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# Prevention of Liquefaction of Saturated Sand Using Biogas Produced by *Pseudomonas stutzeri*

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**ABSTRACT:** Shear strength of sand increases with the decrease of saturation degree. An attempt to develop a more effective biogas desaturation method is made in this study. The microbial denitrification process of *pseudomonas stutzeri*, which is able to produce nitrogen gas, is selected for the desaturation of liquefiable sand. Frist, to demonstrate the feasibility of induced-partial saturation using nitrogen gas bubbles produced by *pseudomonas stutzeri*, research on denitrification condition and efficiency are made. The suitable range of temperature is determined. Comparing with existing method using biogas, this bacteria has advantages including faster average rate of generating gas, shorter initial stagnation period and simple technology. Then, to explore the effect of mitigation using this method, full and partially saturated sand samples are prepared and serial of shaking table simulation tests are performed under applying a series of cyclic loading with different acceleration amplitude. The influences of initial samples saturation on the excess pore water pressure, pore pressure ratio and surface settlement are investigated. Finally, the exponential relationship between volumetric strain and average pore water pressure ratio is established for full and partial saturated samples.

Keywords: liquefaction; desaturation; biogas

## INTRODUCTION

Liquefaction is the loss of shear strength in fully saturated loose sands due to excess pore water pressure build-up during a dynamic cyclic loading. In last decades, many tests about liquefaction resistance of saturated sands have been conducted by researchers. The test results demonstrate that the effect of undesirable air entrapment on the overestimation of the liquefaction strength of fully saturated sands (Martin et al., 1978; Yoshimi et al., 1989; Xia et al., 1991). Therefore, induced partial saturation (IPS) method is proposed. Some attempts have been made to mitigate liquefaction of saturated sand using this method.

IPS aims to increase liquefaction resistance by generating gas in the pores of fully saturated sands. Several methods about generating gas in pores of sands have been proposed. These including air injection (Okamura et al., 2006), water electrolysis (Yegian et al., 2007), drainage-recharge technique (Yegian et al., 2007), biogas (HE et al., 2013). Biogas mainly is nitrogen gas produced by the microbial denitrification process. Comparing the first three methods, $N_2$ is more suitable for this desaturation. The reason is as following. First, $N_2$ is inert. Second, its solubility in water is low. Third, distribution of $N_2$ produced by bacteria is uniform in sands (He, 2013).

Some research have been made on applying biogas to IPS. A strain of denitrification pseudomonas are isolated from anaerobic sludge of municipal wastewater treatment plant. Then, A series of shaking table tests are conducted to demonstrate that $N_2$ can decrease the degree of saturation and increase the liquefaction resistant of sands (He, 2013). *Paracoccus denitrificans* is monitored to assess the effects of nutrient availability, fines content, and pressure-diffusion on the evolution of nitrogen gas generation, and the results support the viability of biogenic gas generation as a tool to increase the liquefaction resistance of soils subjected to cyclic loading (Rebata-Landa et al. ). A method that combines biogas generation with biocement is developed using acidovorax sp. DN1 is proposed, which can enhance stability of the biogas bubbles in sand (Li, 2014).

According to literatures, development of mitigation of liquefaction using biogas is still in a preliminary stage. Especially, there are some negative aspect including low average rate of generating gas, long initial stagnation period of generating gas and complex denitrifying culture. *Pseudomonas stutzeri* which possesses excellent denitrification capability commonly used in sewage treatment (Li, 2012) and aquaculture (Robertson, 1984). In this study, research on denitrification condition and efficiency are made for *pseudomonas stutzeri* to optimize performance of biogas generation and confirm application condition for IPS. After that, the effects of biogas on sand liquefaction are studied by conducting shaking table tests using a laminar box model.

## MATERIALS AND METHODS

### *Sand for tests*

The sand used for this study is from a site of Nanjing in Jiangsu Province. Fig. 1 shows the particle size distribution curve of sand used in the tests. Gradation parameters of sand are summarized in Table 1. The relative density of sand is 2.68. Minimum and maximum void ratio is 0.48 and 0.95 respectively.

**Table 1. Gradation constants of sand.**

| $d_{10}$(mm) | $d_{30}$(mm) | $d_{50}$(mm) | $d_{60}$(mm) | $C_u$ | $C_c$ |
|---|---|---|---|---|---|
| 0.27 | 0.33 | 0.42 | 0.46 | 1.71 | 0.88 |

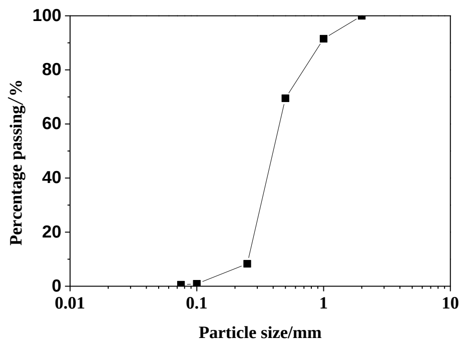



**Figure 1. Particle-size distributions curve of sand sample.**

### *Bacteria and denitrifying cultivation for biogas production*

The strain is *pseudomonas stutzeri* (DSMZ, 5190). For denitrifying cultivation of the bacteria, the medium is composed of the following components: $MgSO_4·7H_2O$ 0.2g; $KNO_3$ 2g; $K_2HPO_4$ 1g; $Na_3C_6H_5O_7·2H_2O$ 5g; and addition of distilled water to 1L.

### *Denitrification characteristics of bacteria*

Potassium nitrate is nitrogen source of denitrification medium, and sodium citrate is carbon source to analyze changes in the content of nitrate and nitrite, then to verify the denitrification capacity of the bacteria. The detail procedures of test are as follows.

(1) A standard curves of nitrate and nitrite are created through Ion Chromatogram.

(2) Using Luria-Bertani cultivation to bacterial growth, centrifuge Luria-Bertani cultivation with 4000r/min when bacteria in logarithmic phase and wipe off supernatant. Add denitrification medium to form treatment liquid of which $OD_{600}$ is 0.1.

(3) Join 1mL treatment liquid into a series of 2mL centrifuge tubes respectively and static culture under 30℃ which is the optimal temperature in official website of DSMZ.

(4) Take samples every other 3h and detect the concentration of nitrate and nitrite and $OD_{600}$ for treatment liquid.

***Gas generation test***

Gas generation test is done to investigate the yield, rate and initial stagnation period of gas generation for the bacteria with different temperature. Schematic diagram of gas measurement device is illustrated in Fig. 2. When gas was generated, the generated gas bubbles would shift the level of liquid paraffin in the burette, which is observed at 3h interval. Samples with sand and without sand are prepared in 20 mL syringe. Treatment liquid is the bacterium suspension of which $OD_{600}$ was 0.1. Parameters of gas generation tests are listed in Table 2.

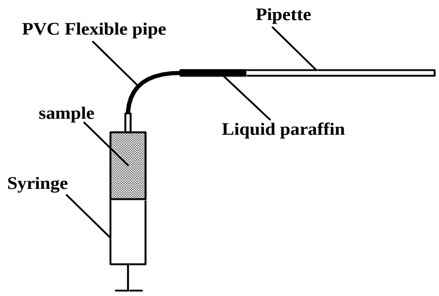



**Figure 2. Schematic diagram of gas measurement device.**

**Table 2. Parameters of gas generation tests.**

| | T(℃) | pH | Volume($cm^3$) | void ratio |
|---|---|---|---|---|
| Test1-1 | 30 | 7 | 9.89 | 0.48 |
| Test1-2 | 20 | 7 | 10.05 | 0.50 |
| Test1-3 | 15 | 7 | 10.05 | 0.51 |
| Test1-4 | 4 | 7 | 9.89 | 0.49 |
| Test1-5 | 30 | 7 | 3.33 | - |

***The shaking table test set-up***

A picture of the test set-up is shown in Fig. 3. It consists of two parts. The upper part is a laminar box and the lower part is an electromagnetic shaking table. The laminar box has an inner size of 50cm×36cm ×40cm (length×width× height). The monitoring indexes for these tests are exceed pore water pressure (pwp1, pwp2, pwp3) and surface settlement (lvdt1, lvdt2, lvdt3). Arrangement of all sensors is shown in Fig. 4.

The samples are prepared using the following steps.

(1) Liner is installed in internal surface of the laminar box to keep water-tightness. 25 liters of treatment liquid (for partial saturated samples) or distilled water (for the fully saturated sample) is poured into the laminar box.

(2) Dry sand is slowly placed into the liquid through a sieve until height of the sand samples reached 30cm. Extra water is removed to make sure that the water tables are around 1 cm above the sand surface.

(3) Saturated samples can be used immediately. However, for desaturated samples, several days is needed to generate biogas until the water level is not change.

**Figure 3. Model test set-up.**

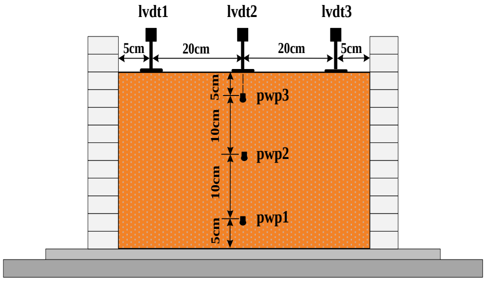



**Figure 4. Schematic diagram of transducer arrangement.**

There are the same five section sine waves applied for one sand sample to verify the effect of biogas. The frequency of sine waves is 2Hz. Start time for sine waves is 65s, 125s 185s, 245s, 305s successively and duration of every sine waves is 15s. The parameters of shaking table tests are given in Table 3.

**Table 3. The parameters of shaking table tests.**

| | Input acceleration(m/s2) | Initial height of sample(m) | Initial degree of saturation (%) | Initial relative density(%) |
|---|---|---|---|---|
| Test2-1 | 1 | 29.6 | 100 | 52.3 |
| Test2-2 | 1.5 | 29.7 | 100 | 51.1 |
| Test2-3 | 2 | 30.1 | 100 | 52.2 |
| Test2-4 | 1 | 30.0 | 85.2 | 49.9 |
| Test2-5 | 1.5 | 30.2 | 84.9 | 51.5 |
| Test2-6 | 2 | 30.1 | 85.1 | 51.4 |

## RESULTS AND DISCUSSION

### *Denitrification and growth of biomass*

Standard curve of nitrate and nitrite are given in Table 3.

**Table 3. Relevant parameter of standard curve of nitrate and nitrite.**

| Analyte | Retention time（min） | Linearity | correlation coefficients |
|---|---|---|---|
| nitrate | 5.52 | y=1.7411x - 0.1684 | 0.999 |
| nitrite | 4.67 | y=2.2505x + 0.0311 | 0.999 |

X is analyte concentration， mmol/L； y is peak area ， µS*min.

The concentrations of nitrate and nitrite and growth of biomass with time are shown in Fig.5. Growth of biomass can be shown by absorbance (optical density at 600 nm) of treatment liquid. The reduction of nitrate completes within 21h. Nitrite accumulates transiently in treatment liquid. When the reaction is carried out for 18 hour, the maximum nitrite accumulation appears and its value is 4.94mmol/L. Thereafter, the peak disappears and residue concentration is 0.82mmol/L. The $OD_{600}$ is 0.94 after 34h and the maximum specific growth rates of 0.08 $hour^{-1}$.

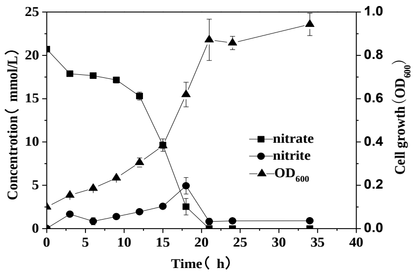



**Figure 5. Denitrification conditions and growth curve of bacteria.**

***Impact of temperatures on gas generation***

The gas generation curve with time under different temperatures is shown as Fig.6. It shows that the curve deviates to the right obviously with the decrease of temperature. Initial stagnation of gas generation is 18h and 15h under 4℃and 15℃ respectively. This is primarily due to the fact that low temperature can result in slow metabolism of bacteria and low enzyme activity involving denitrification. In addition, the final gas quantity of treatment liquid without sand is obviously lower than sand samples under the same temperature, which is because that *pseudomonas stutzeri* belongs to facultative anaerobe whose character is that aerobic respiration conducted to providing energy under aerobic environment and denitrification providing energy and maintaining metabolism itself under anaerobic environment. First, the bacteria conducts aerobic respiration with consuming the dissolved oxygen in pore water. Because of relatively low permeability of sand pore, it is difficult to exchange the dissolved oxygen with the outside. Therefore, the pore of sand samples becomes anaerobic environment as the dissolved oxygen used up, bacterium begins to be denitrification. Since the volume of treatment liquid sample is bigger than sand samples pore, the dissolved oxygen is consumed slow and bacteria is vulnerable to light, and gas generation in Test1-5 is limited (He, 2013). It suggests that the pore structure of sand is the excellent environment to conduct denitrification due to its low permeability and transmittance. Considering the initial stagnate, rate biogas production and et al, the optimum temperature is between 20℃~30℃.

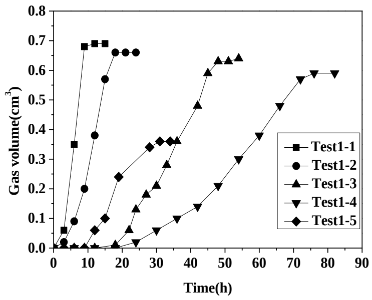



**Figure 6. Gas generation curve with different temperatures.**

***Preponderance of gas generation using pseudomonas stutzeri***

The gas generation tests indicate the feasibility of *pseudomonas stutzeri* decreasing the degree of saturation. But there are some practical problems not to be neglected for the application of microorganism bubbles in actual engineering as follows. First, the ionic concentration difference between treatment liquid and pore water can result in ion diffusion, which will retard bacterium growth and denitrification. Second, groundwater seepage accelerates the decrease of ionic concentration in treatment liquid. Third, formula of denitrification culture is usually complex and expensive. As these problems above, it is important to eliminate earlier stagnate time, increase average gas generation rate and simplify formula of denitrification culture for optimization. Now, denitrification pseudomonas is commonly applied to IPS. Table 4 shows that the comparison of denitrification pseudomonas and *pseudomonas stutzeri* on major parameters. It can be seen that there the problems can be effective solved by this tests in similar environmental conditions. Such as, improving the mean gas produced rate, decreasing the initial stagnate phase and simplifying formula of denitrification culture. Table 4 also indicates that key factors affecting initial stagnate phase and average gas generation rate maybe bacteria, culture medium, initial density of bacteria and sand type. However, this test is an initial exploration, so it is essential to make a further research on optimizing initial condition in the future.

**Table 4. Test conditions and results contrast of gas generation tests.**

| Bacteria | Test conditions | | | | Test results | |
|---|---|---|---|---|---|---|
| | Initial $OD_{600}$ | Initial nitrate concentration (mmol/L) | Temperature（℃） | Component of cultivation | Initial stagnation（h） | Average rate of generating gas of 1cm$^3$ soil (cm$^3$/h） |
| *pseudomonas stutzeri* | 0.100 | 20.17 | 20 | 4 | ＜3 | 0.0037 |
| Pseuomonas denitrifican | 0.005 | 26.74 | Room temperature | 8 | 39 | 0.0017 |

***Excess pore water pressure***

Fig.7 shows the excess pore water pressure generations in samples with fully saturation and partial saturation. Initial relative densities of the two samples are similar and input accelerations are same. A drastic increase in excess pore water pressure in the first section of sine wave is observed in Fig.7 (a). By contrast, the peak of the excess pore water pressure for Test 2-4 is substantially smaller than Test 2-1 at different depth. This could be because by the compressibility of water-biogas mixture is stronger than water. The model of excess pore pressure of full saturated sand during one loading cycle of simple shear tests is established (Finn, 1976), as follows:

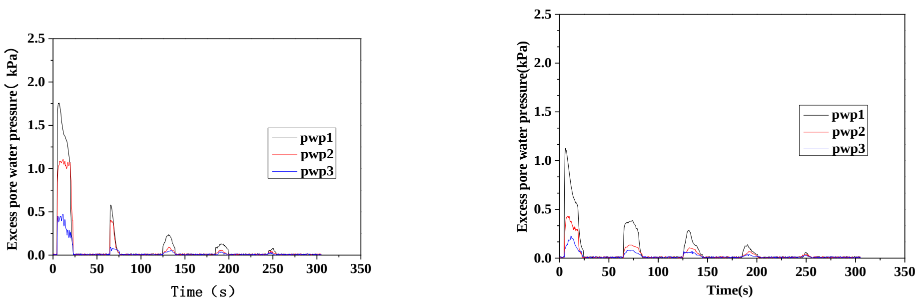
$$\Delta u = \frac{\Delta \varepsilon_{vd}}{\frac{1}{\overline{E}_r} + \frac{n}{K_w}} \tag{1}$$

Where $\Delta u$ is excess pore pressure per load cycle, $\Delta\varepsilon_{vd}$ is net volumetric strain increment corresponding to the decrease in volume occurring during the load cycle in drained case, $\overline{E}_r$ is one dimensional rebound modulus of sand at effective stress, $n$ is porosity of the soil, $K_w$ is the bulk modulus of water. Because $\Delta\varepsilon_{vd}$ is defined as to be the function of the total accumulated strain and the strain cycle amplitude. $\overline{E}_r$ is only related to skeleton characteristics, they are no difference in full and partial saturated sand. Therefore, $K_w$ is the only parameter related to pore water. If samples is partial saturated, $K_w$ can be replaced by the bulk modulus of water-air mixture, and excess pore pressure of partial saturated sand during one loading cycle of simple shear tests is expressed as below (Eseller-Bayat, 2009).

$$\Delta u = \frac{\Delta\varepsilon_{vd}}{\overline{E_r} + n\left[S_r C_w + \frac{(1-S_r)}{u_a}\right]} \tag{2}$$

Where $S_r$ is degree of saturation, $C_w$ is compressibility of water, whose value is 0. Equation (2) shows that $\Delta u$ is decreases with $S_r$ decreasing due to increase in the compressibility of the pore fluid.

The peak of excess pore water pressure is decreasing with time of section of sine wave for these samples. Because the each section of sine wave can result in the increasing of relative density, which can improves liquefaction resistance of sand.

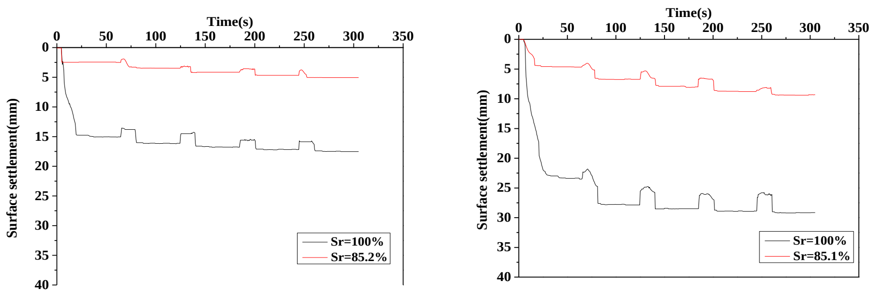



(a) Test2-1 ($S_r$=100%) (b) Test2-4 ($S_r$=85.1%)

**Figure 7. Excess pore water pressure in different depth.**

***Surface settlement***

Fig.8 shows the surface settlement of samples under different acceleration. The surface settlement is the measured by lvdt2 at centerline of the laminar box in Fig.4. In this figure, distinct comparison can be seen between saturated and partial saturated samples. In first section of sine wave as 1m/s$^2$, the surface settlement on fully saturated samples are around 15.03mm, which is the majority of total settlement. In contrast, the total surface settlement in partial saturated samples are much smaller under a=1m/s$^2$. In the first wave, the surface settlement only about 2.48mm after the first wave. With the latter section of sine wave applies, the surface settlements is 0.99mm, 0.68mm, 0.53mm and 0.38mm. After 5 times section of same waves, the total surface settlement for unsaturation samples is less than a third of saturation samples. This comparison demonstrates that desaturation is a good way to control superstructure settlement during earthquake liquefaction. According to Fig.7, because excess pore water pressure in full saturated samples is significantly more than the partial saturated in the first wave, the bigger settlement occurred with the excess pore water dissipate.




(a) a=1m/s$^2$ (b) a=2m/s$^2$

**Figure 8. Surface settlement in lvdt2.**

The settlement in both full and partial saturated samples increase with input acceleration. Because more severe vibrations can result in more soil particles falling off soil skeleton, compressibility of soil skeleton becomes stronger and larger settlement of sample occurs in gravity conditions.

***Pour water pressure ratio and volumetric strain***

A relationship between volumetric strain and average pore water pressure ratio is established in Fig. 9. The volumetric strain and average pore water pressure ratio is respectively calculated by the average of lvdt1, lvdt2, lvdt3 and average of pwp1, pwp2, pwp3 for each test.

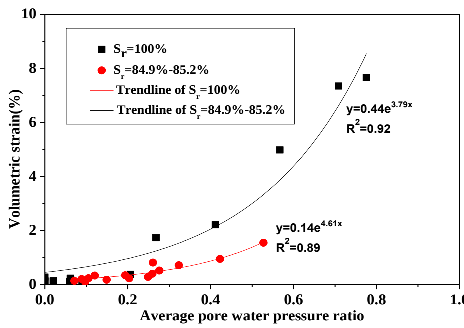



**Figure 9. Plot of volumetric strains against average pore water pressure ratios.**

Volumetric strain increases slowly with increase of average pore water pressure ratio for full and partial samples when average pore water pressure ratio is less than 0.2. However, when average pore water pressure ratio is higher than 0.2, the volumetric strain for full saturation sand samples starts to increase at a more accelerating rate than partial saturated samples. For partial saturated samples, even if the average pore water pressure ratio is 0.52, the volumetric strain is 1.54, which is significantly lower than saturated samples with the similar average pore water pressure ratio. This trend can be explained as follows. The pore water pressure increases after soil particle falling off soil skeleton under vibrating load. The excess air pressure will be all equal excess with water pressures in the voids if the surface tension between air and water is neglected due to air being in bubble form (Fredlund, 1993). Due to strong compressibility of biogas bubbles, volume of bubbles decrease with increase of excess pore water pressure until pressure peak appears. Then, with excess pore water pressure dissipates slowly, volume of bubbles expanses to compensate for the part lost volume of dissipating pore water. Combine this result and surface settlement of partial saturation samples in FIG. 8, it is suggest that the biogas bubbles cannot migrated out of samples with pore water pressure dissipating and characterizes a degree of stability in pore of sand under vibration.

## SUMMARY AND CONCLUSIONS

1 *Pseudomonas stutzeri* has excellent denitrification performance in proposed denitrifying culture. The removal rate of nitrate is 100% when culture time is 21h. A small residue concentration of nitrite is 0.82mmol/L. The optical density is 0.94 after 34h and the maximum specific growth rates of 0.08 $hour^{-1}$ for the media.
2 Biogas can be generated when temperature is between 4℃and 30℃. Average rate of biogas generation increases with temperature increased. Comparing with existing method using biogas, this bacteria has advantages including fast average rate of generating gas, short initial stagnation period and simple denitrifying cultivation.
3 The peak of excess pore water pressure of partial saturation ($S_r$=85.2%) sample is significantly smaller than full sample under acceleration being $1m/s^2$. The final surface settlement of partial saturation (Sr=85.2%) sample is nearly one third of full sample after 5 times sine wave of which acceleration is $1m/s^2$.
4 The exponential relationship between volumetric strain and average pore water pressure ratio is established for full and partial saturated samples. The volumetric strain for full saturation sand samples starts to increase at a more accelerating rate than partial sand samples when average pore water pressure ratio is higher than 0.2.

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