Influence of bacterial activities on cone tip resistance and liquefaction susceptibility of sand

Debnath Mondal
Indian Institute of Technology, Kharagpur, India, debnathmondal92@iitkgp.ac.in

Debasis Roy
Indian Institute of Technology, Kharagpur, India, debasis@civil.iitkgp.ernet.in

Samir Saurav
Indian Institute of Technology (ISM), Dhanbad, India, samsauvik@gmail.com

ABSTRACT: The impacts of exo-cellular metabolic products (EPS) and minerals produced by an endemic species of soil bacteria on cone penetration resistance and liquefaction susceptibility have been investigated. For this, a series of miniature cone penetration tests and shake table tests performed in the laboratory on loose, water pluviated sand samples with live bacterial populations of Lysinibacillus sp. DRG3 minimally sustained to trigger one of three distinct metabolic processes. Comparison of these measurements against those obtained from testing similar but bacteria-free sand samples indicated a 4- to 6-fold increase in the cone tip resistance. Correspondingly, the factors of safety against liquefaction increased by between 7% and 9%, and the pore water pressure ratio decreased by between 49% and 70%. The pattern of these changes was also found to be in agreement with the size of bacterial populations.

Keywords: cone penetration, biological activity, shake table, liquefaction

1. Introduction

Pore water pressure rise due to earthquakes within loose sands and silts results in a significant loss of shear strength and stiffness of such deposits. The loss could be so significant that the soil may start behaving like a viscous liquid. Such a phenomenon is called liquefaction. Metabolic activities of soil bacteria that produce exo-cellular metabolic substances (EPS) and bio-minerals impart intergranular aggregation. Gases produced by the microbes, on the other hand, renders pore fluid compressible. As a result, the loss of shear strength and stiffness is expected to become less significant for sands hosting a live population of soil microbes.

The impacts of the exo-cellular metabolic product (EPS) and minerals and biogas produced by an endemic species of soil bacteria hosted within laboratory sand samples on cone penetration resistance and liquefaction susceptibility have been investigated as described in this paper. The soil bacteria Lysinibacillus sp. DRG3 was minimally sustained within the sand mass so as to trigger one of three distinct metabolic processes. A series of miniature cone penetration tests and shake table tests were performed in the laboratory on loose saturated bacteria-free control sand samples and with live bacterial populations. Cone tip resistance and pore water pressure developments observed in these tests were compared with the corresponding observations from tests on similar sand samples without any bacterial populations.

2. Literature Review

Biological processes have shown to improve soil strength, stiffness, permeability, and liquefaction resistance, [1, 2] although most of these investigations used non-endemic, calcite-producing microbes sustained with elevated nutrient doses.

Microbes sustained with an elevated nutrient dose within soil samples produce an EPS matrix by cementing the soil minerals with carbohydrates, proteins, lipids, nucleic acids, and calcite ($CaCO_3$), struvite ($NH_4MgPO_4.6H_2O$) minerals [3]. The biogenic gases (CO_2, CH_4, N_2) produces from that microbial process affects the compressibility of pore fluid [3-6].

To investigate the impacts of these bio-minerals and biofilm structures on soil stabilization, miniature cone penetration testing setup was developed by modifying the existing tri-axial machine in soil mechanics and foundation engineering laboratory, IIT Kharagpur. There are no studies reported such activities on microbial cementation. Although several previous studies were present, the development and application of miniature cone penetration tests in geotechnical engineering [7].

3. Material and Methods

3.1. Sand

Sub angular siliceous sand (97% Quartz, ~3% feldspar) washed through HCl (0.25N), NaOH (0.25N) successively, and neutralized by distilled water was used for this study summarized in Table 1.

Table 1. Properties of laboratory sand [8]

USCS Classification	SP
Median particle size, D_{50} (mm)	0.55
Uniformity coefficient, C_u	1.99
Coefficient of curvature, C_c	0.66
Specific gravity of soils, G_s	2.65
Maximum void ratio, e_{max}	0.83
Minimum void ratio, e_{max}	0.54

The washed sand particles were dried at 105°C and sterilizing by autoclaving at 121°C with 100 kPa for 15 minutes to avoid the contamination from aerobic micro-organisms.

3.2. Microorganism and Metabolic Pathways

An endemic species of soil bacteria Lysinibacillus sp. abbreviated as DRG3 was used to precipitate calcite and EPS within sand models for three distinct, minimally-sustained metabolic processes summarizes in Table 2. Based upon the microbial growth characteristics of DRG3, a bacterial strain rate of 6 g/L selected to hosting live bacterial population within the soil model under elevated nutrient medium.

Table 2. Metabolic pathways for DRG3 bacterial strain

Metabolic Pathways	Medium Characteristics	Microbial Products
EPS	No $CaCl_2$ and urea	EPS
Non-ureolytic	$CaCl_2$ (Ca^{2+})	EPS and Calcite ($CaCO_3$)
Ureolytic	Urea and $CaCl_2$	EPS and Calcite ($CaCO_3$)

3.3. Microbial Growth Medium

DRG3 biomass was produced in the laboratory from corn steep liquor (CSL)-DRG3 incubated culture by centrifuging at 7000 rpm for 10 min at 4°C. The bacterial growth media within the sand matrix composed of nutritional minerals: glucose (20 g/L), NH_4Cl (3 g/L), $MgSO_4$ (0.6 g/L), NaCl (0.14 g/L), $ZnSO_4$ (2.3 mg/L), $MnSO_4$ (17 mg/L), $CuSO_4$ (10 mg/L), Na_2MoO_4 (4 mg/L), EDTA (10 mg/L), $NiCl_2$ (0.4 mg/L), NaI (6.6 mg/L), KH_2PO_4 (0.14 g/L), K_2HPO_4 (2.2 g/L); reactive minerals: $CaCl_2$ (0.1 g/L) and $CO(NH_2)_2$ (3 g/L) based upon the medium characteristics of metabolic pathways of microbe used for this research [3].

4. Miniature cone penetration tests

The miniature cone penetration setup developed in the laboratory by adopting an existing triaxial loading frame that could accommodate specimens within a small, fixed-wall chamber of 200 mm in diameter and 190 mm in height, as shown in Fig. 1. A miniature cone penetrometer with 2.75 mm diameter driven into the soil model at a rate of 3 mm/min.

The entire set up modeled to a small scale where the inverted weighing machine of 7kg load capacity mounted on triaxial frame act as a load cell to measure the tip resistance of a hand sewing needle penetrometer of 2.75 mm diameter. A cylindrical steel canister of 200 mm inner base diameter and 210 mm hight used to prepare the sample.

A bit of 3.66 mm diameter was attached behind the tip of needle penetrometer at a distance of 4.75 times of needle diameter from cone tip to neglect the sleeve friction on the measured tip resistance. The ratio of bit diameter to needle diameter was limited to 1.25:1.

The penetrometer to chamber diameter ratio kept about 55 to minimize the chamber fixed wall boundary effects.

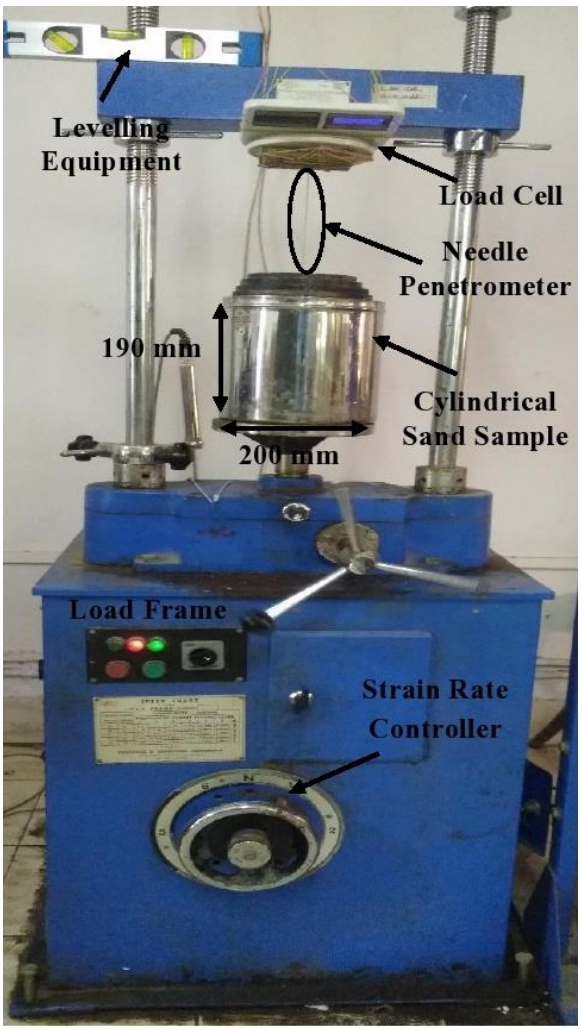

Figure 1. Miniature CPT experimental setup within a triaxial frame

4.1. Sample Preparation

The cylindrical sand samples were prepared within a sterilized steel canister. Sterilized sand of about 9.4 kg was pluviated under 3 L elevated nutrient media within the chamber. The post incubation relative density of 40±2% under a suitable surcharge maintained through adjusting the height and rate of pluviation. For bacteria-inoculated samples, an additional 18 gm centrifuge biomass mixed with this media to hosting live bacterial population. The entire operations were carried out within the laminar airflow chamber to avoid aerobic microbial contamination.

The samples were incubated at 35°C prior to cone testing. It was observed from cyclic triaxial (are not presented in this paper) and shake table study that the bacterial population (CFU) attains a peak value at 36 hours incubation period and getting constant at 72 hours irrespective of the pore volume of the sample. Consequently, the pH of the pore fluid decreases smoothly after 12 hours incubation period. Therefore all the assigned tests were performed on 36 hours incubation period so that the maximum cementation level raises within the sample. The overlying surcharge media on the sand models are ensuring the fully saturated condition, and it removed before the experiment.

4.2. Laboratory Test Programs

The number of laboratory test programs on loose sand samples was distributed primarily based upon the metabolic pathways and the repeatability of the individual program listed in Table 3.

Table 3. Laboratory testing programs

Metabolic Pathways	Miniature CPT	Shake Table Test
Bacteria-free Control sample	2	2
EPS sample	2	2
EPS-NU Sample	2	2
EPS-U Sample	2	2

4.3. Interpretation of cone tip resistance

The samples tested at a uniform strain rate of 0.05 mm/sec, and the load recorded at every 5 sec, i.e., at 0.25 mm depth intervals, which indicate the resistance at cone tip. The resistance profiles were drawn up to 150 mm depth of the soil sample.

As the cone tip resistance (q_c) increases with effective vertical stress, this shallow sounding requires to normalize for effective overburden stresses, which convert the CPT profile to a dimensionless resistance quantity expressed in Eq. (1) [9].

$$q_{c1N} = C_Q \left(\frac{q_c}{P_a}\right) \tag{1}$$

Where C_Q signifies normalizing factor for cone penetration resistance as in Eq. (2).

$$C_Q = \left(\frac{P_a}{\sigma'_{vo}}\right)^n \leq 1.7 \tag{2}$$

Where P_a is the atmospheric pressure = 100 kPa; σ'_{vo} is the effective vertical stress; n is the stress exponent = 0.5 for sand.

5. Liquefaction triggering analysis

In the present investigation, the liquefaction resistance measured from the normalized cone tip resistance based on the CPT-based liquefaction triggering correlation for clean sand at an earthquake magnitude of 7.5 proposed by Robertson and Wride [10].

The liquefaction resistance characterized in terms of normalized penetration resistance. For saturated sandy soils, these approximated by a clean sand base curve and expressed with following Eq. (3) and Eq. (4) [10]:

If $(q_{c1N})_{cs} < 50$

$$CRR_{7.5} = 0.833\left[\frac{(q_{c1N})_{cs}}{1000}\right] + 0.05 \tag{3}$$

If $50 \leq (q_{c1N})_{cs} < 160$

$$CRR_{7.5} = 93\left[\frac{(q_{c1N})_{cs}}{1000}\right]^3 + 0.058 \tag{4}$$

Where $(q_{c1N})_{cs}$ indicated normalized cone penetration resistance for clean-sand.

5.1. Estimation of CSR by simplified method

The equivalent cyclic shear stress induces within the soil model primarily depends on the maximum base acceleration (a_{max}) imposed within the sample. The cyclic stress ratio estimated from the simplified method describes by following Eq. (5) [9].

$$CSR = \left(\frac{\tau_{av}}{\sigma'_{vo}}\right) = 0.65\left(\frac{a_{max}}{g}\right)\left(\frac{\sigma_{vo}}{\sigma'_{vo}}\right) r_d \tag{5}$$

Where g is the gravitational acceleration taken as 9.81 m/s^2, $\sigma_{vo,}$ and σ'_{vo} signify total and effective vertical stresses, respectively, and r_d denotes the stress reduction factor.

Generally, for the present small-scaled model study, the average values of r_d were estimated at a depth z (in m) below the model surface using the following recommendation as in Eq. (6) and Eq. (7).

$$r_d = 1.0 - 0.00765z \; for \; z \leq 9.15m \tag{6}$$

$$r_d = 1.174 - 0.0267z \; for \; 9.15m < z \leq 23m \tag{7}$$

5.2. Factor of safety against liquefaction

For the present sample with low overburden stress and level model surface, the factor of safety against liquefaction (FS_L) is evaluating by Eq. (8) [9].

$$FS_L = \left(\frac{CRR_{7.5}}{CSR}\right) \times MSF \tag{8}$$

Where, MSF signify the magnitude scaling factor, estimated from revised Idriss scaling factors to specific earthquake magnitude, M_w expressed in Eq. (9).

$$MSF = \left(\frac{M_W}{7.5}\right)^{-3.3} \tag{9}$$

6. Shake table test

In the present study, the cubed soil samples vibrated along with two horizontal directions against the specific scaled ground motion to simulating earthquake shaking under the gravitational field. The specimen to platform mass ratio significantly affects the platform motion [11]. Thus the soil model was designed based upon the performance of the shake table under input strong ground motion, which may be affected by the following parameters listed in Table 4.

Table 4. Specification of shake table at IIT Kharagpur

Parameters	Configurations
Table dimension	1m x 1m
Maximum specimen weight	500 kg
Mass of the platform	700 kg
Maximum specimen to platform mass ratio	0.7143
Controlled degree of freedom	6
Translational	X, Y, Z
Rotational	θ_X, θ_Y, θ_Z
Maximum actuator stroke	500 mm
Maximum table acceleration	± 2g to ±5g
Test frequency	50 Hz
Minimum excitation power requirement	3 phase, 410 V

6.1. Design of soil models

The cubed soil samples, as specified in Table 5. were prepared within a watertight polymethyl methacrylate box of 20 mm thick, 560 mm x 560 mm internal base dimension, and 800 mm in height. The model of 300 mm x 300 mm in plan and 400 mm in height was developed with energy-absorbing vertical boundary conditions within the model box mounted on the platform, as shown in Fig. 3. Dry, sterilized sand of about 55.7 kg was pluviated on 15 L nutrient medium within an HDPE membrane. The height and rate of pluviation were adjusted accordingly to achieve a relative density of about 40±2% after incubation at 35^{o}C with a surcharge of 1.5 kg/cm^{2} at the top of the sample (Fig. 2.). For the bacteria inoculated sample, an additional 90 gm centrifuge biomass mixed with nutrient media for microbial activities within the sample. The overlying media was removed before the testing, keeping the sample in a submerged condition.

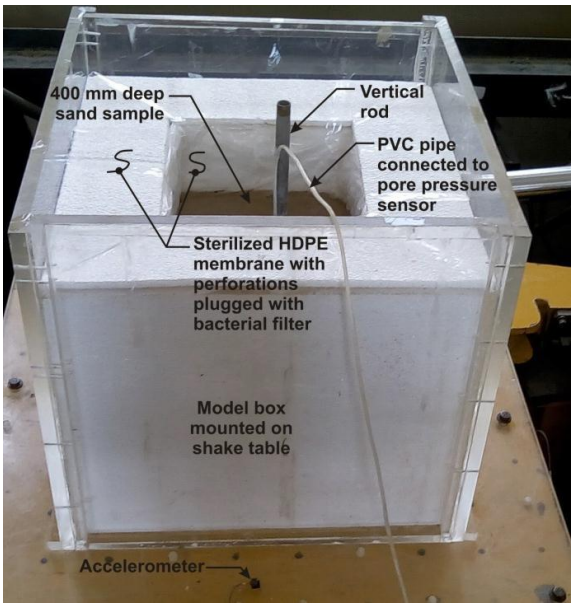

Figure 2. Soil model on the 6-DoF shake table

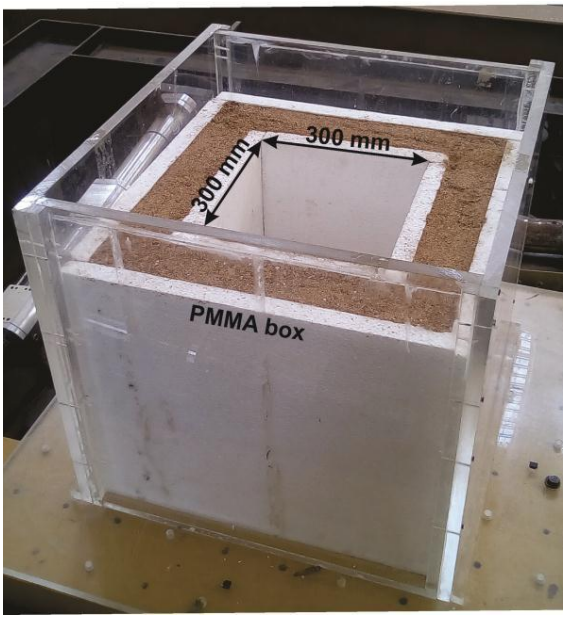

Figure 3. The Energy-absorbing vertical boundary condition

The outlet of the pore pressure sensor placed in the middle of the sample through a vertical rod placed at the center of the box fixed with the PMMA box using a horizontal mild steel plate (Fig. 4).

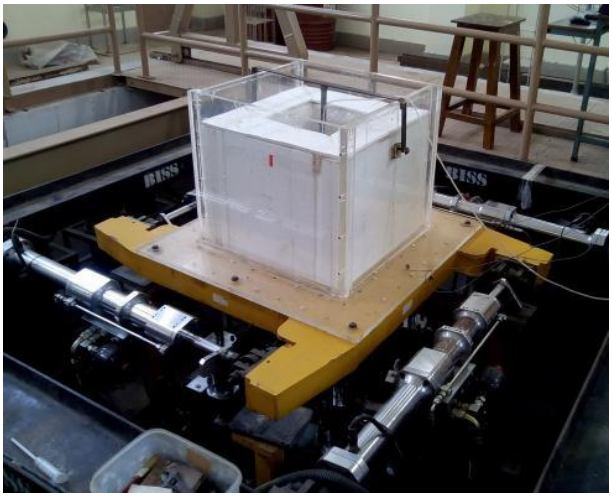

Figure 4. Clamped horizontal mild steel plate attaching vertical rod with model base for pore water pressure measurement

Table 5. Specification of shake table soil model

Dimension (LxBxH)	30cm x 30cm x 40cm
Soil mass	55.7 kg
Sample density	1.5461 gm/cc
Void ratio	0.796
Relative density	40±2%
Payload	175 kg
Specimen to platform mass ratio	0.25

6.2. Bacterial Growth Strategy

Bacterial population size is an important influencing factor for microbial cementation within the soil skeleton. It leads to cementing action on the liquefiable sand matrix by EPS, calcite with biogenic CO_2 production. This activity changed with the bacterial growth strategy over the entire incubation period. Although, the shake table tests of the present research carried out on 36 hours incubated sample indicated the high microbial load level, estimated by the colony-forming unit (CFU), as shown in Fig. 5.

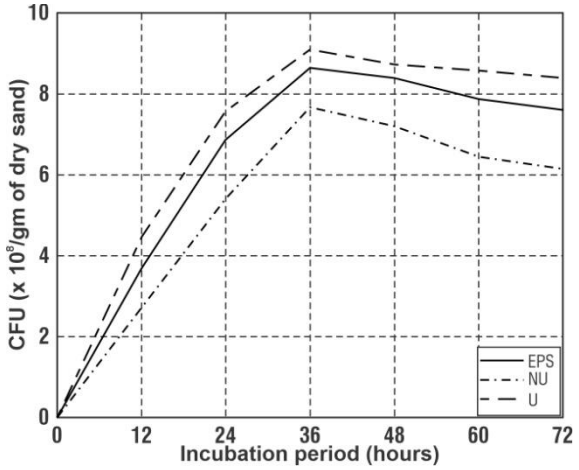

Figure 5. DRG3 population growth within bacteria-inoculated samples at three metabolic pathways.

The colony-forming unit (CFU) estimated by serial dilution spread plate technique [12] and expressed corresponding to the dry weight of the sample. The supernatant of bacteria-inoculated pore fluid collected at 12 hours intervals during incubation was serially diluted to 10^{-16} and 0.1 ml of each diluted solution transfer to the solid nutrient agar media plates and incubated at 35^{o}C for 12 hours. CFU determined from the bacterial colonies on the plate of the corresponding diluted solution that appears within the range of 30 to 300.

Dissolved CO_2 makes the microbial solution acidic (as shown in Fig. 6.) and affects the compressibility of the pore fluid.

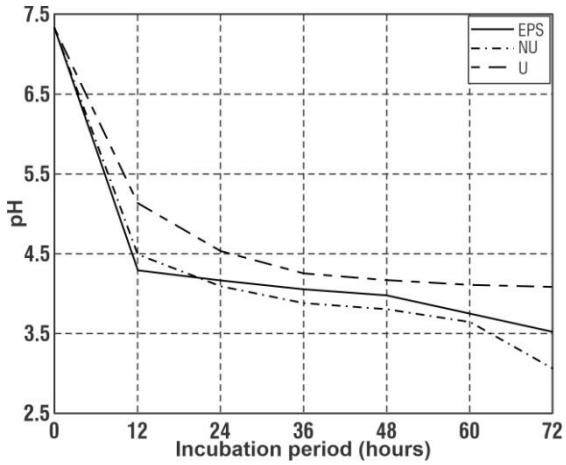

Figure 6. Variation of pH for bacteria-inoculated samples at three metabolic pathways.

6.3. Input Motion

The shake Table was exited with two horizontal components of ALS strong ground motion records- EW (x) and NS (y), as shown in Fig. 7. and Fig. 8. Due to the limitation in actuator stroke, the selected outcropping motion was converted to base motion by scaled-down the displacement of about 50%. In this investigation, a reduced scaled displacement time histories from free field seismograph station A-Li-Shan (ALS: 23.5103^{0}N, 120.8052^{0}E, site class C)during the Chi-Chi earthquake(Mw 7.6, 17:47:15 UTC on September 20, 1999) used to vibrate the soil model horizontally by controller software MTL32 (2011).

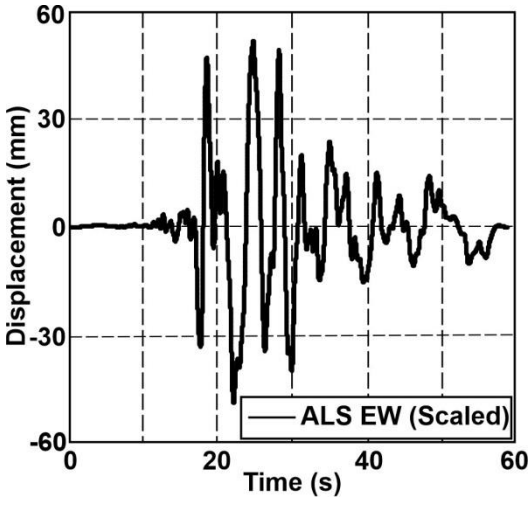

Figure 7. Scaled ALS EW displacement time history.

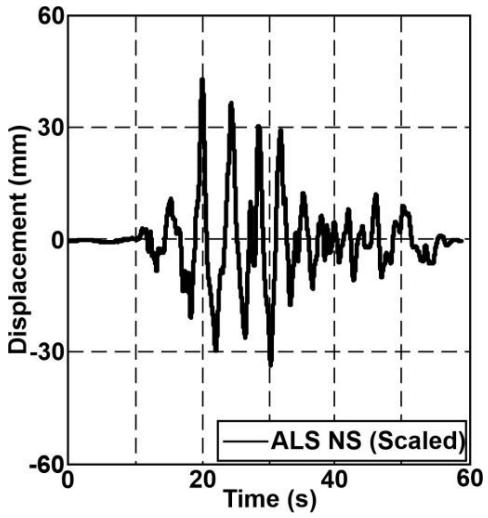

Figure 8. Scaled ALS NS displacement time history

6.4. Acceleration Response

Two piezoelectric, DeltraTron@4507 traditional accelerometer was placed at the base of the model along the orthogonal horizontal direction to measure the base acceleration of the soil model during displacement controlled shaking present in Fig. 9. and Fig. 10. The response was recorded through Pulse LabShop software.

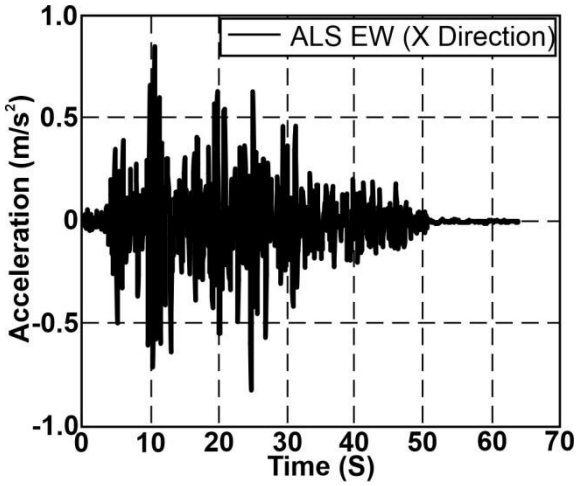

Figure 9. Acceleration response against scaled displacement time history recorded to accelerometer placed along the x-direction.

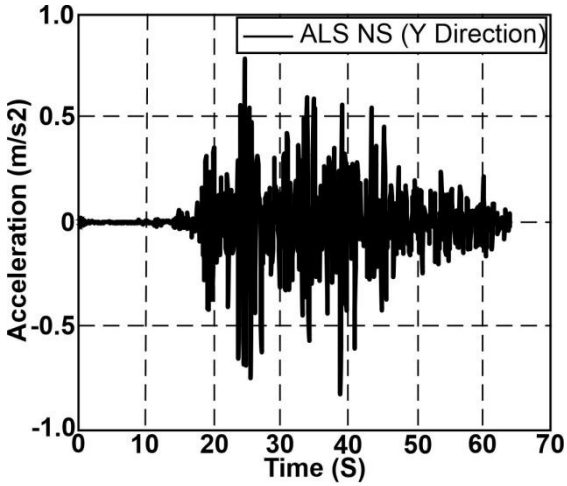

Figure 10. Acceleration response against scaled displacement time history recorded to accelerometer placed along the y-direction.

6.5. Pore pressure Response

A 24 PC Honeywell miniature low-pressure sensor was used to measure the pore pressure response in the middle of the sample. A 24PCAFA6G pressure sensor of 7 kPa capacity was connected with the data acquisition system (DAS) model 34972A and programming numerically and graphically by HP Benchlink Data Logger Pro software of Agilent Technologies.

The pore pressure response obtained from data acquisition measurement is in terms of voltage, which converted to a pressure unit (kPa) through the calibration record of that sensor. The calibration was done using a calibrated burette connected with the pressure sensor and recording the voltage corresponding to a pressure head. The calibration performed triplicate to check the repeatability of the results, which was satisfied with this investigation.

7. Influence of Microbial Activities on cone tip penetration resistance and liquefaction susceptibility

The laboratory tests were conducted on the bacteria-inoculated saturate samples at certain cementation level to show the impact of microbial activities on cone tip resistance and liquefaction susceptibility. The gram staining of bacteria inoculated pore fluid ensured the contamination-free of the tested sample. The repeatability of the results for model cone penetration and shake table tests were comparable for this study.

For the shake table study, the pore water pressure was measured at the mid-height of the sample, i.e., 200 mm. The bacteria inoculated sample may bulge due to biogenic CO_2 gas removal during microbial activities within the sample. Hence the samples were incubated under a surcharge. As the miniature cone penetration sand model is small compared to the shake table model, it has a high probability of bulge due to less vertical overburden pressure against that biogenic thrust. Hence the miniature CPT incubated sand models tested with the incubated surcharge of 2.5 kg/cm^2 over the model surface. The cyclic stress ratio (CSR) for miniature CPT sand model evaluated from the activated base motion of the shake table. The liquefaction resistance is present in terms of factor of safety against liquefaction, which estimated from normalized cone tip resistance against that cyclic stress ratio.

The impacts of microbial products on cone tip resistance are present in terms of cone tip resistance (q_c) ratio and the ratio of factor of safety against liquefaction (FS_L) between bacterial inoculated lightly cemented sand sample (designated as $q_{c\_treated}$ and $FS_{L\_treated}$) and bacteria-free control sample (identified as $q_{c\_control}$ and $FS_{L\_control}$) at cyclic stress ratio induced by shake table input motion present in Fig. 11.

The pore pressure within the soil sample increases up to a maximum value, then it started to decrease or remains constant under cyclic loading. That peak point is called the initiation of liquefaction.

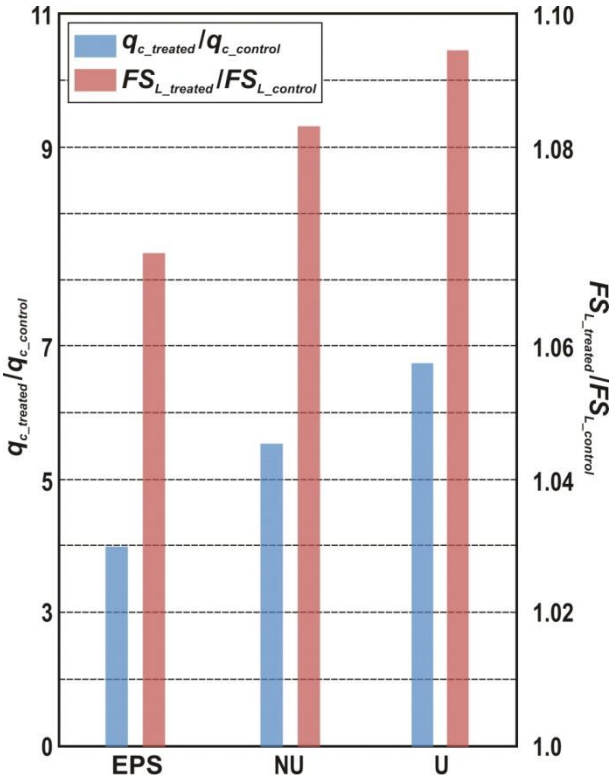

Figure 11. Variation of cone tip resistance ratio and the ratio of the factor of safety against liquefaction of three metabolic pathways.

The impact of microbial activity on the pore water pressure ratio was investigated by shake table study on similar characteristics soil sample as CPT. The variation of excess pore pressure within the soil model during the cyclic loading reflects the impact of three minimally-sustained metabolic processes within the soil mass, presented in Fig. 12.

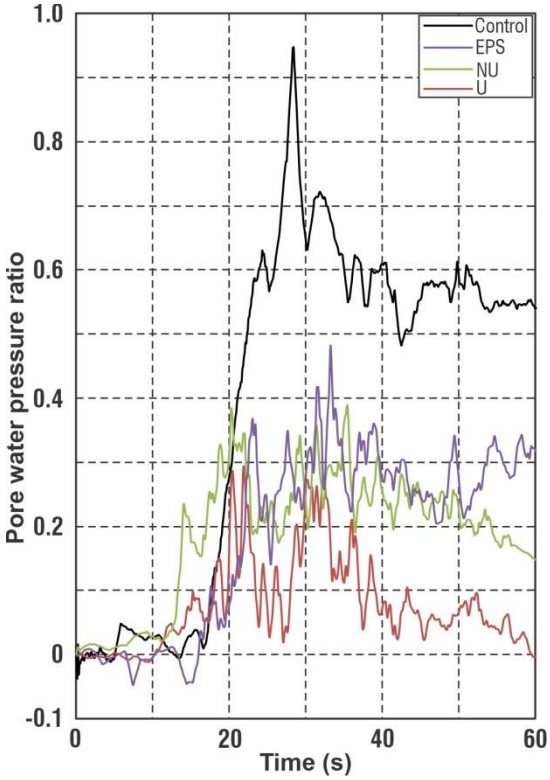

Figure 12. Comparison of pore water pressure ratio under cyclic loading for three minimally sustained metabolic process with control.

7.1. Influence of DRG3 on EPS media

The EPS metabolic pathway showed that the DRG3 microbe primarily produces exo-cellular metabolic substances (EPS). The growth media contained only nutritional minerals. As no reactive solution ($CaCl_2$) mixed with it, the DRG3 microbe was incapable of producing calcite within the saturated sand matrix.

For EPS-DRG3 inoculated saturated sand samples, the cone tip resistance ratio was evaluated for a specific zone corresponding to the bacteria-free control sand model. From Fig. 11., it is shown that the cone tip resistance of the EPS DRG3 sand model improved by 3.73 times than the cone tip resistance. Correspondingly, the factor of safety against liquefaction increased by 6.73%, i.e., 1.07 times than the control sample under the cyclic shear stress for the base acceleration of 0.08g.

From Fig.12. It shows that the excess pore pressure ratio of the bacteria-free control sample attains a peak value of 0.95 at the maximum base acceleration of 0.08g. For the EPS DRG3 inoculated saturated sand sample, this ratio reduced to 0.48 (i.e., 49.17%). The compatibility between the results of two laboratory tests indicates the identical microbial activity developed between the EPS DRG3 inoculated sand samples.

This improvement in liquefaction resistance and cone tip resistance is primarily due to the bonding effects of biofilm structure between EPS and sand matrix.

7.2. Influence of DRG3 on NU media

The Non-Uerolytic growth media contains nutritional minerals with the reactive solution of $CaCl_2$ (0.1 g/L) but no urea. Hence the DRG3 microbe produces EPS and calcite ($CaCO_3$) within the saturated sand matrix in the absence of urea.

From Fig. 11., it is shown that in DRG3 NU saturated sand sample, the cone tip resistance improved by 5.13 times compared to the control sample. Similarly, the factor of safety against liquefaction increased by 8.46%, i.e., 1.08 times the control sample developed under the cyclic shear stress for the base acceleration of 0.08g.

The DRG3 NU saturated sand sample develops the excess pore water pressure ratio of about 0.39 under a maximum base acceleration of 0.08g, which is 59.04% lower than the control sample (Fig. 12.).

In this metabolic path, EPS maintaining a biofilm structure with sand matrix, and calcite ($CaCO_3$) binds the sand particles. As a result, the excess pore pressure ratio decreases, and normalized cone tip resistance increases than the DRG3 EPS pathway.

7.3. Influence of DRG3 on Uerolytic media

The Uerolytic metabolic process contains nutritional minerals and reactive solution of $CaCl_2$ (0.1 g/L) and urea (3 g/L). This pathway facilitates a robust chemical reaction within the sand sample; as a result, the DRG3 microbe produces the EPS and the uerolytic calcite precipitation within the saturated sand matrix as follows [13].

$$CO(NH_2)_2 + 2H_20 \xrightarrow{Urease\ reaction} CO_3 + 2NH_4{}^+$$

$$Ca^{2+} + HCO_3{}^- + OH^- \rightarrow CaCO_3(\downarrow) + H_2O$$

Fig. 11., shows that the cone tip resistance of the DRG3 U inoculated sand model improved by 6.23 times than the control sample. Correspondingly, the factor of safety against liquefaction was increased by 1.095 times, i.e., 9.5% of the control sample at identical cyclic shear stress for the base acceleration of 0.08g.

Consequently, Fig.12., showing that the DRG3 U saturated sand sample develops the excess pore water pressure ratio of 0.29, i.e., 69.27% lower than the control sample.

This metabolic path shows the highest liquefaction resistance compared to DRG3 EPS and DRG3 NU. The EPS contributes bonding effects from biofilm structure with sand matrix and the densification from uerolytic calcite precipitation, which effectively improves the mechanical properties of the sand matrix than the other two metabolic paths. As a result, the excess pore pressure ratio decreases, and normalized cone tip resistance attains the maximum value than DRG3 EPS, DRG3 NU pathways.

8. Relation between pore water pressure and factor of safety against liquefaction (FS_L)

The relation between excess pore water pressure ratio and average factor of safety against liquefaction present graphically to show the effect of bacteria inoculated lightly cemented sand sample on liquefaction parameters against the variable region of level surface saturated sand samples [14,15].

From Fig. 13., it is showing that the excess pore water pressure ratio of lightly cemented sand decreases than the pore water pressure region of the saturated sand sample suggested by Tokimatsu and Yoshimi [16].

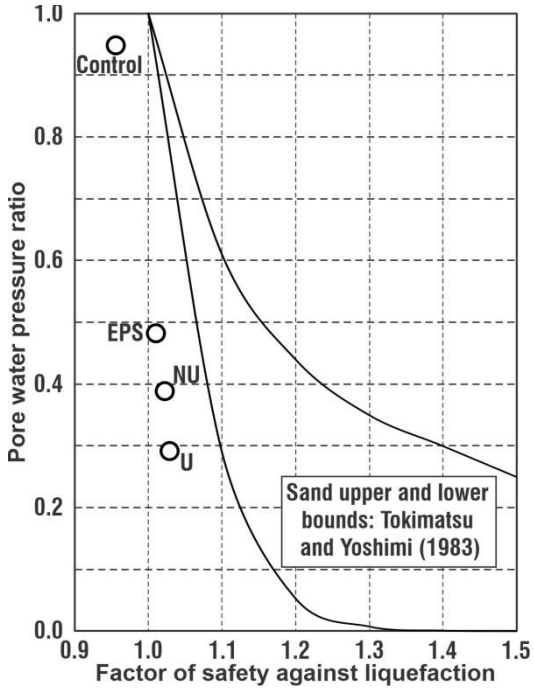

Figure 13. Variation of excess pore pressure ratio with the factor of safety against liquefaction for weakly cemented sand.

9. Conclusions

The conclusions are drawn based on the aim of this investigation with corresponding results. All the results were satisfied with repeatability.

The Uerolytic DRG3 inoculated saturated sand sample exhibits maximum cone tip resistance, liquefaction resistance, and minimum excess pore pressure ratio (i.e., 0.29) than the other two metabolic pathways. It is primarily due to effectiveness for bacterial growth and resulting EPS production, uerolytic calcite precipitation within the saturated sand matrix, which reflected from CFU records of the corresponding samples.

The microbial activities on Non-Uerolytic DRG3 inoculated saturated sand sample precipitate the calcite due to the effect of reactive solution $CaCl_2$ beside the EPS production, which make this pathway more effective than EPS DRG3. Although the bacterial population density (CFU) in the EPS DRG3 inoculated samples is higher than the NU DRG3 samples.

The pore water pressure ratio decreases due to lightly cementation levels within the sand matrix and exhibits a minimum value under the uerolytic metabolic pathway of DRG3 soil bacteria.

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