2014

Mitigation of sand liquefaction using in situ production of biogas with biosealing

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Mitigation of sand liquefaction using *in situ* production of biogas with biosealing

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Civil Engineering (Environmental Engineering)

Program of Study Committee:
Jian Chu, Co-Major Professor
James E. Alleman, Co-Major Professor
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Iowa State University
Ames, Iowa
2014

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ACKNOWLEDGEMENTS

I would like to thank my co-major professor, Dr. Jian Chu, for his patience, motivation, enthusiasm, immense knowledge. Without his support and mentorship this dissertation would not have been possible.

I would like to thank my co-major professor, Dr. James E. Alleman, and my committee member, Dr. Shihwu Sung, for their encouragement and providing with all the necessary facilities.

In addition, a thank you to Dr. Volodymyr Ivanov, whose guidance helps me all the time of research and writing of this thesis.

ABSTRACT

Sand liquefaction refers to a phenomenon whereby sand loses its strength and stiffness. It is responsible for many of the damages associated with earthquake. Partial desaturation of soil using bacterial production of biogas is a new method for mitigation of sand liquefaction. However, there is a concern that whether biogas in sand can be stable over a long duration when there is groundwater flow. In this study, a new method that combines biogas generation *in situ* with biosealing of the biogas bubbles in sand using a small quantity of biocement was developed. Biogas bubbles were produced in the form of nitrogen gas during microbial denitrication process by denitrifying bacteria *Acidovorax sp.* DN1 in fully saturated sand. It was experimentally observed of complete removal of biogas under flow conditions with a hydraulic gradient of 0.1 within three days. On the other hand, if sand with biogas bubbles was biosealed with calcium carbonate crystals produced by urease-producing bacteria *Sporosarcina pasteurii* DSMZ 33, the stability of biogas bubbles was improved by 40% - 70%. Therefore, the sequential biogas production in saturated sand and biosealing of the gas bubbles in sand pores could be useful for sustainable mitigation of sand liquefaction under groundwater flow. The cost of the sequential biogas production in saturated sand and biosealing of biogas bubbles in sand pores could be significantly lower than the cost of biocementation of the saturated sand.

CHAPTER I

INTRODUCTION

1.1 Problem Statement

Earthquake is one of the most devastating geo-hazards on earth causing damages to infrastructures and properties resulting in great economic losses and even life losses. Many of the damages were related to sand liquefaction – a phenomenon in which saturated sand loses its strength and acts as liquid. Development of excess pore water pressure in saturated sand due to cyclic load during earthquakes decreases effective stress of sand to its liquefaction so that structures founded on saturated sand can be settled and collapsed (Frydman et al., 2009). Conventional ground improvements for mitigation of sand liquefaction are vibroreplacement, compaction grouting, and deep dynamic compaction methods (Wijewickreme and Atukorala, 2005; Chu et al., 2009). However, these methods are energy-consuming and expensive. Cost-effectiveness becomes the most important challenge in developing new liquefaction mitigation methods.

Energy-effective alternative for compaction grouting of loose sands is induced-partial saturation (IPS) that can be performed by introduction of gas in saturated sand and entrapment of gas bubbles there (Yegian et al., 2007). Microbiological production of gas in soil were proposed to introduce small gas bubbles in saturated soil (Rebata-Landa, 2007; He et al., 2013).This method is an introduction of nitrogen gas bubbles into soil using biochemical reduction of nitrate (denitrification) *in situ.* However, previous literatures and studies have shown that biogas bubbles are not stable under conditions of vertical or

horizontal flows of groundwater. In this scenario, a new method which combines the biogas production *in situ* with the sealing of the biogas bubbles in sand using small quantity of biocement has been developed.

1.2 Study Scope

The scope of this study includes:

1) Study the feasibility and method of combining of biogas generation and biosealing of the gas bubbles.

2) Select and cultivate the favorable denitrification bacteria and urease-producing bacteria.

3) Apply the biogas generation and sequential biosealing into saturated sand in small scales to test the effectiveness.

4) Conduct biogas stability tests under seepage in sand columns to further evaluate.

1.3 Outline of Report

This thesis consists of six chapters. Chapter 2 includes a literature review on application of two microbial methods to mitigate sand liquefaction. Chapter 3 discusses the selection of bacteria strains and cultivation of urease-producing bacteria. Chapter 4 describes the experimental designs for analysis of biogas stability in saturated sand. Chapter 5 presents the test results and evaluation of biogas stability with and without biosealing of gas bubbles. The conclusions and the recommendations are explained in Chapter 6.

CHAPTER II

LITERATURE REVIEW

2.1   Introduction

This chapter will start with a literature review on the development of the induced partial saturation method and the evaluation of its effectiveness. Furthermore, a brief review on the microbially induced calcite precipitation technology will help to understand the feasibility and mechanism of biosealing of the gas bubbles.

2.2   Induced partial saturation

The induced partial desaturation (IPS) in loose saturated sands can decrease excess pore water pressure and increase the bearing capacity and shear strength of the soil, which is beneficial in foundation design and roadway construction (Seagren and Aydilek, 2010). It has been demonstrated that the liquefaction resistance of saturated sand can be significantly increased when the sand is slightly desaturated with some voids displaced by gas (Yegian et al., 2007; Chu, 2011; He et al., 2013; He and Chu, 2014). Even a small decrease in the degree of saturation of sand to 99 - 97% increases resistance of water-saturated sand to liquefaction by 30 - 40% (Xia and Hu, 1991; Yang et al., 2003), while reduction of the sand saturation to 90% can increase resistance of water-saturated sand to liquefaction twice (Chaney, 1978; Yoshimi et al., 1989). An injection of air into ground to desaturate the sand and increase its liquefaction resistance has been done in the real scale (Yoshimi et al., 1989; Okamura et al. 2006; 2011 Shiraishi, 2007; Okamura, 2006). However, gas injected in this way may be not

evenly distributed. Yegian et al. (2011) has proposed a method to generate oxygen bubbles in-situ using a chemical compound sodium percarbonate ($Na_2CO_3.3H_2O_2$). However, oxygen tends to react with minerals in soil. Thus, the amount of gas may reduce with time. Electro-chemical (Hocking, 2003) also produces chemically active gases that diminish the stability of gas microbubbles.

2.2.1 *Induced partial saturation using microbial denitrification process*

Microbiological production of gas in soil were proposed to introduce smaller and more stable gas bubbles in saturated soil (Rebata-Landa, 2007; He et al., 2013).This method is an introduction of nitrogen gas bubbles into soil using biochemical reduction of nitrate (denitrification) *in situ.* It is most suitable approach because nitrogen gas is a chemically inert substance (Rebata-Landa and Santamarina, 2006; Seagren and Aydilek, 2010). Different organic and inorganic substances can be biooxidized by nitrate but ethanol (C_2H_5OH), acetic acid (CH_3COOH), or glucose ($C_6H_{12}O_6$) that can be as used as 75% (w/v) syrup from corn, are most suitable electron donors because of their low cost, availability, and high solubility in water. Their biooxidation by nitrate (denitrification), is shown below:

$$1.67\ C_2H_5OH + 2\ NO_3^- \rightarrow N_2\uparrow + 3.33\ CO_2 + H_2O + 2\ OH^- \quad (1),$$

$$1.25\ CH_3COOH + 2\ NO_3^- \rightarrow N_2\uparrow + 2.5\ CO_2 + 1.5\ H_2O + 2\ OH^- \quad (2),$$

$$0.42\ C_6H_{12}O_6 + 2NO_3^- \rightarrow N_2\uparrow + 2.5\ CO_2 + 1.5\ H_2O + 2\ OH^- \quad (3).$$
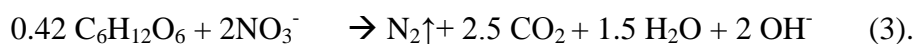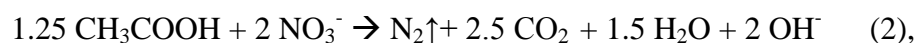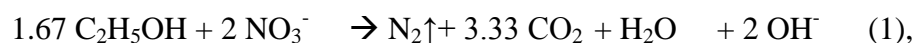
The stoichiometrical parameters of these reactions are almost same: consumption of electron donor is 3.4 kg/m^3 of N_2 and consumption of electron acceptor (sodium nitrate) is

7.6 kg/m^3 of N_2. The consumption of electron donor and acceptor for 10% (volume of gas/volume of water) desaturation of soil with porosity 50% is 0.55 kg/m^3 of saturated soil. Production of carbon dioxide in reactions 1-3, which is from 120 to 159 g/m^3 of N_2 or from 12 to 16 g/m^3 of water in saturated soil with 50% porosity, is not accounted for desaturation of soil because solubility of CO_2 in water at 10°C is 2500 g/m^3.

There is almost no cost difference between these electron donors: the cost of electron donor is from \$0.5 to \$0.7/kg, the cost of electron acceptor (sodium nitrate) is from \$0.4 to \$0.5/kg, so the estimated cost of electron donor and acceptor for partial desaturation is from \$5.1 to \$6.2/m^3 of N_2. The estimated cost of electron donor and acceptor for 10% (volume of gas/volume of water) desaturation of soil with porosity 50% is from \$0.25 to \$0.31/m^3 of saturated soil. However, even stoichiometrical and economic parameters of the electron donors are similar, ethanol could be more preferable electron donor then acetic acid or glucose syrup for geotechnical applications because it is liquid with neutral pH and not corrosive substance with low viscosity.

Biogenic gas generation can increase the liquefaction resistance of soils subjected to cyclic loading at earthquake (Seagren and Aydilek, 2010; He and Chu, 2014). The using of denitrifying bacteria to generate nitrogen gas *in-situ* (Chu et al. 2009; He et al. 2013; He and Chu, 2014) has two major advantages over the other gas-introducing methods: (1) the gas bubbles generated by denitrifying bacteria are tinny and thus the bubbles are more stable underground; and (2) nitrogen gas is inert and very low in solubility. The studies by He et al. (2013), He and Chu (2013) using a series of laboratory experiments including shaking table tests have proven that the production of biogas in situ is a feasible method for mitigation of liquefaction of sand.

2.2.2 *Long-term sustainability problem with Induced partial saturation*

A problem of a long-term sustainability of gas bubbles in sand during vertical or horizontal flows of groundwater is remained most important for practical application of IPS. Hypothetically, stability of nitrogen gas bubbles can be ensured by low hydraulic conductivity of soil due to the decrease in the size of the water-conducting pores because of soil pores clogging by the gas bubbles (Baveye et al., 1998; Seagren and Aydilek, 2010). Diffusion and dissolution of such gases as oxygen and nitrogen in water is also low. Experiments of Yegian et al. (2007) showed that under hydrostatic conditions the degree of saturation of sand with introduced gas bubbles slightly increased from about 83 to 84% after 442 days. Similar results for hydrostatic conditions were obtained by He (2012). Field data of Okamura et al. (2006) showed that gas bubbles introduced in sand during sand compaction piling remained entrapped there for 26 years.

Meanwhile, there are controversial data on the stability of gas bubbles in partially saturated sand in case of vertical or horizontal flow of water. It is known that from the work of He (2012, 2013) on the production of biogas *in situ* using denitrifying bacteria that nitrogen gas bubbles are not stable in 1 m length sand column during upward or downward flows of water with hydraulic gradient 0.1. These bubbles disappeared from sand after 2 - 4 days increasing degree of sand saturation up to 100%. For one day of experiment, saturation of sand increased only from 89 to 92%. Other data (Eseller-Bayat et al., 2012) showed that after 18 h of a vertical upward flow with hydraulic gradient from 0.05 to 0.5 with average hydraulic retention time 0.36 h the degree of saturation increased only from 82.6% to 83.6%. Long-term sustainability of the gas bubbles in sand under hydrostatic conditions has no doubts but under conditions of vertical or horizontal flows of groundwater in partially

saturated sand the gas bubbles will be probably not stable. Therefore, for practical implementation of induced partial saturation (IPS) in loose sands using biogas production *in situ* some additional technological solutions must be developed to ensure long-term sustainability of IPS under conditions of groundwater flows in sand.

2.3 Mitigation of sand liquefaction using biocementation

Another biological approach to mitigate saturated sand liquefaction is biocementation of loose sand using microbially induced calcite precipitation (MICP) (DeJong et al., 2006; Montoya et al., 2012). In this process, calcite is produced from calcium chloride and urea solution due to hydrolysis of urea by urease-producing bacteria (UPB) according to the following equations:

$$(NH_2)_2CO + 3H_2O + \text{urease of UPB} \rightarrow 2NH_4^+ + HCO_3^- + OH^- + \text{urease} \qquad (1)$$

$$CaCl_2 + HCO_3^- + OH^- \rightarrow CaCO_3\downarrow + H_2O + 2Cl^- \qquad (2)$$
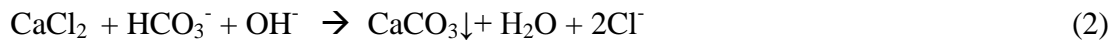
Calcite is formed on the surfaced of urease-producing bacterial cells which are adsorbed on soil particles and thus binds soil particles together as shown in **Figure 2.2**.

It was shown that the resistance of the saturated sand to liquefaction, measured by decrease of excess pore water pressure ratio, significantly increased after MICP. MICP binds sand grains by calcite crystals resulting in higher strength of treated saturated sand. However, sufficiently strong biocementation of saturated sand, at the level of unconfined compressive strength 250 - 500 kPa, could be at the content of precipitated calcium carbonate of 75 -100 g/kg of sand (Ivanov et al., 2012; Cheng et al., 2013). Therefore, it could be material-

consuming process requiring about 88 kg $CaCl_2$ and 96 kg of urea per 1 m^3 of sand, which will cost at least $41/$m^3$ of saturated soil. This value is about 140 times higher than 10% desaturation of soil using biogas production *in situ*. Therefore, biocementation of soil to mitigate liquefaction is too expensive to be applicable for large-scale geotechnical practice. However, **Figure 2.1** shows that small quantity of biocement can significantly lower the hydraulic conductivity of soil even though large quantity of biocement is needed to enhance the unconfined compressive strength of soil. So, biocementation with much smaller quantity of used biocement probably can be used to seal the biogas bubbles in sand to increase stability of mitigation of soil liquefaction under conditions of groundwater flow.
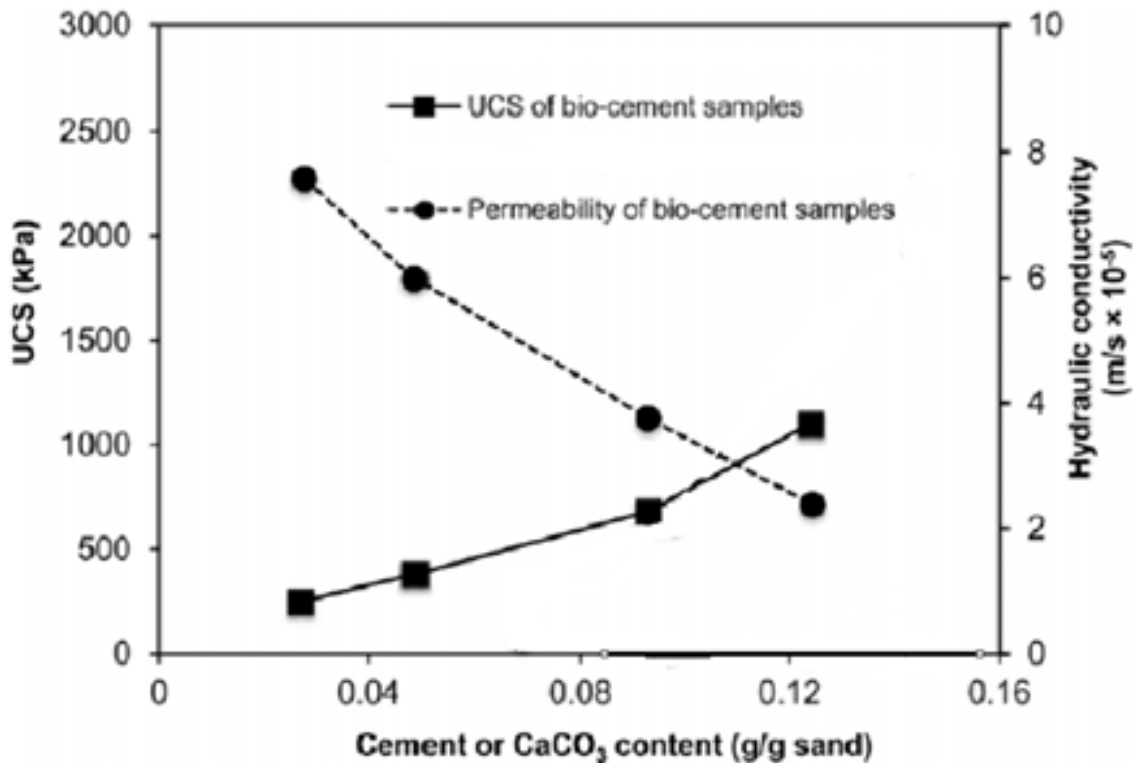
Figure 2.1 UCS and permeability of biocemented sand samples (after Cheng, 2013)
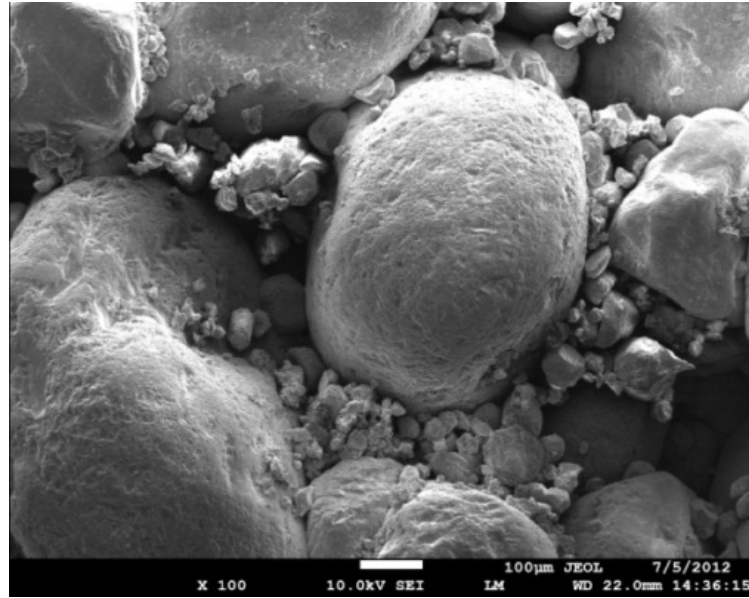
(a)
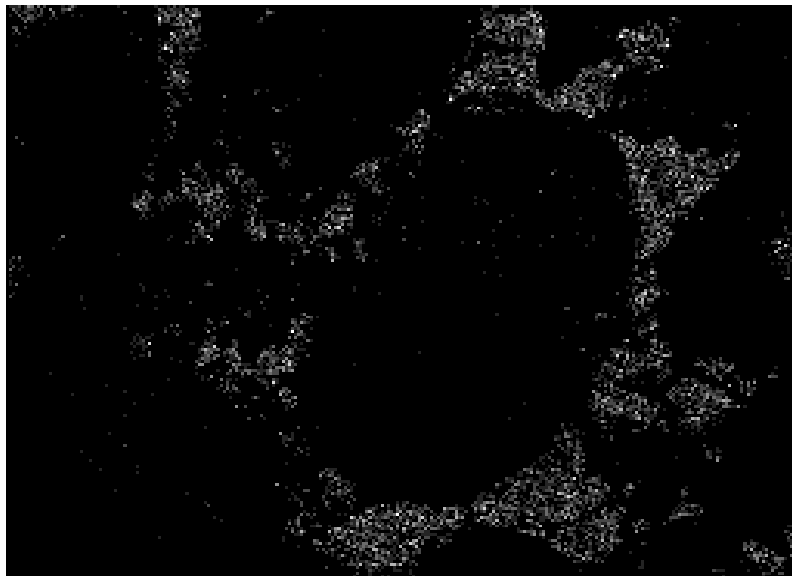
Ca Ka1

(b)

Figure 2.2 Microscopic image of biotreated sand: (a): SEM image of pores in sand filled

with calcite crystals; (b): DESEM/FDX image of calcite crystals around sand grains (after Li,

2014)

CHAPTER III

MICROBIAL STRAINS AND MEASUREMENTS

3.1 Denitrification bacterial strain and medium

The isolated strain *Acidovorax sp.* DN1 (He et al., 2013) that was able to oxidize ethanol by nitrate has been used for the production of gas bubbles in saturated sand. The isolation was done from denitrifying enrichment culture inoculated with sewage sludge of municipal wastewater treatment plant.

Denitrifying bacteria have been grown anaerobically on the denitrifying medium of the following composition: C_2H_5OH, 0.5 g (0.63 mL); KNO_3, 1.01 g; NH_4Cl, 0.12 g; KH_2PO_4, 0.75 g; K_2HPO_4, 2.5 g; $MgSO_4 \bullet 7H_2O$, 0.1g; $FeSO_4 \bullet 7H_2O$, 0.01 g; $CaCl_2 \bullet 2H_2O$, 0.015 g; trace element solution 1ml; and addition of de-ionized water to 1 L. 1ml of trace element solution contains: EDTA-2Na, 10 mg; $MnCl_2 \bullet 4H_2O$, 0.12 mg; $ZnSO_4 \bullet 7H_2O$, 0.12 mg; $CuSO_4 \bullet 5H_2O$, 0.03 mg; $(NH_4)_6Mo_7O_{24} \bullet 4H_2O$, 0.05 mg; $NiCl_2 \bullet 6H_2O$, 0.1 mg; $CoCl_3 \bullet 6H_2O$, 0.1 mg; $AlCl_3 \bullet 6H_2O$, 0.05 mg; H_3BO_3, 0.05 mg. The solid culture contained additionally 12 g/L Bactoagar Difco. The medium was purged with nitrogen gas before sterilization. Cultivation was at 30^oC for 5 days. The concentration of biomass in the suspension after cultivation was measured by filtration of 50 ml of bacterial suspension through a membrane with 0.2 µm pores and drying the filter paper at 60^oC for 12 h. The content of bacterial biomass introduced into the sand for biogas production was about 13 mg of dry biomass/kg of sand.

3.2   Cultivation of biocementation bacterial strain

3.2.1   *Enrichment culture of urease-producing bacteria*

Five samples of upper layer of soil in Iowa from different locations were combined for production of enrichment culture. Liquid medium for enrichment culture of ureolytic, alkalophilic and halotolerant bacteria had the following composition: Tryptic Soya Broth DIFCO™, 30 g; urea, 20 g; NaCl, 100 g, $MnSO_4 \cdot H_2O$, 12 mg; $NiCl_2 \cdot 6H_2O$, 24 mg, phenol red, 10 mg/l, distilled water 1 l. The factors of selection were high concentration of NaCl and presence of urea giving increase of pH after hydrolysis. All components of this medium, for exemption of urea, were sterilized at 121^oC for 15 minutes. Stock solution of urea, 100 g/l, was filter sterilized by using 0.2 μm Whatman™ nitrocellulose membrane to avoid decay of urea during autoclaving. Two ml of the trace elements stock solution was added to 1 l of sterile medium. The trace elements stock solution consists of the following components: $ZnSO_4 \bullet 7H_2O$, 0.1 g; $MnSO_4 \bullet H_2O$, 0.085 g; H_3BO_3, 0.06 g; $CoCl_2 \bullet 6H_2O$, 0.02 g; $CuCl_2$, 0.004 g; $Na_2MoO_4 \bullet 2H_2O$, 0.04 g; $FeCl_2$, 0.3 g, deionized water, 1 l. The pH was adjusted to 2.0 using 1 N HCl. This medium was inoculated with soil sample in dosage 10 g of soil per 0.5 L. Cultivation was on the shaker with 150 rpm at 30^oC for six days.

3.2.2   *Measurement of urease activity*

Urease activity can be measured by production of CO_2, ammonium ions, changes of pH, or by the changes of electric conductivity of urea solution due to production of ammonium. The common method for urease activity through the measurement of electric conductivity was used in our experiments. Urease activity was defined as the amount of

ammonium produced from 1 M solution of urea per minute. 5 ml of bacterial suspension was added to 45 ml of 1 M urea solution. Concentration of ammonium produced from urea was determined using an electric conductivity meter showing linear correlation ($R^2 = 0.99$) between the difference of molar concentrations of NH_4^+ and the changes of electric conductivity of solutions in mS/cm (**Figure 3.1**).
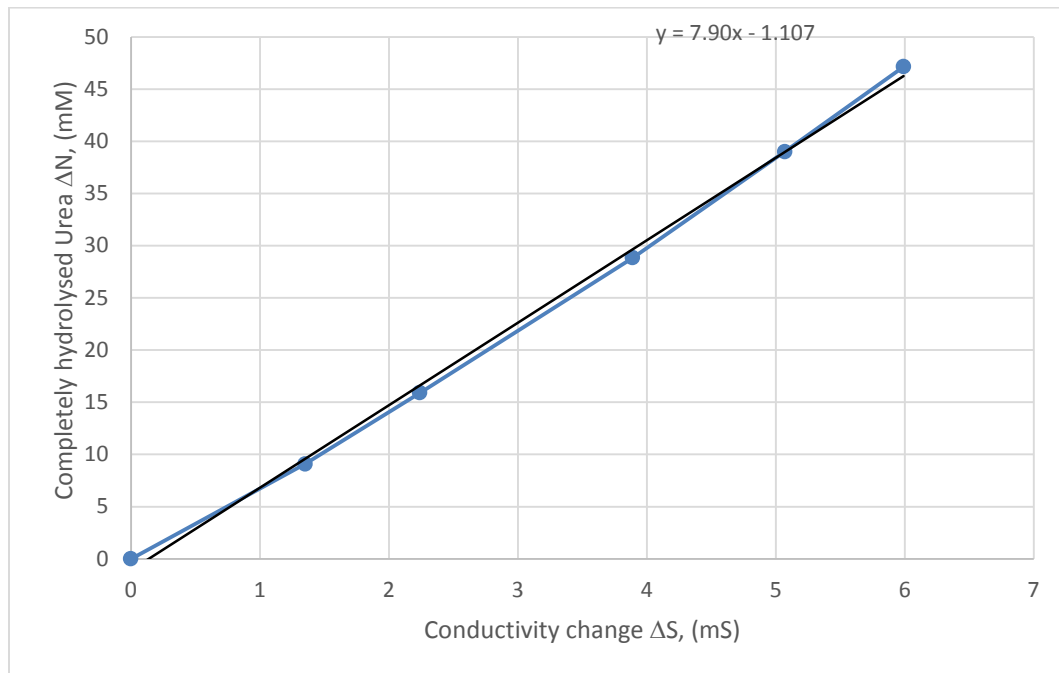
Figure 3.1 Calibration for urease activity

Enrichment culture, even having high initial urease activity, showed its decrease with each culture transfer to fresh medium after every six days of cultivation (Stabnikov et al., 2013). Urease activity of enrichment culture of urease-producing bacteria (UPB) decreased by 20 times after 5 transfers to fresh medium (**Figure 3.2**).
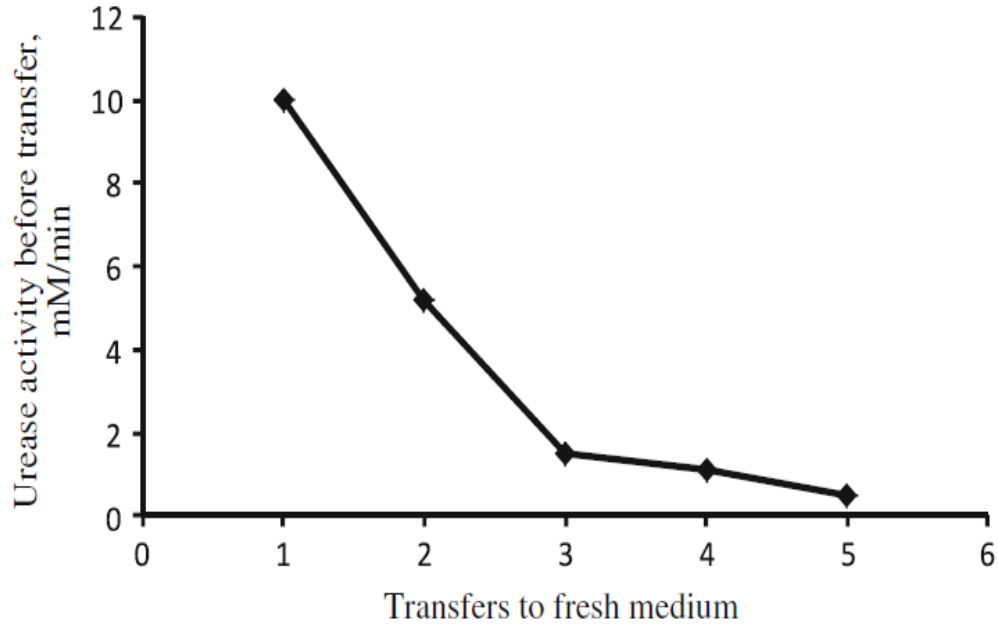
Figure 3.2 Change of urease activity of enrichment culture during transfers

Considering that one cycle of batch cultivation included approximately four generations, average calculated rate of elimination of urease activity was about 5% per one generation, so urease activity is an unstable feature of enrichment culture of urease-producing bacteria. The changes of urease activity in enrichment culture after transfers could be hypothetically explained by the genetic changes of the strains in this enrichment culture or the changes of microbial diversity of this enrichment culture. Therefore, enrichment cultures are not too suitable for MICP because of instability of urease activity.

3.2.3 *Cultivation of pure culture of UPB*

Pure culture of halophilic, alkaliphilic urease-producing bacteria *Sporosarcina pasteurii* DSMZ 33 (ATCC 11859, CCM 2056, NCIB 8841, and NCTC 4822) was purchased and used for further studies. Synonym of this strain is *Bacillus pasteurii*, grown on the Medium 220 + urea (20g/l), 30°C. Content of the Medium 220 (CASO AGAR, Merck 105458) is: peptone from casein 15.0 g; peptone from soymeal 5.0 g; NaCl 5.0 g; Agar 15.0 g; Distilled water 1000.0 ml. Adjust pH to 7.3. Medium is identical with Tryptone Soy Agar (Oxoid Cm131).

Cultivation of the bacteria *Sporosarcina pasteurii* DSMZ 33 has been done in the Fermentation Facility, Center for Crops Utilization Research, Iowa State University using 50 L sterilizable-in-place fermenter as shown in **Figure 3.3** (ABEC, Inc., Bethlehem, PA, USA). Centrifugation of biomass was done in CEPA Z-41 high-speed Centrifuge (Eppendorf AG, Hamburg, Germany), then biomass was suspensded in 1% of NaCl and freeze dried in VirTis Ultra 35 L Pilot Lyophilizer (VirTis SP Scientific, Stone Ridge, NY, USA). Medium for thermal sterilization included, g/L:

Tryptic Soy Broth DIFCO™, 30; Yeast Extract, 10g; NaCl, 20 ; $MnCl_2 \cdot 4H_2O$, 14 mg/L; $NiCl_2 \cdot 6H_2O$, 24 mg. Urea, 10 g/L was added to the sterile medium using the Millipore system for membrane sterilization. Cultivation was at 30^oC, aeration rate 1.5 L of air /L of

liquid/min with addition of silicone antifoam, and pH control at 7.3 with 1M HCl.

Consumption of oxygen started 6 h after inoculation and stopped after 24 h of cultivation

(**Figure 3.4**).
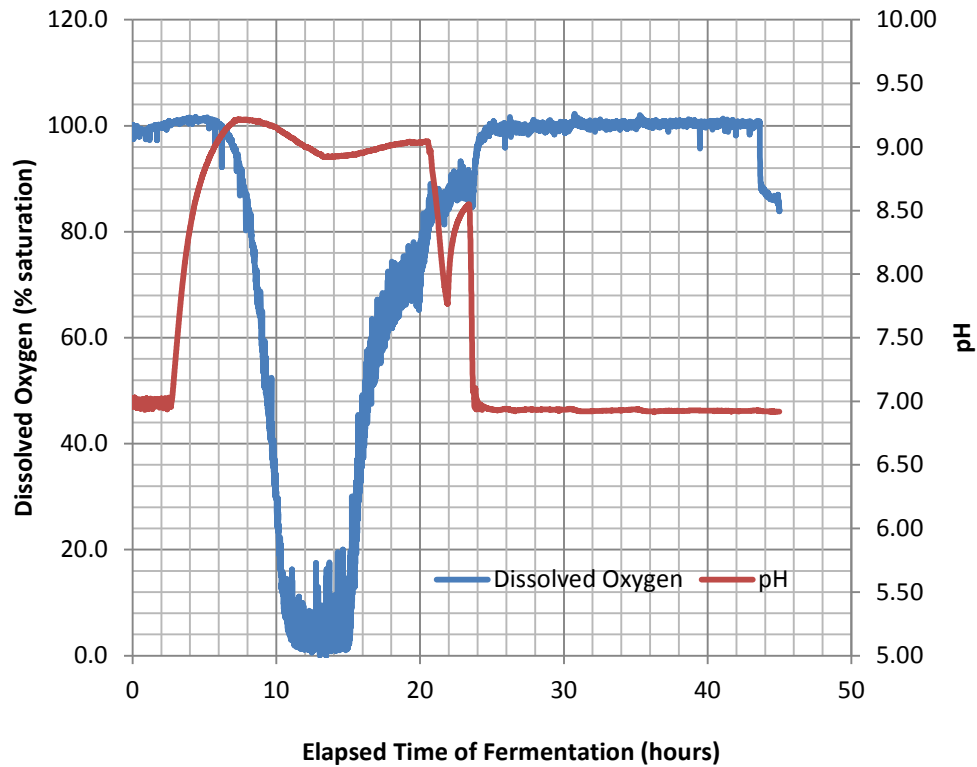
Figure 3.4 Dynamics of dissolved oxygen concentration and pH during cultivation of S.

pasteurii DSMZ 33.

Growth of biomass measured by OD at 650 nm and urease activity of culture (30 min

period of measurement) is shown in **Figure 3.5**. Maximum of biomass concentration was at

24 h, while urease activity increased even after 72 h of cultivation (**Figure 3.5**).
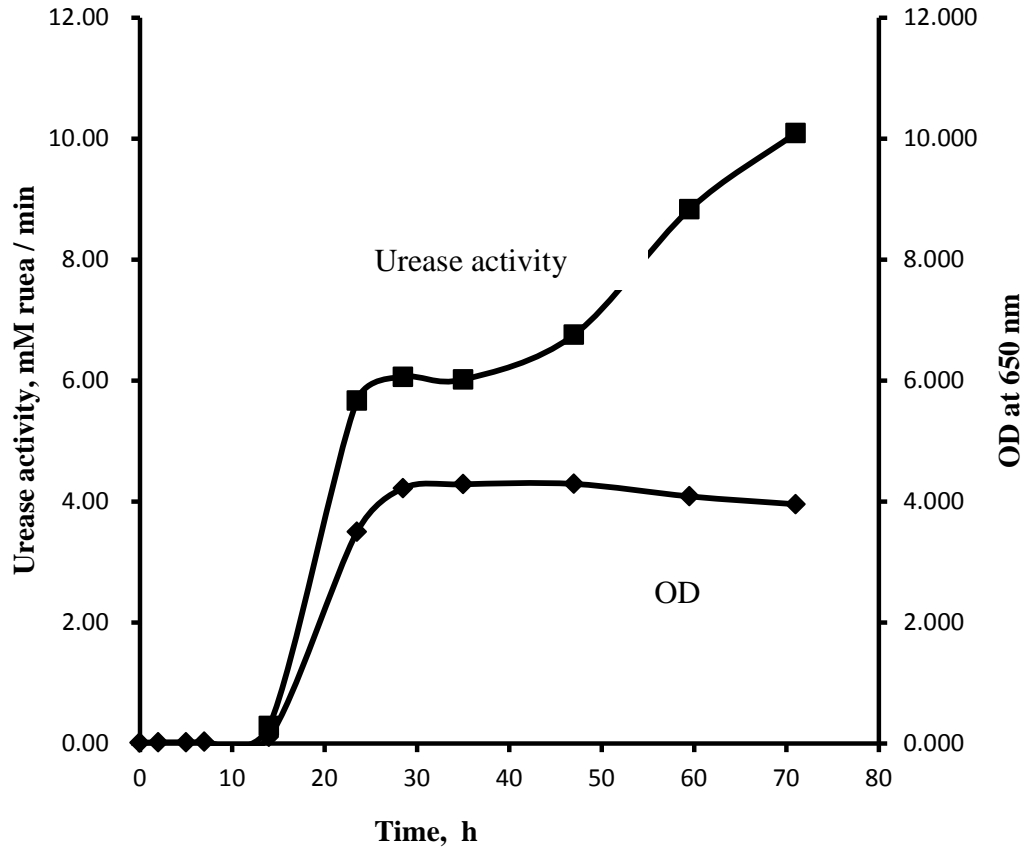
Figure 3.5 Growth of biomass measured by OD at 650 nm and urease activity of culture (30 min period of measurement)

pH of biomass after centrifugation was 6.1, initial urease activity was 14.4 mM/min but decreased twice for about 220 min (**Figure 3.6**).
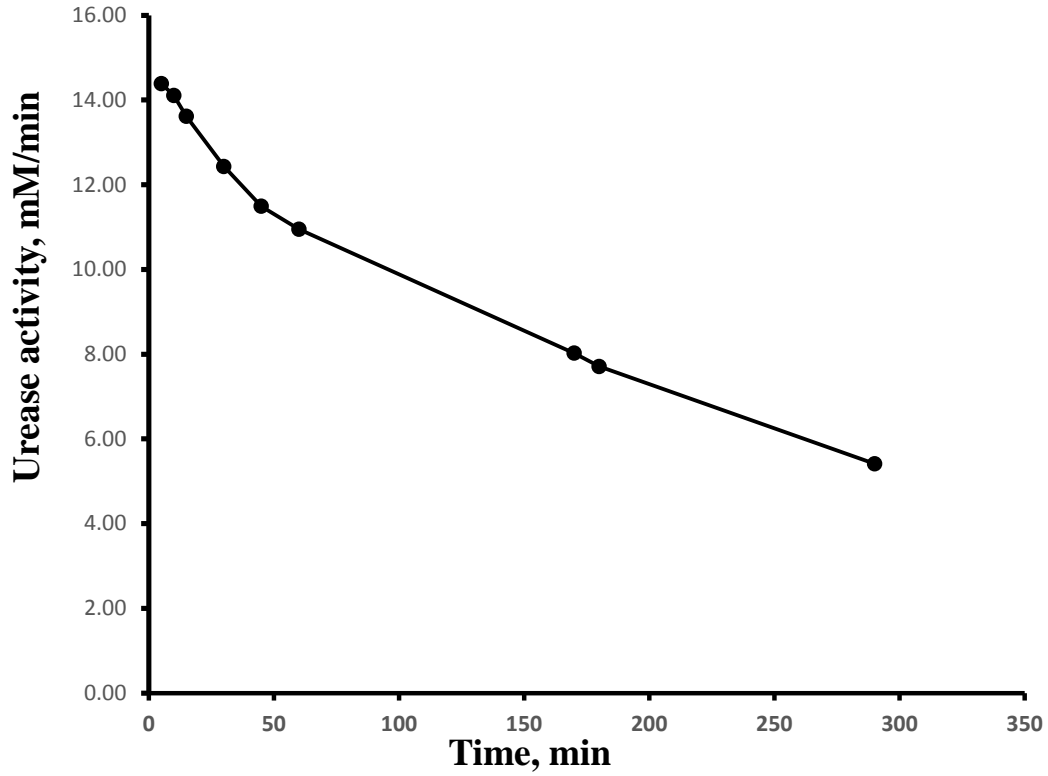
Figure 3.6 Urease activity of biomass before freeze drying (concentration of dry biomass in

1% NaCl was about 10 g/L).

However, urease activity of the bacterial suspension after freeze drying

(concentration of dry biomass 2 g/L) increased to 2 – 3.5 mM/min (**Figure 3.7**).
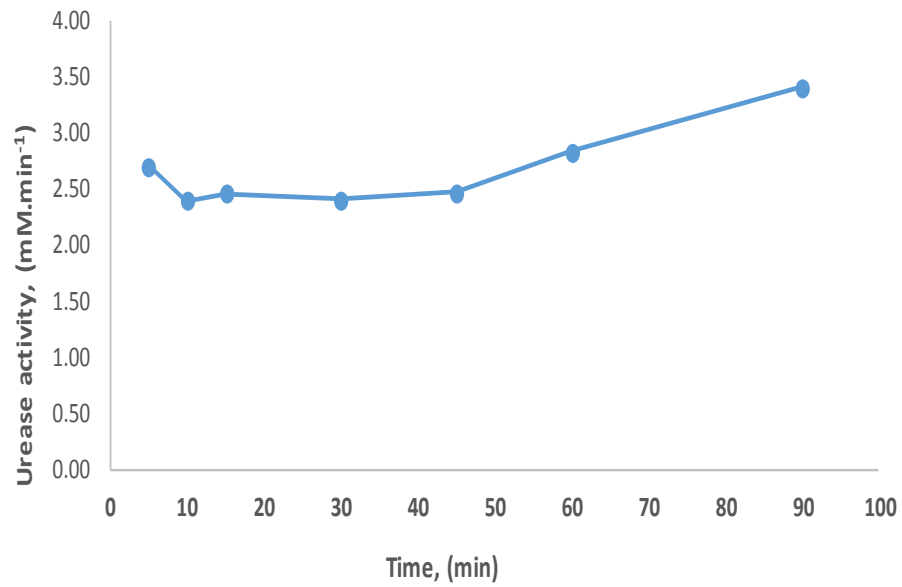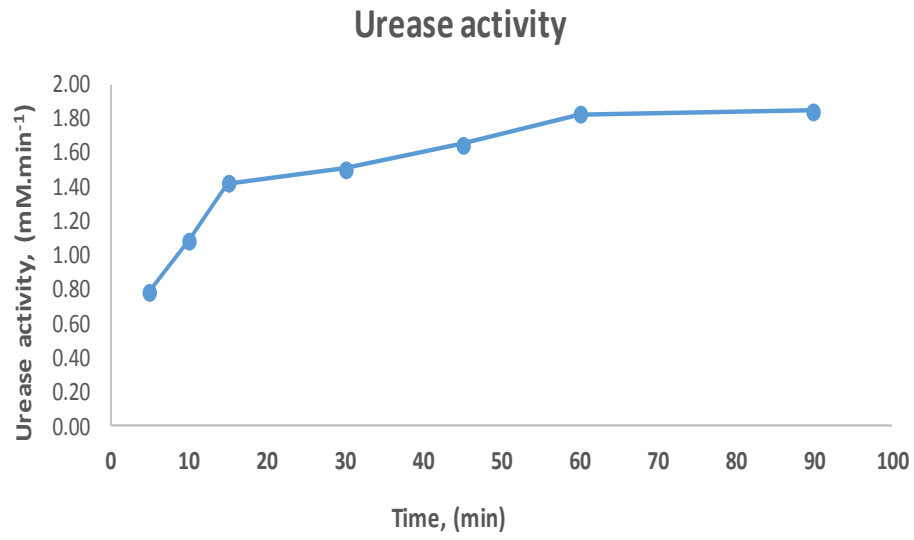
Figure 3.7 Urease activity after freeze drying (for two batches of cultivation in 50L

fermenter)

This dry biomass can be used as suspension in 1% of NaCl, or in dentiryfing medium, or urea solution for the biosealing of the gas bubbles in partially saturated sand.

3.3   Testing of microbial strains for simultaneous denitirification and biocementation

In principle, a strain of denitrifying bacteria can perform biocementation and biosealing at the same time because bioreduction of nitrate increase pH of medium and produce CO_2 for precipitation of $CaCO_3$. However, biogas production from nitrate by the strain *Acidovorax sp.* DN1 was inhibited in the solution of 0.75M CaCL2 and 1.5M of urea used for the bioseling. The following set of halophilic and alkaliphilic strains of bacteria with denitrifying ability and urease activity for aerobic growth on the urease-producing bacteria (UPB) medium and anaerobic growth on the denitrifying medium (DM) with ethanol (electron donor) and nitrate (electron acceptor) was tested: 1) *S. pasteurii* DSMZ33; 2) *Bacillus cohnii* DSMZ 6356; 3) *Bacillus sphaericus* (*Lysinibacillus sphaericus)* DSMZ 28; 4) *Paracoccus denitrificans* DSMZ 1405; and *Halomonas denitrificans* DSMZ 735 (ATCC 13511; NCIMB 700). The results are shown in the **Table 3.1**.

3.4   Measurements

Nitrite and nitrate ions were measured using WQ nitrate and nitrite sensors (NexSens Technology, Inc., Beevercreek, OH, USA), as well as using cadmium reduction method 8039 provided by the Hach Company with Hach DR 3900 spectrophotometer (Hach Company, Loveland, CO). pH and conductivity were measured using Fisher Scientific™ accumet™ AP85 Portable Waterproof pH/Conductivity Meter. Oxidation–reduction potential (ORP) was measured using Milwaukee MW500 ORP Monitor (Milwaukee Instruments, Inc., Rocky Mount, NC, U.S.A.).

Table 3.1 Parameters of the strains after 5 days of growth

Parameter after 5 days of cultivation	Strain				
	DSMZ 33	DSMZ 6356	DSMZ 28	DSMZ 1405	*Acidovorax sp.* DN1
Anaerobic growth in denitrifying medium (non-halophilic denitrification ability)	+	-	+	+	+
Production of gas bubbles Anaerobic growth in denitrifying medium with (non-halophilic ability to produce N_2 from nitrate)	-	-	+	+	+
Anaerobic growth in denitrifying medium with 2 M of NaCl and production of gas bubbles (ability for halophilic denitrification)	n.d.	n.d.	n.d.	n.d.	-
Anaerobic growth in denitrifying medium with 1 M of $CaCl_2$ and 1 M of urea and production of gas bubbles (ability for halophilic denitrification in biocementing solution)	n.d.	n.d.	n.d.	n.d.	-
Final concentration of nitrate in denitrifying medium, mg/L	79	122	1	57	0
Final pH after anaerobic growth in denitrifying medium (a sign of denitrification)	9.4	7.3	9.3	7.6	7.5
Final pH after aerobic growth in UPB medium (a sign of urease activity)	9.2	9.2	9.0	9.0	7
Urease activity of bacteria grown in UPB medium, mM/min	1.12	0.33	0.04	0	0
Urease activity of bacteria grown anaerobically in denitrifying medium, mM/min	0.4	0	0	0	0

CHAPTER IV

EXPERIMENTAL STUDY

4.1 Introduction

Two preliminary experiments on biogas generation and gas bubbles stability in saturated sand using centrifugation analysis were conducted in 15 ml and 50 ml tubes separately. As discussed in Section 3.2, denitrification bacteria must not be injected together with biocementation solution which has high concentrations of $CaCl_2$ and urea. Therefore, in 15 ml tubes denitrification bacteria and denitrification medium were added first followed by the injection of biocementation bacteria suspended in biocementation solution. For 50 ml tubes experiment, another sequential treatment method was developed. In this method, mixture of denitrification bacteria, denitrification medium and biocementation bacteria was firstly injected followed by the injection of biocementation solution. Control and experiments were done in triplicates.

In order to further verify the improvement of the stability of the biogas bubbles with biosealing, sand columns were tested under water flow conditions for 10 days. The biosealing the bacterial suspension of *S. pasteurii* DSMZ33 was introduced altogether with the suspension of *Acidovorax sp.* DN1 and denitrification medium.

4.2 Centrifugation analysis of biogas production and gas bubbles stability

To analyze biogas production and gas bubbles stability in sand nine Corning®15 ml screw cap conical bottom centrifuge plastic tubes were filled with 4.3 ml of denitrifying medium, then 0.2 mL of suspension of enrichment culture of denitrifying bacteria. 10 mL of

sand was slowly added from the tube top. When 50 mL- tubes have been used for experiment, 14.4 mL of denitrifying medium was mixed with 0.6 mL of denitrifying bacteria suspension, and filled in with 30 mL of sand that was slow added from the tube top.

The level of liquid was about 1 cm higher than level of sand which is referred as zero-level in the following discussion. The desaturation of sand was monitored by the increase of the level of liquid above zero-level due to production of gas bubbles in sand voids and water displacement from the voids. As shown in Fig. 4.1, the initial level of wet sand (volume S_0) in the tube was bigger that the volume of added dry sand (S_d) and was slightly below the zero-level of liquid (volume W_0). The gas space of the tubes was flashed with nitrogen gas and the tubes were sealed with the caps. After 5 days of the denitrification process, the level of water (volume W_1) increased due to production of biogas ($= W_1 - W_0$) but the level of sand (volume S_1) did not changed ($S_1 = S_0$). Specific production of biogas ($N_2 + CO_2$) was calculated as ($W_1 - W_0)/S_d$, mL of gas/mL of dry sand.

After biogas production in saturated sand, three control tubes were centrifuged for 20 min at $600 \times g$. The level of water decreased slightly (volume W_2) due to release of gas bubbles from sand ($= W_1 - W_2$), and the level of sand (volume S_2) was decreased also due to compression of sand particles ($= S_1 - S_2$). The loss of gas bubbles after denitrification and centrifugation was calculated as 100% x ($W_1 - W_2)/(W_1 - W_0$). Loose sand settlement after biogas production and centrifugation was calculated as 100% x ($S_1 - S_2)/S_1$.

In first option of the biosealing – surface biosealing, the biocementation bacteria suspended in biocementation solution was applied only on the surface of the sand approximate 1 cm below the level of sand. In the second option of the biosealing – bulk biosealing, the biocementation bacteria suspended in biocementation solution was injected

slowly, approximately 1 ml/min, from the bottom of the tube with sand. The volume of liquid

during injection for biosealing was maintained constant at zero-level ($W_3 = W_0$) by the

removal of liquid to 1 cm above the level of sand. After three days of incubation the level of

liquid was increased (W_4) showing production of biogas (CO_2) during biocementation. The

experimental tubes were centrifuged for 15 min at $600 \times g$. The level of liquid in the tubes

after centrifugation (volume W_5) decreased due to release of gas bubbles from sand, and

level of sand (volume S_5) decreased also due to compression of partially desaturated sand.

The loss of gas bubbles after biosealing and centrifugation was calculated as 100% x (W_4 -

W_5)/(W_1 - W_0). Sand settlement after biosealing and centifugation was calculated as 100% x

(S_3 - S_2)/S_2. The differences between the tubes and the procedures described above are shown

in **Figure 4.1**. The changed levels of liquid (W) and sand (S) are shown in **Table 4.1** and

Table 4.2. Control and experiments have been done in triplicates. Mean value ± standard

deviations are shown in the data comparisons.
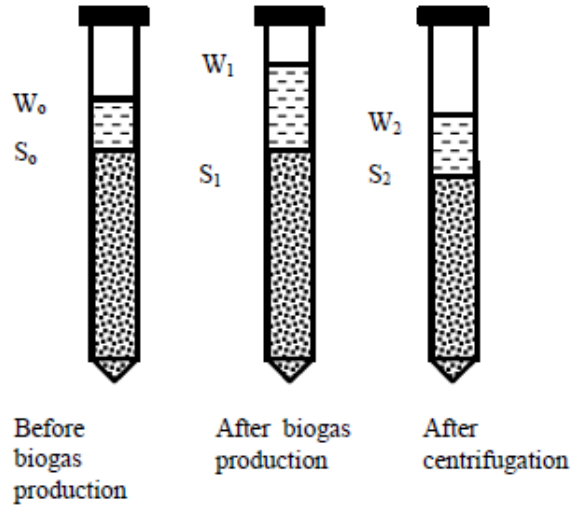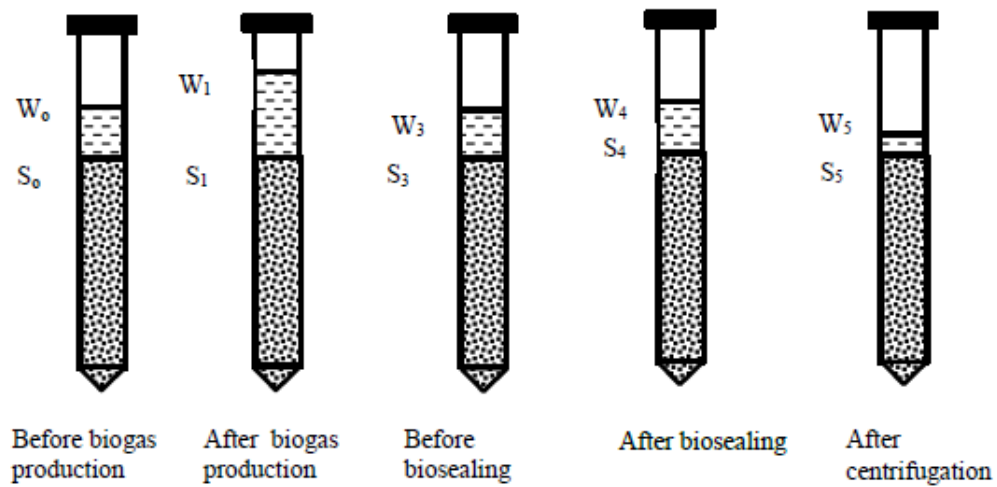
Figure 4.1 Measurement of biogas production and biogas bubbles stability after centrifugation.

Table 4.1 Centrifugation analysis of biogas production and gas bubbles stability in 15 ml tubes

Centrifugation analysis of biogas production and gas bubbles stability in 15 ml tubes										
1a, 2a, 3a - no biosealing, 1b, 2b, 3b - bulk biosealing, 1c, 2c, 3c - surface biosealing										
S.L. - Sand Level, W.L. - Water Level (ml)										
		1a	2a	3a	1b	2b	3b	1c	2c	3c
Initial	S.L.	10.8	9.6	10.4	10.6	9.8	10.4	10.5	9.5	10
	W.L	11.5	10.5	11	11.1	10.5	11	10.9	10.4	10.7
After denitrification	S.L.	10.8	9.6	10.4	10.6	9.8	10.4	10.5	9.5	10
	W.L.	11.8	10.8	11.5	11.4	10.9	11.5	11.4	10.7	11
After biocementation	S.L.	10.8	9.7	10.5	10.4	9.5	10.1	10.5	9.5	10.1
	W.L.	11.7	10.7	11.5	10.7	9.5	10.1	11.6	10.7	11
After centrifugation	S.L.	10.7	9.5	10.3	10.4	9.5	10.1	10.4	9.5	10
	W.L.	11.6	10.5	11.4	10.4	9.5	10.1	11.5	10.7	11

Table 4.2 Centrifugation analysis of biogas production and gas bubbles stability in 50 ml tubes

Centrifugation analysis of biogas production and gas bubbles stability in 50 ml tubes							
1a, 2a, 3a - no biosealing, 1b, 2b, 3b - bulk biosealing							
S.L. - Sand Level, W.L. - Water Level (ml)							
		1a	2a	3a	1b	2b	3b
Initial	S.L.	36.25	36	35.5	32.5	33	33
	W.L	38	38	37.5	36	36	36.25
After Denitrification	S.L.	37.5	37	36.5	35	35	35
	W.L.	40	39.5	38.75	37	37	37.5
Biogas Production (ml of gas/ml of sand)		0.055	0.042	0.035	0.031	0.03	0.038
After biosealing	S.L.	37	36.5	36.5	34	34.5	35
	W.L.	40	40	39.5	36.25	36.25	37
After centrifugation	S.L.	36	36	35.5	34	34.5	35
	W.L.	39	39	39	36.25	36.25	37
Loss of gas bubbles		50%	67%	40%	0%	0%	0.00%
Sand settlement		2.80%	1.40%	2.80%	0.00%	0%	0.00%

4.3 Stability of biogas bubbles in sand columns under seepage

Six acrylic columns filled with saturated sand were set up as shown in **Figure 4.2** to examine the effect of water flow on biogas bubbles stability after denitrification only and after sequential denitrification and biocementation.

The length of each column was 150 cm and the inner diameter was 7.62 cm. The length of the sand sample was 100 cm as shown in **Figure 4.2**(a). A 15 cm-thick layer of gravel was placed on the bottom of the columns. 1.9 L of water and then 7 kg of dry Ottawa sand were added into each column so that an initial sand height was 100 cm. Dry sand was added slowly, approximately 200 g/min so that the initial degree of saturation could be assumed as 100%. Two water tanks with a water head difference of 10 cm were connected to the bottom and the top of the columns in order to create either upward or downward seepage with a hydraulic gradient of 0.1. Another water reservoir was used to supply water and collect water for the two water tanks.

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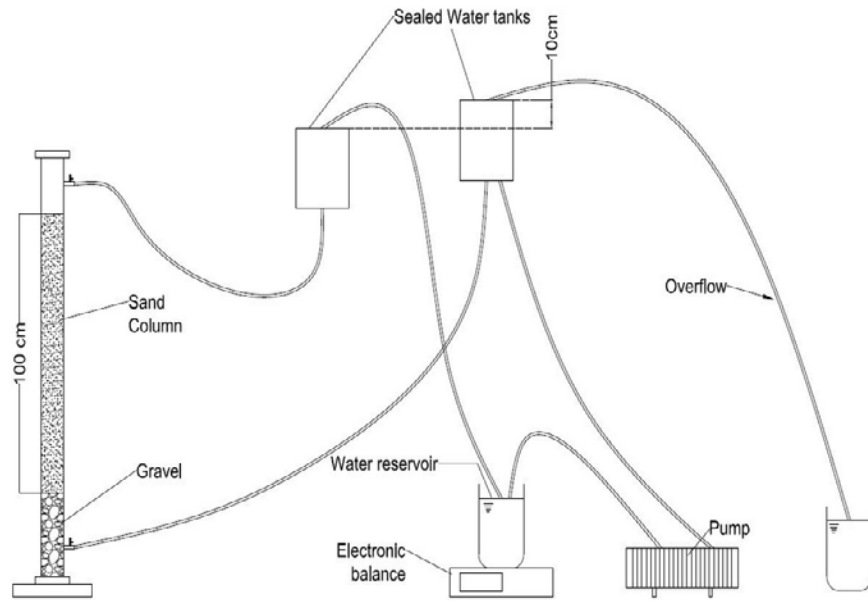
(a)

(b)

Figure 4.2 Set up of the test for biogas bubbles sustainability: a) schematics of one column;

b) picture of the facility.

The conditions of the sand treatment and gas bubbles stability testing are shown in **Table 4.3**.

Table 4.3 The conditions of the sand treatment and gas bubbles stability testing

Column No	Sand treatment	Flow of water during gas bubbles stability test
Column 1a	Denitrification process only	Hydrostatic condition
Column 2a	Denitrification process only	Downward flow condition
Column 3a	Denitrification process only	Upward flow condition
Column 1b	Denitrification followed by biosealing	Hydrostatic condition
Column 2b	Denitrification followed by biosealing	Downward flow condition
Column 3b	Denitrification followed by biosealing	Upward flow condition

The volume of generated biogas bubbles during denitirification was calculated by the increase of the water level in the columns. Two parameters were used to evaluate biogas bubbles stability: 1) the permeability of sand, and 2) the degree of saturation. The permeability was determined by the measuring the amount of water outflow for a known duration under a constant head. The reduced degree of sand saturation was determined by measuring the decrease of the sand level and the decrease of water volume in the water reservoir. This water volume replaced the volume of biogas bubbles in sand.

The first step for reducing the degree of saturation of the sand sample was to initiate the denitrification process to generate biogas. The bacterial suspension and the components of the media for denitrification and biosealing were the same as described in the test with the tubes. However, the concentrations of nitrate and ethanol in the denitrification medium were doubled to achieve the target volume of gas generation and the target degree of sand saturation. A mixture of 1.6 L of denitrification medium and 0.3 L of denitrifying bacterial culture *Acidovorax sp.* DN1 were added to each of the six columns. Additionally, 2g of

freeze dried biomass of the biosealing bacteria *S. pasteurii* DSMZ 33 were added to the columns 1b, 2b, and 3b.

The level of water and the level of sand in each column were monitored. When the water level became constant and the denitrification was completed, biocementation solution was injected slowly to generate bioseal. In the columns 1b, 2b, and 3b, the biocementation solution containing 1 M $CaCl_2$ and 2 M urea which replaced the denitrification medium in the columns using downward flow at a flow rate of 10 ml/min. Four of the six samples (Column 2a, 3a, 2b, and 3b) were tested under either downward flow or upward flow conditions.

CHAPTER V

RESULTS AND DISCUSSIONS

5.1 Centrifugation analysis of biogas production and biosealing of the gas bubbles in 15 mL tubes

As shown in **Table 4.1**, production of biogas was 0.04 ± 0.01, mL biogas /mL of dry sand. Degree of saturation of sand after biogas production decreased from 100% to 92% $\pm$ 2%. The loss of gas bubbles after denitrification was 26 ± 6 % but the biosealing of wet sand decreased gas bubbles loss to zero. The sand settlement after denitrification was 1.6 ± 0.5 % but biosealing resulted in no sand settlement. **Figure 5.1** shows a microscopic image of one sample with small quantity of biocementation biosealing the sand grains.

1C top 150x
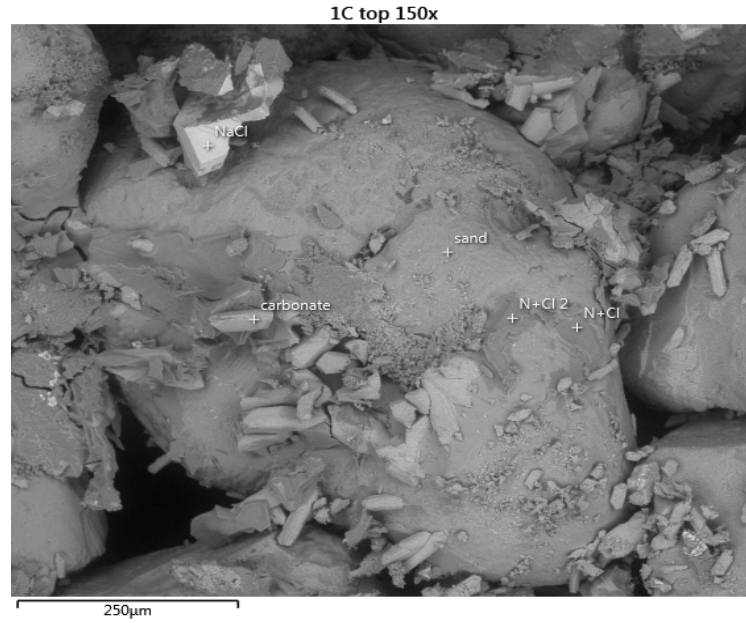
(a)
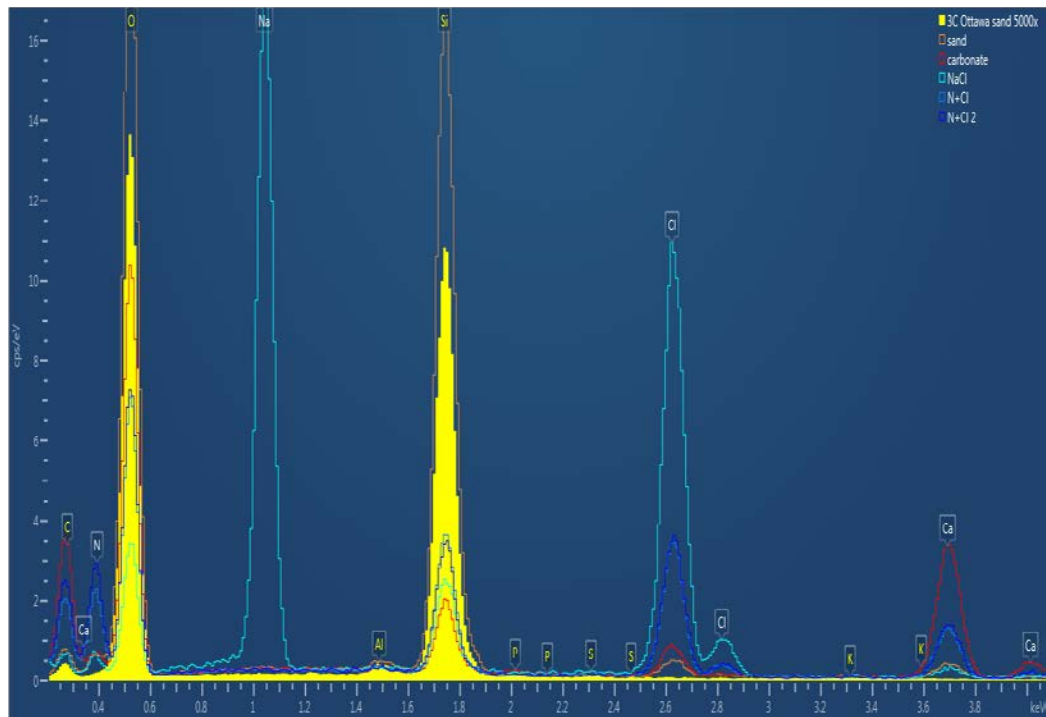
(b)

Figure 5.1 Microscopic image of biocemented sand:a) SEM image; and b) Chemical analysis

(Ca shows the carbonate location and it is correlated with C and O)

5.2   Biogas production and biosealing of biogas bubbles in 50 mL tube

During biogas production, water level increased for 70 h of incubation then slightly

decreased, probably due to solubility of CO_2 in water (**Figure 5.2**).
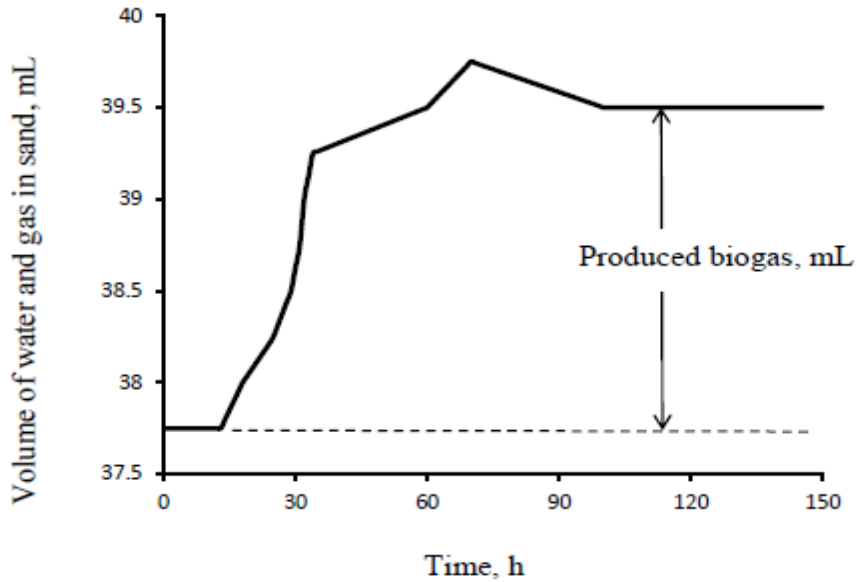
Figure 5.2 Dynamics of biogas production in saturated sand.

The data of biogas production, biosealing, and stability of biogas bubbles in sand in

the 50 mL tube are shown in **Table 4.2**. The loss of gas bubbles after denitrification was $53 \pm$

13 % while the biosealing of wet sand decreased gas bubbles loss to zero. The sand

settlement after denitrification was 2.3 ± 0.8 % while biosealing resulted in no sand

settlement.

5.3   Stability of biogas bubbles in sand columns under seepage

Level of water in the columns reached maximum after 2 days in case when denitrifying bacteria were introduced into the columns altogether with UPB and after 4 days in case when denitrifying bacteria were introduced into the columns without UPB (**Figure 5.3**). The curves 1b, 2b, and 3b show the process where denitrifying bacteria and UPB were added altogether; the curves 1a, 2a, and 3a show the process where only denitrifying bacteria added into the columns with saturated sand. The conditions in six sand columns after denitrification process are shown in the **Table 5.2**.

Table 5.1 Biogas production and biosealing of the gas bubbles

Parameter	Biogas production by *Acidovorax sp.* DN1without sealing (control)	Biogas production and sealing made by simultaneous introduction of *S. pasteurii* DSMZ33 and *Acidovorax sp.* DN1	Biogas production and sealing made by separate introduction of *S. pasteurii* DSMZ33 and *Acidovorax sp.* DN1
Biogas production, mL of gas/mL of dry sand	0.044 ± 0.008	0.033 ± 0.003	0.041 ± 0.006
Degree of Saturation of sand,%	91 ± 2	93 ± 6	92 ± 1
Loss of biogas bubbles in sand, %	52 ± 11	0	0
Sand settlement, %	2.3 ± 0.7	0	0
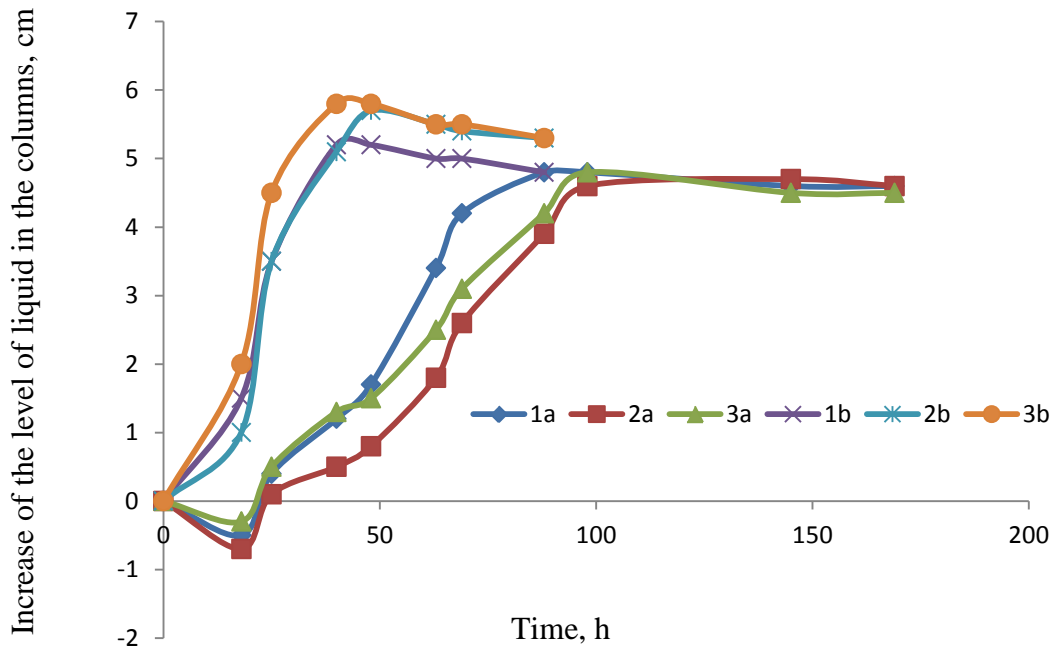
Figure 5.3 Changes of water level during denitirification.

Table 5.2 The degree of sand saturation and pH in six sand columns after denitrification

process

Column	Degree of saturation, %	pH
1a	88.5	6.5
2a	89.7	7
3a	89.6	7.2
1b	89.8	7.3
2b	87.2	7.1
3b	87.9	7.4

Even though water level slightly decrease after denitrification process was completed as shown in **Figure 5.3** which could be explained by the dissolution of CO_2 gas, there was no variation in water level any more in 10 days for column 1a and 1b under hydrostatic condition. Under downward and upward flow conditions, without biosealing of the gas bubbles the sand column became fully saturated in 3 days. Meanwhile with only one treatment of biocementation there was 4.5% reduction in degree of saturation in the first 2 days and the gas bubbles remained steady in another 8 days under downward flow. A little more gas bubbles lost under upward flow condition than that under downward flow condition but still there were half of gas bubbles remained. The change in degree of saturation and the change in the coefficient of permeability are shown in **Figure 5.4** and **Figure 5.5**.
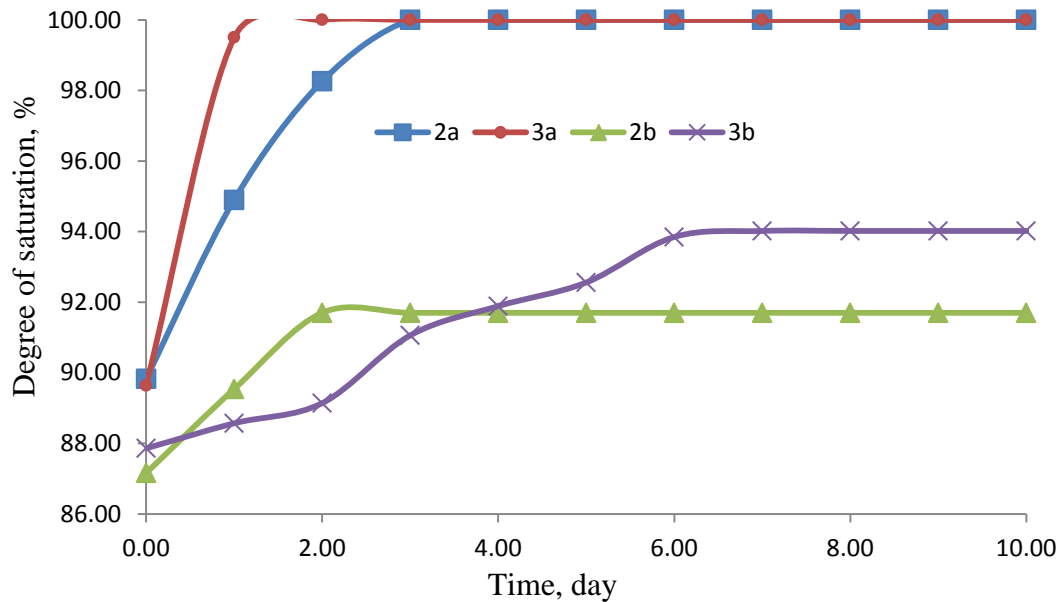
Figure 5.4 Changes in degree of saturation.
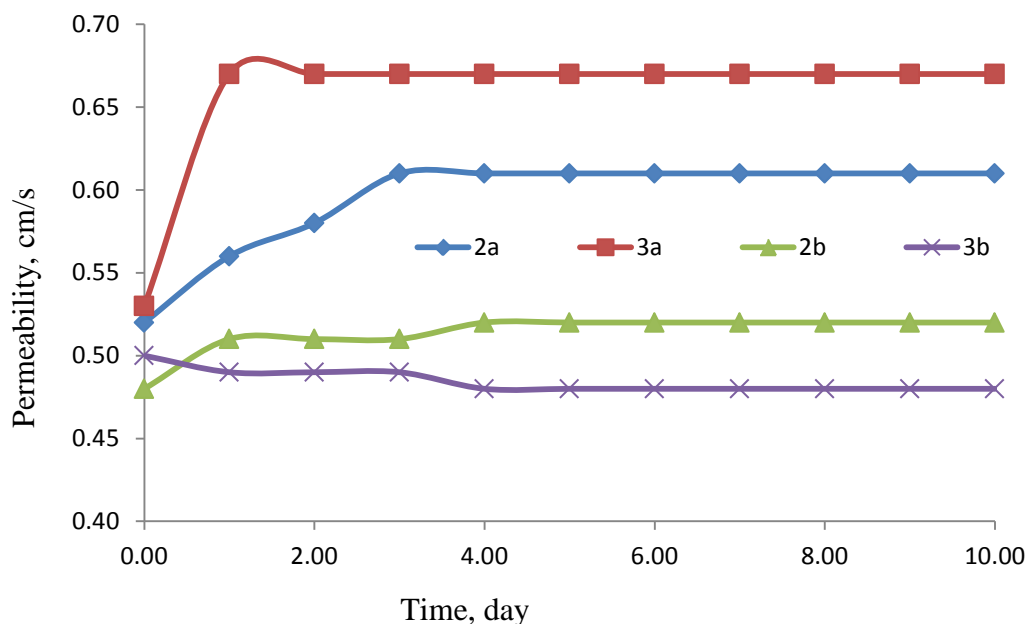
Figure 5.5 Changes in the coefficient of permeability.

5.4 Discussion

There are known experimental data on the movement of inert gas bubbles in saturated porous soil (He et al., 2013), which is very sensitive to changes in pressure and in the terminal velocity as described by the Stokes formula (Etiope and Lombardi, 1996). Biosealing of partially saturated sand showed high efficiency of stabilization of the biogas bubbles that were produced in saturated sand. So, the production of biogas in situ and biosealing of the gas bubbles could be useful for sustainable mitigation of sand liquefaction in case of groundwater flow through saturated sand that can remove gas bubbles from the sand pores. The difference between biosealing and biocementation is only in the quantity of the added reagents. So, at low quantity of the added reagents the precipitation of calcium carbonate is going mainly in the sites of sand grain contacts, which is sealing the microchannels (**Figure 5.6** a, c). However, at higher quantity of added reagents precipitation

will be also in the sand pores creating high strength of the biocemented sand (**Figure 5.6 b,c**).

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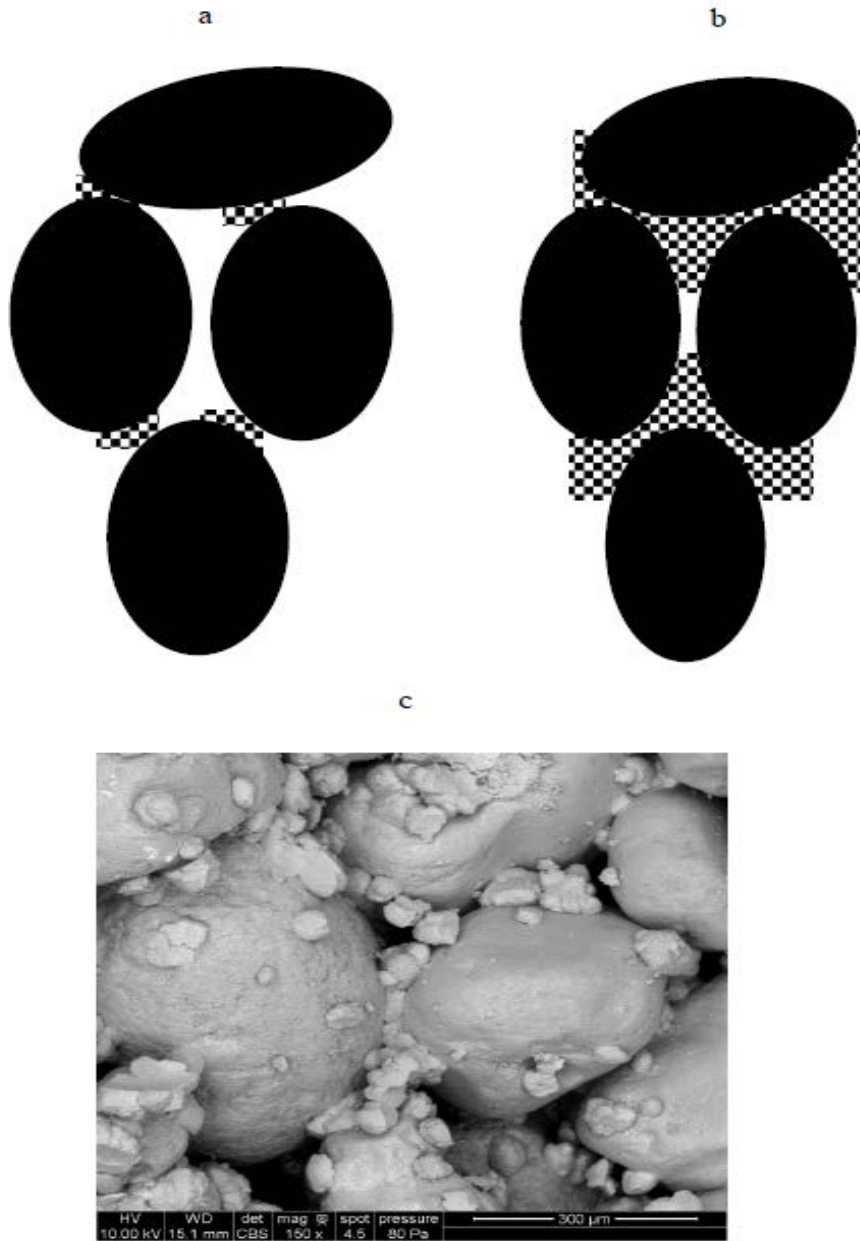
Figure 5.6 Biosealing and biocementation in saturated sand. a: biosealing of the channels in sand; b: biocementation of the pores in sand; c: SEM picture of biosealing and biocementation in saturated sand.

The content of precipitated calcium carbonate in dry sand for complete biosealing of biogas in our experiments was about 2 % (w/w), which is four times lower than content of calcium carbonate to create the unconfined compressive strength of biocemented sand at the level 250 kPa (Ivanov et al., 2012). Therefore, the cost of the biogas production *in situ* combined with the biosealing of the gas bubbles is significantly lower than the cost of saturated sand biocementation for mitigation of sand liquefaction.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1   Conclusions

Two types of experimental studies were carried out. One used centrifugation using test tubes and another model tests with sand columns.

1). Biogas bubbles produced in partially saturated sand in quantity about 4% (v/v) were unstable under centrifugation acceleration 600×g. The biogas bubbles were stabilized in the sand pores after biosealing using microbially-induced carbonate precipitation (MICP). The settlement of saturated sand was around 2% but was decreased to zero after MICP with 2% of precipitated calcium carbonate in partially saturated sand.

2). The results of the sand column tests have shown that gas bubbles were stable under hydrostatic conditions. However gas bubbles in sand were not stable under upward or downward flows if biosealing is not applied.

3). With the use of biosealing, the stability of the gas bubbles can be significantly enhanced. Even after 240 h of a vertical upward flow with hydraulic gradient 0.1 the degree of saturation increased only from 87.2% -87.9% to 91.7%-94.0%.

4). Therefore, the sequential biogas production in saturated sand and biosealing of the gas bubbles in sand pores could be useful for sustainable mitigation of sand liquefaction in case of groundwater upflow through saturated sand that can remove gas bubbles from sand pores. The cost of the sequential biogas production in saturated sand and biosealing of biogas bubbles in sand pores could be significantly lower than the cost of biocementation of the saturated sand.

6.2   Recommendations

Two injections of the solutions for mitigation of sand liquefaction have been used in our experiments. Meanwhile, one simultaneous injection of all reagents and bioagents into saturated sand could be the best option from the practical point of view. However, simultaneous injection of the suspensions of biogas-producing strain *Acidovorax sp.* DN1 and biosealing strain *S. pasteurii* DSMZ33 altogether with denitrifying and biosealing media did not produce biogas because of high salinity of the medium that was used for biosealing. Therefore, two injections of the different solutions for mitigation of sand liquefaction are essential for biogas production and its biosealing in sand. The halophilic denitrification bacteria could be the solution to the problem.

Another effective way of biomitigation of sand liquefaction could be application of one bacterial species for both biogas production and biosealing. Even though strain *S. pasteurii* DSMZ33, which was used for the biosealing of the gas bubbles, was able to reduce nitrate with ethanol, the final product of denitrification was nitrite instead of nitrogen gas. More halophilic and alkaliphilic bacteria strains need to be studied to find the preferable strain with both denitrification and urease-producing ability.

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APPENDIX

Biogas generation rate in sand columns						
	Water Level Change (cm)					
Time (hrs)	1a	2a	3a	1b	2b	3b
0	0	0	0	0	0	0
18	-0.5	-0.7	-0.3	1.5	1	2
25	0.4	0.1	0.5	3.5	3.5	4.5
40	1.2	0.5	1.3	5.2	5.1	5.8
48	1.7	0.8	1.5	5.2	5.7	5.8
63	3.4	1.8	2.5	5	5.5	5.5
69	4.2	2.6	3.1	5	5.4	5.5
88	4.8	3.9	4.2	4.8	5.3	5.3
98	4.8	4.6	4.8	4.8	5.3	5.3
145	4.6	4.7	4.5	4.8	5.3	5.3
169	4.6	4.6	4.5	4.8	5.3	5.3

Biogas stability test under seepage in sand columns				
2a, 3a: denitrification process only; 2b, 3b: denitrification followed by biosealing				
	2a: downward flow	3a: upward flow	2b: downward flow	3b: upward flow
Initial Water Height (cm)	104.00	103.00	101.30	102.30
Initial Sand Height (cm)	102.50	101.00	99.00	101.50
Initial Degree of Saturation (%)	100.00	100.00	100.00	100.00
Initial pH	7.00	7.00	6.80	7.00
Water Level Increase after den. (cm)	4.60	4.50	5.30	5.30
Sand Level Increase after den. (cm)	0.00	0.35	0.20	0.10
Gas Generation (cm3)	209.76	205.20	241.68	241.68
Degree of Saturation after den. (%)	89.68	89.64	87.16	87.86
pH after den.	7.00	7.20	7.10	7.40
pH after biosealing	n/a	n/a	9.60	9.50
0 day				
Measured Degree of Saturation (%)	89.83	89.64	87.16	87.86
Permeability (cm/s)	0.52	0.53	0.47	0.5
1 day				
Measured Degree of Saturation (%)	94.90	99.60	89.54	88.57
Permeability (cm/s)	0.56	0.67	0.51	0.49
2 day				
Measured Degree of Saturation (%)	98.26	100.00	91.70	89.14
Permeability (cm/s)	0.58	0.67	0.51	0.49

Continuned				
3 day				
Measured Degree of Saturation (%)	100.00	100.00	91.70	91.06
Permeability (cm/s)	0.61	0.67	0.51	0.49
4 day				
Measured Degree of Saturation (%)	100.00	100.00	91.70	91.89
Permeability (cm/s)	0.61	0.67	0.52	0.48
5 day				
Measured Degree of Saturation (%)	100.00	100.00	91.70	92.56
Permeability (cm/s)	0.61	0.67	0.52	0.48
6 day				
Measured Degree of Saturation (%)	100.00	100.00	91.70	93.85
Permeability (cm/s)	0.61	0.67	0.52	0.48
7 day				
Measured Degree of Saturation (%)	100	100.00	91.70	94.02
Permeability (cm/s)	0.61	0.67	0.52	0.48
8 day				
Measured Degree of Saturation (%)	100	100.00	91.70	94.02
Permeability (cm/s)	0.61	0.67	0.52	0.48
9 day				
Measured Degree of Saturation (%)	100	100.00	91.70	94.02
Permeability (cm/s)	0.61	0.67	0.52	0.48
10 day				
Measured Degree of Saturation (%)	100	100.00	91.70	94.02
Permeability (cm/s)	0.61	0.67	0.52	0.48