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Undrained cyclic triaxial testing on sand with non-plastic fines content cemented with microbially induced $CaCO_3$

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Abstract

Sand containing non-plastic fines is susceptible to liquefaction, and there seems to be room for more efficient soil improvement for such soils. Microbially Induced Carbonate Precipitation (MICP) is a promising soil cementing technique in which bacteria's urease enzyme is utilized to catalyze $CaCO_3$ precipitation in the intergranular void, reinforcing the soil structure without disturbance and strong adverse environmental effects. In the present study, Urayasu sand, which is liquefaction ejecta in Urayasu City, Japan, produced by the 2011 Great East Japan Earthquake, was treated with MICP as well as other materials with various amounts of non-plastic fines content, to investigate the effectiveness of MICP in improving liquefaction resistance of such sand. The result of undrained cyclic triaxial shear test on the microbially cemented Urayasu sand specimen showed that its number of cycles to liquefaction can be improved from 1 cycle to 21 cycles with 3 % $CaCO_3$ when the cyclic stress ratio and relative density are 0.3 and 45 %, respectively. However, it was also found that the improvement for sand with non-plastic fines is much less pronounced relative to that for clean sand. There are several possible mechanisms behind the hindered improvement and they are analyzed and discussed in this paper.

Authors' keywords: Liquefaction; Non-plastic fines content; Soil stabilization; Calcium carbonate ($CaCO_3$); Microbially induced carbonate precipitation (MICP); Undrained cyclic triaxial shear test

International Geotechnical Classification Numbers: D07, K06

Introduction

Microbially induced carbonate precipitation (MICP) is a novel soil improvement technique where bacteria mediate the precipitation of calcium carbonate crystal ($CaCO_3$) between soil particles, thereby increasing the strength of the soil. There are several different mechanisms to produce $CaCO_3$ (van Paassen et al., 2010) among which the one that utilizes ureolytic bacteria seems to be the most promising technique. In this method, urease enzymes produced by bacteria act as a catalyst to facilitate precipitation of $CaCO_3$ by generating carbonate ions via urea hydrolysis which then react with artificially added or naturally present calcium ions to precipitate $CaCO_3$ in the interparticle voids (Stocks-Fischer et al., 1999). The urease method is advantageous over conventional soil improvement techniques in such aspects as applicability to the soil under existing buildings and minimal adverse effects on the environment.

Past research has shown that the urease method is effective in reinforcing soils. Increase in shear strength of fine sand was observed with an increasing amount of precipitated $CaCO_3$ (Whiffin et al., 2007; Chou et al., 2011; Al Qabany and Soga, 2013; Feng and Montoya, 2015; Lin et al., 2015). Stiffness of fine sand was also enhanced by interparticle bonds created by $CaCO_3$ (van Paassen, 2009; Feng and Montoya, 2015; Lin et al., 2015). Dilation of fine sand was also enhanced by MICP (Chou et al., 2011;Feng and Montoya, 2015; Lin et al., 2015). Moreover, undrained strength was improved to the point where loose sand was no longer collapsible under undrained monotonic loading (DeJong et al., 2006; Montoya and DeJong, 2015). In addition, undrained cyclic shear strength was significantly improved due to $CaCO_3$ precipitation (Burbank et al., 2013; Montoya et al., 2013).

Currently, MICP is considered feasible to gravels, sands and silty sands (DeJong et al., 2013). However, it seems that research on MICP on silty sand (i.e. sand with fines) is rather limited. Unconfined compressive strength of residual soil samples containing more than 60% of silt and clay content was successfully enhanced by MICP (Lee et al., 2013; Ng et al., 2013; Ng et al., 2014). Undrained cyclic shear resistance of natural river sand samples was improved by MICP involving indigenous bacteria (Burbank et al., 2013). MICP was also effective in increasing erosion resistance of sand having 20% of kaolin content (Jiang and Soga, 2014; Jiang et al., 2014). Nevertheless, unconfined compressive strength of silty sand might not be representative of either drained or undraied strengths since permeability of silty sand may not guarantee completely drained or undrained conditions. Also, it is not certain whether enhanced erosion resistance of sand with fines can be correlated with increase in strength. Finally, effects of fines content on undrained cyclic shear resistance of MICP-treated silty sand are not investigated sufficiently.

Sand with fines is often vulnerable to liquefaction, particularly when the fines are of non-plastic nature (Polito and Martin Ⅱ, 2001); for example both Urayasu City soil and Christchurch soil contained a moderate to substantial amount of non-plastic fines at the locations where severe liquefaction took place during the 2011 Great East Japan Earthquake and 2010-2011 Christchurch earthquakes, respectively (Tokimatsu and Katsumata, 2012; Towhata et al., 2013; Orense et al., 2011). Therefore, it is of critical importance to investigate whether MICP with the urease method is effective to sand with fines content in reducing liquefaction potential.

In the present study, sandy materials with or without non-plastic fines content, including sand recovered from Urayasu City after the 2011 Great East Japan Earthquake, were employed and solidified with $CaCO_3$ produced by a bacterial species named *Sporosarcina pasteurii*. The solidified materials were prepared in a triaxial cell and were then subjected to undrained cyclic loading. The results were analysed to provide an insight into the effectiveness of MICP to sand with non-plastic fines content in mitigating liquefaction potential.

Materials and Methods

Microbial Solution

In this study a liquid media which was prepared to grow *S. pasteurii* is named microbial solution. Microbial solution was produced by inoculating an arbitral amount of *S.pasteurii* from a petri dish into nutrient solution in a sterile chamber. The dish and the nutrient solution were made of the same ingredients except for agar that was added only to the former to make it gel. The shared chemical compounds were 20 g of yeast extract, 10 g of $(NH_4)_2SO_4$ per litre of 0.13 M Tris buffer whose pH was adjusted to 9.0. The nutrient solution was sterilized in an autoclave at 121 ^{o}C for 20min before it was mixed with the bacteria in a sterile condition and then stored in an air-conditioned chamber at 30 ^{o}C for about 24 hours to obtain microbial solution. It was then used to treat the triaxial specimen. The bacteria count of microbial solution was measured sporadically and the typical value was 10^7 per milligram of the solution.

Cementation Solution

Cementation solution is liquid media prepared in this study that contain chemicals necessary to induce the precipitation of $CaCO_3$. These chemicals are 3.000 g of nutrient broth, 30.030 g of $(H_2N)_2CO$, 73.505 g of $CaCl_2 \cdot 2H_2O$, 10.000 g of NH_4Cl, and 2.120 g of $NaHCO_3$per litre of distilled water. As a result, cementation solution consists of 0.5 M of urea and calcium chloride. The solution was prepared as follows: firstly $CaCl_2 \cdot 2H_2O$ was placed alone in a flask while the other compounds were put in a separate flask. Measured amounts of distilled water were poured into the two flasks. After the chemical powders were dissolved into the distilled water in each flask, the two solutions were mixed together to produce cementation solution. This care was taken because of a tendency that mixing all the ingredients in one flask with water resulted in the coagulation of the chemicals. Cementation solution was not sterilized prior to its use in the triaxial experiment. In addition, pH of cementation solution was not adjusted prior to injection and it remained around 7.0. This ensured that reaction of calcium with carbonate in the solution to produce insoluble $CaCO_3$ was minimal without the bacteria, as pH close to 9.0 is required to do so (Stocks-Fischer et al., 1999).

Tested Soils

Three types of soils were tested in the triaxial experiment. Their properties are summarized in Table 1 and Fig. 1. Toyoura sand is a clean sand and thus does not contain fines (Fig. 2(a)). Also, Toyoura sand is poorly graded and is very susceptible to liquefaction. Urayasu sand is a material recovered from Urayasu City in Chiba Prefecture, Japan, after enormous amounts of sand gushed from the ground due to severe liquefaction that occurred following the 2011 Great East Japan Earthquake. Hence, Urayasu sand is a product of real liquefaction and has little resistance against liquefaction despite the presence of large amounts of fines content. The nature of the fines content of Urayasu sand was examined and it was identified as non-plastic silt. A SEM image of Urayasu sand is provided in Fig. 2(b). The third material used in the experiment is an artificial mixture of Toyoura sand and a non-plastic silt branded as DL Clay whose SEM image is supplied in Fig. 2(c). The mixture with three levels of fines content was prepared and used to make the triaxial specimen.

Table 1. Material properties of the sands

Material	G_s	e_{min}	e_{max}	F_c(%)
Toyoura sand	2.656	0.632	0.992	0
Urayasu sand	2.685	0.78	1.40	32.4
Toyoura sand+DL clay	2.656	0.562	0.971	5
Toyoura sand+DL clay	2.657	0.438	0.894	15
Toyoura sand+DL clay	2.659	0.366	0.834	30

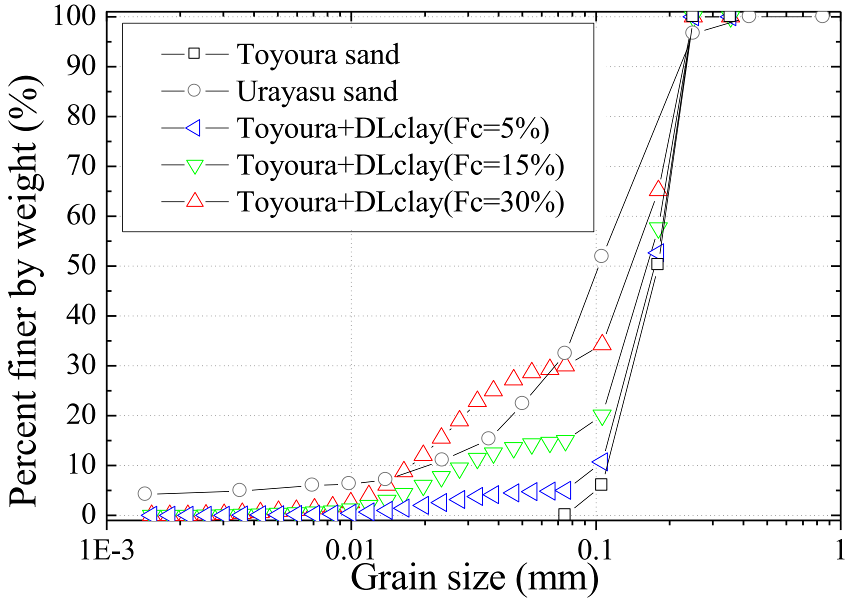
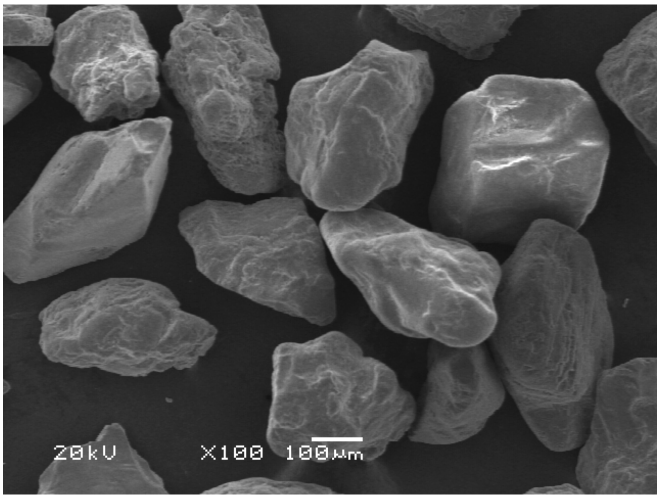
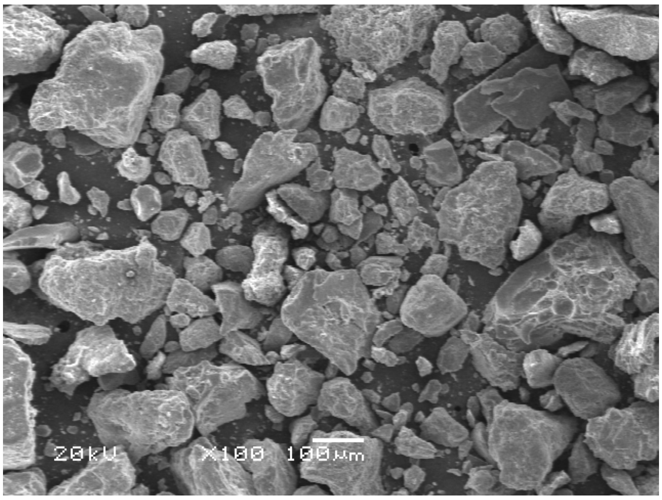


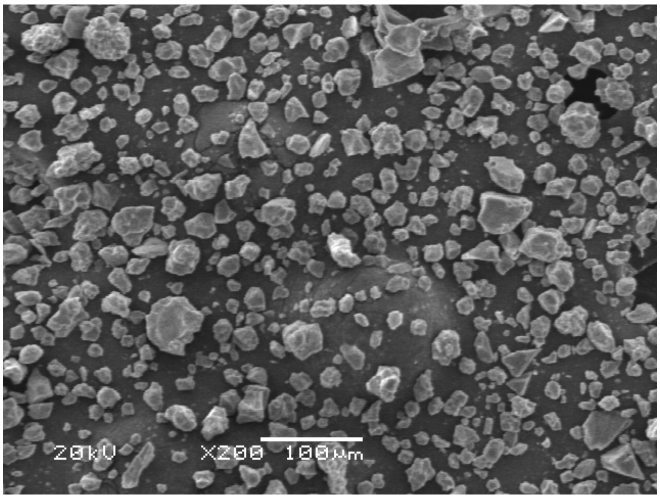
Fig. 1. Grain size distribution of the materials



(a) Toyoura sand (×100)



(b) Urayasu sand (×100)



(c) DL Clay (×200)

Fig. 2. SEM images of the tested materials

Specimen Setup

The triaxial specimen was prepared by air-pluviating an oven-dried material into a mould from a prescribed height to achieve relative density of around 45 %. The dimensions of the specimen are 100 mm in height and 50 mm in diameter. The specimen was covered with a rubber membrane whose thickness was 0.3 mm, and the material was capped with a pair of filter papers and porous stones at both ends to prevent a loss of fine particles during the infiltration of the solutions. Negative air pressure of about -30 kPa was then applied to the pore of the specimen to stabilize it without the mould. Cell pressure was then gradually applied while the pore pressure was increased until the former reached 30kPa and the latter was reduced to zero. The axial load was adjusted with appropriate dead weights for a given cell pressure level to ensure no deviator stress was generated throughout the rest of the preparation process.

Microbial Treatment of the Specimen

Firstly, approximately 100 mL of microbial solution was provided to the specimen from the bottom end by reducing the air pressure inside the specimen to about -5 kPa. A hundred mL corresponded to 1.0-1.3 pore volume (PV) of the triaxial specimen with the upper and lower bounds represented by Toyoura sand samples with 30 % DL Clay content and Urayasu sand samples, respectively. The cell pressure was also decreased to 25 kPa in the meantime. The infiltration rate was not controlled. Following the first infiltration, the second infiltration of microbial solution was carried out from the top end of the specimen in an attempt to distribute the bacteria uniformly in the specimen. Right after the second infiltration, a 100 mL of cementation solution was provided to the specimen from the bottom end under the same conditions as the infiltration of microbial solution. After the provision of the first cementation solution, the pressure inside the pore was brought back to zero and the specimen was cured with a confining pressure of 30kPa for about 12 hours. This retention time is considered to be the shortest to achieve high precipitation efficiency with the given urea-calcium concentration (Al Qabany et al., 2012). Following the 12-hour retention period, the second infiltration of cementation solution was conducted from the top end of the specimen. The specimen was then cured for another 12 hours before the void was washed by providing 1.5 PV of distilled water to halt the precipitation process of $CaCO_3$. Note that during the infiltration of cementation solution, the flow rate decreased significantly for several specimens due to the reduction of the pore space by the production of $CaCO_3$ and/or biomass from microbial activities (Nemati et al., 2005; Rebata-Landa, 2007). In such cases, the pore pressure was further reduced from -5 kPa up to -20kPa while adjusting the cell pressure so that the confining pressure was kept at 30kPa. As a result, all infiltrations were completed within 2 hours.

In addition, during the retention periods the valves on the fluid passage leading to the pore space of the specimen were closed and air was not provided from an outside source to the specimen. However, air bubbles are usually present in the pore space of soil specimens unless specific efforts, such as replacing the pore air with CO_2 or applying the vacuum pressure, are made. It was thus assumed that the remaining air bubbles would provide enough oxygen for the bacteria. A high precipitation efficiency of $CaCO_3$was recorded for most of the specimens following the abovementioned treatment procedure, indicating the validity of the assumption.

Specimen Saturation, Consolidation and Undrained Cyclic Shear

After the precipitation process, the pore pressure was reduced to -100 kPa while the cell pressure was also decreased to -70 kPa. This condition was maintained for about 2 hours to ensure a good de-airing of the specimen. De-aired water was then imbibed (i.e. not circulated) into the vacuum void by connecting a de-aired water tank, in which the pressure was also reduced to -100 kPa, to the specimen and lifting the tank up such that approximately 1 m of hydraulic head was created between the specimen and the water level in the tank. Up to half an hour was taken to facilitate a complete saturation. The pore pressure and the cell pressure were then increased gradually until they reached 0 kPa and 30 kPa, respectively. The pressures were further raised to achieve a cell pressure of 230 kPa and a pore pressure (i.e. referred to as back pressure thereafter) of 200 kPa. Skempton's B-value was measured once at this point to evaluate the saturation level, and if the value was less than 0.95 the back pressure was increased to 300 kPa to diminish the remaining air bubbles. To start isotropic consolidation the cell pressure was increased to 260kPa while the back pressure was maintained at 200 kPa, creating a confining pressure of 60kPa. The consolidation was continued until the measured volume change became stable. Following the termination of consolidation Skempton's B-value was measured again. The specimen was then sheared cyclically under undrained condition with the loading frequency of 0.1 Hz. The specimen was deemed liquefied when the axial strain exceeded 5 % in double amplitude. The axial strain was measured with an external displacement transducer, and the axial load, excess pore water pressure and the cell pressure were monitored with a pressure compensated load cell placed inside the triaxial chamber, differential pressure transducer and a high-performance bourdon gauge, respectively. Estimation of Precipitated $CaCO_3$ Distribution

The treated specimen was cut into four to five pieces after it was detached from the testing apparatus. They were then dried at 105 ℃ for about 24 hours and their weights were measured before hydrochloric acid (0.5 M) was added to remove $CaCO_3$. Approximately 100 mL of hydrochloric acid was used for a single fragment which was around 60 g in dry weight. Each piece was then washed by tap water while making sure no soil particles were lost in the process. The pieces were dried again for about 24 hours and their weights were recorded. The gap in weight before and after the addition of hydrochloric acid was considered to be the quantity of precipitated $CaCO_3$. Note that the dissolution of soil particles by hydrochloric acid was evaluated and no change in weight was observed, ensuring the weight difference was solely due to the removal of $CaCO_3$.

Scanning Electron Microscopy

Tiny test pieces were taken from each of the precipitated specimens for Scanning Electron Microscopy (SEM). The fragments were oven-dried prior to their use in SEM and they were then placed on a metal setting and covered with a thin platinum paste, in order to facilitate the conduction of electricity. Two opposing points on the perimeter of the sample was also applied with silver paste for the same purpose. The sample was observed with an accelerating voltage of 20 kV. Energy Dispersive X-ray Spectrometer (EDS) was also utilized to identify elements constituting the cemented samples such as calcium and silica.

Results

Undrained Cyclic Shear Strength of the Specimen

All the test cases examined in the study are listed in Table 2 with details on their testing conditions. The letters ‘T’, ‘U’ and ‘DL’ mean the Toyoura sand specimen, Urayasu sand specimen, and the specimen of Toyoura sand and DL Clay mixture, respectively. The ‘Bio’ indicates the specimen was treated with microbial $CaCO_3$ precipitation, and the percentage behind the 'DL' shows the value of fines content. Only the results of selected test cases are provided in detail (i.e. stress-strain relationship and the stress path) and the rest of the cases are summarised in Fig. 3. In addition, although several treated specimens (i.e. T_Bio1, T_Bio2 and DL5%_Bio1) did not achieve a B-value greater than 0.95, which is a default standard in undrained cyclic shear test, a B-value of 0.94 would hardly render overly exaggerated liquefaction resistance and the presented results would thus remain valid within the typical error range of soil mechanics testing.

Table 2. Test conditions of each specimen in undrained cyclic triaxial test

Specimen	Relative Density, *Dr* (%)	Cyclic Stress Ratio, $\sigma'_d/2\sigma'_c$	Confining Stress, σ'_c(kPa)	Back Pressure (kPa)	B-value
T_Bio1	47	0.25, 0.50*	60	200	0.94
T_Bio2	46	0.75	60	300	0.94
T_1	45	0.30	60	300	0.97
T_2	48	0.15	60	200	0.99
U_Bio1	44	0.30	60	200	0.96
U_Bio2	45	0.50	60	200	0.96
U_1	49	0.15	60	200	0.97
U_2	48	0.30	60	200	0.96
DL5%_Bio1	38	0.30, 0.50*	60	300	0.94
DL5%_1	50	0.30	60	200	0.96
DL15%_Bio_1	49	0.30	60	200	0.95
DL15%_1	50	0.30	60	200	0.96
DL30%_Bio_1	55	0.30	60	200	0.96
DL30%_1	59	0.30	60	200	0.99**

*Cyclic stress ratio was raised to 0.5 after a negligible increase in excess pore water pressure with 0.3.
**Measured before consolidation.

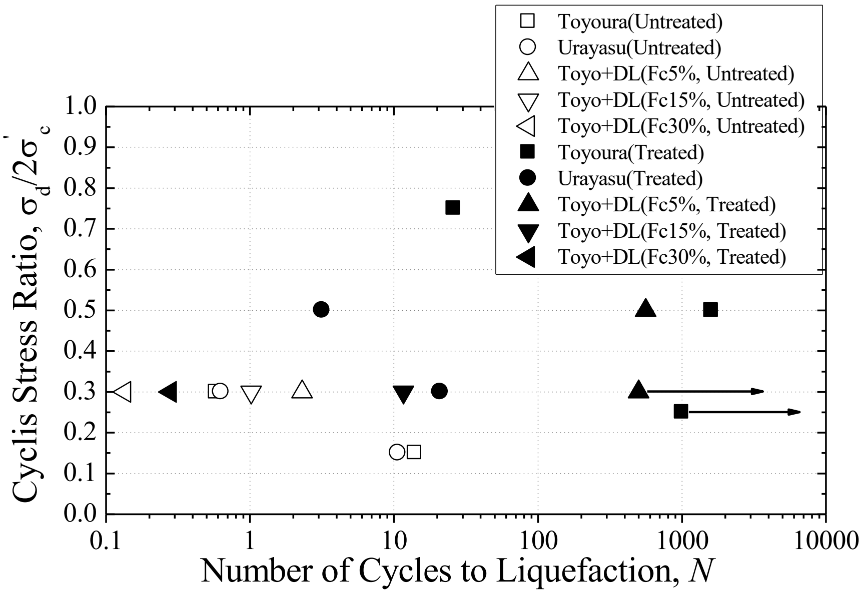
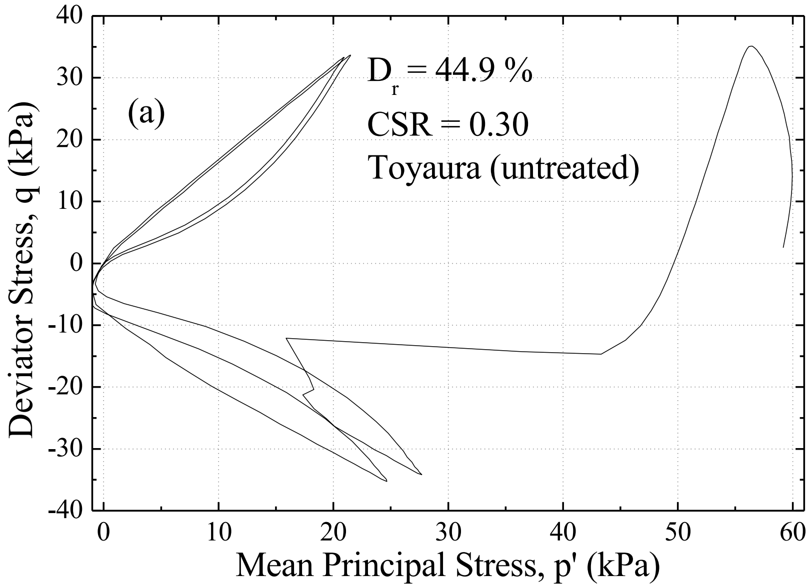


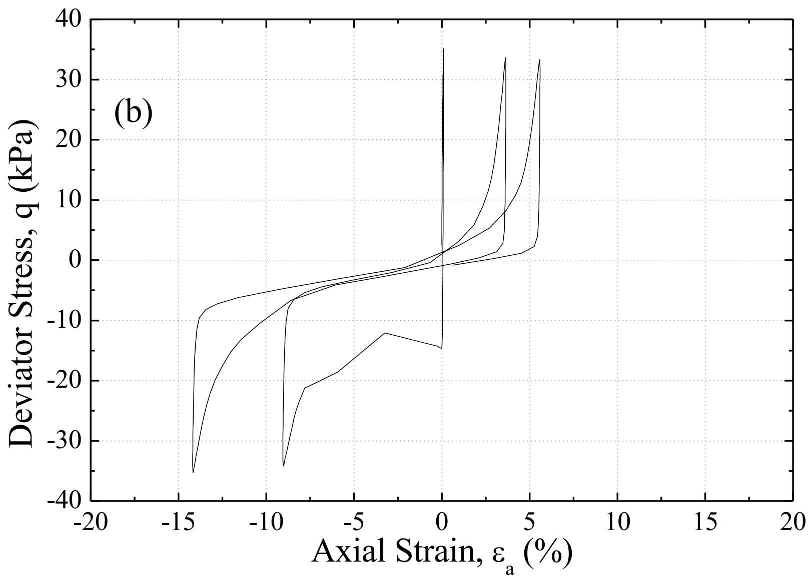
Fig. 3. Summary of all the test results in undrained cyclic triaxial test; the arrow indicates the test was terminated before liquefaction

Toyoura Sand Specimen

The untreated Toyoura sand specimen experienced liquefaction in about 0.6 cycles (Fig. 4). Axial strain developed suddenly from 0 % to around -9 % in the first cycle, and the effective stresses vanished. In contrast, the microbially improved specimen with a CSR of 0.25 (i.e. T_Bio1) did not liquefy in a thousand loading cycles. The specimen was thus subjected to a further loading with a raised CSR of 0.50 without carrying out consolidation, and the specimen did not liquefy until the 1600th cycle (Fig. 3). In order to liquefy a treated specimen with less loading cycles, a CSR of 0.75 was employed for another microbially cemented specimen (i.e. T_Bio2) and its test result is illustrated in Fig. 5. This time the specimen reached an axial strain of 5 % in double amplitude in about 26 cycles. The manner the axial strain developed was similar to the other treated specimen, with the strain growing only in the direction of extension, and the axial strain increment in a single loading cycle was very small. Also, substantial negative excess pore water pressure was generated (Fig. 5(a)), indicating a strong positive dilatancy of the treated Toyoura sand specimen.

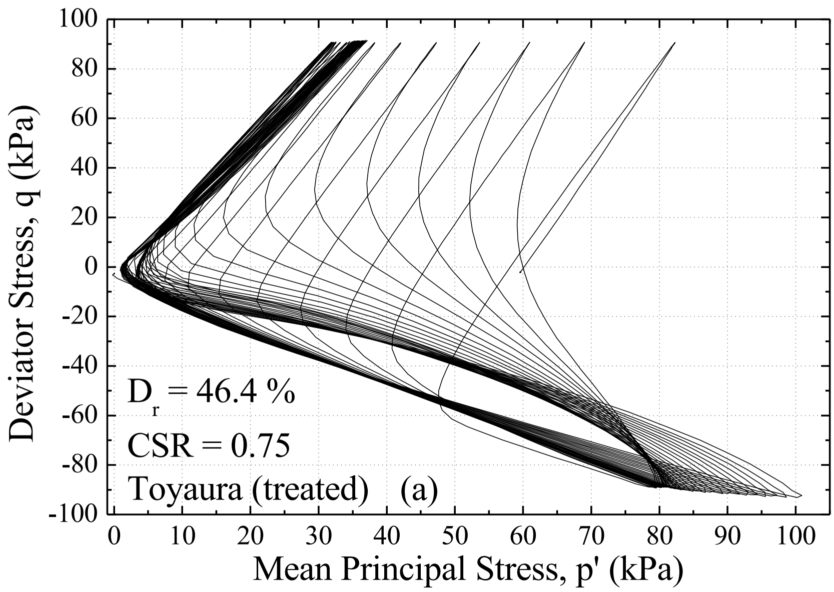


(a) Stress path

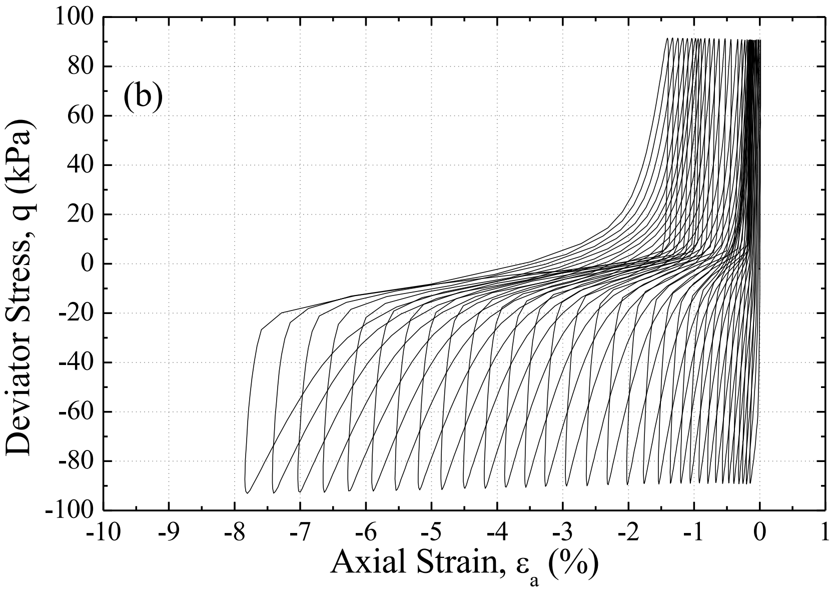


(b) Deviator stress-axial strain relationship

Fig. 4. Test result of untreated Toyoura sand specimen



(a) Stress path

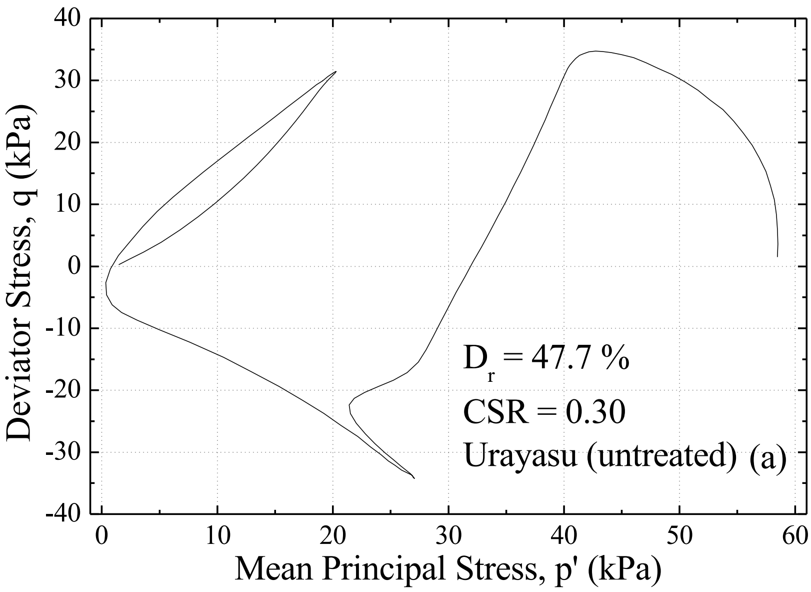


(b) Deviator stress-axial strain relationship

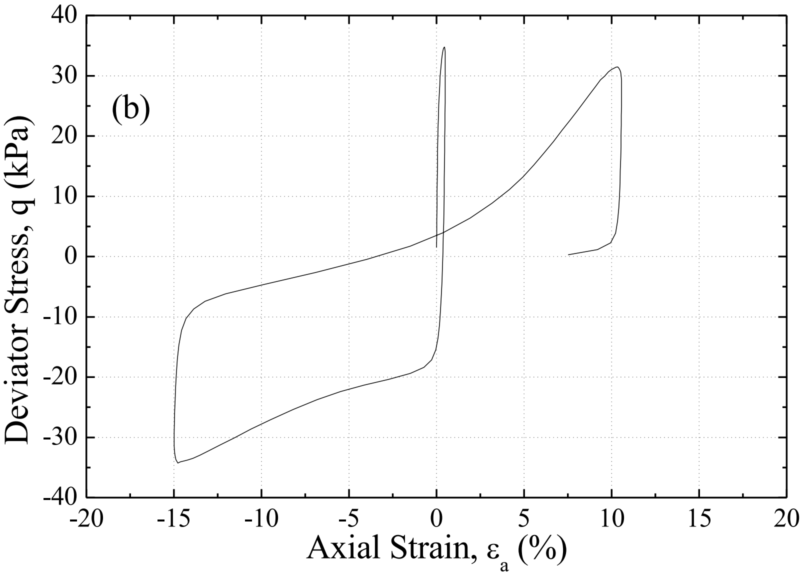
Fig. 5. Test result of microbially improved Toyoura sand specimen

Urayasu Sand Specimen

The test result of the untreated specimen is provided in Fig. 6. The untreated specimen liquefied very quickly in about 0.65 cycles with a CSR of 0.30.The microbially improved specimens, on the other hand, demonstrated a robust behaviour. Fig. 7 presents the case of CSR 0.3 and it shows that the specimen endured over 20 cycles before it liquefied. The axial strain increment in a single loading cycle was substantially smaller than that of the untreated specimen, resembling the undrained cyclic behaviour of a dense sandy specimen. However, when a CSR of 0.50 was applied, a microbially cemented Urayasu sand specimen liquefied in three cycles (Fig. 3) in a sharp contrast to the improved Toyoura sand specimen that endured over 1600 loading cycles with the same CSR.

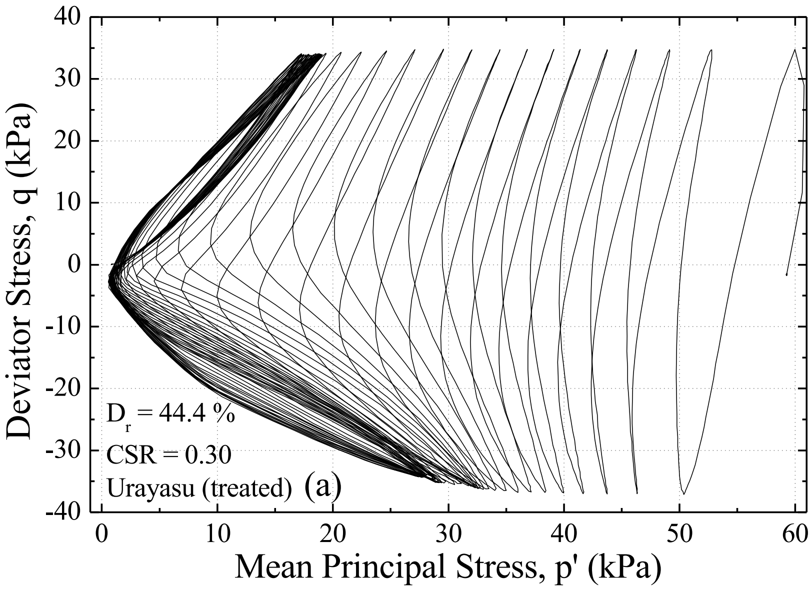


(a) Stress path

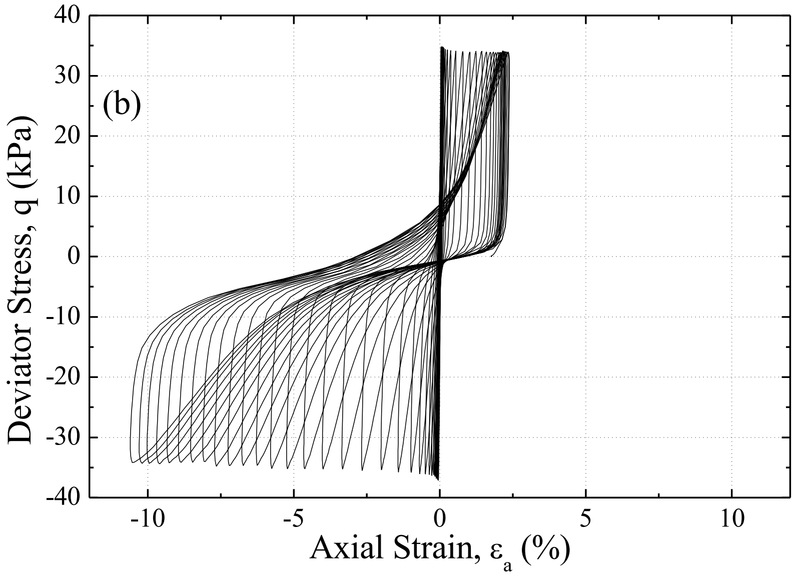


(b) Deviator stress-axial strain relationship

Fig. 6. Test result of untreated Urayasu sand specimen



(a) Stress path

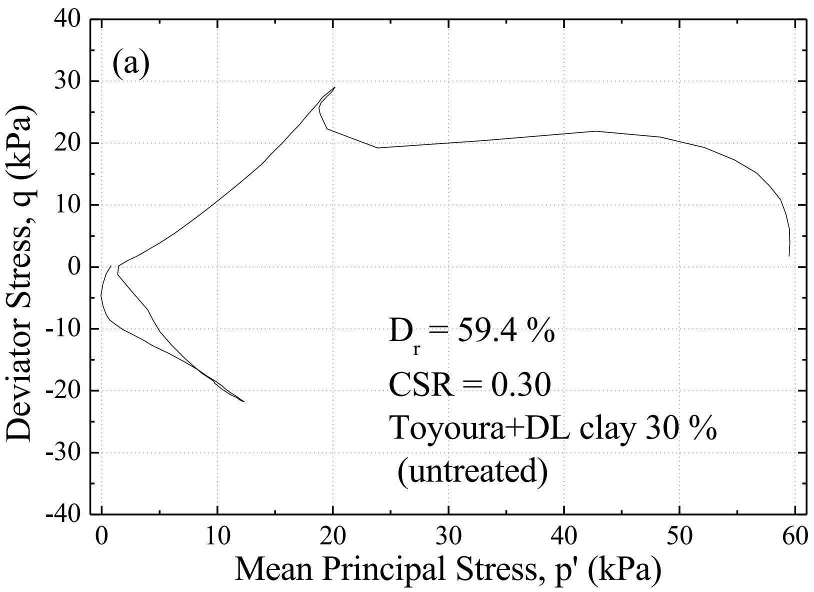


(b) Deviator stress-axial strain relationship

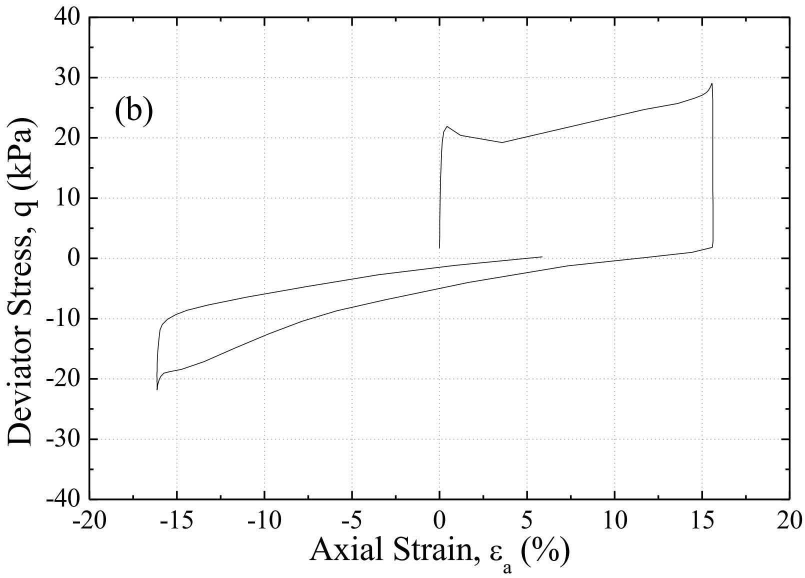
Fig. 7. Test result of microbially improved Urayasu sand specimen

Toyoura Sand and DL Clay Mixture

Only the result with 30 % fines content is presented; the summary of the remaining test cases for the material are provided in Fig. 3. The untreated specimen with 30 % of DL clay exhibited the most vulnerable behaviour against liquefaction, instantaneously developing a huge axial strain as soon as the loading was initiated (Fig. 8). As to the behaviour of the microbially cemented version, it experienced liquefaction in approximately 0.25 cycles (Fig. 9), showing no improvement in liquefaction resistance. The reason for this minimal improvement is discussed in later sections. At least the axial strain did not exceed the limit of the measurement (i.e. about 15 % for both directions) in half a cycle unlike the untreated specimen.

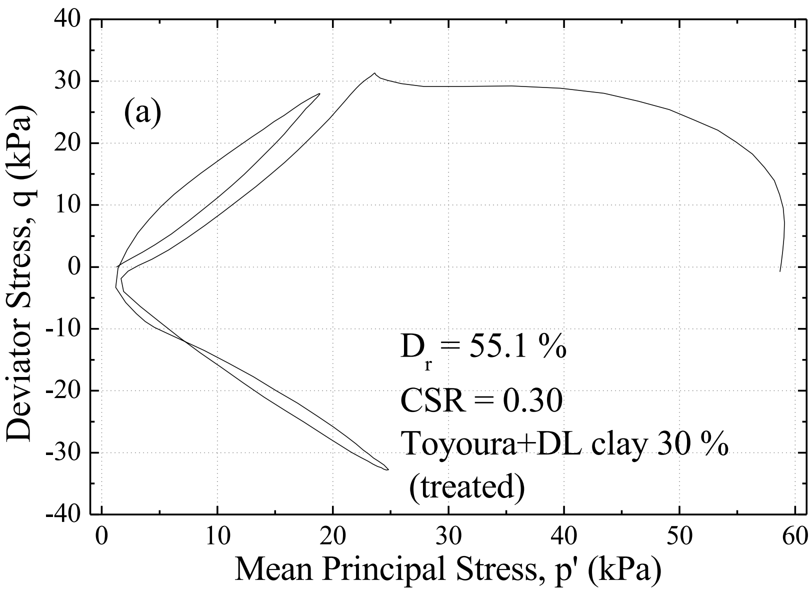


(a) Stress path

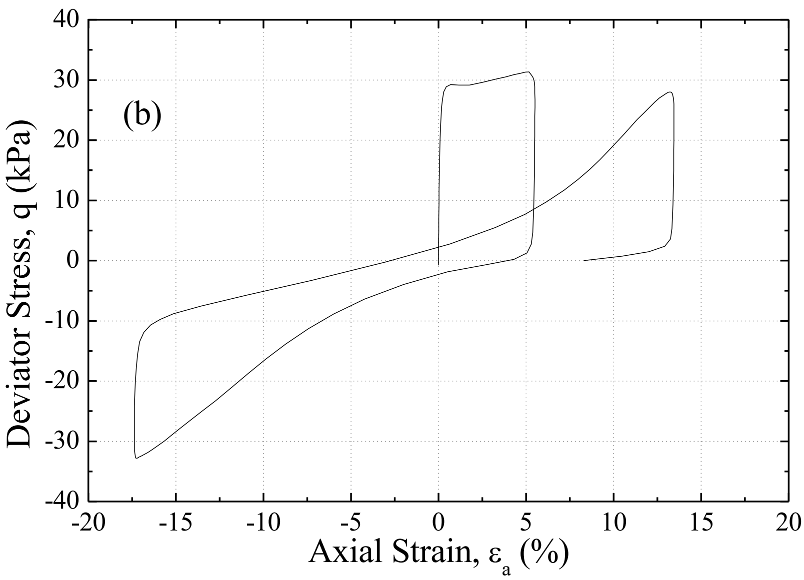


(b) Deviator stress-axial strain relationship

Fig. 8. Test result of untreated Toyoura sand-DL Clay mixture (F_c = 30 %)



(a) Stress path



(b) Deviator stress-axial strain relationship

Fig. 9. Test result of microbially improved Toyoura sand-DL Clay mixture (F_c = 30 %)

Distribution of Precipitated $CaCO_3$

The distribution of microbially precipitated $CaCO_3$ for each treated specimen is shown in Fig. 10. The horizontal scale indicates the proportion of precipitated $CaCO_3$ to the dry weight of the host soil. The results shown as Toyoura sand and Urayasu sand are those of T_Bio2 and U_Bio1, respectively. The most heavily precipitated part of the Toyoura sand specimen appeared at around 7 cm from the top of the specimen, with approximately 3 % of $CaCO_3$. Both top and bottom ends were cemented with the least amount of $CaCO_3$ that is almost half the peak value. As to the Urayasu sand specimen, the quantity of $CaCO_3$ peaked near the top of the specimen, with about 6 % of $CaCO_3$, and the remaining parts seem to have uniform amounts of $CaCO_3$. The results of Toyoura sand mixed with DL clay revealed a clear trend; the more fines the specimen contained the less $CaCO_3$ precipitation propagated. While the sand with a marginal amount of DL clay (i.e. F_c = 5 %) showed nearly uniform precipitation, the one with 15 % fines content had $CaCO_3$ concentrated at the proximity of both ends and less $CaCO_3$ was obtained around the middle. The specimen with the highest amount of DL clay (i.e. F_c = 30 %) intensified this trend by showing a negligible quantity of precipitated $CaCO_3$ at the locations positioned 3-7 cm from the top of the specimen. By and large, clean sand tends to have more uniform distribution of $CaCO_3$, with the peak value often found near the first injection point of bacteria (Martinez et al., 2011; Martinez et al., 2013; Mortensen et al., 2011; Whiffin et al., 2007).

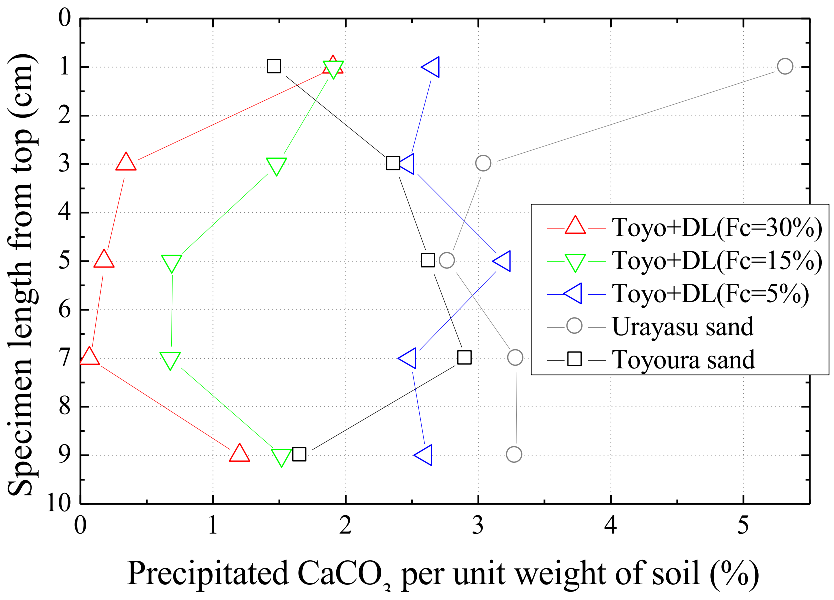
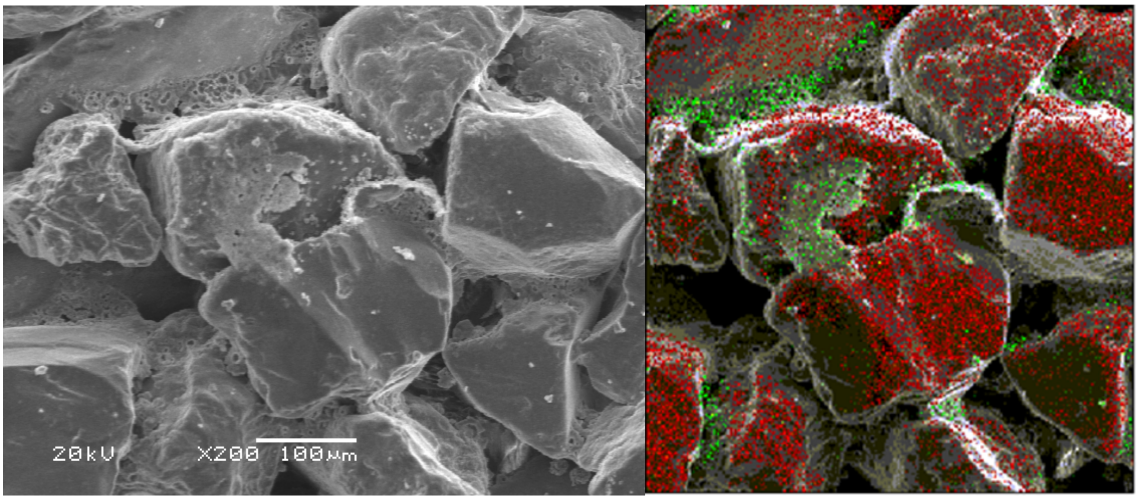


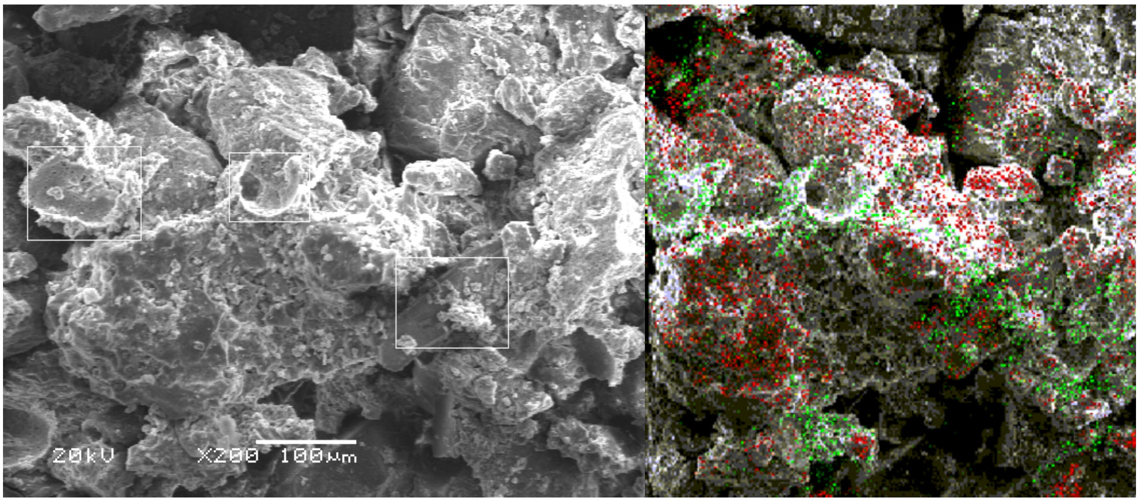
Fig. 10. Distribution of precipitated $CaCO_3$ for each material

SEM and EDS Analysis

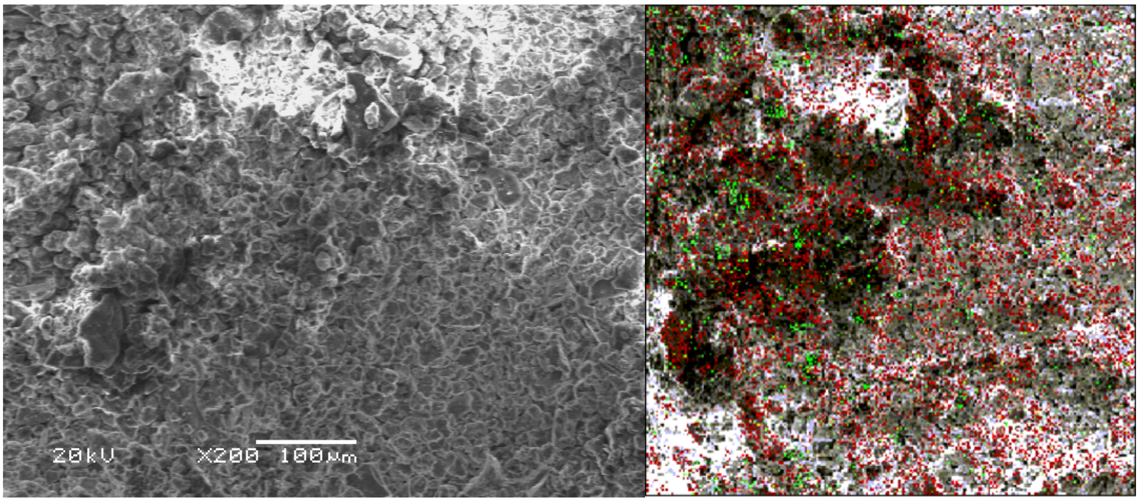
SEM images of the treated specimen for each material are provided in Fig. 11. The one shown as the mixture of Toyoura sand and DL Clay is the one with 30 % fines content. The Toyoura sand specimen, which demonstrated the greatest improvement in liquefaction resistance, seems to be cemented in a very efficient way, with $CaCO_3$ precipitated exactly at the particle-particle contact (Fig. 11(a)). For the treated Urayasu sand specimen, $CaCO_3$ was not as conspicuous as those found in the Toyoura sand specimen. The size of $CaCO_3$ appeared to be smaller than the ones captured in the Toyoura sand specimen, and the location of precipitated $CaCO_3$ was not necessarily at the particle contact, indicating inefficient cementation due to the presence of fines (Fig. 11(b)).When it comes to the mixture with 30 % fines content, few $CaCO_3$ crystals as large as those found in the other specimens were detected by EDS analysis (Fig. 11(c)), supporting the result of undrained cyclic shear test in which the specimen showed no improvement in liquefaction resistance. Note that although the Urayasu sand specimen and the DL Clay mixture had a similar level of fines content, Urayasu sand had greater void ratio than the mixture. The samples shown in the pictures were taken from the most heavily precipitated part of the specimen of each material.



(a) Toyoura sand with precipitated $CaCO_3$



(b) Uraysu sand with precipitated $CaCO_3$



(b) Toyoura sand-DL Clay mixure (F_c = 30 %) with precipitated $CaCO_3$

Fig. 11. SEM images with EDS analysis results; each pair shows the same area with the part coloured in green in the right image indicating the presence of $CaCO_3$

Discussion

Liquefaction Resistance of Microbially Improved Sand with Fines Content

Comparing the test results of the Toyoura sand and Urayasu sand specimens, the resistance of the former was enhanced much greater than the latter. This outcome was obtained despite the fact that Urayasu sand was precipitated with greater amount of $CaCO_3$ per unit soil weight (Fig. 10) and the resistance of both types of the specimens before the treatment was quite similar. The result thus indicates that the inclusion of non-plastic fines in the soil skeleton of sand might impair the degree of improvement, as smaller $CaCO_3$ was found in the Urayasu sand specimen at locations which are not particle contact points (Fig. 11). Al Qabany and Soga (2013) demonstrated that sand precipitated with uniformly distributed $CaCO_3$ at the particle contact had greater unconfined compression strength compared to the ones in which $CaCO_3$ was produced non-uniformly at arbitral locations on the sand grain. Thus, the poor distribution of $CaCO_3$ in the Urayasu sand specimen might have led to the inhibited increase in liquefaction resistance. A potential cause of the ineffective $CaCO_3$ precipitation would be the presence of iron oxides, which was inferred from observations where a small portion of Urayasu sand grains were attached to a magnet. Iron oxides are positively charged in the soil skeleton under common soil pH levels, enhancing the attachment of bacteria, and iron oxides tend to be concentrated at fine-grained areas of soil (Ginn et al., 2002). Hence, iron oxides in the fines of Urayasu sand might have preferentially induced $CaCO_3$ precipitation at locations ineffective to increase soil strength. Nevertheless, more reliable testing than sticking a magnet is necessary to confirm that the grains attached to the magnet were iron oxides, in order to validate the hypothesis.

Another mechanism with which effective MICP has been prevented in sand with fines seems to be clogging of bacteria. In other words, if the void ratio is reduced below a certain level due to the presence of fines content, clogging of bacteria might occur, leading to non-uniform precipitation of $CaCO_3$ or even no improvement at all of the target soil (Fig. 10). Careful considerations of soil grain properties would thus be necessary to ensure the mobility of bacteria through the pore space (Rebata-Landa, 2007). Direct use of urease enzymes, which are much smaller than the microbe, has the potential to resolve this problem (Yasuhara et al., 2012).

Montoya et al. (2013) employed Ottawa 50-70 sand (D_{50} = 0.22, e_{min} = 0.55, e_{max} = 0.87, D_r = 40 %) and microbially precipitated the sand with 3% $CaCO_3$. Their results of cyclic direct simple shear test indicated that the improved Ottawa sand could endure well beyond a thousand loading cycles when CSR was 0.30, which is significantly higher than the number of cycles the improved Urayasu sand specimen withstood. Although the definition of the onset of liquefaction in their study differed from the present study's definition (i.e. 3 % strain was used in their study as opposed to 5 % in this study) and there might be differences involved in the choice of the test apparatus, their result still strongly supports the trend obtained in the present study that the addition of fines content in sand hinders the increase in liquefaction resistance. Burbank et al. (2013) retrieved natural soil samples from the shore of a river, and carried out undrained cyclic triaxial shear test to the samples that were microbially precipitated with $CaCO_3$. Their result showed that the improved sample took 10 cycles of loading to liquefaction under a CSR of 0.23 when the relative density of the soil was around 35 % and the amount of produced $CaCO_3$ was somewhere between 2.2-2.6 %. Although they did not clarify the presence of fines in the sample, it is strongly suspected that fines were included more or less since it was taken from a natural setting. As such, their result appears to be comparable to that of the Urayasu sand specimen in the present study. In fact the degree of improvement was equivalent to each other under similar relative density and similar quantity of precipitated $CaCO_3$. Therefore, there seems to be a tendency that the inclusion of fines content in soils hinders the improvement in liquefaction resistance by the microbial precipitation of $CaCO_3$.

It should be acknowledged, however, that limited types of materials were tested in the present study and little external research was available to validate the assumption. Also, $CaCO_3$ bond might have been degraded to some extent during the consolidation of the specimens. Although $CaCO_3$ precipitated in clean sand would not be degraded easily by an increase in confining pressure (Montoya et al., 2013; Feng and Montoya, 2015; Lin et al., 2015), that might not be the case for sand with fines. Monitoring of shear wave velocity during consolidation would have ensured the state of $CaCO_3$ bond before shearing began. Nevertheless, if degradation of $CaCO_3$ during consolidation did occur it would indicate weak cementation of $CaCO_3$ in the sand with fines relative to $CaCO_3$ bond in clean sand, supporting the idea that fines would inhibit increase of strength of MICP-treated sand. The hindered improvement for sand with fines indicates that greater quantity and/or repeated injections of cementation solution (i.e. urea and calcium) would be required for such soils to achieve improved liquefaction resistance comparable to that of clean sands.

Morphology of Precipitated $CaCO_3$

The $CaCO_3$ precipitated in Urayasu sand specimens might have been vaterite, which is less stable form of $CaCO_3$ relative to calcite (Turnbull, 1973), and fines contained in Urayasu sand might have caused it. In fact, $CaCO_3$ formed in residual soil via MICP possessed somewhat disparate morphology compared to calcite generated in clean sand (Ng et al., 2014). Coal minerals such as silica in the colloidal form or iron pyrites could affect the crystallization process of $CaCO_3$ to create vaterite (Brunson and Chaback, 1979). In addition, Rodriguez-Navarro et al. (2007) provided examples of microbially produced vaterite and calcite crystals and demonstrated that vaterite, instead of calcite, could be formed and stabilized in the presence of organics including ones produced by microbial activities. Organic compounds such as glycine and serine could also lead to the formation of vaterite (Kitano and Hood, 1965). Moreover, Sondi and Salopek-Sondi (2005) demonstrated that the urease of *S. pasteurii* tends to produce vaterite rather than calcite when it is used alone. Hence, some minerals contained in Urayasu sand and/or organics in the microbial and cementation solutions as well as those produced by bacteria might have stabilised $CaCO_3$ as vaterite, and it might have led to the weak improvement of undrained cyclic shear resistance of Urayasu sand owing to the instability of vaterite.

However, presence of vaterite in MICP-treated Urayasu sand was not confirmed through reliable testing such as powder X-ray diffraction. Thus, the morphology of $CaCO_3$ precipitated in Urayasu sand remains uncertain. Even if it was vaterite, it may not be because of fines in Urayasu sand as vaterite could also be produced in clean sand as a result of MICP (Lin et al., 2015). Also, calcite alone has different morphologies depending on the concentration of CO_2 around the crystal (Cizer et al., 2008), and effects of $CaCO_3$ morphology on improvement of strength of MICP-treated soils are not well known. Therefore, further research is required to clarify the effect of minerals constituting fines in soils on the morphology of $CaCO_3$ precipitated through MICP, and the effect of $CaCO_3$ morphology on strength improvement of MICP-treated soils, in order to substantiate the hypothesis.

Conclusions

Sand with non-plastic fines content is quite vulnerable to liquefaction regardless of the amount of the contained fines and is more analogous than clean sand to the soil found in natural settings. The soil improvement of such soil was attempted in this study by employing *S. pasteurii*, which is a bacterial species possessing urease enzymes, and Toyoura sand (F_c = 0 %), Urayasu sand (F_c = 32.4 %) and the mixture of Toyoura sand and DL Clay (F_c = 5, 15, 30 %) as the test material, in order to reveal the effectiveness of the microbial treatment to sand containing fines with particular emphasis on liquefaction resistance. The results of undrained cyclic triaxial test showed that Urayasu sand, which is the product of actual liquefaction in the 2011 Great East Japan Earthquake, could be improved to the point where a loading of CSR equalling 0.3 took 21 cycles to achieve an axial deformation of 5 % in double amplitude. However, the result of the Toyoura sand and DL Clay mixture with a comparable amount of fines (i.e. 30 %) gained no increase in liquefaction resistance by the microbial precipitation of $CaCO_3$. This was because the void ratio of the mixture was much smaller than that of Urayasu sand at a similar relative density, causing the clogging of the bacteria near the areas where bacteria were injected. In addition, the Toyoura sand specimen treated with microbial $CaCO_3$ precipitation recorded a massive increase in liquefaction resistance, becoming almost unliquefiable with a loading of CSR up to 0.50; it liquefied in 26 cycles of loading when CSR was raised to 0.75.

This indicates that the inclusion of fines content in sand impairs the degree of the improvement by microbial precipitation of $CaCO_3$. Apart form the clogging of bacteria, the main cause of the hindered improvement for sand with fines seems to be fines curtailing efficient precipitation of $CaCO_3$ at particle-particle contacts, thereby generating $CaCO_3$ at locations where no structural reinforcement of the soil is expected. Morphology of $CaCO_3$ might also have been affected by the presence of fines content. The findings of the present research point toward the necessity of a greater amount of urea and calcium among other chemicals to solidify sand with non-plastic fines content to a comparable level of liquefaction resistance of the clean sand improved in the same way. Also, direct use of urease enzymes might help resolve the clogging issue.

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