addition. e-4: (a1-a3); Ly.xy: (b1-b3); PS-1: (c1-c3)

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

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

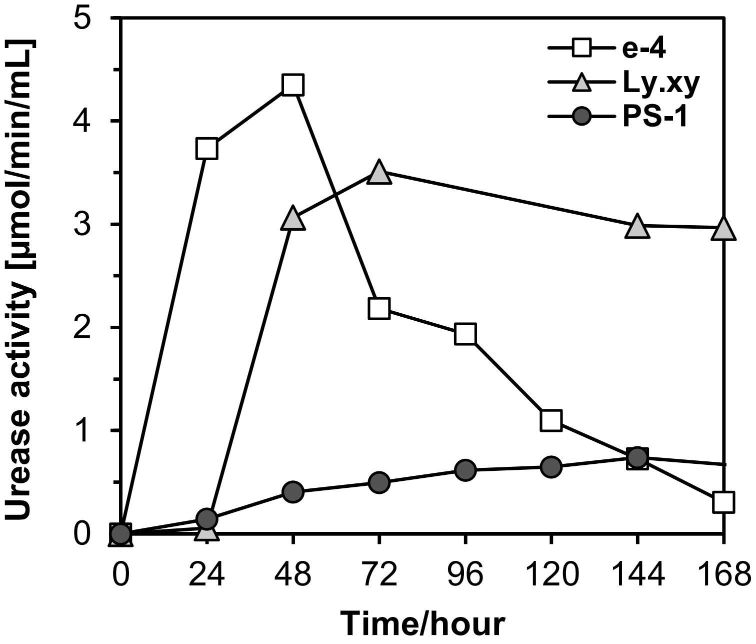
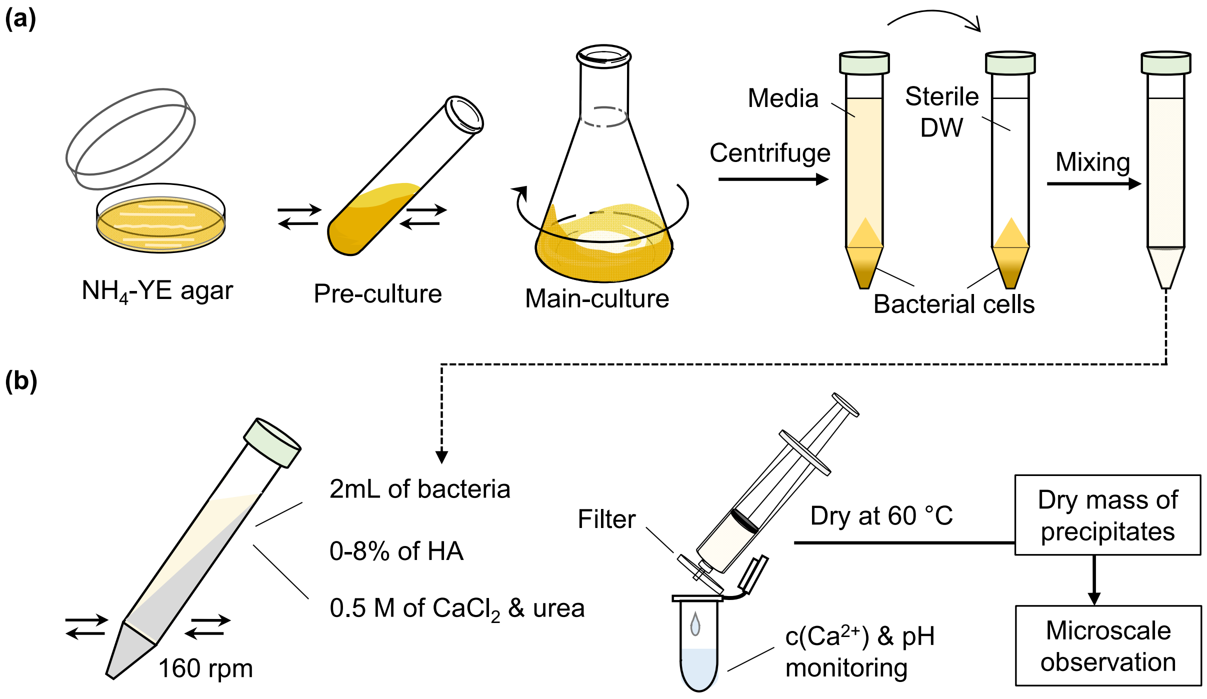


Figure 2. Experiment process: (a) Preparation of bacteria culture; (b) $CaCO_3$ precipitation tests



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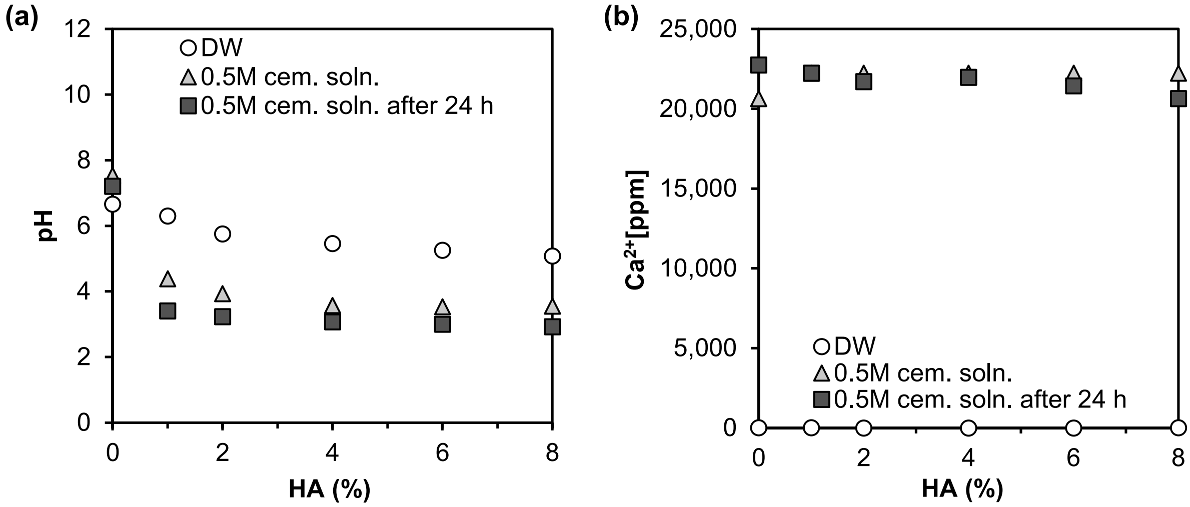

Figure 4. Monitoring during the precipitation tests: (a) concentration of calcium ions and (b) pH changes with 0, 1%, 2%, 4%, 8% of HA addition (measurement after 1-hour, 18-hour, 24-hour, and 48-hour incubation)

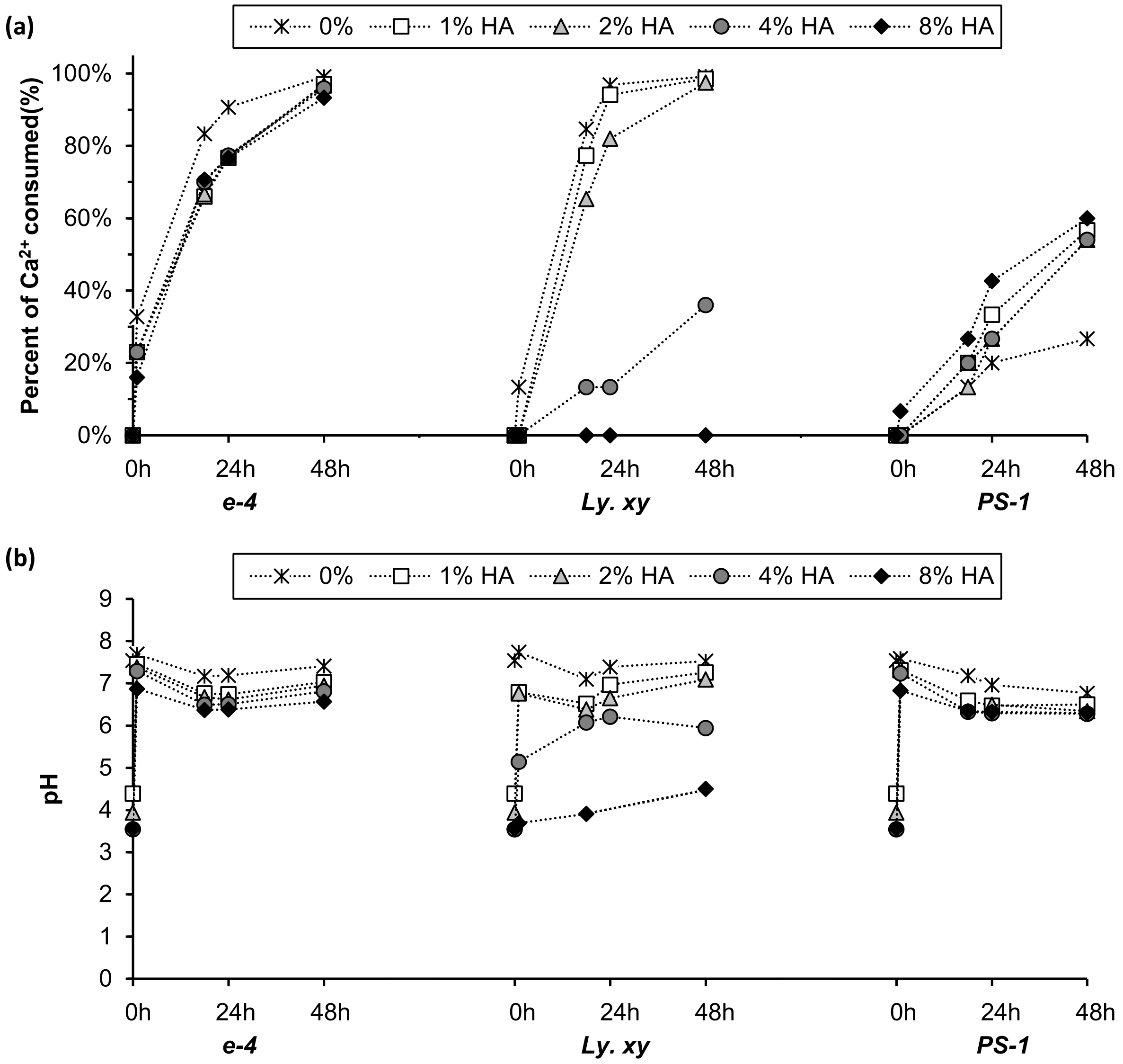

Figure 5. (a) Percent of calcium ions consumed after 24 hours; (b) Percent decrease based on the consumption rate of control cases: e-4 & Ly. xy (c) Percent increase based on the consumption rate of control cases: PS-1

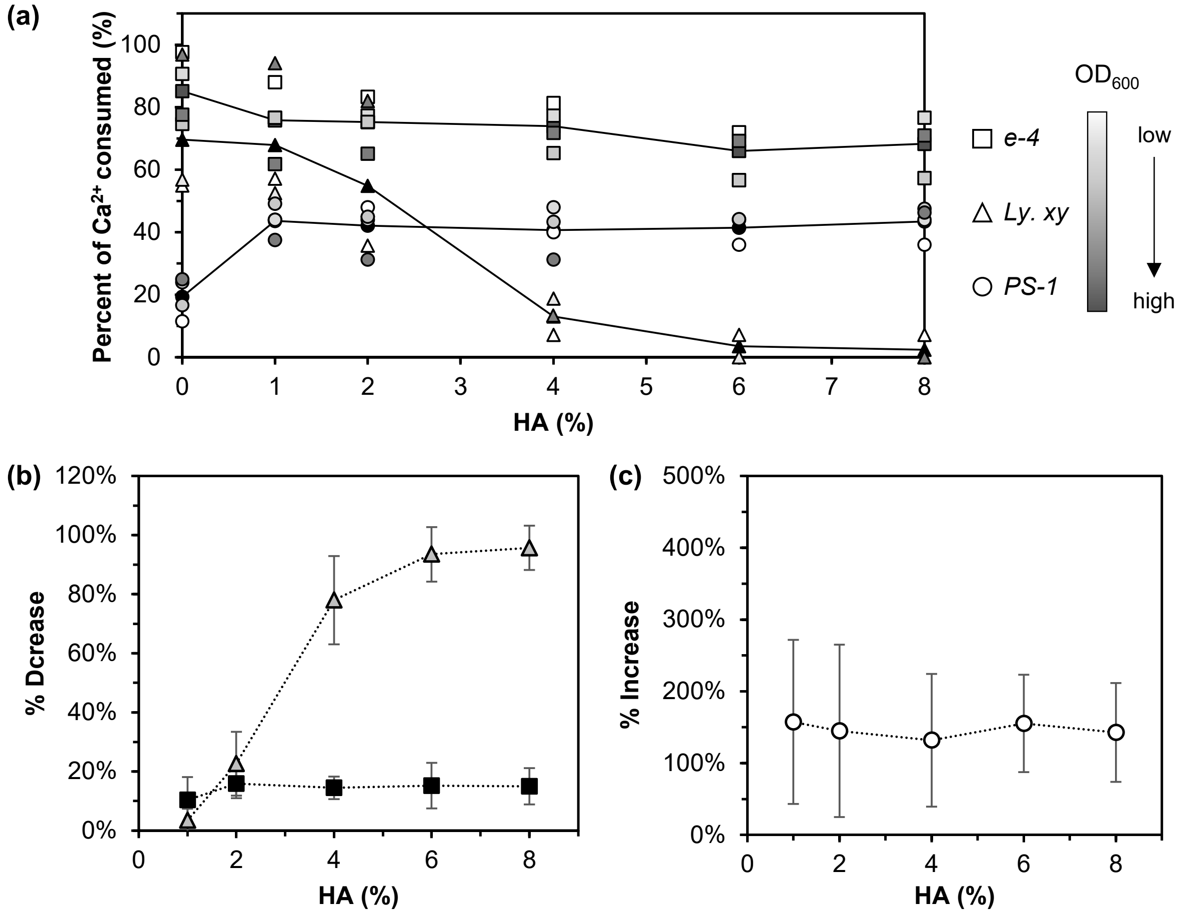

Figure 6. Dry mass of precipitates calculated based on weight changes in each case

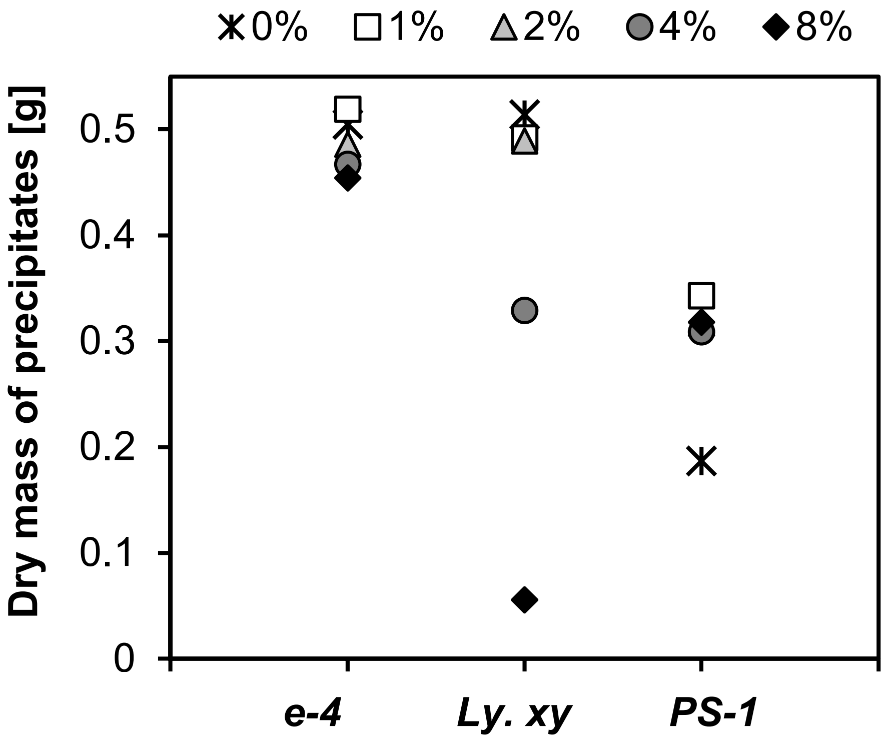

Figure 7. $CaCO_3$ induced by three strains in precipitation tests with 0, 1%, and 4% HA addition. e-4: (a1-a3); Ly.xy: (b1-b3); PS-1: (c1-c3)

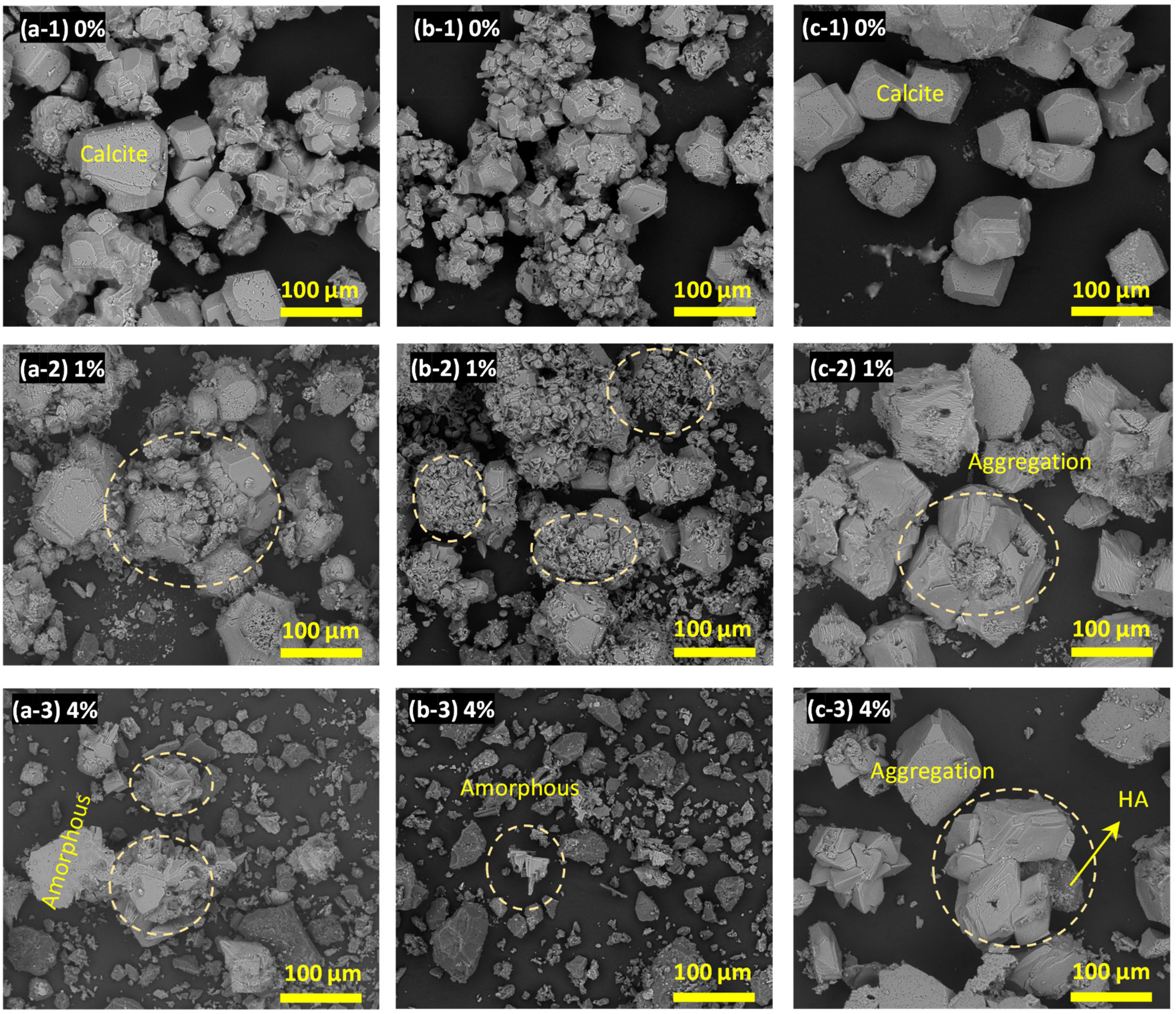

Figure 8. Illustration of potential effects of HA on microbial induced carbonate precipitation: from microbes and HA-X complexation (hypothetical structure of HA modified from Stevenson, 1994)