Soil biocementation using a new one-phase low pH injection method

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Abstract

Soil biocementation via Microbially Induced Carbonate Precipitation (MICP) has been extensively studied as a promising alternative technique to traditional chemical cementing agents for ground improvement. The multiple-phase injection methods are currently well-adopted for MICP treatment, but it is rather complex and requires excessive number of injections. This paper presents a novel one-phase injection method using low pH all-in-one biocement solution (i.e., a mixture of bacterial culture, urea, and $CaCl_2$). The key feature of this method is that the lag period of the biocementation process can be controlled by adjusting the biomass concentration, urease activity, and pH. This process prevents the clogging of bio-flocs formation and thus allows the biocement solution to be well distributed inside the soil matrix before biocementation takes effect, allowing a relatively uniform MICP treatment to be achieved. Furthermore, the ammonia gas release would be reduced by more than 90%, which represents a significant improvement in the environmental friendliness of the technology. The new one-phase method is also effective in terms of the mechanical property of MICP treated soil; an unconfined compressive strength (UCS) of 2.5 MPa was achieved for sand after six treatments.

KEYWORDS: Microbially induced carbonate precipitation; Ground improvement; Biocementation; One-phase; Microscopy

1 INTRODUCTION

In recent years, intensive studies have been made to develop a new approach for the use of Microbially induced carbonate precipitation (MICP) in soil improvement [1-5]. Currently, the most effective MICP is achieved through microbiologically or enzymatically catalysed urea hydrolysis, whereby soluble calcium source is converted into insoluble calcium carbonate crystals that bind individual sand grains together, leading to increased soil shear strength and stiffness. The fundamental mechanism of MICP process can be simply described through urea hydrolysis pathway by the following equation [6] (Eq. 1):

$$CO(NH_2)_2 + Ca^{2+} + 2H_2O \xrightarrow{Ureolytic\ bacteria} CaCO_3 \downarrow + 2NH_4^+ \quad \text{(Eq.1)}$$

The urea hydrolysis reaction produces ammonia (NH_3) and carbamic acid (H_2NCOOH) (Eq. 2), which rapidly decomposes to yield another molecule of ammonia and carbon dioxide (CO_2) (Eq. 3) [7-8]. In solution, the released one molecule of CO_2 and two molecules of ammonia consequently equilibrate with their deprotonated and protonated forms, resulting in an increase in the pH (Eqs. 4 and 5) [9].

$$CO(NH_2)_2 + H_2O \xrightarrow{Ureolytic\ bacteria} NH_3 + H_2NCOOH \quad \text{(Eq. 2)}$$

$$H_2NCOOH \rightarrow NH_3 + CO_2 \quad \text{(Eq. 3)}$$

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- \rightarrow 2H^+ + CO_3^{2-} \quad \text{(Eq. 4)}$$

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad \text{(Eq. 5)}$$

From the above listed equations, it can be concluded that both the concentration of dissolved inorganic carbon (DIC) and the pH of the environment influence the concentration of the carbonate ions and thus the calcium carbonate precipitation [10].

There are several treatment strategies available in the literature for soil improvement using MICP. Whiffin et al. [1] developed the two-phase injection method (i.e., injection of bacterial culture followed by injection of the cementation solution) that has been used in most subsequent biocementation studies. This injection strategy has the advantage of avoiding the rapid flocculation and clogging of the pore voids near the injection end. As a modification of the two-phase injection method, the so-called staged injection method was also developed [4]. In this method, a retention period was applied after the injection of bacteria to allow for better bacterial fixation. This method prevented the accumulation of the $CaCO_3$ precipitates around the injection points and thus improved the uniformity of the distribution of the $CaCO_3$ crystals precipitation [11]. Harkes et al. [12] demonstrated that using a three-phase injection procedure, which includes injection of bacteria, followed by a fixation solution and finally a cementation solution, more homogeneous distribution of bacteria and $CaCO_3$ can be obtained. Alternative to exogenous bacteria injection, the bio-stimulation approach using the in-situ enriched indigenous ureolytic bacteria was also tested [13-15]. This method includes a first phase of injection of growth media for the in-situ bio-stimulation followed by a second phase of multiple injections of cementation solution for biocementation. It should be noted that the aforementioned multiple-phase injection methods are usually complex and difficult to predict the interactions between the different phases during injection. Therefore, it is desirable to simplify the injection process to a proposed one-phase injection, with all necessary ingredients included in one solution.

In fact, Stocks-Fischer et al. [16] experimented biocementation of sand columns via a single stage method through injecting a mixture of bacterial culture and cementation solution (urea and $CaCl_2$) into the sand matrix. However, the mixing caused an instant and intensive ex-situ bioflocculation and rapid precipitation of $CaCO_3$ in the aqueous phase prior to the injection, leading to a severe clogging during the biocementation treatment. To avoid the instant interaction between the bacterial cells and chemical reagents, the bacterial culture and cementation solution were simultaneously injected via separate injection tubes but again, rapid clogging of pore voids around the injection points was observed. Shahrokhi-Shahraki et al. [11] further explained that the single-stage MICP treatment leads to massive precipitation of the reagents near the injection point due to the immediate reaction between the dissolved Ca^{2+} and microbially induced CO_3^{2-} ions, resulting in severe surface clogging.

To overcome the aforementioned one-phase clogging problem, a new biocementation methodology using a one-phase injection of low pH, all-in-one solution, is proposed in the current study. The new method implies injecting low pH biocement solution that is comprised of suspended ureolytic bacteria, chemical reagents of urea and soluble calcium such as $CaCl_2$. The key feature of the new method is that the bio-flocculation can be mitigated at low pH, and the lag period of the MICP process can be controlled by adjusting the biomass concentration, urease activity, and pH. This new process allows the biocement solution to be distributed uniformly within the soil matrix (assuming no preferential flow paths) before the MICP process starts as the urea-hydrolysis reaction must first buffer the pH upwards before the carbonate can precipitate. This process also potentially avoids the surface bio-clogging as occurred in the previous one-phase injection method. In the current study, several parameters in relation to the use of the new

one-phase injection method are examined and discussed. These include the urease activity and retention, microstructure of the produced $CaCO_3$ content, mechanical behaviour of MICP treated soil, uniformity of treatment, and amount of ammonia gas release. The predictability of the new method as measured by the repeatability of the test results is also evaluated.

2 MATERIALS AND METHODS

2.1 Bacterial culture and cementation solution

The urease active microorganism used in current study was *Bacillus sp.* isolated by Al-Thawadi and Cord-Ruwisch [17]. The microorganism was cultivated sterile aerobic batch growth medium (200 mL growth medium placed in 1 L flask shaken at 170 rpm) consisting of 20 g/L yeast extract, 15 g/L ammonium chloride, and 0.1 mM $NiCl_2$, at pH = 9.25. The biomass concentration was recorded as dry weight per volume. Because of the good correlation between biomass concentration and optical density (Eq. 6), the biomass monitoring was carried out by optical density measurements using a spectrophotometer (600 nm). All samples were diluted to a range of 0.2–1 of absorbance prior to measuring. A correlation of biomass concentration (dry weight) and optical density was established and expressed as the following equation (Eq. 6):

$$C \text{ (biomass concentration, g/L)} = 0.438 \times OD \text{ (600 nm)} \ (R^2 = 0.998) \quad (6)$$

The originally harvested bacterial culture had a biomass concentration of about 1.84±0.08 g/L (OD_{600} = 4.2 ± 0.2) and the urease activity was about 20 ± 1 U/mL, which means that the amount of the urease enzyme contained in 1 mL of culture can hydrolyse 20 ± 1 μmol of urea per minute. The urease activity in the current study was determined through the

following three steps:1) mix 5 mL of culture with 9 mL of urea solution to a final urea concentration of 1.5 M; 2) incubate the above mixture for 5-10 mins at 25 ^{o}C to allow the urea hydrolysis reaction to happen; 3) determine the ammonia concentration before and after the urea hydrolysis reaction for calculation of urease activity. The cementation solution (CS) used in this study consisted of equal moles of urea and $CaCl_2$.

The effect of pH on the bioflocs formation induced by Ca^{2+} was tested by a series of mixture of the raw ureolytic bacterial culture with the $CaCl_2$ solution at different proportions. The final biomass concentration (g/L) of the prepared mixtures were 0.981 (OD_{600} = 2.24), 0.539 (OD_{600} = 1.23), 0.267 (OD_{600} = 0.61) and 0.145 (OD_{600} = 0.33), and the concentration of $CaCl_2$ was maintained constant at 1 M. The pH of the mixture was adjusted ranging from 3.5 to 6 using 1 M HCl solution. The mixture was kept undisturbed for 30 mins until all coagulated bacterial flocs settled completely. The amount of suspended biomass was obtained by measuring the OD_{600} value of the supernatant so that the percentage of the flocculated bacterial cells can be calculated.

2.2 Preparation of all-in-one solution

The all-in-one solution (referred herein as biocement solution) proposed in this paper is defined as a mixture of the ureolytic bacterial culture and cementation solution. Before the all-in-one solution was applied to soil treatment, the solution characteristics was examined, including the biomass concentration, pH, and urease activity, for its stability assessment (i.e., occurrence of bio-flocs or precipitates). A series of the all-in-one solution was prepared by mixing the bacterial culture, deionized water and cementation solution (2 M urea and $CaCl_2$) with different proportions to gain desired initial concentrations of urease activity (i.e., 10, 5, 2.5, and 1.25 U/mL, respectively). The

concentration of the urea and $CaCl_2$ of all the prepared all-in-one solutions was 1 M. The initial pH of the all-in-one solution was then adjusted to be acidic using 1 M HCl solution. The pH evolution of the all-in-one solution was tested by continuously measuring the pH of the all-in-one solution (initial pH = 4). During the measurement the solution was kept stirring at a rate of 400 rpm.

The chemical conversion efficiency of the all-in-one solution with different urease activities (biomass concentrations) and initial pH values was tested. A series of all-in-one solution (100 mL) was prepared with various initial urease activities ranging from 1.25 to 19.5 U/mL and various initial pH ranging from 2.5 to 6 . The solution was kept stirring (400 rpm) for 24 hours. Then, the produced crystals in the all-in-one solutions were carefully collected, dried and weighted. The chemical conversion efficiency was then obtained by calculating the percentage of injected urea and $CaCl_2$ that precipitate as $CaCO_3$.

The ammonium gas release from the biocement solution was tested in a 500 mL of Schott bottle, which was filled with 200 mL of the low pH biocement solution (initial pH = 4, cementation solution = 1 M, biomass density = 0.254 g/L (OD_{600} = 0.58), urease activity = 2.5 U/mL). The atmospheric ammonia was collected by blowing air though the headspace of the rubber bung sealed bottle into an absorption unit, which was filled with H_2SO_4 solution (1 mol/L, 0.5 L). The air flow rate was kept at 0.5 L/min. The final concentration of ammonium in the H_2SO_4 solution was measured after 24 hours, which was then converted into the total amount of ammonia gas that was released into the air of the headspace. A control experiment was also conducted using the all-in-one solution without pH adjustment.

2.3 Setup and testing of treated sand columns

In order to evaluate the proposed one-phase injection strategy using the low pH all-in-one solution, a series of identical sand columns were prepared. The columns used were made of Poly Vinyl Chloride (PVC) tubing (internal diameter = 50 mm, and length = 120 and 360 mm), which were packed with pure dry silica sand (Cook Industrial, Minerals Pty. Ltd. Western Australia). The short and long columns were prepared for reproducibility and uniformity tests, respectively. The sand used has the following grading: > 0.425 mm (0.53%); 0.3–0.425 mm (50.78%); 0.15–0.3 mm (45.96%); and < 0.15 mm (2.73%). An inlet (bottom) was connected to a peristaltic pump to allow for injecting the solution. The sand was packed into each column in six consecutive layers, ensuring that each layer was compacted evenly so as to achieve at least 95% of the maximum dry density (16.35 kN/m^3) to maintain consistency of experiments. All experiments were conducted at the room temperature (25 ± 1 ^oC).

During the process of biocementation treatment, the sand columns were just simply loaded with the prepared all-in-one solutions (90 mL for 120 mm columns and 270 mL for 360 mm columns) from the bottom at a constant flow rate of about 1 L/hour until the soil is fully saturated. Then, the columns were kept at the room temperature (25 ± 1^oC) for 24 hours. Repeated injection of the prepared all-in-one solution every 24 hours was applied to reach various levels of cementation. The effluent of each treatment was collected for urease activity, ammonium and biomass concentration measurement.

To investigate the uniformity of biocementation, the 360-mm sand columns were treated using the all-in-one solution at pH = 4 with various urease activities ranging from 1.25 to 10 U/mL. After four treatments, the cemented sand samples were removed from the PVC

columns and cut into three sections: top (0-10 cm), middle (10-20 cm), and bottom (20-30 cm). The unconfined compressive strength (UCS) values for each cut section of sample were measured. Prior to the UCS tests, the bio-cemented sand specimens were flushed with at least five void volumes of tap water to wash away any excess soluble salts. The UCS tests were conducted in accordance with the ASTM Standards D2166 [18], on samples of diameter-to-height ratios ranging between 1:1.5 and 1:2 with an applied axial load at a constant rate of 1.0 mm/min.

The calcium carbonate content of bio-treated sand samples was determined by adding hydrochloric acid (HCl) solution into crushed samples according to the previous published method [19]. For each bio-treated sand sample, measurements of the $CaCO_3$ were carried out at least three times so as to obtain an average level of $CaCO_3$ precipitation.

Microscopy analysis using the scanning electron microscopy (SEM, Tescan Mira3 XMU) was conducted on dried crushed cemented soil samples after the UCS measurement. Light microscopy (Olympus IX51) was also used to examine the behaviour of bio-flocculation under various conditions (e.g., presence and absence of Ca^{2+} and low pH environment). The all-in-one solution was gently mixed at a stirring speed of 30 rpm throughout the light microscopy measurements.

3 RESULTS

3.1 Characterisation of low pH all-in-one solution

In the current study, the behaviour of biomass flocculation induced by the $CaCl_2$ was tested with various pH values ranging from 3.5 to 8. It was found that the addition of

220 $CaCl_2$ (concentration of 1 M) to the raw bacterial culture led to an instant bio-flocculation
221 within seconds, resulting in 99% of biomass precipitated as bio-flocs. By varying the pH
222 value, it was found that the flocculation could be mitigated at low pH levels, and the
223 percentage of coagulated biomass was decreased with the decrease in pH, while it was
224 increased with the increase of the biomass concentration (Fig. 1). For low concentration
225 of biomass (i.e., OD_{600} = 0.33), a pH lower than 5.5 resulted in almost complete
226 dissociation of the bio-flocs, which would be beneficial for the injection of biomass into
227 the deep location of soil and prevention of the surface bio-clogging. For high
228 concentration of biomass (i.e., OD_{600} = 2.24), a pH lower than 4 was essential to gain a
229 homogeneous suspension. It should be noted that, although the homogenous bacterial
230 suspensions in the presence of Ca^{2+} ions were obtained, the stability of the different
231 homogenous suspensions and the uniformity of their treatment outcomes need to be
232 further investigated.

233 Although the obstacle of the instant bio-flocculation induced by the Ca^{2+}, which usually
234 caused surface bioclogging in the conventional one-phase injection, was solved by
235 lowering the pH of the solution, it is expected that the pH would increase when urea is
236 added to the system due to the urea hydrolysis, which gives rise to the pH increase [20].
237 Such an increase in the pH and bicarbonate concentration (also due to urea hydrolysis)
238 might lead to an unwished ex-situ flocculation and crystal precipitation. Therefore, it is
239 important to ensure a sufficient lag period that the injection of solution can be completed
240 before the bio-flocculation occurs and the MICP process is fully activated.

241 It can be seen from Fig. 2 that the rate of pH increase varied with different concentrations
242 of the bacterial culture and urease activity. The higher the initial urease activity resulted

in slower pH increase, while the lower the initial urease activity led to faster pH rise. This is somehow contradicting the general principal that high urease activity can lead to fast urease hydrolysis, thus, quick pH increase [21]. Although the pH increases faster in the diluted bacterial culture with lower initial urease activity and biomass concentration, the system was more stable (longer lag period) due to the higher pH threshold of flocs occurrence compared to the higher initial concentration of biomass, as shown earlier in Fig. 1. For example, the development of large bio-flocs was suppressed for about 35 mins in case of the lowest initial concentration of urease activity and biomass (i.e., OD = 0.3, urease activity = 1.25 U/mL) (see t4 in Fig. 2). This is in line with the previous results (see Fig. 1), which indicated that for low concentration of biomass, high pH was needed to induce the bacterial flocs large and heavy enough to precipitate and settle.

The evolution of bio-flocs was further investigated by the light microscopy. The all-in-one solution (OD_{600} = 1.25, urase activity = 5 U/mL, CS = 1 M) was sampled at different time after the pH was lowered to 4. No or minor bacterial flocculation can be observed for the first 15 minutes after lowering the pH (see Fig. 3a-d), demonstrating that a stable and relatively homogeneous all-in-one solution was achieved. The bacterial flocculation continuously developed with time leading to a strong flocculation with flocs size eventually larger than 200 μm (see Fig. 3e & 3f). This development of bacterial flocculation is likely due to the increase in the solution pH as discussed earlier.

3.2 MICP driven by low pH all-in-one solution

The process of MICP biocementation driven by the low-pH all-in-one solution were assessed according to the following five aspects: (a) ammonia gas release; (b) chemical conversion efficiency; (c) treatment reproducibility; (d) biocementation uniformity; and (e) microstructure analysis. The above aspects are discussed in some detail below.

3.2.1 Ammonia gas release

The production of atmospheric ammonia using the new low pH treatment method was examined. The results show that in comparison with the traditional method without pH adjustment more acidic environment was achieved using the low-pH treatment approach, as indicated by the evolution of the pH in Fig. 4. It was also found that the amount of atmospheric ammonia was significantly reduced by about 90% compared to the conventional method, which is due to the more acidic environment in which the produced ammonia was associated with proton to form soluble ammonium ions. It is also important to note that the overall chemical conversion efficiency over the testing period of 24 hours was not significantly affected by the initial pH adjustment to a low level of 4, suggesting similar process efficiency of the new method to the conventional approach.

3.2.2 Chemical conversion efficiency

In this study, the chemical conversion efficiency (24 hours of reaction period) of the biocement solution was tested at different levels of initial pH levels and urease activities. It can be seen from Fig. 5 that the chemical conversion efficiency was reduced with the decrease in the initial pH. The conversion of urea and $CaCO_3$ precipitates were not detectable when the initial pH was lower than 3. This is probably due to the acid stress

285 that inhibits the urease activity of alkaliphilic ureolytic bacteria. An initial pH higher than
286 4 had minor effect on the chemical conversion efficiency. This pH adjustment eliminates
287 the formation of bio-flocs, generates a stable all-in-one solution, and at the same time
288 achieves high chemical conversion efficiency. Therefore, it is recommended that the
289 minimum initial pH of the biocement solution should not be adjusted to lower than 4 prior
290 to the application of the one-phase injection strategy for soil stabilisation.

291 The results also show that the chemical conversion efficiency decreases with the decrease
292 in the urease activity (see Fig. 6). For the low urease activity of 1.25 U/mL, only about
293 60% of cementation solution (i.e., 1 M) was converted into $CaCO_3$ precipitates. It is well-
294 known that the urease activity decreases with the increase in the amount of $CaCO_3$
295 precipitation as a result of compounded effect of biological degradation and chemical
296 reaction [19, 22]. This affects the chemical conversion efficiency of the all-in-one
297 solution and in turn the cost effectiveness. Therefore, the result suggests that in order to
298 improve the efficiency and reduce the amount of waste (unconverted chemicals), lower
299 concentration of cementation solution (e.g., 0.5 M) should be applied given the low urease
300 activity used. In this case, more flushes are needed to reach a target level of cementation.

301 **3.2.3 Treatment reproducibility**

302 As an engineering solution, the outcome of improvement needs to be predictable to allow
303 reliable engineering design. One way to assess the predictability of a method in a
304 laboratory is to check the repeatability of the test results. Fig. 7 shows the UCS values of
305 two groups of identical sand columns treated equally using the one-phase injection of the
306 low pH all-in-one solution. The results of the two samples were quite comparable with
307 low and medium cementation levels (variation less than 50 kPa), indicating a good

reproducibility. However, when the sand columns were treated further to reach a higher level of cementation (i.e., 6 treatments) the variation became greater. Overall, the difference in UCS value between the two sand columns was less than 10%. It should be noted that almost no urease activity (urease activity < 0.1 U/mL) and biomass (OD_{600} < 0.02) were detected in the effluent during the repeated treatments, suggesting biomass retention of almost 100%. In comparison, the traditional two-phase injection method can only achieve biomass retention of about 30%-80% [4].

3.2.4 Biocementation uniformity

In order to achieve a uniform treatment, the all-in-one solution needs to have a sufficient lag period to allow the biocement solution to be well distributed. However, according to the results presented earlier, the lag period of the all-in-one solution varied according to the system pH increase rate, which is a function of the urease activity.

It can be seen from Fig. 8 that the sand columns treated with low urease activity shows a relatively homogeneous strength distribution. For example, for the urease activities equal to 1.25 and 2.5 U/mL, the strongest section of the treated sand columns was found at the bottom part, which gained strength of about 700 and 760 kPa, respectively (Fig. 6), which are about 160 kPa higher than the weakest section of treated sand columns (top part). For the 5 U/mL, the difference between the strongest section (bottom part) and weakest section (top part) increased to about 330 kPa (Fig. 8). For the highest urease activity of 10 U/mL, the difference between the strongest and weakest parts became even larger to about 1190 kPa (Fig. 8). The UCS obtained from each test is also plotted versus the average $CaCO_3$ content in the sample (see Fig. 9). It can be seen that the UCS obtained

330 is highly correlated to the $CaCO_3$ content as previously established [3]. The large
331 variation in the UCS is related to the large variation in the $CaCO_3$ content.

332 **3.2.5 Microstructure analysis**

333 Fig. 10 portrays the SEM results of the sand particles cemented with $CaCO_3$ crystals
334 produced using the low pH all-in-one solution with various urease activities. It can be
335 seen from Fig. 10a & b that the $CaCO_3$ produced at low urease activity (1.25 U/mL) are
336 majorly accumulated at the gaps between the sand grains and the sand grains surface
337 possesses minimum crystals precipitation (indicated by the red circle area in Fig. 10a)
338 compared to those produced at high urease activities (see Fig. 10d-f). This is possible due
339 to the deposition or entrapment of bio-flocs at the connecting points of sand grains. For
340 higher urease activity, more biomass was present leading to larger amount of bio-flocs,
341 which were not only precipitated at the gaps but also possibly on the sand grains surface
342 (see Fig. 10d-f). Therefore, the individual $CaCO_3$ crystals were well distributed spatially
343 and covered the surface of the sand grains as a coating-like layer.

344 The average size of individual crystals was similar for all urease activities, ranging from
345 10 to 25 µm. However, the shape of crystals was found to be remarkably different in
346 relation to the urease activity, especially for the highest urease activity and biomass
347 concentration. This is likely due to the presence biopolymer or amino acids, especially
348 the contained carboxylic acid or sulfate functional groups, in the all-in-one solution. It
349 was found that specific binding of natural polypeptides to particular calcite crystal faces
350 was responsible for the modification in the calcite crystals morphology [23]. The
351 morphology of $CaCO_3$ was also influenced by the concentration of biopolymers, such as
352 lysozyme and collagen[24, 25]. It has been shown that high urease activity increase the

353 saturation at which crystals nucleate and grow, which results in more likely occurrence
354 of metastable precursor minerals such as vaterite. The urease activity and organic
355 molecules will also change the onset and rate of crystal nucleation, thus the morphology
356 of mature crystals. Therefore, the different crystal texture could be attributed to the types
357 and concentration of organic polymers in the all-in-one solution. The effect of such
358 different shapes of crystals on the chemical bonding and final mechanical strength
359 performance is still unclear and will be worthwhile investigated in a future study.

360 # 4 DISCUSSION

361 ## 4.1 Low pH enables stable all-in-one solution

362 In addition to the rapid calcium carbonate precipitation, instant coagulation of bacterial
363 cells induced by a trace amount of calcium ions was another major reason for the surface
364 bio-clogging in the use of all-in-one solution without pH adjustment [26]. These bio-flocs
365 were unable to be injected into the sand columns due to the large particle size [1, 16]. Due
366 to the negative charge of the extracellular biopolymer substance (EPS) attached to the
367 bacterial cells, divalent cations, such as Ca^{2+} and Mg^{2+}, can bridge the negative sites on
368 the biopolymer network, resulting in bacterial flocculation and settlement [27]. Several
369 studies have suggested that the bivalent cations such as Ca^{2+} and Mg^{2+} play a role in the
370 flocculation process at high pH [28, 29]. The change in pH to a low level probably alters
371 the EPS structure, bacterial surface properties, surface charges and accordingly the
372 microbial flocculation behaviour [30], resulting in the elimination of bio-flocculation.

373 The low pH also enables a lag phase of MICP process. The $CaCO_3$ precipitation is
374 controlled mainly by the Ca^{2+} and DIC concentration and the pH [31]. Keeping the pH of

the all-in-one solution at a low level is essential to achieve an adequate lag period of MICP process as the urea-hydrolysis reaction must first buffer the pH upwards before $CaCO_3$ can precipitate. In the all-in-one system, the pH evolution is determined by the buffer capacity of the solution, which, in the current study, is attributed to the concentration of the chemicals in the bacterial culture, such as amino acids, NH_4^+, EPS, etc. The low urease activity obtained by diluting the raw bacterial culture with deionised water resulted in a dilution of the buffer capacity accordingly. Therefore, the heavily diluted bacterial culture (i.e., lowest urease activity) could not buffer the produced hydroxide ions as much as the undiluted or moderately diluted culture, resulting in the fastest increase in pH (see Fig. 2). In this case, adding chemical acidic buffers to the all-in-one solution to slow down the pH increase and improve its lag period will be beneficial for a large-scale treatment.

4.2 Advantages of one-phase low pH injection strategy

During the MICP process, the urea-driven process produces toxic end product of ammonia. Ammonia is highly water-soluble and can largely remain in the water in the dissociated form as ammonium (NH_4^+). Only that part which is present in the unionised form (NH_3) can become volatile and be released as a gas. The impact of ammonium on the environment can be mitigated by extracting the solution out of the ground and treated separately. However, the atmospheric ammonia usually causes unpleasant smell and is toxic for a long-term exposure at concentrations as low as 25 ppm [32]. The higher pH, the more ammonia is present in the water in volatile form, thus more atmospheric ammonia is released [33]. By lowing the pH of the all-in-one solution, the produced

ammonia during the MICP process remained largely as ionised form of NH_4^+, thus, significantly diminished the atmospheric ammonia production.

The proposed low pH approach injects all chemical ingredients, including bacteria, urea and $CaCl_2$, into the soil in one phase, leading to a homogenous reaction over the entire treatment zone. Although there is a trend of greater variation in UCS for a high level of cementation, which is likely due to the self-enhanced and enlarged inhomogeneity during the repeated treatments [34], the reproducibility with overall variation less than 10%. was achieved.

The uniformity of the cementation using the one-phase low pH injection strategy is strongly related to the homogeneity of the mixture and the lag period of MICP. Because low urease activity provided substantial period of lag phase enabling complete injection and uniform distribution of all the chemical ingredients within the sand column, a relatively homogeneous strength distribution was achieved (see Fig. 6). In contrast, the higher non-uniformity in the $CaCO_3$ content was related to the short lag period associated with the high urease activities. For example, for the sand column treated with the highest urease activity (i.e., 10 U/mL), the strongest section was achieved at the middle part of the sand column. The short lag period of the all-in-one solution resulted in a rapid bio-flocs formation before the suspended biomass reaching the end (top part) of the sand columns. This in-situ formed bio-flocs probably accumulated inside of the sand columns and acted as filter to prevent further penetration of the following injected biomass, resulting in limited amount of biomass that reached the top part of the sand column. The accumulated biomass in the middle part of the sand columns would not only consume the urea and $CaCl_2$ from the local area but also the urea and $CaCl_2$ diffused from the top part

of the sand column at which limited amount of biomass was found, enabling substantial precipitation, hence, gaining the highest strength (Fig. 6). Theoretically, the period of lag phase of the prepared all-in-one solution can be also be enhanced by increasing its acidic buffer capacity through addition of chemical buffer, such as weak acid of acetic acid.

It is also interesting to note that for the low urease activities (i.e., 1.25, 2.5, and 5 U/mL), the slightly stronger section obtained at the bottom part was likely due to the higher amount of biomass. This is because when the suspended bacteria cells travelled through the soil pore space, they were likely to be filtered through the soil grains with long linear reduction of microbe concentration along the injection path [35].

4.3 Limitation of current research

Although the study has successfully demonstrated the feasibility of this new method for bio-cementation in short sand columns, the process has yet to be tested for soil at a meter scale. Thus, the effect of applying the proposed method for large scale soil improvement is still unknown. Furthermore, only one type of ureolytic bacteria was tested in the current study. For practical applications, it is necessary to test the bioflocculation behaviour of other ureolytic bacteria species, such as *Sporosarcina pasteurii*, *B. cereus*, *B. sphaericus*, etc. We have, in fact, also tested the commonly used ureolytic bacterial strain *Sporosarcina pasteurii* (DSM 33). The preliminary results show similar bio-flocculation behaviour to the strain used in this study. Nevertheless, the proposed method with fast injection speed associated with prolonged lag phase will enable a much larger regime to be loaded with the all-in-one solution prior to the formation of bio-flocs and precipitation and thus improve the uniformity of the treatment. In future work, advanced measurements such as 3D X-ray microtomography would be conducted to investigate the fundamental

mechanism of the crystal bonding produced by the all-in-one biocement solution and the results would be compared with traditional biocement published elsewhere [36].

5 CONCLUSIONS

This paper presents a new soil bio-cementation method based on MICP process using one-phase injection of low pH all-in-one biocement solution. The biocement solution provides a lag period, which is a function of several parameters (i.e., pH, biomass concentration, and urease activity), to allow the solution to be distributed evenly within the soil before large amount of bio-flocculation and MICP occurs. The new one-phase approach was proved to be able to provide a relatively uniform soil strength distribution. By lowering the pH of the biocement solution, the lag period was able to be controlled to up to 35 mins, enabling an easy injection of the biocement solution without facing clogging issues. This period of lag phase can be theoretically enhanced by increasing the acidic buffer capacity of the all-in-one solution. The UCS values of the bio-cemented samples were significantly improved to about 2.5 MPa after 6 treatments. More importantly, the proposed one-phase method reduced the production of ammonia gas by 90% compared to the unchanged MICP methods, overcoming one of the major limitations of the application of MICP in practice. Therefore, the proposed method represents a considerable advance in the use of biocementation for soil improvement.

Future research on this topic may include a thorough investigation on the correlation between the soil mechanical response and various compositions of the all-in-one solution used. Theoretically, the biocementation using the proposed one-phase injection approach is majorly related to the distribution of the one phase biocement solution, which can be

beneficial for future study of the establishment of reliable analytical and/or numerical models that can predict the outcomes of soil improvement more precisely.

6 ACKNOWLEDGEMENT

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570 biocemented sand using 3D X-ray microtomography. Acta Geotechnica 12(5):
571 955–970
572

573 Figures Captions:

574 **Fig. 1** Biomass flocculation of ureolytic bacteria as a function of pH in presence of 1 M
575 $CaCl_2$

576 **Fig. 2** Evolution of pH of all-in-one solution with initial pH of 4 in presence of different
577 urease activity (t1-t4 indicates the time when the bio-flocs started to appear; UA = urease
578 activity

579 **Fig. 3** Evolution of bacterial flocs in an acidified all-in-one solution (OD_{600} = 1.21, urase
580 activity = 5 U/mL, cementation solution = 1 M urea and 1 M $CaCl_2$). The samples were
581 taken at time: (a) 0 min; (b) 5 min; (c) 10 min; (d) 15 min; (e) 20 min; and (f) 25 min, for
582 the light microscopy measurements (the scale bar = 30 μm). Magnified suspended
583 bacterial cells were indicated in the rectangle area in image (a)

584 **Fig. 4** Comparison of the total amount of atmospheric ammonia produced (24 hours)
585 between the conventional and newly invented low-pH method.

586 **Fig. 5** Effect of initial pH on chemical conversion efficiency of the all-in-one solution
587 (urease activity was about 5 U/mL, OD_{600}=1.28) (N.D. = not detectable)

588 **Fig. 6** Effect of urease activity on chemical conversion efficiency of bio-cementation
589 using the one-phase injection of low pH all-in-one solution (CS = 1 M and pH = 4)

590 **Fig. 7** Reproducibility of bio-cemented sand columns (120 mm long) treated with low pH
591 all-in-one solution using the one-phase injection strategy (OD_{600} = 0.58, urease activity
592 = 2.5 U/mL, CS = 1 M, pH = 4)

Fig. 8 Effect of urease activity of all-in-one solution on strength distribution of the sand columns

Fig. 9 Correlation between the UCS and $CaCO_3$ of cemented sand columns treated with different urease activities

Fig. 10 SEM images of bio-treated sand samples using one-phase injection strategy of low pH all-in-one solution (i.e., pH = 4 and cementation solution = 1 M): (a) 1.25 U/m; (b) 2.5 U/mL; (c) 5 U/mL; and (d) 10 U/mL

Figure 1

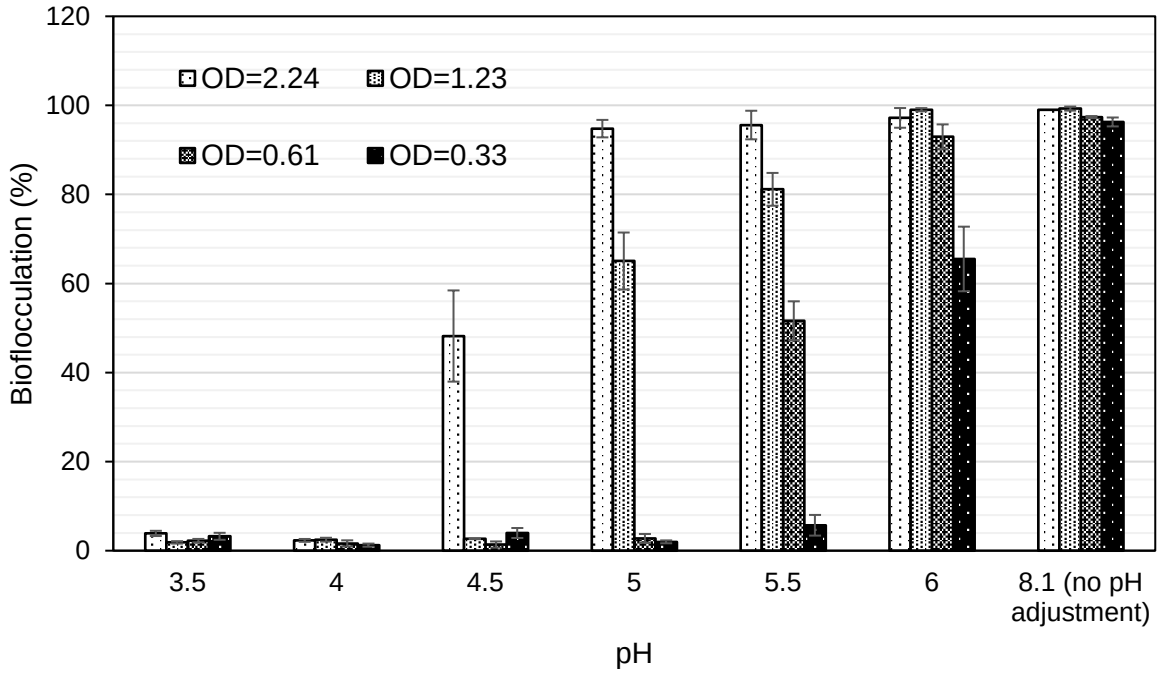

120
100
80
60
40
20
0
Bioflocculation (%)
OD=2.24
OD=1.23
OD=0.61
OD=0.33
3.5
4
4.5
5
5.5
6
8.1 (no pH adjustment)
pH

Figure 2

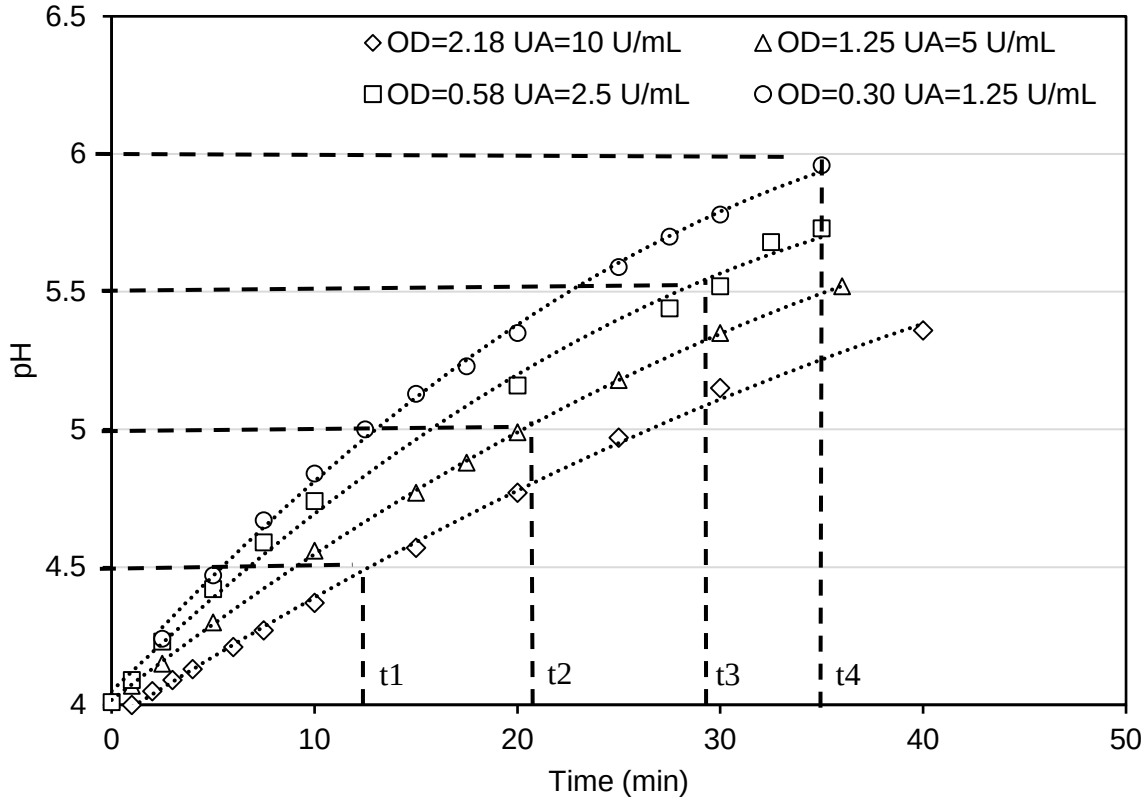

OD=2.18 UA=10 U/mL
OD=1.25 UA=5 U/mL
OD=0.58 UA=2.5 U/mL
OD=0.30 UA=1.25 U/mL
6.5
6
5.5
5
4.5
4
pH
0
10
20
30
40
50
Time (min)
t1
t2
t3
t4

Figure 3

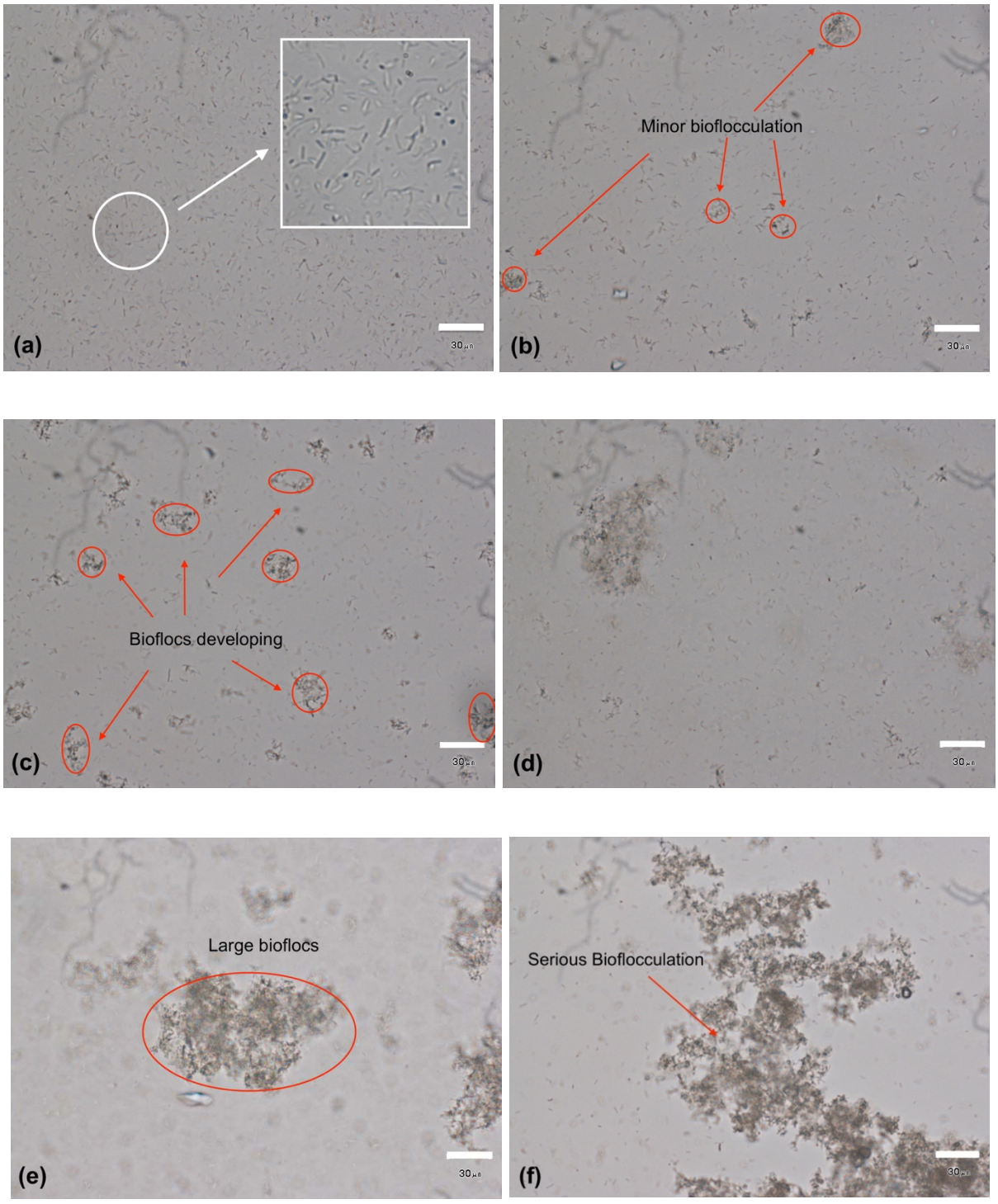

(a)
30 μm
Minor bioflocculation
(b)
30 μm
Bioflocs developing
(c)
30 μm
(d)
30 μm
Large bioflocs
(e)
30 μm
Serious Bioflocculation
(f)
30 μm

Figure 4

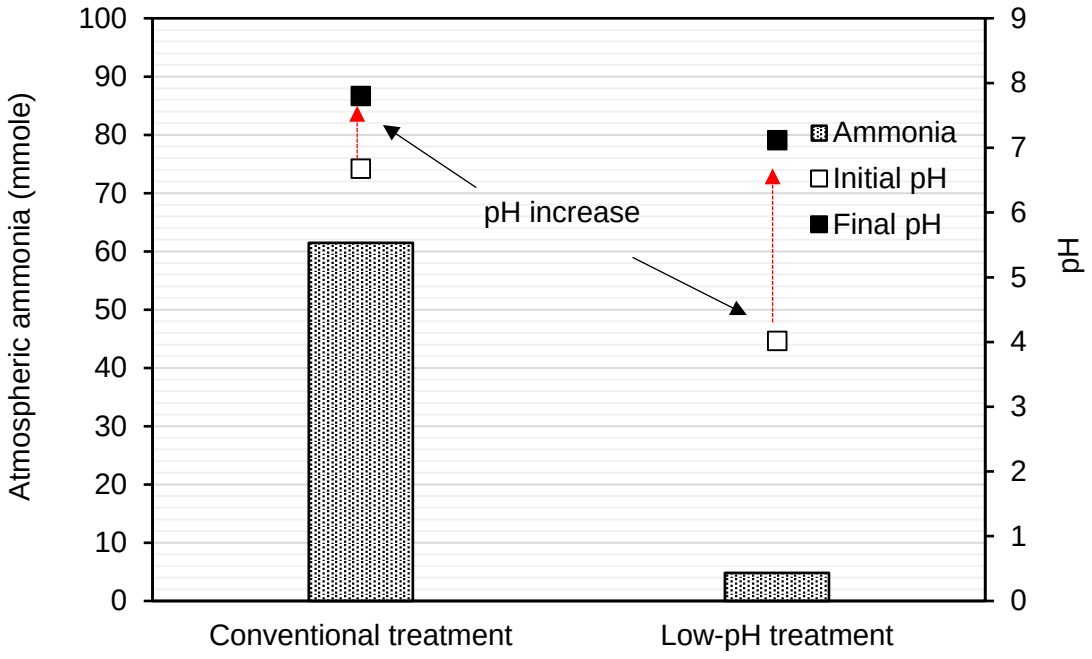

Atmospheric ammonia (mmole)
pH
100
90
80
70
60
50
40
30
20
10
0
9
8
7
6
5
4
3
2
1
0
Ammonia
Initial pH
Final pH
pH increase
Conventional treatment
Low-pH treatment

Figure 5

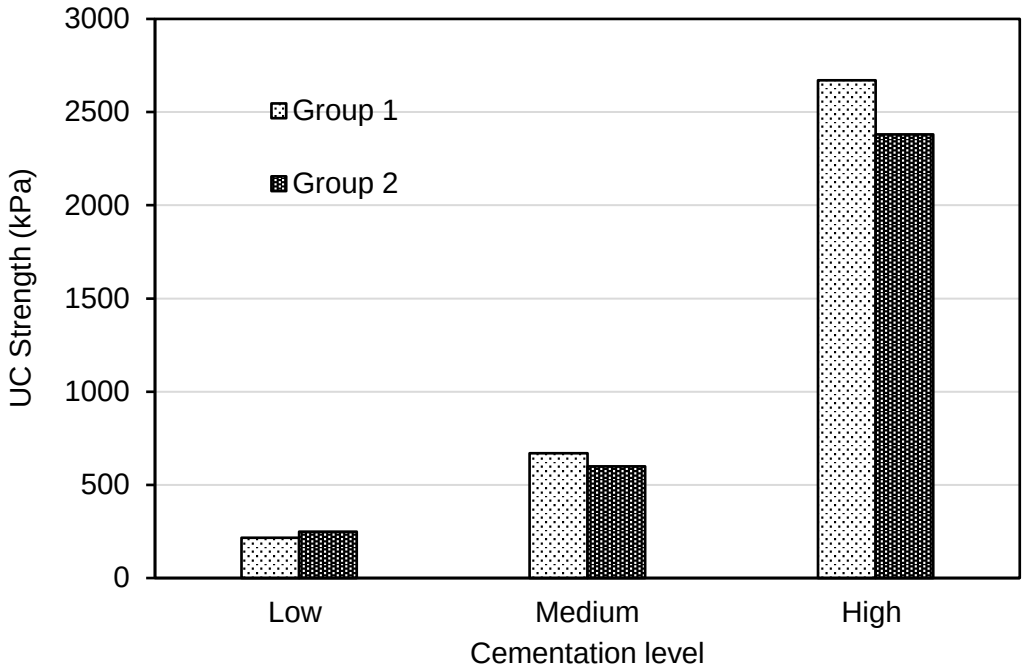

3000
2500
2000
1500
1000
500
0
UC Strength (kPa)
Group 1
Group 2
Low
Medium
High
Cementation level

Figure 6

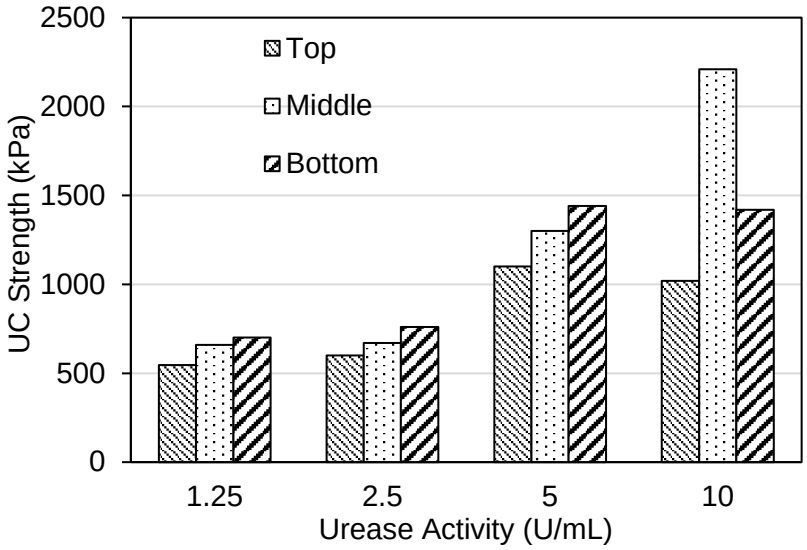

2500
2000
1500
1000
500
0
UC Strength (kPa)
Top
Middle
Bottom
1.25
2.5
5
10
Urease Activity (U/mL)

Figure 7

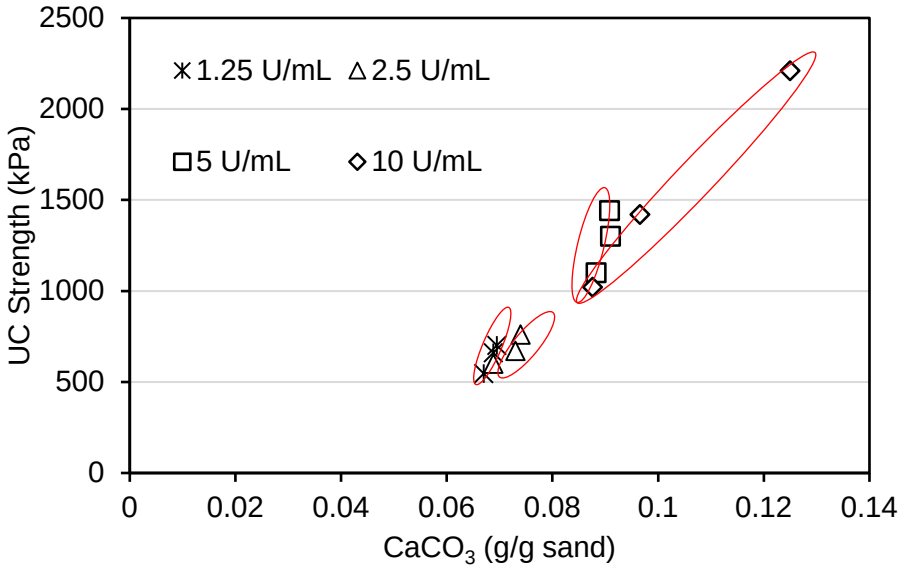

2500
2000
1500
1000
500
0
UC Strength (kPa)
1.25 U/mL
2.5 U/mL
5 U/mL
10 U/mL
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
CaCO3 (g/g sand)

Figure 8

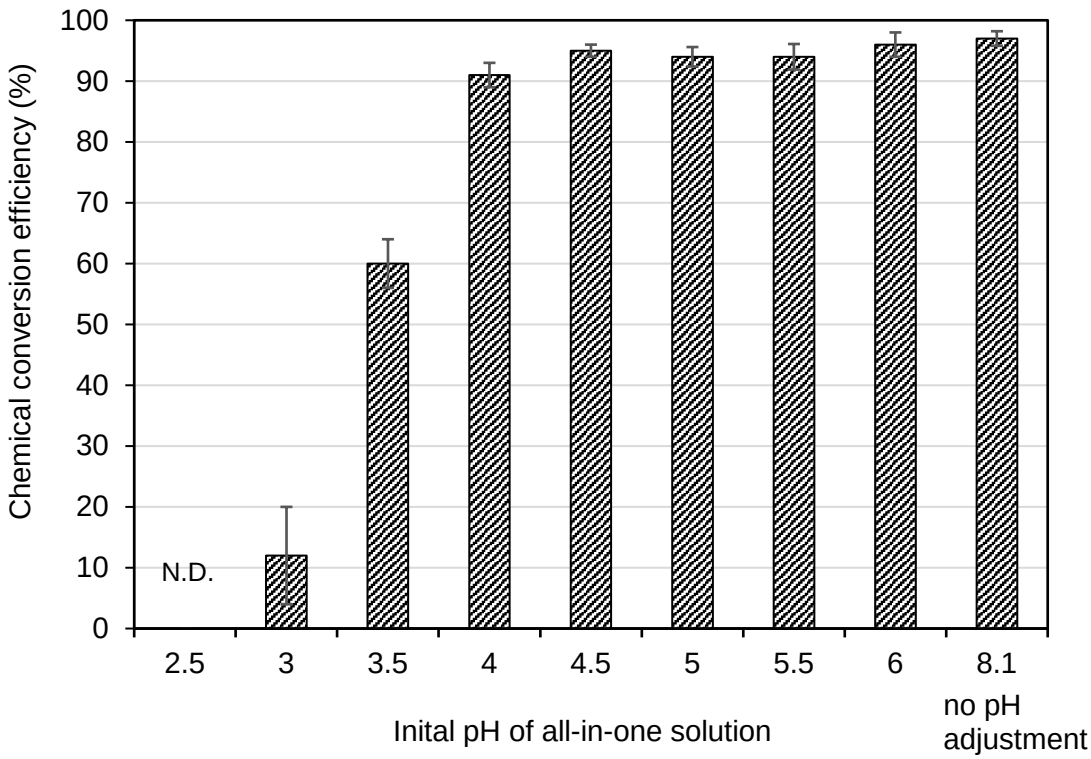

Chemical conversion efficiency (%)
100
90
80
70
60
50
40
30
20
10
0
N.D.
2.5
3
3.5
4
4.5
5
5.5
6
8.1
no pH adjustment
Inital pH of all-in-one solution

Figure 9

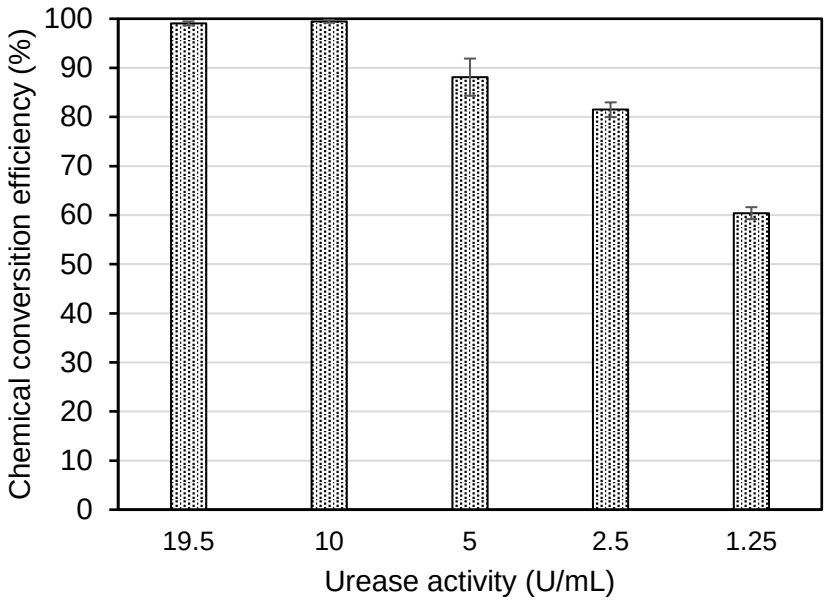
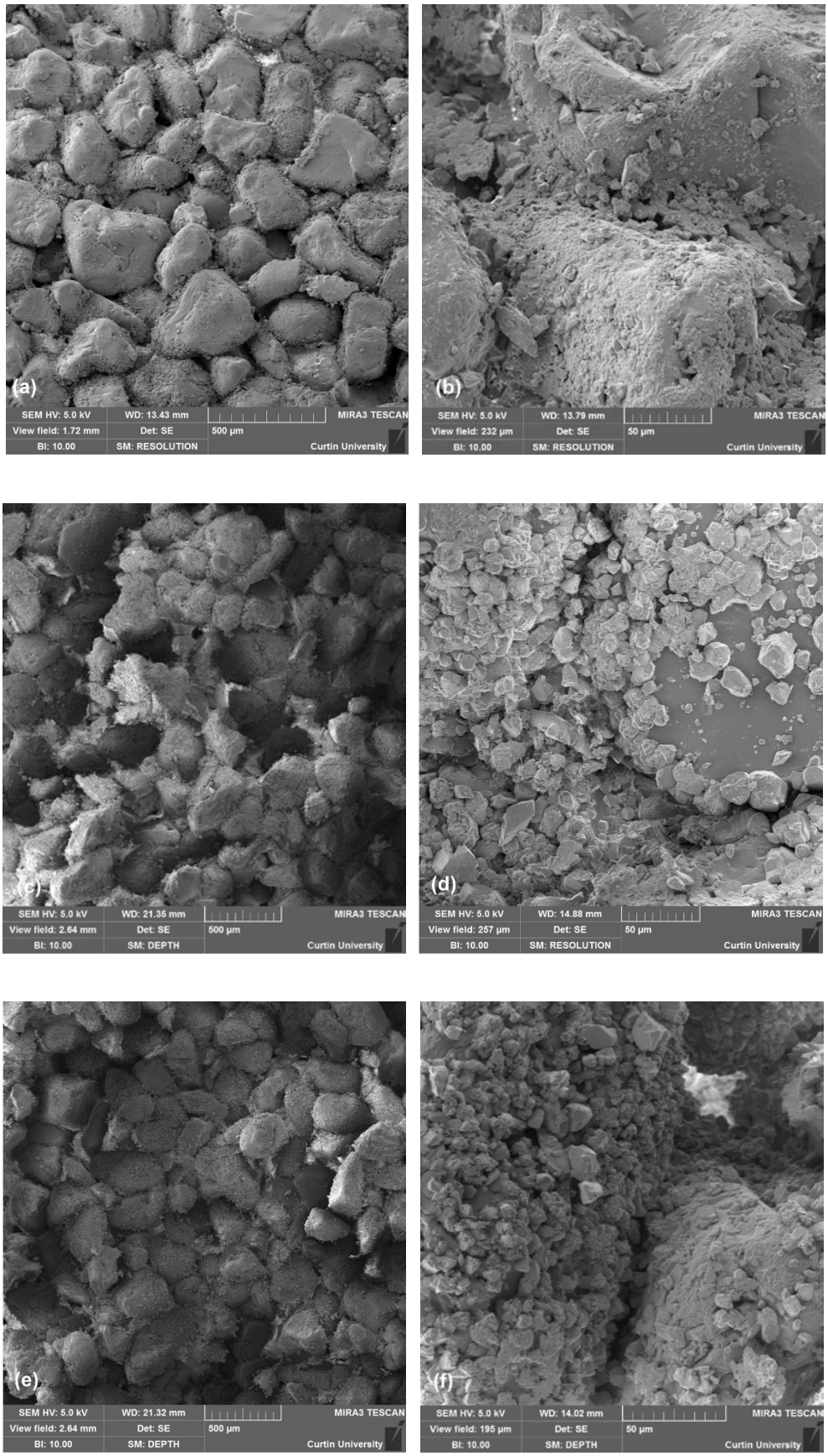
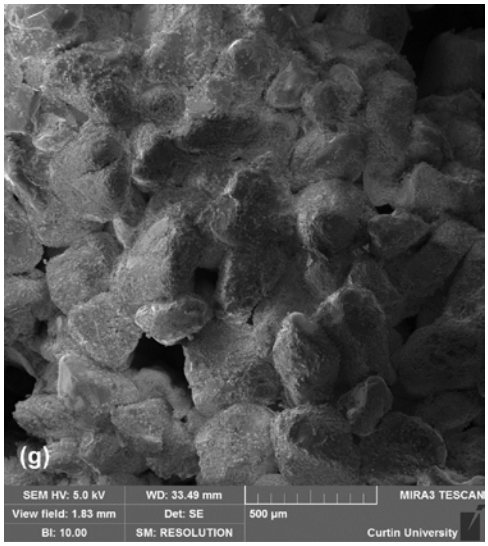
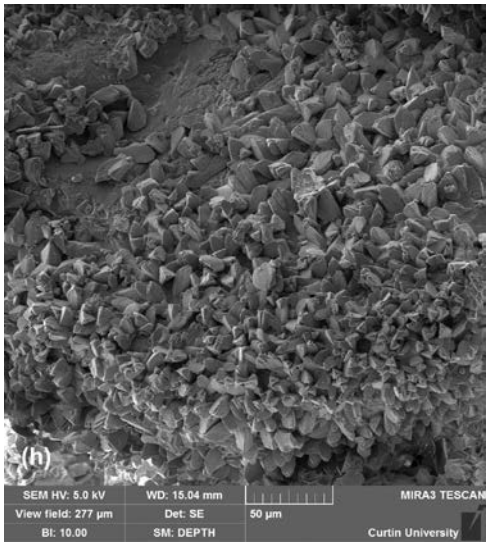

Chemical conversition efficiency (%)
100
90
80
70
60
50
40
30
20
10
0
19.5
10
5
2.5
1.25
Urease activity (U/mL)

Figure 10


(a)
SEM HV: 5.0 kV
WD: 13.43 mm
MIRA3 TESCAN
View field: 1.72 mm
Det: SE
500 µm
BI: 10.00
SM: RESOLUTION
Curtin University
(b)
SEM HV: 5.0 kV
WD: 13.79 mm
MIRA3 TESCAN
View field: 232 µm
Det: SE
50 µm
BI: 10.00
SM: RESOLUTION
Curtin University
(c)
SEM HV: 5.0 kV
WD: 21.35 mm
MIRA3 TESCAN
View field: 2.64 mm
Det: SE
500 µm
BI: 10.00
SM: DEPTH
Curtin University
(d)
SEM HV: 5.0 kV
WD: 14.88 mm
MIRA3 TESCAN
View field: 257 µm
Det: SE
50 µm
BI: 10.00
SM: RESOLUTION
Curtin University
(e)
SEM HV: 5.0 kV
WD: 21.32 mm
MIRA3 TESCAN
View field: 2.64 mm
Det: SE
500 µm
BI: 10.00
SM: DEPTH
Curtin University
(f)
SEM HV: 5.0 kV
WD: 14.02 mm
MIRA3 TESCAN
View field: 195 µm
Det: SE
50 µm
BI: 10.00
SM: DEPTH
Curtin University


(g)
SEM HV: 5.0 kV
WD: 33.49 mm
MIRA3 TESCAN
View field: 1.83 mm
Det: SE
500 µm
BI: 10.00
SM: RESOLUTION
Curtin University


(h)
SEM HV: 5.0 kV
WD: 15.04 mm
MIRA3 TESCAN
View field: 277 µm
Det: SE
50 µm
BI: 10.00
SM: DEPTH
Curtin University