Influence of key environmental conditions on microbially induced cementation for soil stabilization

Liang Cheng, Mohamed A. Shahin, M. ASCE and Donovan Mujah

Liang Cheng (corresponding author): Postdoctoral Research Associate, Department of Civil Engineering, Curtin University, Kent Street, Bentley, Perth, Western Australia 6102. Email: liang.cheng@curtin.edu.au

Mohamed A. Shahin: Associate Professor, Department of Civil Engineering, Curtin University, Kent Street, Bentley, Perth, Western Australia 6102. Email: m.shahin@curtin.edu.au

Donovan Mujah: PhD Scholar, Department of Civil Engineering, Curtin University, Kent Street, Bentley, Perth, Western Australia 6102 (corresponding author). Email: donovanm@postgrad.curtin.edu.au

ABSTRACT: Microbially induced calcite precipitation (MICP) is a sustainable biological ground improvement technique that is capable of altering and improving soil mechanical and geotechnical engineering properties. In this paper, laboratory studies were carried out to examine the effects of some key environmental parameters on ureolytic MICP mediated soils, including the impact of urease concentrations, temperature, rainwater flushing, oil contamination and freeze-thaw cycling. The results indicate that effective crystals precipitation pattern can be obtained at low urease activity and ambient temperature, resulting in high improvement in soil unconfined compressive strength (UCS). The microstructural images of such crystals showed agglomerated large clusters filling the gaps between the soil grains, leading to effective crystals formation. The rainwater flushing was detrimental to the bio-cementation process. The results also indicate that although traditional MICP treatment by the two-phase injection method did not succeed in treatment of oil contaminated soils, and the proposed premixing of bio-flocs with soil can significantly improve UCS and stiffness of oil contaminated soils. Finally, MICP treated soils showed a high durability to the freeze-thaw erosion, which is attributed to the inter-particle contact points and bridging of crystals formation.

27 **Author Keywords:** Microbially induced calcite precipitation (MICP); bio-cemented sand;

28 ground improvement; microstructural analysis; unconfined compressive strength (UCS).

Introduction

Due to its versatility and sustainable application, microbially induced calcite precipitation (MICP) has recently gained much attention from the geotechnical engineering researchers worldwide. MICP is a naturally driven biological technique that harnesses the metabolism of bacteria to create an in-situ cementation agent known as calcium carbonate or calcite. Although there are many biological processes that can lead to MICP, the use of urea hydrolysis by ureolytic bacteria is gaining more interest, and the current study will thus focus on MICP via urea hydrolysis pathway. In this technique, aerobically cultivated bacteria with highly active urease enzyme (*Sporosarcina pasteurii*, formerly known as *Bacillus pasteurii*) are introduced to a targeted soil. The urea hydrolysis takes place when the active ureolytic bacteria catalyze the reaction of urea hydrolysis to produce ammonium (NH_4^+) and carbonate ions (CO_3^{2-}). In the presence of calcium from a supplied calcium source (i.e. $CaCl_2$), crystals of calcium carbonate (i.e. calcite) form inside the soil matrix. The chemical reactions involved in this technique are as follows:

$$CO(NH_2)_2 + H_2O \rightarrow 2NH_4^- + CO_3^{2-} \qquad (1)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \ (s) \downarrow \qquad (2)$$
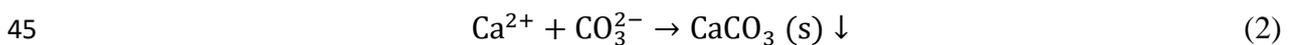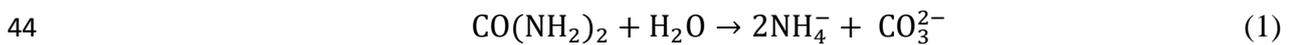
The bonding of soil particles by calcite provides an improvement to the mechanical and geotechnical engineering properties of bio-treated soils (DeJong et al. 2010). For example, bio-cemented sand treated by MICP has been reported to provide a reduction in soil settlement (DeJong et al. 2006; van Paassen et al. 2010a), an increase in soil shear strength (Ismail et al. 2002a; DeJong et al. 2006; Chou et al. 2011), and an improvement in soil stiffness (Montoya and DeJong 2015; Feng and Montoya 2015; Lin et al. 2015). In an attempt to advance the MICP technique for field implementation, van Paassen et al. (2010b) applied MICP to treat 100 m^3 of sand in a large-scale experiment, and the results demonstrated that

55   MICP was able to improve large spatial areas of treated sand. DeJong et al. (2014) developed

56   a repeated five-spot treatment model to simulate a more realistic soil volume treatment in the

57   field, and it was found that the proposed model was able to show spatial and temporal trends

58   in the microbial content, which indicates that the model has the means to capture the complex

59   treatment scenario involving MICP.

60

61   Mortensen et al. (2011) studied the environmental factors affecting MICP, namely the

62   aqueous environmental conditions, various soil particle sizes and soil gradation. The results

63   showed that the natural aqueous environments (freshwater and seawater) and myriad soil

64   particle sizes and gradation (gravel, well-graded sand, poorly-graded sand and silt) were

65   equally favorable to calcite precipitation. Mortensen et al. (2011) also proposed that the

66   calcite precipitation rate and nutrient concentrations must be kept to their minimum in order

67   to achieve treatment homogeneity across the bio-cemented soil columns. Similar findings

68   were reported by Al Qabany and Soga (2013). However, the correlation between the

69   mechanical and geotechnical behavior and amount of calcite formed under the above

70   conditions has not been yet investigated. Earlier studies on the correlation between the shear

71   strength of bio-cemented sand and amount of calcite content enveloping the sand particles

72   have been investigated by Fujita et al. (2000) and Okwadha and Li (2010), showing that the

73   shear strength of sand may not be directly proportional to the amount of calcite content

74   (Whiffin et al. 2007). This was confirmed and explained by Cheng et al. (2013) who observed

75   deposition of calcite crystals in the contact points between the sand grains, forming effective

76   bridges that contribute to the shear strength improvement of bio-cemented soil.

77

78   The calcite precipitation pattern is another factor that greatly influences the target application

79   of bio-cementation in the field as it influences the flow properties of porous media, which

80   may lead to treatment homogeneity by shaping the preferential flow path according to the

size, shape, and structure of the pore throats affected by the accumulation of calcite crystals (Al Qabany et al. 2012). The calcite precipitation pattern also affects the load transfer mechanism between the soil particles, through the area of contact points developed by the precipitated calcite, leading to variation in strength and stiffness of bio-cemented soils (Ismail et al. 2002b).

This paper aims to investigate the impact of some key environmental parameters on the efficacy of MICP for its in-situ implementation and optimization process. The parameters studied include the effects of urease enzyme concentration, degree of temperature, rainwater flushing, oil contamination, and freeze-thaw weathering in winter alpine regions. The impact of these parameters on the performance and effectiveness of MICP technique was related to both the shear strength improvement of bio-treated soil and $CaCO_3$ precipitation pattern at the micro-scale level. In this study, the introduction of urease enzyme to the soil samples was achieved by the injection of cultivated exogenous pure ureolytic bacteria, which can also be achieved by other methods, such as enrichment of indigenous ureolytic active microorganisms (Burbank et al. 2011) or premixing ureolytic bacteria with soil (Duraisamy and Airey 2015). The aim of the current study is to understand the effect of micro-scale level behavior of MICP (crystal pattern, crystal bonding, bacteria attachment, etc.) under various environmental conditions on the macro-scale mechanical behavior of soil. This is applicable to the MICP process carried out through various introduction methods of urease active bacteria including injection, pre-mixing, and in-situ enrichment. Therefore, the current study is crucial as it provides a deeper understanding of MICP treatment for site conditions, which is crucial before the technique can be applied successfully in the field.

Materials and Methods

Soil used

The soil used in the current study was silica sand obtained from Cook Industrial Minerals Pty Ltd, Western Australia. A number of four different particle size distributions, i.e. three poorly-graded and one well-graded, were utilized (Fig. s1). The characteristics of the sand used are listed in Table 1. Sand-1 (uniform sand with 99.6% less than 0.425 mm) was used to investigate the influence of urease enzyme concentration, degree of temperature, rainwater flushing, and oil contamination. Sand-2 (uniform sand with 99.6% less than 0.15 mm), Sand-3 (uniform sand with 99.9% less than 1.18 mm), and Sand-4 (well-graded sand with grain size ranges from 0.053 mm to 2.36 mm) were used to study the impact of freeze-thaw weathering.

Laboratory set-up

The laboratory setup comprised of polyvinyl chloride (PVC) column (45 mm in diameter and 180 mm in length) which was cut into half vertically and then glued together using a silicon glue with an inlet (top) connected to a peristaltic pump to allow injection of the reagents into the PVC column and an outlet (bottom) affixed to a U-type tubing. Scour pads and 10 mm thick gravel were placed at the top and bottom of the column, acting as filters to facilitate the spread of the solution during treatment and to prevent the fine sand particles from being flushed out during the treatment cycles. The sand was packed into the column in three consecutive layers, ensuring that each layer was compacted evenly to achieve at least 95% of the maximum dry density (Table 1) so as to maintain consistency of experiments.

Bacterial suspension and cementation solution

The urease active bacteria used in the current experiments were *Bacillus sp.* isolated from a previous work carried out by Al-Thawadi and Cord-Ruwisch (2012). The bacteria were

133   cultivated in a sterile aerobic batch growth medium consisting of 20 g/L yeast extract, 0.17 M

134   ammonia sulphate, and 0.1 mM $NiCl_2$, with a pH value of 9.25. The cultivated bacteria were

135   collected at the stationary phase of the culture growth after 24 hours of incubation at 28°C.

136   The optical density (OD_{600}) of the harvested culture varied between 2–2.5, and the urease

137   activity was approximately 10 U/mL (1 U = 1 μmol urea hydrolyzed per minute). The culture

138   was stored at 4°C refrigerator for no longer than one week prior to use. The cementation

139   solution used in the present study consisted of 1 M anhydrous calcium chloride (110.98 g/L)

140   and 1 M urea (60.06 g/L).

141

142   *MICP treatment process*

143   Except elsewhere stated, the process of MICP treatment was as follows. The PVC column

144   was fully saturated with water at 100% degree of saturation prior to MICP treatment to

145   ensure a relatively controlled flow field during the treatment injections. This was carried out

146   using the upward flow method that facilitates the removal of the air voids from inside of the

147   soil sample. Then, the MICP treatment was employed using the down flow injection method.

148   In order to keep the soil sample fully saturated throughout the treatment, the water level in

149   the external U-type tubing attached to the bottom of the PVC column was maintained equally

150   to the top of the PVC column.

151

152   A two-phase injection scheme was employed for the MICP treatment (except elsewhere

153   stated) by injecting a half void volume of bacteria culture followed by injecting a half void

154   volume of cementation solution (bacterial placement phase), as recommended by Martinez et

155   al. (2013) and Cheng and Cord-Ruwisch (2014). Then the column was cured for 24 hours to

156   allow for bonding of bacteria with the sand particles. Full void volume of cementation

157   solution was then injected into the sand column and was left to cure for 24 hours to allow for

158   calcite precipitation. For each individual sand column, treatment through the cementation

159 solution was repeated several times to gain different degrees of cementation, and the

160 ammonium content and bacterial activity in the effluent of each treatment was monitored.

161

162 *Determination of CaCO₃ content*

163 The calcium carbonate content of treated soil samples was determined by adding solution of 2

164 mL of 2M hydrochloric acid (HCl) to 1–2 g of dry crushed cemented sample, and the volume

165 of CO_2 gas was then measured using a U-tube manometer under standard conditions of 25°C

166 at 1 atm (Whiffin et al. 2007). The actual amount of $CaCO_3$ was calculated based on an

167 established relationship between the volume of CO_2 gas and amount of pure analytical grade

168 $CaCO_3$ powder.

169

170 **Soil Properties Investigated**

171 *Permeability*

172 The permeability tests were carried out using the constant head permeability method in

173 accordance with the Australian Standards (2001). The tests were conducted inside the PVC

174 column, where the treated soil samples were still intact, instead of using the standard mold

175 specified in the guidelines. The permeability of soil samples was taken before and after

176 treatment.

177

178 *Unconfined compressive strength (UCS)*

179 After MICP treatment, the treated sand samples were flushed with at least five void volume

180 of tap water to wash away all excess soluble salts prior to UCS experiments. Before the tests

181 were carried out, the sand samples were dried at 105°C for at least 24 hours. The UCS tests

182 were conducted on specimens with selected aspect diameter-to-height ratios of 1:1.5 to 1:2.

183 The axial load was applied at a constant rate of 1.0 mm/min.

184

Microstructure analysis

To characterize the shapes and location of precipitated $CaCO_3$ and to investigate the bonding behavior between the grain hosts and bio-cementation agent, microscopy analysis was conducted on the bio-treated soil samples. Before conducting the microscopy investigation, all bio-treated samples were flushed with tap water and dried at 60°C for 24 hours, and were then crashed to small pieces and coated with gold under vacuum conditions. The microscopy investigation was carried out using the available scanning electron microscopy (SEM), PHILIPS XL20 scanning electron microscope (Eindhoven, The Netherlands).

Environmental Parameters Investigated

In order to investigate the influence of various environmental conditions on the mechanical and geotechnical response of bio-cemented soils, some key parameters including the urease enzyme concentration (i.e. bacterial cell concentration), degree of temperature, rainwater flushing, oil contamination, and freeze-thaw cycles were examined and procedure of each parameter is explained below.

Urease enzyme concentrations

Hammad et al. (2013) observed that more rapid $CaCO_3$ crystals were formed under higher urease activity in agar plates. Earlier study carried out by Nemati et al. (2003) indicated that an increase in the urease concentration enhances the extent of $CaCO_3$ precipitation. However, no study is available in the literature directly linking the effect of different urease enzyme concentrations on the effective crystal bridging formation and the final strength of bio-cemented soils. In the present study, by either diluting the raw bacterial culture (10 U/mL) with deionized (DI) water or concentrating it via centrifuge, a series of bacterial culture with different urease enzyme concentrations (indicated by various levels of urease activity of 5, 10 and 50 U/mL) was obtained and utilized for bio-cementation of Sand-1. The treatment

process followed the same procedure of that used for the raw bacterial culture, as stated

earlier. The urease activity of bacterial culture was determined by measuring the urea

hydrolysis rate of the tested bacterial culture. This was made by recording the change in the

ammonium concentration of mixture over 30 mins of reaction period, which consisted of 10

ml of 3 M urea solution, 2 ml of bacterial culture, and 8 ml of DI water. The ammonium

concentration was determined using the Nessler method (Greenburg et al. 1992), and the

correlation between the UCS and $CaCO_3$ content of bio-cemented soils was examined using

different urease enzyme concentrations (i.e. urease activities).

Temperature

The subsurface temperature surrounding MICP treated soils was previously investigated by

Ng et al. (2012). It was found that the urease activity, which leads to the rate of $CaCO_3$

crystal precipitation, was increased with the increase in temperature from 10°C to 60°C.

Nemati et al. (2003) demonstrated that an increase in temperature (from 20°C to 50°C)

enhanced both the production rate of $CaCO_3$ and extent of the cementation solution

conversion in a batch system. However, the effectiveness of crystals formed at different

degrees of temperature was not investigated. In the current study, three different temperature

scenarios were examined for Sand-1. The temperature values were selected to simulate the

subsurface soil temperature in cold regions (4°C), tropical regions (25°C), and arid regions

(50°C). During the process of MICP treatment, the sand samples used were placed as

follows: (1) inside 4°C refrigerator; (2) room temperature of 25°C; and (3) inside 50°C oven,

and the UCS and SEM analysis were conducted for the bio-treated samples.

Rainwater flushing

In the current study, tap water (pH ranges from 6.8-7.2) was used to simulate the effect of

heavy rain with rainfall intensity of 50 mm/h (National Meteorological Library 2015) on the

237　MICP process. Sand columns made from Sand-1 were subjected to tap water flushing for

238　about 12 hours. The tap water flushing was conducted immediately or 24 hours after the

239　bacterial placement, which was consisted of sequential injections of a half void volume of

240　bacterial culture (45 mL) and a half void volume of cementation solution (45 mL), as stated

241　earlier. All sand columns were then treated with cementation solution (full void volume) for

242　two times. After treatment, the sand samples were extracted from the PVC columns (by

243　gently cutting the silicon glue and opening the PVC column for sand column extraction) for

244　final UCS (if applicable) and $CaCO_3$ content measurements.

245

246　*Oil contamination*

247　Hydrocarbon contaminants from oil exploration, transportation, production, and processing

248　can change the behavior of soil and may alter the soil engineering properties, resulting in

249　safety issues in civil engineering structures (Nicholson and Tsugawa 1997; Shroff et al. 1998).

250　In the current study, for the first time, the stabilization of oil-contaminated soil using MICP

251　technology was investigated and evaluated through measurements of UCS and $CaCO_3$

252　content.

253

254　Dried Sand-1 was contaminated with 5% diesel engine oil (w/w), which obtained a maximum

255　dry density of 17.8 kN/m^3 and corresponding optimum moisture content of 7%. The oil-

256　contaminated soil was treated using two procedures, including the two-phase injection

257　method as described above and the premixing method in which bacterial culture was

258　premixed with sand prior to the application of cementation solution. The latter process will be

259　further explained later.

260

261

262

263 *Freeze-thaw cycles*

264 Destruction of porous materials caused by freezing and thawing has been of a great concern

265 to engineers for more than 200 years (Johnson 1952). As suggested by Litvan (1980), soil

266 grain size distribution and permeability affect soil durability against the freeze-thaw (FT)

267 cycles. Therefore, in the present study, sand samples with three different grain size

268 distributions (i.e. Sand-2 to Sand-4, see Fig. 1 and Table 1), and different permeability values

269 (stated in the result section) were examined. Each bio-cemented sand column was cut into

270 half to obtain diameter-to-height aspect ratios of 1:1.5 to 1:2, followed by exposure to 4-FT

271 and 10-FT cycles prior to UCS experiments. Each procedure of FT cycle was subjected to a

272 12h freeze at $-14°C$ followed by a 12h thaw at the room temperature ($25\pm1°C$). To ensure

273 that full saturation was achieved, all treated samples were completely immersed in water

274 throughout the FT cycles. The effect of FT cycles on the bio-cemented samples was

275 evaluated by comparing the UCS values before and after the FT cycles.

276

277 **Results and Discussion**

278 *Effect of urease enzyme concentrations*

279 The influence of different urease concentrations on UCS of bio-cemented soil samples was

280 examined by plotting the measured UCS values against $CaCO_3$ contents (Fig. 1a). It can be

281 seen that for all urease activities, the UCS increases exponentially with the increase of the

282 $CaCO_3$ content, which is in line with previous results reported by van Paassen et al. (2010b)

283 and Cheng et al. (2013). It can also be seen that for the same amount of $CaCO_3$ precipitation,

284 the UCS of bio-cemented soil treated with higher urease activity is less than that of the

285 samples treated with lower activity. For instance, at an amount of 0.04 g/g $CaCO_3$

286 precipitation, the sand sample treated with 50 U/ml urease activity achieved UCS value of

287 around 250 kPa, whereas the sand samples treated with 10 and 5 U/ml urease activity

288 achieved higher UCS values of around 500 and 850 kPa, respectively. This suggests that the

289    $CaCO_3$ formed slowly, which is due to the slower hydrolysis of urea caused by the lower

290   urease activity, are more effective to form "bridges" that bond the sand grains together in a

291   more effective way. It should be noted that the efficiency in UCS improvement was only

292   slightly improved using the 2 U/ml activity compared to the 5 U/ml (data not shown), and the

293   use of such low urease activity required a significant extension of treatment period to allow

294   for completion of the MICP reaction.

295

296   In Fig. 1b, the measured permeability values of soil samples after MICP treatment were

297   normalized with the initial permeability values and plotted against the $CaCO_3$ content. As can

298   be seen, all samples show a reduction in permeability with the increase in the amount of

299    $CaCO_3$ precipitation. Fig. 1b also indicates that the reduction in permeability of bio-cemented

300   samples was mainly controlled by the amount of $CaCO_3$ precipitation, whereas the level of

301   urease activity used in treatment has a minor impact.

302

303   The micro-feature of precipitated crystals within the sand matrix was examined using SEM

304   (Fig. 2). It can be seen in Fig. 2(a-c) that the $CaCO_3$ produced at high urease activity formed

305   small crystals of a typical crystal size of 2-5 µm. The agglomerated small crystal precipitates

306   formed thin coating layers ($CaCO_3$ envelope) with a thickness of around 5 µm covering the

307   individual sand grain surface and bridging the adjacent sand grains. This type of crystal

308   precipitation might not be strong enough to bear high shear strength, which is probably due to

309   the thin and weak "bridging layer" and remaining large gaps between the sand grains. In

310   contrast, the crystals produced at low urease activity were agglomerated and formed large

311   clusters of size of around 20-50 µm [Fig. 2(d-f)]. One important feature in this regard is that

312   although large crystal clusters precipitated on the sand grain surface, the gaps between the

313   sand grains were almost completely filled with crystals (Fig. 2d). It should be noted that the

314   samples presented in Fig. 2 demonstrate similar UCS values but have different $CaCO_3$

315 contents, suggesting that the crystals pattern formed at lower urease activity is more effective

316 in gaining strength.

317

318 It has been reported in the literature that there are two mechanisms for crystal precipitation in

319 MICP (Stocks-Fischer et al. 1999; DeJong et al. 2006). The first mechanism is that bacterial

320 cells act as nucleation sites for $CaCO_3$ precipitation. The second mechanism is that the urea

321 hydrolysis produces CO_3^{2-} ions and raises the pH around the cells, favoring the precipitation

322 of $CaCO_3$ by lowering the solubility. In the current study, it is apparent that development of

323 $CaCO_3$ is vastly different under different urease activities, in terms of location, size, and

324 shape of crystals. Because the same cementation solution was applied to all samples, it was

325 expected that the bacterial urease activity would be the only factor that can have an impact on

326 the precipitation patterns.

327

328 An explanation of the noted difference in the precipitation patterns may be attributed to the

329 competition between the crystal growth and crystal nucleation. Gandhi et al. (1995) reported

330 that the nucleation of new crystals would compete with the process of crystal growth if

331 nucleation of new crystals prevails over the growth of those exist. In case of high urease

332 enzyme concentration, a high number of bacterial cells were introduced to the soil samples,

333 which were accumulated at the pore "throat" (contact points) and also attached to the sand

334 grain surface. With the presence of abundant bacterial cells acting as nucleation site, the

335 produced carbonate ions may be consumed mainly by nucleation of new $CaCO_3$ crystals

336 rather than growth of existing crystals, resulting in an abundance of small crystals. These

337 numerous small crystals would develop to form dense crystal layers with continuous supply

338 of cementation solution. In contrast, in case of low urease enzyme concentration (i.e. low

339 urease activity/bacteria concentration), the nucleation site was limited by the small number of

340 bacterial cells. Otterstedt and Brandreth (2013) stated that the final crystal size is inversely

341 related to the number of nuclei. For low urease activity, the presence of high concentration of

342 calcium (i.e. 1 M in the current study) and increased super-saturation in the solution due to

343 urea hydrolysis would result in accumulating of precipitation over the initial small crystals,

344 leading to formation of larger crystal sizes rather than new small crystals. It is worthwhile

345 noting that using different ureolytic bacteria, with either higher or lower specific urease

346 activity (urase activity per cell) compared to the current strain, might lead to different results

347 to those presented herein. This is because in order to obtain the same urease activity as

348 obtained in the current study, different amount of biomass might be required, leading to

349 different number of nucleation site and hence, affecting the patterns of precipitated crystals

350 potentially.

351

352 The findings obtained from this study indicate that the efficacy (strength per mass of calcite)

353 of larger calcite crystals precipitated at the gaps between the sand grains is higher than that

354 obtained from smaller crystals randomly precipitated throughout the sand matrix. This is

355 consistent with the described phenomena in the literature. For example, Cheng et al. (2013)

356 found that bridging crystals were formed by restricting the pore water at the contact points

357 between the sand grains, showing more efficiency in terms of strength improvement

358 compared to crystals randomly precipitated. Al Qabany and Soga (2013) demonstrated that

359 using cementation solution of low concentration may yield to higher sample strengths, which

360 may be attributed to the larger amount of precipitation at the particle contacts, similar to the

361 findings of Okwadha and Li (2010) and Cheng et al. (2014). In particular, the significance of

362 calcium carbonate precipitation pattern on properties of MICP treated soils was highlighted

363 by many researchers (e.g., Al Qabany and Soga 2013; Okwadha and Li 2010; Cheng et al.

364 2013). Martinez and DeJong (2009) reported that the degradation of bio-cemented sand

365 particles at the micro scale level was governed by the calcite bonds between the silica sand

366 grains. Accordingly, it was expected that the shear strength of sand may not be directly

15

proportional to the amount of calcite content. Those crystals filling the gaps between the sand grains and forming effective bridges contribute to the pathway of load transferred between the soil particles; thus, improve the soil stiffness and strength.

Effect of degree of temperature

The effect of various degrees of temperature equal to 4, 25 and 50°C on strength improvement of bio-cemented soil was examined and presented in Fig. 3. It can be seen that all UCS values of bio-treated sand samples were exponentially increased with the increase in $CaCO_3$ content, irrespective of the applied temperature. However, the strength improvement as per the amount of crystals produced was higher at 25°C compared to that at temperature of either extremely low (4°C) or high (50°C). The crystals formed at the highest temperature of 50°C was the least efficient to gain strength improvement.

The crystals microstructure analysis indicates that MICP treatment at 50°C results in crystal distribution over the entire sand grain surface, with a typical individual crystal size of 2-5 µm [Fig. 4(e-f)]. For such samples, the individual $CaCO_3$ crystals possessed similar size and were well-distributed spatially and also covered the surface of the sand grains as a coating-like layer. However, the sand grains were not effectively connected because of the remaining large gaps [Fig. 4(e-f)]. For samples treated at the ambient temperature, it was found that the average crystal size increased by 10 times (individual crystals size between 20 to 50 µm) compared to that formed at 50°C. These large crystals were found to precipitate on the grain surface, covering the contact areas of the sand grains [Fig. 4(c-d)]. This type of crystals distribution pattern was also found in the samples treated at 4°C, and small crystal size was observed [Fig. 4(a-b)].

393   The kinetics of crystallization indicates that activated energy, which is a function of

394   temperature and relative supersaturation degree, has a strong impact on the rate of nucleation

395   and crystal growth. It is the relationship between the competing kinetic rates of nucleation

396   (birth of new crystal nuclei) and crystal growth (increase in size of crystals) that determines

397   the size distribution of crystals. The higher the temperature the lower the activation energy

398   barrier as well as the faster the rate of nucleation of $CaCO_3$ precipitation (Wojtowicz 1998).

399   The faster nucleation rate may induce excess nucleation sites, which would cause smaller

400   average crystal size as it was found in the samples treated at 50°C. It is worthwhile noting

401   that although the nucleation rate was low at low temperature of 4°C, small size of crystals

402   was also observed. This is possibly due to the slow crystal growth at low temperature due to

403   the low relative supersaturation degree as a result of the low urease activity at low

404   temperature (Sahrawat 1984). A decrease in the relative supersaturation led to a decrease in

405   both the crystal nucleation rate and growth rate. As stated earlier, the final crystal size

406   distribution is dependent on the competition between these two rates.

407

408   The precipitation of $CaCO_3$ in MICP is a very complex process because of the involvement

409   of bacteria acting as nucleation site and CO_3^{2-} ion producer. Therefore, if different amount of

410   bacteria is applied, the precipitation pattern can be different from what was presented in the

411   current study. It is evident from Fig. 2(a-c) that large amount of bacteria (high activity 50

412   U/ml) at the ambient temperature would result in abundant nucleation sites (numerous

413   bacterial cells) and high relative supersaturation degree (high urease activity). As a result, the

414   precipitation pattern obtained under this environmental condition could be similar to that

415   obtained at 50°C with low amount of bacteria (10 U/ml) [Fig. 4(e-f)]. Generally, temperature

416   can affect many physical, chemical, and biological properties of MICP system. For example,

417   temperature can affect the urease activity, which in turn influences the urea hydrolysis rate,

418   CO_3^{2-} production rate, and consequent crystal growth rate. The solubility of $CaCO_3$ crystals

17

419 also varies with temperature. Therefore, further investigation on this complex process is

420 worthwhile to carry out in future development of the current study. Overall, based on the

421 results obtained from the current study of different urease concentrations and temperatures, it

422 can be concluded that large size crystals located at the gaps between the sand grains are

423 deemed to be effective crystals, which contribute the most to the strength gain of bio-

424 cemented soils.

425

426 *Effect of rainwater flushing*

427 In the current study, tap water flushing was made to determine the impact of intensive rainfall

428 towards $CaCO_3$ precipitation and corresponding soil stabilization. The control sample (i.e. no

429 water flushing) was fully cemented (Fig. 5a). In contrast, the samples encountered with water

430 flushing during the treatment process were partly cemented or completely non-cemented [Fig.

431 5(b-c)]. For the control sample, the chemical conversion efficiency, which is defined as the

432 percentage of injected urea and calcium chloride that precipitate as $CaCO_3$, was found to

433 reduce from about 95% to 50% throughout the MICP process (Fig. 6a). The decrease in

434 chemical conversion efficiency was probably explained by the loss of urease activity due to

435 the encapsulation of the bacterial cells by precipitated crystals. The water flushing caused

436 significant decrease in the chemical conversion efficiency down to less than 5%, irrespective

437 of the waiting period applied to the bacterial attachment. The negative impact of water

438 flushing on bio-cementation was also demonstrated by the low degree of cementation and

439 final $CaCO_3$ content measurements. Less than 0.3% (0.003 g/g sand) of crystals content was

440 detected in the samples subjected to water flushing, whereas 10 times more (0.043 g/g sand)

441 crystals were found in the control sample with a detectable UCS of about 260 kPa (Fig. 6b).

442

443 The low chemical conversion efficiency and $CaCO_3$ precipitation after water flushing may be

444 due to the washout of bacteria and substrate. For successful bio-cementation, it is necessary

445 that the bacteria are introduced into the soil first and immobilized, then followed by

446 application of cementation solution that contains urea and calcium chloride. Many authors

447 have shown that the adsorption of bacteria increases with increasing the salinity of bacterial

448 suspension (Scholl et al. 1990; Torkzaban et al. 2008). By flushing low salinity solutions

449 after the bacterial suspension, a large part of the adsorbed bacteria can be remobilized from

450 the solid surface into the liquid phase (Harkes et al. 2010). This suggests that in real

451 application the rainwater flushing during MICP bio-cementation can impact the effectiveness

452 of MICP treatment by washing out the immobilized bacterial cells and reducing the chemical

453 conversion efficiency. In order to overcome this problem, a new way of bacterial fixation,

454 which can prevent bacterial cells from being washed out by the low salinity solution (i.e.

455 rainwater) will be investigated in future work.

456

457 *Effect of oil contamination*

458 Oil contamination can alter the soil engineering properties, resulting in safety issues in

459 relation to civil engineering infrastructures. Also, oil-contaminated soil represents the worst

460 case scenario of hydrophobic soil particle surface property, which might have a negative

461 impact on bacteria immobilization. As expected, the initial trial of stabilization of oil

462 contaminated soils using MICP via the normal two-phase injection method did not succeed

463 (data not shown). This suggests that the traditional bacterial immobilization method does not

464 work effectively for oil-contaminated soils due to the hydrophobic surface of oil-

465 contaminated soil particles, resulting in poor bacterial attachment. In fact, the impact of

466 hydrophobic oil on bacteria attachment is a function of the bacterial culture properties. The

467 bacterial cells (i.e. oil-degrading strain), which are hydrophobic can attach to the hydrophobic

468 surfaces (Rosenberg 2006); however, bacteria with hydrophilic characteristics prefer

469 hydrophilic surface (An and Friedman 1998). In order to improve the bacterial retention,

470 premixing of bacteria with soil in the presence of 100 mM $CaCl_2$ was applied. The presence

471   of $CaCl_2$ acted as flocculent and induced cells coagulation. This coagulation of bacterial cells

472   was found not to significantly lower the urease activity, as reported by Al-Thawadi (2008).

473   To provide a sufficient urease activity, 180 mL of bacterial culture containing 100 mM $CaCl_2$

474   was used and concentrated into 25 mL volume using centrifuge. The concentrated bacterial

475   flocs were then mixed with the oil-contaminated soil to reach the optimum moisture content.

476   The mixture of bacterial flocs and soil was compacted thoroughly in the PVC column to gain

477   the maximum dry density, which was then repeatedly treated 5 times by the cementation

478   solution.

479

480   The UCS results indicated that by increasing the $CaCO_3$ content from 0.035 to 0.054 g/g

481   sand, strength of oil contaminated soil was improved significantly from about 150 kPa to 400

482   kPa (see Fig. s2). Accordingly, soil stiffness was also improved from 10.7 MPa to 68.3 MPa.

483   The failure mechanisms of bio-treated oil contaminated soil were consistent with previous

484   observations on cemented pure silica sand (Cheng et al. 2013). For the weak sample of UCS

485   = 150 kPa, the broken cores completely lost strength at the grain scale around the failure

486   plane. On the other hand, the strong sample of UCS = 400 kPa, longitudinal tensile cracks

487   along the sample were clearly observed.

488

489   *Effect of freeze-thaw cycles*

490   Litvan (1980) found that porous solids with high porosity or permeability usually have a good

491   service record after FT action. Cheng et al. (2013) found that microbially induced $CaCO_3$

492   crystals covered the connecting points between the sand grains and thus provided key

493   bonding force within the soil matrix against the FT cycles. This bonding behavior might be

494   different for different PSD sand samples. Therefore, before carrying out the FT cycles, it was

495   necessary to investigate the mechanical behavior of different PSD sand samples after MICP

496   treatment.

497   Fig. 7 shows that the UCS increases exponentially with the increase in the $CaCO_3$ content. At

498   low level of $CaCO_3$ content (below 0.04 g/g sand), similar UCS improvement was obtained

499   for all different PSD samples; however, the improvement varied at high level of crystal

500   content (above 0.05 g/g sand). At high $CaCO_3$ content, Sand-3 showed the least effective

501   strength improvement because it required the highest amount of crystals to gain strength

502   similar to that of the other PSD samples. For the two types of uniform sand tested herein, the

503   crystals were more effective in improving the strength of soil of smaller grain size, which is

504   consistent with previous findings obtained by Ismail et al. (2002b). This is probably due to

505   the increase in number of contact points, which provide better location for crystals to

506   precipitate, and the decrease in stress acting per particle contact. For Sand-4 (well-graded),

507   the effectiveness of crystal precipitation in terms of strength improvement was greater than

508   that of the uniform coarse sand (i.e. Sand-3) but less than the uniform fine sand (i.e. Sand-2).

509   According to Schiffman and Wilson (1958), the greater the percentage of the coarser fraction,

510   the smaller the available grain surfaces for grout adhesion and the lower the internal tension

511   in the grouted mass. Although the used well-graded sand has the highest density compared to

512   the fine sand, it possesses less contact points and grain surface due to its large percentage of

513   coarse particles. Therefore, the crystals formed for well-graded sand were less effective

514   compared to those formed for fine sand.

515

516   The effect of FT cycles on the mechanical performance of different PSD sand samples treated

517   with MICP is illustrated in Fig. 8. Generally speaking, it can be observed that an increase in

518   the number of FT cycles is associated with a decrease in the compressive strength, for all

519   uniform sand specimens (Fig. 8 (a-b)). The main reason for the strength decrease after FT

520   cycles is due to the formation and growth of micro-cracks generated due to the tensile

521   stresses around the soil particles when the pore water turns into ice during the freezing

522   process. In theory, higher porosity and permeability allow more rapid water mass transfer in

523   the sand matrix, which can increase the FT resistance. However, in the current study, the fine

524   uniform sand (0.15 mm in size) of less porosity and smaller pore size was more durable

525   against the FT cycles compared to the coarse sand (1.18 mm in size) of higher porosity and

526   larger pore size. Smith et al. (1929) found that the average number of contacts per sphere

527   increases with the decrease in porosity. Therefore, for finer sand, the larger number of inter-

528   particle contact points favored more bridging crystals formed at the contact points, which

529   reduced the acting tensile stress per particle contact; hence, resulted in higher durability. Fig.

530   8c shows that the FT cycles have a minor impact on the well-graded sand. The main reason

531   for the high durability of MICP well-graded sand is probably due to the unique characteristics

532   of having high number of inter-particle contact points (attributed to the presence of fine sand)

533   and high permeability, as well as large pore size (attributed to the presence of coarse sand).

534   Overall, it can be concluded that the influence of FT cycles on soil strength and durability

535   depends on the soil porosity, pore size, and bonding behavior of MICP in the soil matrix.

536

537   *Implication for civil engineering applications*

538   In civil engineering applications such as soil stabilization for transportation subgrades and

539   embankments, adequate MICP strength improvement can be achieved by forming sufficient

540    $CaCO_3$ bonds between the soil grains. The ability to produce $CaCO_3$ crystals with high

541   efficacy is thus highly desirable for cost minimization. The findings obtained from the current

542   study show that different urease activities can result in different micro-scale crystal

543   precipitation patterns, leading to varied efficacy in obtaining the macro-scale strength values.

544   This potentially has implications in terms of how MICP treatment can be applied more

545   economically in engineering practice. Low level of urease activity associated with an

546   optimum operating temperature could result in larger calcium carbonate crystals precipitated

547   in the voids of soil grains, which can lead to a more effective strength improvement with less

548   consumed chemicals.

22

549 In other civil engineering applications such as control of seepage-induced internal erosion of

550 dams, it is important to immobilize the urease active bacteria and urease activity within the

551 target zones so that sufficient $CaCO_3$ can be produced throughout the entire treated soil. The

552 experimental observations shown in this paper indicated poor immobilization through the

553 two-phase injection method after tap water flushing, which implies that the current bacteria

554 fixation method might not be applicable to field application of MICP during rainy days. It is

555 thus considered essential to develop a new bacteria fixation method, which can further extend

556 MICP application to continuous water flow regions.

557

558 Soils at high latitude or elevation usually freeze during winter. Under this environmental

559 condition, geotechnical engineering foundations that are exposed to FT cycles can be subject

560 to significant structure damages. In such a case, FT cycles induce uneven stresses within the

561 soil, resulting in a decrease in soil stability. Given the improvement in UCS values of the

562 sand tested in the current study, it can be confirmed that MICP can be used as a viable

563 solution to improve the properties of uncemented soils by creating cemented soil bodies that

564 have high durability against FT cycles. The high durability of MICP treated soils against FT

565 cycles is attributed to the sufficient contact points in the soil matrix and large pore size and

566 permeability of soil. This characteristic enhances the efficacy of MICP cementing agents in

567 bridging the particle-to-particle contacts, and in the meanwhile allows a rapid water mass

568 transfer in the sand matrix.

569

570 **Conclusions**

571 This paper presented a series of experimental results in relation to the mechanical behavior of

572 silica sand stabilized using microbially induced calcite precipitation (MICP) under different

573 environmental conditions. The formation of large agglomerated crystal clusters filling the

574 gaps between the sand grains showed high efficiency in improving the compressive strength

23

575 of bio-treated soils. Such optimum crystal precipitation pattern was found to be obtained at

576 low urease concentration and ambient temperature of 25°C. Trials of "rain" flushing and oil

577 contaminated soil stabilization have clearly shown the bacterial attachment using the two-

578 phase injection method can be deteriorated by flushing of low ionic strength water (i.e. tap

579 water, rain water) or hydrophobic surface of sand grain particles (i.e. oil-contaminated soils).

580 Alternatively, premixing of bio-flocs with oil-contaminated soil was able to achieve sufficient

581 retention of bacteria and urease activity, leading to successful soil stabilization of significant

582 UCS improvement. The FT cycle tests have shown that the durability of bio-cemented soils

583 was varied depending on the particle size distribution. The soil matrix of well-graded sand

584 allowed sufficient amount of contact points for effective crystals that create strong bonding,

585 large pore size, and high permeability, leading to a rapid water mass transfer in the sand

586 matrix and contributing to the resistance of the FT cycles.

587

592

593 **Supplemental Data**

594 Figs. s1-s2 are available online in the ASCE library (ascelibrary.org)

595

596 **References**

597 Al Qabany, A., Soga, K., and Santamarina, C. (2012). "Factors affecting efficiency of

598   microbially induced calcite precipitation." *J. Geotech. Geoenviron. Eng.,*

599   10.1061/(ASCE)GT.1943-5606.0000666, 992–1001.

Al Qabany, A., and Soga, K. (2013). "Effect of chemical treatment used in MICP on engineering properties of cemented soils." *Géotechnique,* 63(4), 331–339.

Al-Thawadi, S. M. (2008). "High strength in-situ biocementation of soil by calcite precipitating locally isolated ureolytic bacteria." Ph.D. thesis, Murdoch Univ., Perth, Australia.

Al-Thawadi, S. M., and Cord-Ruwisch, R. (2012). "Calcium carbonate crystals formation by ureolytic bacteria isolated from Australian soil and sludge." *J. Adv. Sci. Eng. Science Res.,* 2(1), 13–26.

An, Y. H., and Friedman, J. R. (1998). "Concise review of mechanisms of bacterial adhesion to biomaterial surface." *J. Biomed. Mater. Res.,* 43(3), 338-348.

Australian Standards. (2001). "Methods of testing soils for engineering purposes - Soil strength and consolidation tests - Determination of permeability of a soil - Constant head method for a remoulded specimen." Australia.

Burbank, M. B., Weaver, T. J., Green, T. L., Williams, B. C., and Crawford, R. L. (2011). "Precipitation of calcite by indigenous microorganisms to strengthen liquefiable soils." *Geomicrobiol. J.,* 28(4), 301-312.

Cheng, L., Cord-Ruwisch, R., and Shahin, M. A. (2013). "Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation." *Can. Geotech. J.,* 50(1), 81–90.

Cheng, L., and Cord-Ruwisch, R. (2014). "Upscaling effects of soil improvement by microbially induced calcite precipitation by surface percolation." *Geomicrobiol. J.*, 31(5), 396–406.

Cheng, L., Shahin, M. A., Cord-Ruwisch, R. (2014). "Bio-cementation of sandy soil using microbially induced carbonate precipitation for marine environments." *Géotechnique,* 64(12), 1010–1013.

625 Chou, C. W., Seagren, E. A., Aydilek, A. H., and Lai, M. (2011). "Biocalcification of sand

626 through ureolysis." *J. Geotech. Geoenviron. Eng.,* 10.1061/(ASCE)GT.1943-

627 5606.0000532, 1179–1189.

628 DeJong, J. T., Fritzges, M. B., and Nüsslein, K. (2006). "Microbially induced cementation to

629 control sand response to undrained shear." *J. Geotech. Geoenviron. Eng.,*

630 10.1061/(ASCE)1090-0241(2006)132:11(1381), 1381–1392.

631 DeJong, J. T., Mortensen, B. M., Martinez, B. C., and Nelson, D. C. (2010). "Bio-mediated

632 soil improvement." *Ecol. Eng.,* 36(2), 197–210.

633 DeJong, J. T., Martinez, B. C., Ginn, T. R., Hunt, C., Major, D., and Tanyu, B. (2014).

634 "Development of a scaled repeated five-spot treatment model for examining microbial

635 induced calcite precipitation feasibility in field applications." *Geotech. Test. J.,* 37(3),

636 424–435.

637 Duraisamy, Y., and Airey, D. W. (2015). "Performance of biocemented Sydney sand using ex

638 situ mixing technique." *J. Deep. Found. Inst.,* 9(1), 48-56.

639 Feng, K., and Montoya, B. M. (2015). "Influence of confinement and cementation Level on

640 the behavior of microbial-induced calcite precipitated sands under monotonic drained

641 loading." *J. Geotech. Geoenviron. Eng.,* 10.1061/(ASCE)GT.1943-5606.0001379,

642 04015057.

643 Fujita, Y., Ferris, F. G., Lawson, R. D., Colwell, F. S., and Smith, R. W. (2000). "Subscribed

644 content calcium carbonate precipitation by ureolytic subsurface bacteria." *Geomicrobiol.*

645 *J.,* 17(4), 305–318.

646 Gandhi, K. S., Kumar, R., and Doraiswami, R. (1995). "Some basic aspects of reaction

647 engineering of precipitation processes." *Ind. Eng. Chem. Res.,* 34(10), 3223–3230.

648 Greenburg, A.E., Clesceri, L.S., and Eaton, A.D. (1992). *Standard Methods for the*

649 *Examination of Water and Wastewater*, 18th ed. American Public Health Association,

650 Washington.

Hammad, I. A., Talkhan, F. N., and Zoheir, A. E. (2013). "Urease activity and induction of calcium carbonate precipitation by Sporosarcina pasteurii NCIMB 8841." *J. App. Sci. Res.,* 9(3), 1525–1533.

Harkes, M. P., van Paassen, L. A., Booster, J. L., Whiffin, V. S., and van Loosdrecht, M. C. M. (2010). "Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement." *Ecol. Eng.*, 36(2), 112–117.

Ismail, M. A., Joer, H. A., Sim, W. H., and Randolph, M. F. (2002a). "Effect of cement type on shear behavior of cemented calcareous soil." *J. Geotech. Geoenviron. Eng.,* 10.1061/(ASCE)1090-0241(2002)128:6(520), 520–529.

Ismail, M.A., Joer, H.A., Randolph, M.F., and Meritt, A. (2002b). "Cementation of porous materials using calcite." *Géotechnique,* 52(5), 313–324.

Johnson, A. W. (1952). "Frost action in roads and airfield-a review of the literature." *Special Report No. 1*, Highway Research Board, Washington, USA.

Lin, H., Suleiman, M. T., Brown, D. G., Kavazanjian, E. (2015). "Mechanical behavior of sands treated by microbially induced carbonate precipitation." *J. Geotech. Geoenviron. Eng.,* 10.1061/(ASCE)GT.1943-5606.0001383.

Litvan, G. G. (1980). "Freeze-thaw durability of porous building materials." *Proc., 1st international conference of durability of building materials and components,* P. J. Sereda, and G. G. Litvan, ed., Ottawa, Canada, 455-463.

Martinez, B. C., and DeJong, J. T. (2009). "Bio-mediated soil improvement: Load transfer mechanisms at micro- and macro- scales." *Proc. of the 2009 U.S.-China Workshop on Ground Improvement Technologies,* ASCE, Reston, VA, 242–251.

Martinez, B., J. DeJong, T. Ginn, B. Montoya, T. Barkouki, C. Hunt, B. Tanyu, and D. Major. (2013). "Experimental optimization of microbial-induced carbonate precipitation for soil improvement." *J. Geotech. Geoenviron. Eng.,* 10.1061/(ASCE)GT.1943-5606.0000787, 587–598.

677   Montoya, B. M., and DeJong, J. T. (2015). "Stress-strain behavior of sands cemented by

678   microbially induced calcite precipitation." *J. Geotech. Geoenviron. Eng.*,

679   10.1061/(ASCE)GT.1943-5606.

680   Mortensen, B. M., Haber, M., DeJong, J. T., Caslake, L. F., and Nelson, D. C. (2011).

681   "Effects of environmental factors on microbial induced calcite precipitation." *J. Appl.*

682   *Microbiol.,* 111(2), 338–349.

683   National Meteorological Library (2015). "Water in the atmosphere."

684   <http://www.metoffice.gov.uk/media/pdf/4/1/No._03_-_Water_in_the_Atmosphere.pdf>

685   (Jul. 22, 2015).

686   Nemati, M., and Voordouw, G. (2003). "Modification of porous media permeability, using

687   calcium carbonate produced enzymatically in situ." *Enzyme Microb. Technol.,* 33(5), 635–

688   642.

689   Nicholson, P. G., and Tsugawa, P. R. (1997). "Stabilization of diesel contaminated soil with

690   lime and fly ash admixtures." *Fuel Energy Absrt.*, 38(4), 269–269.

691   Ng, W. S., Lee, M. L., and Hii, S. L. (2012). "An overview of the factors affecting microbial-

692   induced calcite precipitation and its potential application in soil improvement." *World*

693   *Acad. Sci. Eng. Technol.,* 6(2), 683–689.

694   Okwadha, G. D., and Li, J. (2010). "Optimum conditions for microbial carbonate

695   precipitation." *Chemosphere,* 81(9), 1143–1148.

696   Otterstedt, J. E., and Brandreth, D. A. (2013). *Small Particles Technology*, Springer, USA.

697   Rosenberg, M. (2006). "Microbial adhesion to hydrocarbons: twenty-five years of doing

698   MATH." *FEMS Microbial. Lett.,* 262, 129-134.

699   Sahrawat, K. (1984). "Effects of temperature and moisture on urease activity in semi-arid

700   tropical soils." *Plant Soil*, 78(3), 401–408.

701   Schiffman, R., and Wilson, C. (1958). "The mechanical behaviour of chemically treated

702   granular materials." *Proc. Am. Soc. for Test. Mater.*, 58, 1218–1244.

703 Scholl, M. A., Mills, A. L., Herman, J. S., and Hornberger, G. M. (1990). "The influence of

704   mineralogy and solution chemistry on the attachment of bacteria to representative aquifer

705   materials." *J. Contam. Hydrol.,* 6, 321–336.

706 Shroff, A. V., Shah, D. L. and Shah, S. J. (1998). "Characteristics of fuel oil contaminated

707   soil and remedial measures–A case study." *Proc. of Indian Geotech. Conf.*, India, 49–51.

708 Smith, W. O., Foote, P. D., and Busang, P. F. (1929). "Packing of homogeneous spheres."

709   *Phys. Rev.,* 34(9), 1271-1274.

710 Stocks-Fischer, S., Galinat, J. K., and Bang, S. S. (1999). "Microbiological precipitation of

711    $CaCO_3$." *Soil Biol. Biochem.,* 31(11), 1563–1571.

712 Torkzaban, S., Tazehkand, S. S., Walker, S. L., and Bradford, S. A. (2008). "Transport and

713   fate of bacteria in porous media: coupled effects of chemical conditions and pore space

714   geometry." *Water Resour. Res.,* 44, 1–12.

715 van Paassen, L. A., Daza, C. M., Staal, M., Sorokin, D. Y., Van der Zon, W., and Van

716   Loosdrecht, M. C. M. (2010a). "Potential soil reinforcement by microbial denitrification."

717   *Ecol. Eng.,* 36(2), 168–175.

718 van Paassen, L.A., Ghose, R., van der Linden, T.J.M., van der Star, W.R.L., and van

719   Loosdrecht, M.C.M. (2010b)." Quantifying biomediated ground improvement by

720   ureolysis: large-scale biogrout experiment." *J. Geotech. Geoenviron. Eng.,*

721   10.1061/(ASCE)GT.1943-5606.0000382, 1721–1728.

722 Whiffin, V. S., van Paassen, L. A., and Harkes, M. P. (2007). "Microbial carbonate

723   precipitation as a soil improvement technique." *Geomicrobiol. J.,* 24(5), 417–423.

724 Wojtowicz, J. A. (1998). "Factors affecting precipitation of calcium carbonate." *J. Swimming

725   Pool Spa Ind.*, 3(1), 18-23.

726

727

728 Captions:

729 **Fig. 1.** Effect of the different urease activity on (a) UCS and (b) permeability of the bio-

730 cemented samples

731 **Fig. 2.** SEM images of bio-cemented samples treated with different urease activities (a-c: 50

732 U/mL, UCS = 713 kPa, and $CaCO_3$ content = 0.061 g/g sand; d-f: 5 U/mL, UCS=709 kPa,

733 and $CaCO_3$ content = 0.039 g/g sand)

734 **Fig. 3.** Effect of the temperature on the UCS of the bio-cemented samples

735 **Fig. 4.** SEM images of bio-cemented samples treated at different temperatures (a-b: 4°C,

736 UCS = 108 kPa and $CaCO_3$ content = 0.021 g/g sand; c-d: 25°C, UCS=245 kPa and $CaCO_3$

737 content = 0.028 g/g sand; e-f: 50°C, UCS=121 kPa and $CaCO_3$ content = 0.034 g/g sand)

738 **Fig. 5.** Images of MICP treated samples (removed from the PCV columns prior to UCS test)

739 subjected to water flushing during the treatment process: (a) control with no water flushing;

740 (b) water flushing after 24 hours of bacterial placement; and (c) water flushing immediately

741 after bacterial placement

742 **Fig. 6.** MICP treated samples subject to water flushing during the treatment process: (a)

743 chemical conversion efficiency; and (b) UCS and $CaCO_3$ content

744 **Fig. 7.** UCS of different PSD sand samples treated with different amount of MICP

745 **Fig. 8.** Effect of FT cycles on UCS of different PSD sands treated with different amount of

746 MICP: (a) 0.15 mm uniform sand; (b) 1.18 mm uniform sand; and (c) well-graded sand

747

748

749

750

751

752

753

754

755   Fig. 1.
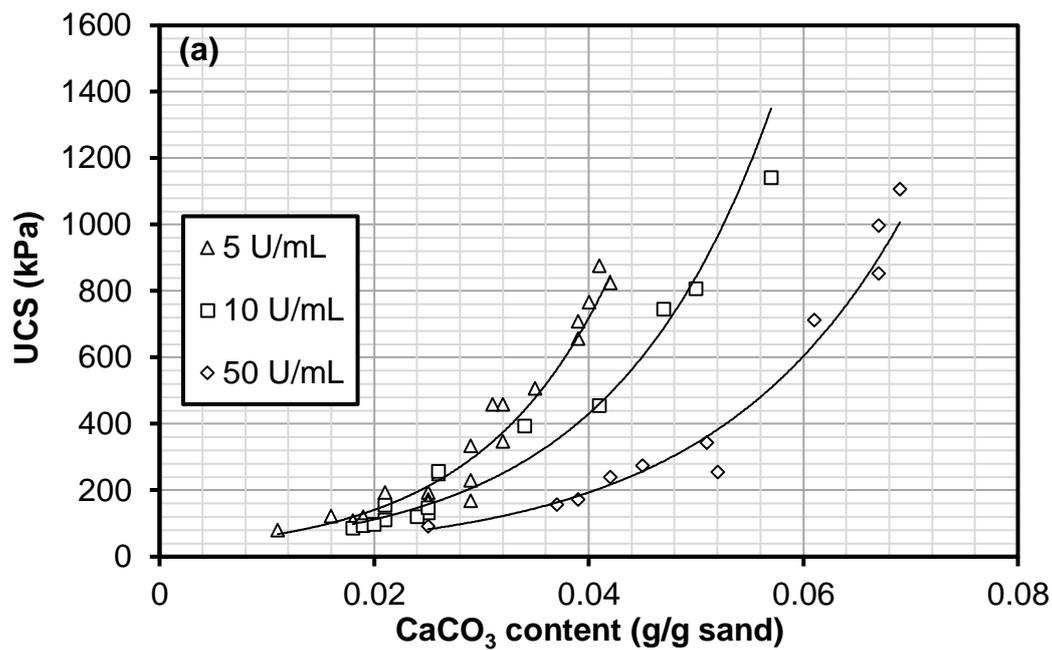
756
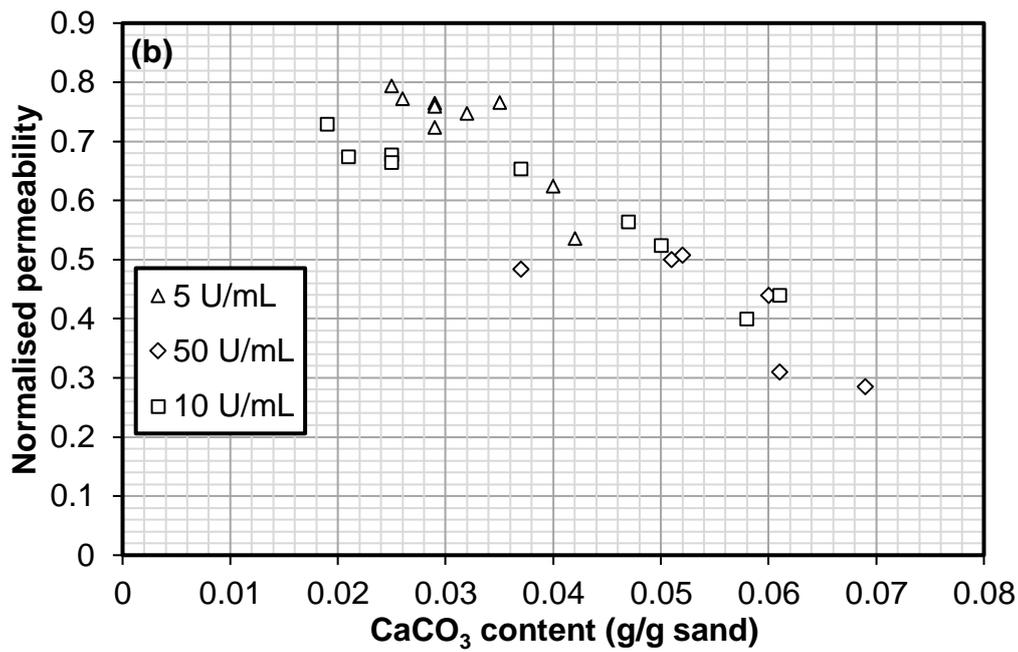
757

758

759   Fig. 2
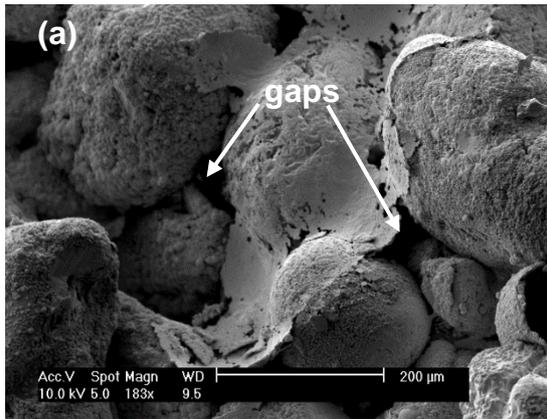

760
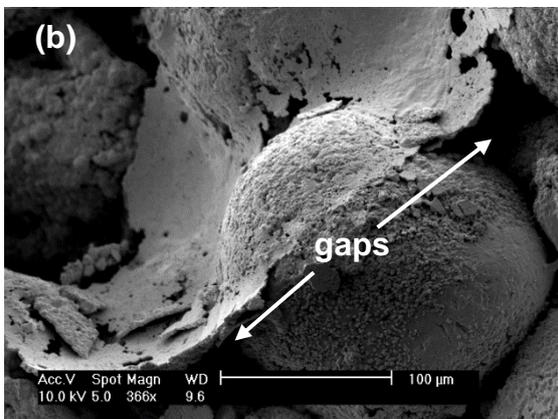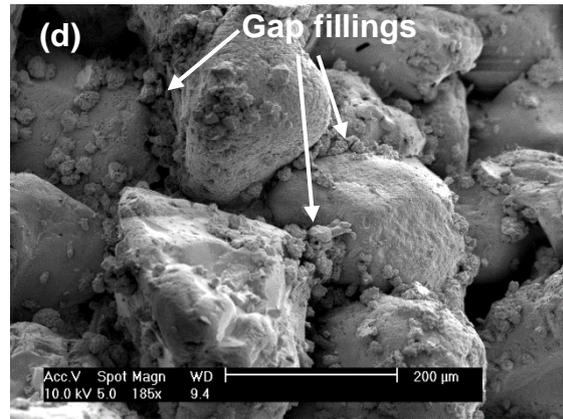
761
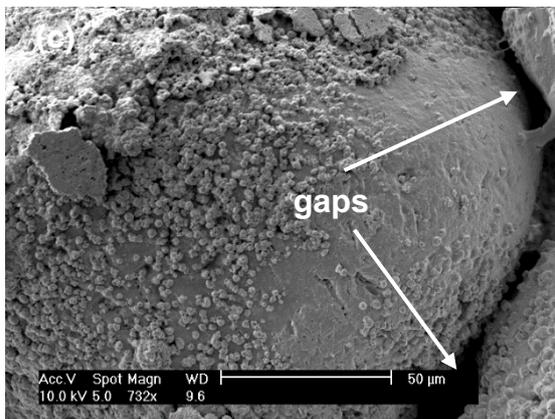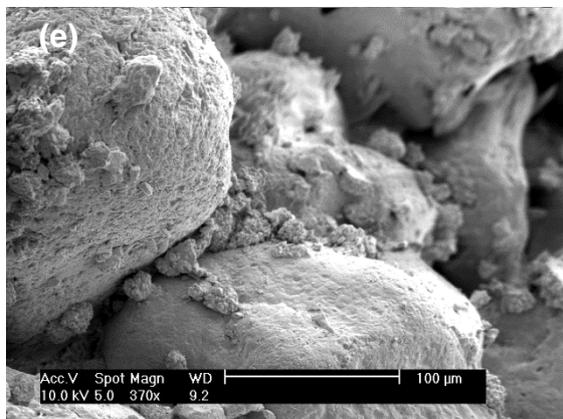
762
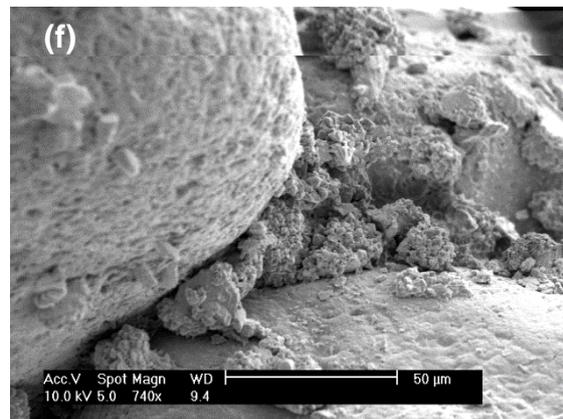
763

764

765   Fig. 3.
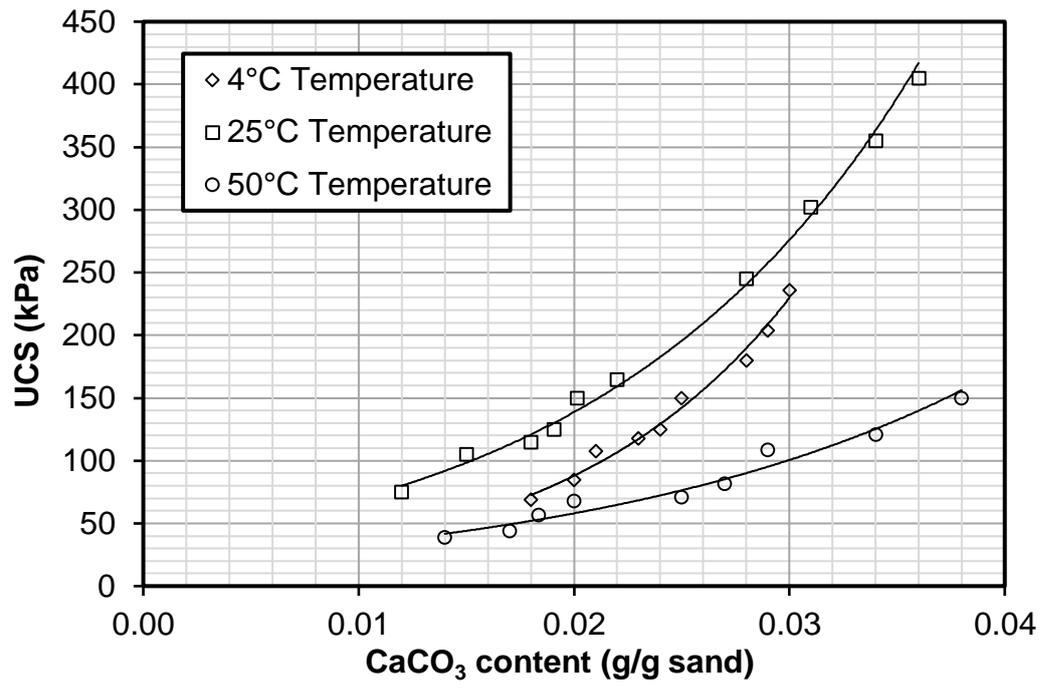
766

767

768 Fig. 4.
769
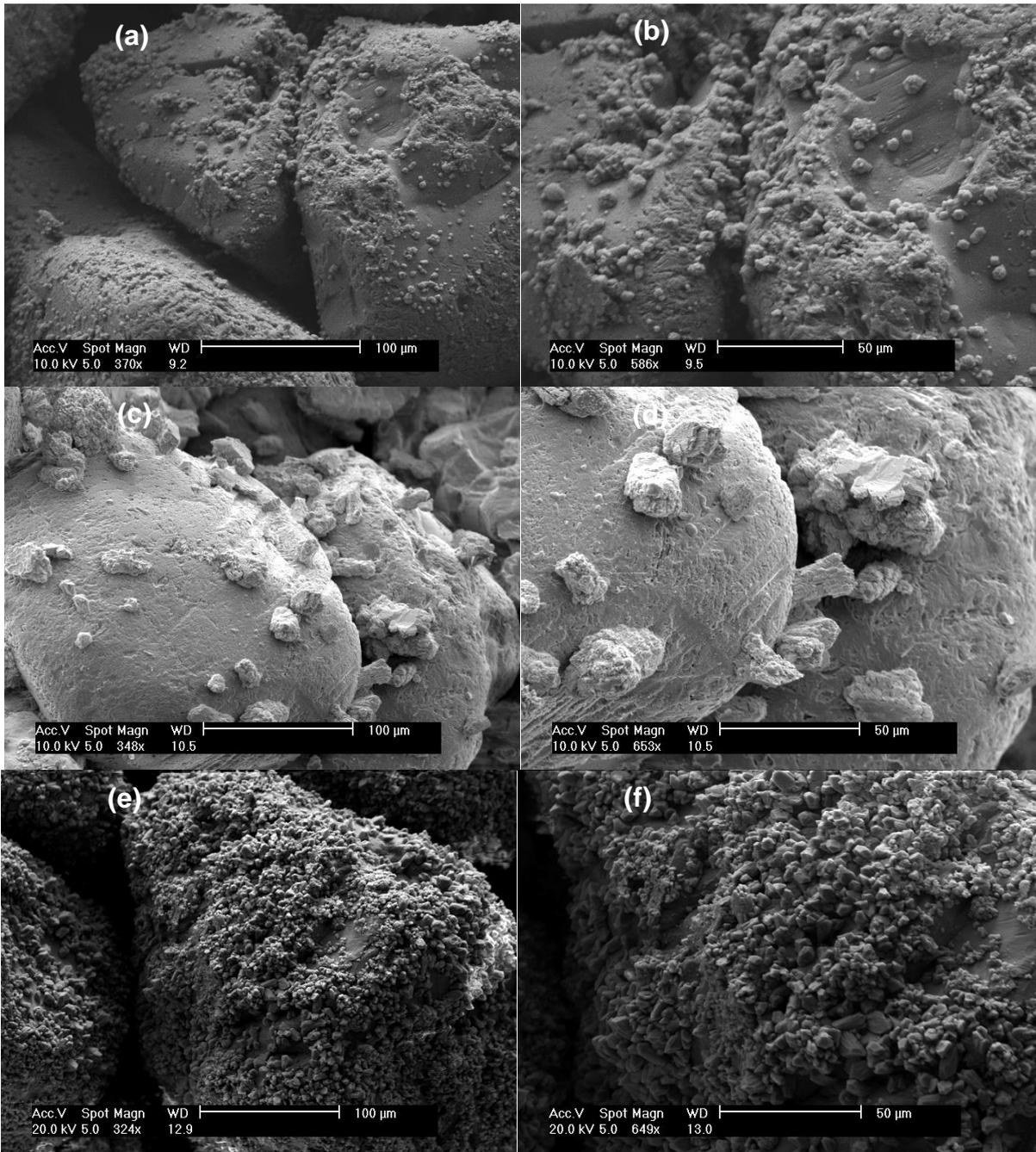
770

771

772
773

774
775

776   Fig. 5

777

782   Fig. 7

783

784