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Performance of biocemented sand under various environmental conditions

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SUMMARY: Microbially induced calcite precipitation (MICP) is a naturally driven biological technique that employs the use of ureolytic bacteria to hydrolyse urea, producing carbonate ions. In the presence of a calcium rich medium, carbonate ions react with calcium ions to form a solid compound called calcium carbonate or calcite ($CaCO_3$). The precipitated $CaCO_3$ would act as a cementing agent that binds the soil grains together by means of effective bridging at the soil pore throats, leading to increased strength and stiffness of biocemented soil. In this study, the performance of biocemented uniform silica sand (0.425 mm in diameter) under extreme environmental conditions were investigated, including: (1) low, ambient and high surficial temperatures; (2) different soil pH; and (3) two freeze-thaw (FT) cycles conditions (4 FT and 10 FT). The study was carried out through a series of laboratory tests including the determination of the $CaCO_3$ content, the pre and post hydraulic conductivity and the unconfined compressive strength (UCS). The crushed biocemented samples were observed at the microscale level using the scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS), to analyse the microstructure and chemical characterisation of the precipitated $CaCO_3$. The UCS results indicated that soil biocementation is more effective at the ambient temperature and neutral soil pH. Moreover, the results also demonstrated that the biocemented sand was able to withstand up to 10 FT cycles. Although all biocemented samples recorded reduction in their hydraulic conductivity values, regardless of the environmental parameters tested, the ability to retain sufficient permeability while ensuring fairly adequate strength compared to traditional chemical treatment by Portland cement was a clear advantage of MICP treated soil. The SEM images of the biocemented samples confirmed the formation of effective bridges that connect the voids of soil grains, leading to improved soil strength and stiffness. The EDS analysis verified the existence of calcium (Ca), carbon (C) and oxygen (O) elements as unique set of peaks on its EDS spectrum denoting the presence of mainly $CaCO_3$ crystals in the biocemented samples. The understanding of these fundamental extreme environmental parameters through a laboratory scale model is crucial because it can have great implications towards employing MICP in field applications.

KEYWORDS: Biocementation, microbially induced calcite precipitation (MICP), soil stabilisation, ground improvement.

1 INTRODUCTION

The addition of chemical additives such as ordinary Portland cement (OPC) (Pourakbar et al. 2015), lime (Obuzor et al. 2012), and fly ash (Cristelo et al. 2012; Mujah, 2016) is the most commonly used technique for soil stabilisation. However, over the years, the use of chemical grouts has been under scrutiny because of their detrimental effects toward the environment (Ariyanti et al. 2011). Moreover, the production of chemical additives requires substantial utilization of energy, which emits unwarranted carbon dioxide to the environment. Therefore, the exploration of new soil stabilisation techniques that can take into consideration the economic-viability, cost-effectiveness and sustainability are crucial and timely.

Microbially induced calcite precipitation (MICP) is an emerging and promising soil stabilization technique in which are utilized microorganisms to hydrolyse urea to produce carbonate ions, which react with calcium ions (calcium chloride) to produce calcite (calcium carbonate). The chemical reactions of this process are shown in Equations 1 and 2. The precipitated calcite crystals bind the soil particles together, and thus improve the mechanical properties (strength and stiffness) of the biocemented soil.

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

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad (2)$$

In the present study, the performance of biocemented soil samples under the effects of different environmental condition, including degree of temperature, soil pH, and freeze-thaw (FT) cycles were investigated. The mechanical behaviour of biocemented soil samples was measured by recording the shear strength and stiffness using unconfined compressive strength (UCS) tests. The constant head permeability and calcite content tests were also adopted to examine the effect of calcite content on the hydraulic conductivity of bio-treated soil. The crushed biocemented soil samples were observed using the scanning electron microscope (SEM). The energy dispersive X-ray spectroscopy (EDS) was also performed on the crushed bio-cemented soil samples to identify the elements present. The current study is crucial in the sense that it simulates the extreme environmental conditions that affect the MICP treatment.

2 MATERIALS AND METHOD

2.1 Soil used

Natural silica sand obtained from Cook Industrial Minerals Pty. Ltd Western Australia was used in this study. The sand is classified as poorly graded according to the unified soil classification system (USCS) with particle size of 0.425 mm. The particle size distribution of the sand used is shown in Figure 1 and its characteristics are given in Table 1.

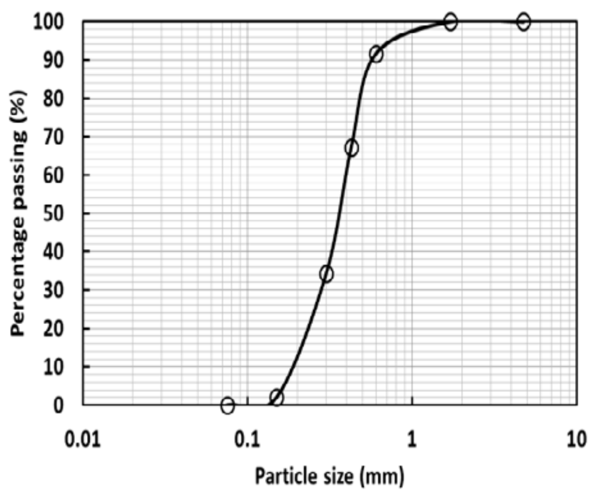

Figure 1. Particle size distribution of the sand used.

Table 1. Characteristics of the sand used.

Property (unit)	Value
D_{10} (mm)	0.17
D_{30} (mm)	0.2
D_{60} (mm)	0.28
C_U	1.65
C_C	0.84
G_S	2.71
e_{min}	0.48
e_{max}	0.71

2.2 Bacteria culture and cementation solution

The microorganism used in this study was *Bacillus sphaericus* strain (MCP-11 DSM 23526) (Al-Thawadi and Cord-Ruwisch, 2012). Under sterile aerobic condition, the bacteria strain was cultivated in a growth medium consisting of yeast extract and ammonium sulphate. After 24 hours of cultivation at 32°C, the bacteria culture was stored in refrigerator at 4°C prior to MICP treatment. The optical density (OD_{600}) of the culture varied between 2–2.5 and the urease activity of the culture was about 10 U/mL (1 U = 1 μmol urea hydrolysed per min). Urea-calcium chloride of 1 M was used as the cementation solution to facilitate the production of calcite. The recipes for the growth medium and cementation solution are given in Table 2.

Table 2. Solution recipes.

Solution	Constituents
Growth medium	20 g/L yeast extract
	16 g/L ammonium sulphate 1 L of deionised water
	0.05 mM nickel (II) chloride hexahydrate adjust pH to 9.3 by addition of sodium hydroxide
Cementation solution	60 g/L urea 147 g/L calcium chloride dehydrate 1 L of deionised water

2.3 Sand column preparation

The sand was compacted into a polyvinyl chloride (PVC) pipe of an inner diameter of 42 mm and length of 180 mm. To ensure uniform compaction throughout all tests, the sand was compacted to at least 95% of its maximum dry density (MDD = 16.2 kN/m^3 and OMC = 12%) by applying 20 blows per layer for three consecutive layers. The final dry densities of compacted samples ranged between 15.5–16.1 kN/m^3. To allow for the chemical injections to flow into the soil samples, the inlet (top) of the mould was connected to a peristaltic pump while the outlet (bottom) was connected to vacuum. Scour pads and 10 mm thick sand were placed at the top and bottom of the mould, acting as filter to ensure a smooth spreading of the solution during treatment and also to prevent the sand particles from being flushed out during the treatment cycles.

2.4 MICP treatment

The PVC mould was up-flushed with 1 void volume of deionised water to remove the air voids inside the sand samples, which were then left to be fully saturated (S = 100%) for 24 hours prior to MICP treatment. The sand column was then down-flushed with 0.5 void volume of bacteria culture, followed by 0.5 void volume of cementation solution. A retention time of 24 hours was adopted for each treatment cycle, to ensure the bacteria attachment to the sand particles. The column was left at the ambient temperature (25±1°C) (otherwise stated differently in Section 2.7.1) during the treatment and full saturation condition was maintained by ensuring that the liquid level inside the outlet tube was level with the column's top cap. After 24 hours, the column was then repeatedly treated with 1 void volume of the cementation solution every 24 hours to achieve different degrees of cementation, as proposed by Cheng and Cord-Ruwisch (2014). The flow rate of all injections was kept constant at 1 L/hour.

2.5 Permeability and calcite content tests

The permeability measurement in this study was carried out based on the constant head permeability test as outlined in the Australian Standards AS1289.6.7.1 (2001). The reduction in permeability in terms of the percentage of precipitated calcite crystals was determined through the difference in values taken before and after treatment. Permeability is an important factor in soil mechanics as it governs the behaviour of porous materials in many geotechnical engineering applications.

The calcite content was determined by adding 2 mL of 2 M hydrochloric acid solution into 2 g of dried crushed sample. This reaction produces carbon dioxide gas, as shown in Equation 3, and its volume can be measured using the U-tube manometer. The actual amount of calcite per gram of sand (g/g) was calculated based on an established relationship between the volumes of carbon dioxide gas and amount of pure analytical grade calcite powder (Cheng and Cord-Ruwisch, 2012).

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + H_2O + CO_2 \quad (3)$$

2.6 UCS tests

The samples were trimmed into 80 mm in length and 40 mm in diameter to maintain an aspect length-to-diameter ratio of 2. The UCS test was conducted using the GCTS STX-300 fully automated apparatus according to the Australian Standards AS5101.4 (2008). The axial load was applied at a constant speed of 1 mm/min. Termination of the experiment was done when either: (1) clear shear failure plane was observed along the column; or (2) the axial displacement reached 20 mm.

2.7 Environmental parameters investigated

2.7.1 Temperature

Subsurface temperature surrounding MICP treated soil strata is important to ensure the success of biocementation in the field. In this study, the temperature values were selected to simulate the subsurface soil temperature in cold region (4°C), tropical region (25°C) and arid region (50°C). During the MICP treatment process, the sand samples were placed: (i) inside the refrigerator at 4°C; (ii) at ambient temperature of 25°C; and (iii) inside a 50^{o}C oven for 24 hours.

2.7.2 Soil pH

Harkes et al. (2010) pointed that the pH value can influence the bacteria transport and attachment in the targeted treatment environment. In this study, the sand was flushed with 2 L each solution containing citric acid pH = 3.5 (acidic) and sodium hydroxide pH = 9.5 (basic) whereas pH = 7 (neutral) acts as control.

2.7.3 Freeze-thaw cycle

Freeze-thaw (FT) action has been the cause of soil failure in cold regions. In this study, an attempt was made to replicate the effect of FT cycle on bio-cemented samples experimentally. The bio-cemented samples were cut into 80 mm in length and 40 mm in diameter to maintain an aspect length-to-diameter ratio of 2. Then, the sample was exposed to 4 FT and 10 FT cycles prior to UCS tests. Each FT cycle procedure was subjected to 12 hours freeze at -14°C inside the freezer followed by 12 hours thaw at the room temperature (25±1 °C). To ensure full saturation, all samples were immersed in deionised water throughout the FT cycles.

2.8 SEM and EDS analyses

The crushed bio-cemented samples from the UCS tests were oven-dried at 105°C for 24 hours before undergoing microstructure analysis. SEM and EDS (Tescan Mira3 XMU) were conducted on the samples. The SEM analysis is an important part of the present study because it helps to characterise the crystalline formation of the calcite crystals and can specify the location of calcite precipitation, which contributes to the soil strength. On the other hand, the EDS analysis indicates the elemental composition presents in the bio-cemented sand matrix.

3 RESULTS AND DISCUSSION

3.1 Permeability

Figure 2 shows the measured permeability values at different cement content of MICP treated (current study) and OPC treated (Ismail et al, 2002) samples. It can be seen that MICP treated samples have higher permeability value at all cement content compared to OPC treated samples. Cheng et al. (2013) explained that the significant loss of permeability in OPC samples was due to the formation of water-insoluble hydrates as a result of the cement hydration reaction, occupying the pore space while the formation of calcite crystals in MICP causes smaller volume change compared to the hydrates. Also, Figure 2 confirms that MICP bio-cemented soil is still classified as a good drainage material since it retains sufficient permeability (> 1 x 10^{-5} m/s) (Bear, 1972) at high cement content (> 0.1 g/g). This aspect of MICP would certainly benefit several geotechnical engineering applications such as the consolidation of embankment and railway subgrade where provision of adequate drainage must be maintained at all time.

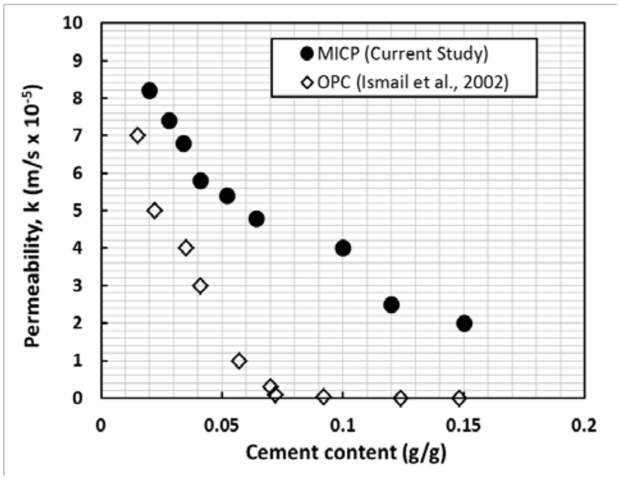

Figure 2. Comparison of permeability.

3.2 Environmental parameters

3.2.1 Effect of temperature

Figure 3 shows the effect of different degrees of temperature on the bio-cemented soil samples. The figure suggests that MICP treatment in the ambient temperature (25°C) offers the most effective strength improvement compared to at temperatures of 4°C and 50°C. This is attributed to the precipitation of the largest calcite crystals (20-50 μm) at 25°C treated samples (refer to Section 3.3.1 for the SEM image). The size of calcite crystals was determined by the competition between the rate of nucleation (birth of new crystal nuclei) and crystal growth rate (increase in size of crystal). Based on the calcite crystal size precipitated in the ambient temperature, the latter takes precedence.

Wojtowicz (2001) stated that at higher temperature (in this case 50°C), the rate of calcite nucleation becomes faster, thus, producing smaller calcite crystals (2-5 μm). Small calcite crystals could not effectively connect the sand particles together and this leads to the lowest observed strength improvement. Although the rate of nucleation was low at 4°C, the size of individual crystal was fairly small (5-10 μm) due to the slow crystal growth rate. This was because of the slow urea hydrolysis process controlled by the low urease activity at low temperature.

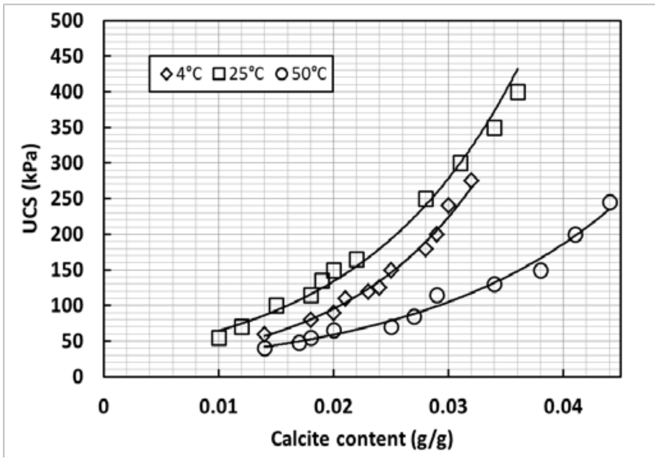

Figure 3. Effect of temperature.

3.2.2 Effect of soil pH

Figure 4 shows the effect of different soil pH on the bio-treated soil samples. It can be seen that the neutral pH condition provides the highest UCS strength with an effective calcite content of 0.013 g/g. Both acidic and basic conditions have negative impact on the MICP treatment, resulting in lower strength compared to that obtained from the neutral pH condition. It was observed in all tests that regardless of the initial soil pH condition, the final pH value after treatment ranged between 9–10 and this is due to the urea hydrolysis process during MICP.

Ferris et al. (2004) found that MICP process favoured an initial pH environment of 6.5–9.3 (neutral to basic condition). During urea hydrolysis, the pH level of MICP environment was increased due to the release of hydroxyl ions (OH^-) into the treatment environment from the dissociation of water (H_2O) (DeJong et al., 2010). It is believed that the mechanism of improvement in different soil pH conditions is governed by the calcite distribution along the biocemented samples as a result of bacteria transport and attachment to sand particles (McWhirter et al., 2002; Harkes et al., 2010).

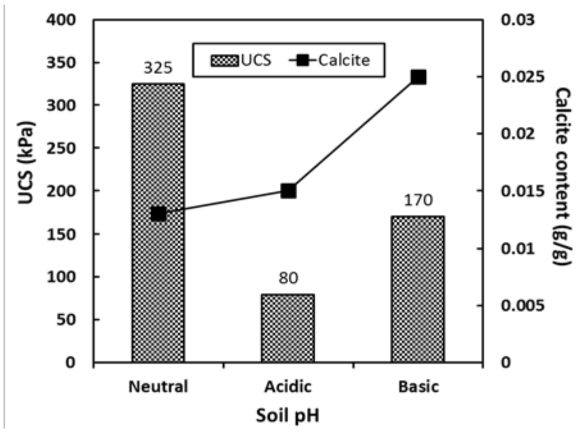

Figure 4. Effect of soil pH.

Figure 5 shows the calcite distribution along the length of bio-cemented soil column for different pH conditions. Clearly, the neutral pH condition exhibits more homogeneous distribution of calcite as opposed to acidic and basic pH conditions. The lower UCS values displayed by both the acidic and basic pH conditions can also be explained by the spatial accumulation of calcite which is concentrated at the top part of the samples. This results in fewer bacteria transportation deep into the sand column. The concentrated calcite precipitates would not contribute towards the global UCS values of soil samples, hence, explaining their lowest strength.

Although more calcite crystals were precipitated at the top part compared to the bottom at the basic pH condition, the overall calcite content at the bottom (which gives global UCS 170 kPa) was still higher than the calcite content at the neutral pH condition (which gives global UCS 325 kPa). This indicates that the strength of the biocemented sand samples was not necessarily proportional to the amount of the precipitated calcite content. Rather, the formation of the effective calcite crystals that is large and connecting two or more sand particles together at the pore throat that contributes towards higher strength. Therefore, higher UCS value in the neutral pH condition can be explained by the formation of effective calcite precipitation formed at the neutral pH (refer Figure 7).

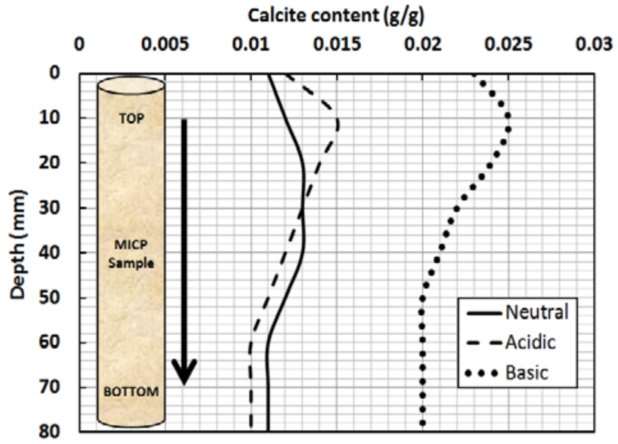

Figure 5. Calcite distribution in different pH conditions.

3.2.3 Effect of freeze-thaw (FT) cycle

Figure 6 shows the effect of 10 FT cycles on bio-cemented soil samples. The results were compared with OPC treatment derived from Cheng et al. (2013). After 10 FT cycles, about 10% and 40% reduction in UCS values were observed in MICP and OPC treated samples, respectively. As indicated earlier in Section 3.1, MICP treated samples have a high permeability retention which favour the samples endurance towards FT cycles action. This is supported by Litvan (1980) who stated that the severity of the damage due to FT cycles action is proportional to the water content of porous solids. Porous solids with high permeability usually have higher FT resistance because they allow more rapid water mass transfer within the soil matrix.

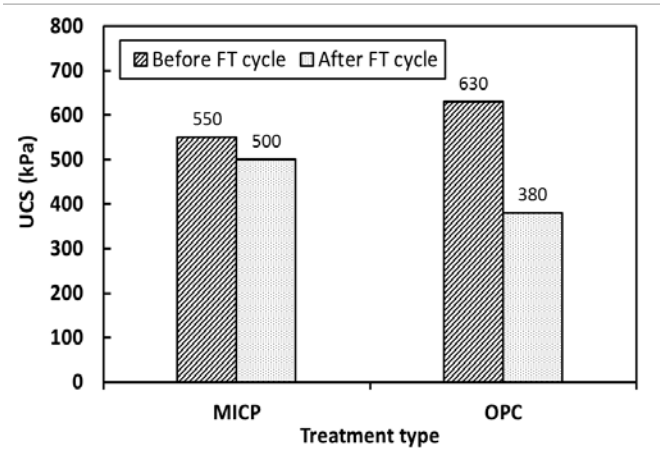

Figure 6. Effect of 10 FT cycles.

3.3 SEM and EDS analyses

Figure 7 shows the calcite precipitation pattern under the optimised treatment conditions (i.e. ambient temperature and neutral pH). The SEM image clearly shows that large calcite crystals (20-50 μm) are precipitated at the soil pore throats covering the gap between the soil particles. This type of calcite formation in MICP is called “effective calcite” because the size of calcite is large enough to link the sand particles together, thus, providing high strength.

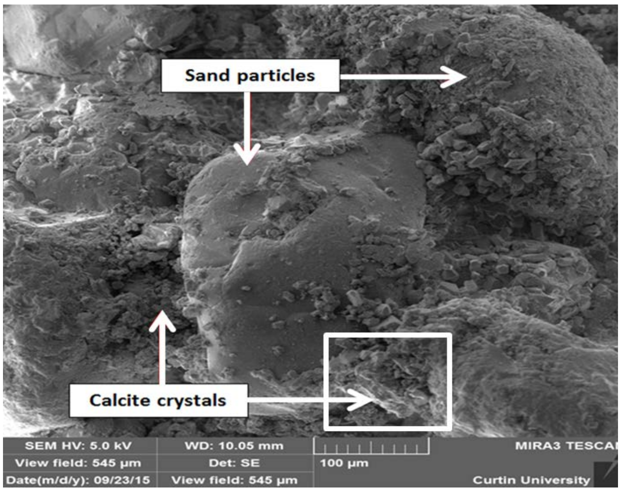

Figure 7. SEM image showing effective calcite precipitation.

Figure 8 shows the EDS analysis taken at the square box region indicated in Figure 7. The EDS analysis confirms the presence of calcium (Ca, 16.2%), carbon (C, 20.55%) and oxygen (O, 20.99%) elements, which made up the calcite ($CaCO_3$) in the biocemented sand matrix. Silicon (Si, 40.22%) was derived from the quartz (SiO_2), which is the main component of silica sand used in the current study.

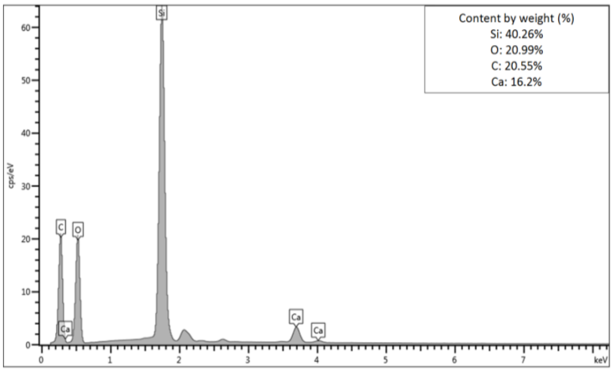

Figure 8. EDS spectrum of MICP treated sample.

3.4 Strength improvement comparison

Figure 9 shows strength comparison of the optimised MICP treatment conditions adopted in the current study (i.e. ambient temperature and neutral pH), compared to MICP treatment conducted by Al-Qabany and Soga (2013), OPC (Ismail et al., 2002) and iron-based biogrout (Ivanov et al., 2010). It can be seen that the MICP treatment adopted in the current study performs better than the MICP treatment by Al-Qabany and Soga (2013) and OPC treatment by Ismail et al. (2002), and is as good as the iron-based bio-grout by Ivanov et al. (2010). Although Al-Qabany and Soga (2013) used exactly the same concentration of cementation solution (1 M), their UCS values were considerably lower than those obtained from the current study. This is probably because of the use of much lower OD_{600} bacteria value (0.8–1.2) compared to the current study (2–2.5).

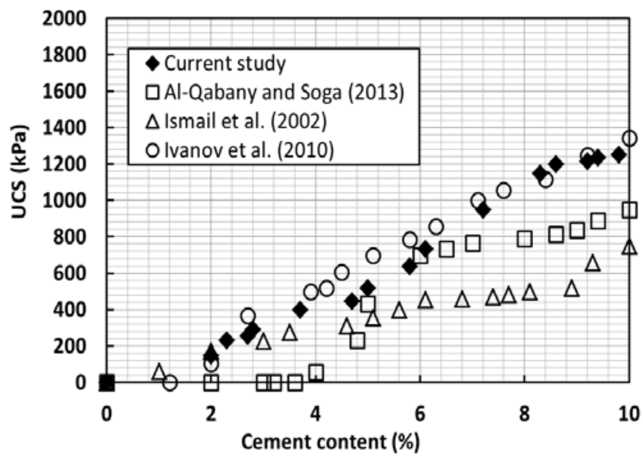

Figure 9. Strength improvement comparison with other treatments.

It should be noted that compared to OPC and iron-based bio-grout treatment, MICP promises long term benefits in the sense that the existing active microorganism in the sample can sustain repeated treatment of cementation solution. This novelty of MICP has been used to self-heal soils post-seismic excitation (Montoya and DeJong, 2013). Also, MICP can offer sustainable approach in its application by substituting the use of artificial calcium chloride ($CaCl_2$) with natural salt found in seawater (Cheng et al., 2014).

4 CONCLUSIONS

Based on the results obtained from the current study, the following conclusions are drawn:

- MICP treated sand achieved the highest strength in the ambient temperature and neutral pH level and this is attributed to the effective calcite precipitation and calcite distribution homogeneity.
- Bio-cemented soil is highly durable towards FT cycles action (up to 10 FT cycles) due to the treatment high permeability retention characteristic.
- SEM and EDS analyses confirmed the precipitation of calcite crystals ($CaCO_3$) in the calcite-sand matrix.

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