Carbonate Cementation via Plant Derived Urease

Cimentation carbonatée par l'utilisation d'uréase issue de plantes

Hamdan N., Kavazanjian Jr. E., O'Donnell S.
School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ 85287-5306; PH: (480) 965-3997

ABSTRACT: The use of plant-derived urease enzyme to induce calcium carbonate ($CaCO_3$) cementation has been demonstrated through laboratory column tests. Benefits of the use of plant-derived urease over the use of microbially-generated urease to induce carbonate cementation include the small size of the enzyme, which permits penetration into finer grained soils and makes the process less sensitive to bioplugging, and the availability of 100% of the carbon in the substrate for conversion to $CaCO_3$. The laboratory column tests employed both Ottawa 20-30 silica sand and finer-grained F-60 silica sand. The laboratory column specimens were prepared in a variety of manners and showed varying degrees of cementation and carbonate yield. Triaxial tests performed on cemented specimens showed significant strength increases over non-cemented specimens. These tests confirm the feasibility of using plant-derived urease to induce carbonate cementation in sand and provide valuable insight into the factors that must be considered in developing practical applications for ureolytic carbonate precipitation using plant-derived urease enzyme.

RÉSUMÉ : La cimentation de sable par du carbonate de calcium ($CaCO_3$) produit par l'enzyme uréase obtenue à partir de plantes a été réalisée en laboratoire. Les avantages d'utiliser de l'uréase obtenue de plantes plutôt que de l'uréase produite microbilogiquement pour produire la cimentation carbonatée sont la petite taille de l'enzyme qui permet la pénétration dans les sols fins et rend le processus moins sujet au colmatage biologique et la disponibilité à 100% du carbone présent dans le substratum pour conversion en $CaCO_3$. Des essais en colonnes ont été réalisés sur deux sables de silice dits Ottawa 20-30 et F-60 (plus fin). Les échantillons ont été préparés de différentes manières et ont atteint des degrés de cimentation variés et des productions de carbonate différentes. Les résultats des essais de compression triaxiale sur des échantillons cimentés et des échantillons non-cimentés indiquent que les premiers sont beaucoup plus résistants. Ces essais confirment que l'uréase obtenue à partir de plantes peut être utilisée pour induire une cimentation carbonatée dans les sables. De plus ces essais ont permis de d'identifier les facteurs à considérer pour développer des applications pratiques pour l'utilisation de la précipitation carbonatée « uréolytique » en utilisant l'uréase issue de plantes.

KEYWORDS: carbonate, cementation, urease, calcite, soil improvement

1. INTRODUCTION

1.1 *Background*

The potential for using plant-derived urease enzyme to cement sands by inducing calcium carbonate ($CaCO_3$) precipitation has been demonstrated through a series of laboratory column tests on two different gradations of silica sand. The use of microbially induced carbonate precipitation (MICP) to cement cohesionless soils has recently received substantial attention from geotechnical researchers (Burbank et al. 2012, Chou et al. 2011, Dejong et al. 2010, Harkes et al. 2010, van Paassen et al. 2010). The MICP mechanism most often discussed in the literature and most advanced in terms of field application is hydrolysis of urea (ureolytic hydrolysis). MICP via ureolytic hydrolysis relies on microbes to generate urease enzyme, which then serves as a catalyst for the precipitation reaction. The use of plant-derived urease (enzymatic ureolytic hydrolysis) to induce $CaCO_3$ precipitation eliminates the need for microbes in the $CaCO_3$ precipitation process.

Besides eliminating the need to nurture urease-producing microbes, enzymatic ureolytic hydrolysis offers several other advantages over ureolytic MICP. Applications of ureolytic MICP on clean sands in laboratory column tests and limited field tests have encountered significant practical difficulties, including bioplugging (permeability reduction accompanying induced mineral precipitation) and generation of a toxic waste product (ammonium salt) (Harkes et al. 2010, van Paassen et al. 2008). Bioplugging not only limits the distribution of precipitation agents within the soil but also makes flushing of the waste product from the soil a difficult, energy intensive task. Due to these limitations, mass stabilization of soil using ureolytic MICP remains problematic. Furthermore, the microbes that produce the urease enzyme cannot readily penetrate the pores of soils smaller than medium to fine sand, limiting the minimum grains size of soils amenable to ureolytic MICP to clean fine sands or coarser graded soils. The small size (on the order of 12 nm) of the urease enzyme suggests that $CaCO_3$ precipitation by enzymatic ureolytic hydrolysis will be less susceptible to bio-plugging and will be able to penetrate finer grained soils, perhaps into the silt-sized particle range, compared to MICP processes.

1.2 *Sustainability of Ground Improvement Practices*

Finding effective solutions to ground improvement challenges is becoming increasingly complex due to sustainability considerations. Established materials and methods often need to be either replaced or supplemented by innovative materials and environmentally-friendly practices to address sustainability considerations. One example of a common building material that poses significant sustainability concerns is Portland cement. Portland cement is widely used in ground improvement applications. Unfortunately, Portland cement production is extremely energy intensive and a major source of emissions of carbon dioxide (CO_2), as well as of sulfur and nitrogen oxides. MICP has been explored recently as an alternative to Portland cement for ground improvement. Reductions in the use of

Portland cement through either direct substitution or complementary use of MICP could contribute considerably towards reduction in CO_2 emissions. Research suggests that cementation using MICP can address a number of important geotechnical problems in granular soils, including slope stability, erosion and scour, under-seepage of levees, the bearing capacity of shallow foundations, tunneling, and seismic settlement and liquefaction (Dejong et al. 2010, Harkes et al. 2010, Kavazanjian and Karatas 2008, van Paassen et al. 2010).

1.3 *Ureolytic MICP*

MICP attempts to create a cemented soil mass by precipitating calcium carbonate from the pore fluid such to form cementation bonds at the interparticle contacts (van Paassen et al. 2010, DeJong et al. 2006). Karatas et al. (2008) have identified several mechanisms for MICP. The MICP mechanism that has garnered the most attention and is most advanced in terms of development is ureolytic hydrolysis, or ureolysis (Chou et al. 2011, DeJong et al. 2006, van Paassen et al. 2010, Whiffin et al. 2007). Ureolytic MICP has typically been accomplished using a technique best described as biogrouting (Harkes et al. 2010, van Paassen et al. 2010), wherein bacteria and nutrients are mixed in a tank ex-situ and then injected into the soil followed by a fixation fluid to foster microbial attachment to soil particles and, finally, by a calcium-laden cementation fluid. Ureolytic MICP by stimulation of indigenous bacteria has also been reported in the literature (Burbank et al. 2012).

1.4 *Agricultural Urease*

Urease is a widely occurring hexameric protein found in many microorganisms, higher order plants, and some invertebrates. The enzyme is approximately 12 nm in dimension (Blakely & Zerner 1984). The small size of a solubilized urease enzyme affords it a distinct advantage over carbonate cementation methods that employ ureolytic microbes in cases that require penetration into very small pore spaces as nearly all known bacteria are greater than 300 nm in diameter, with the majority in the range of 500-5000 nm. Several families of common plants are very rich in urease, including some varieties of beans, melons and squash, and the pine family (Das et al. 2002). Extraction of urease enzyme from most urease containing plants has been shown to be very simple (Srivastava et al. 2001) and the enzyme is readily available from laboratory suppliers.

It is well-established that urease can occur as both an intra- and extra-cellular enzyme (Ciurli et al. 1996, Marzadori et al. 1998). Free soil urease (i.e. urease not bound to any living organism), generally derived from dead and decaying microorganisms and possibly from plant sources, readily occurs apart from the host microorganism and, upon absorptive association with soil particles, can persist for long periods of time without degradation or loss of function (Pettit et al. 1976). By contrast, exogenously added urease (i.e. urease added as a free enzyme) has a limited lifespan and its activity and function decrease with time (Marzadori et al. 1998, Pettit et al. 1976). This limited lifespan is potentially advantageous in some engineering applications as the enzyme can naturally degrade thereby eliminating long term impacts to the ecosystem.

2. METHODS

2.1 *Ottawa 20-30 Sand*

Laboratory column tests were conducted using plant derived urease to induce $CaCO_3$ precipitation in Ottawa 20-30 sand These tests were carried out in 6”x 2” (152 mm x 51 mm) acrylic tubes and membrane-lined 2.8” x 6” (71 mm x 152 mm) split molds (for creating specimens for triaxial testing). Three acrylic tubes and two columns for triaxial testing were filled with 20-30 Ottawa silica sand (mean grain size 0.6 mm, coefficient of uniformity 1.1) and treated as follows: tube #1: the sand was dry pluviated via funnel at ≈3” (76 mm) drop height and then received 5 applications of a cementation solution containing urea and calcium chloride mixed with 1.4g/L enzyme (total solution volume ≈ 300 ml); tube #2: sand was added in same manner as tube #1 and then received 2 applications (≈ 150 ml total) of the same cementation solution mixed with 1.4g/L enzyme; tube #3: the lower-third of tube was filled with sand and dry enzyme (≈ 3g), the remainder of the tube contained dry pluviated sand without enzyme, and the tube then received 2 applications (≈ 150 ml) of the cementation fluid with no enzyme added. The cementation fluid composition was based upon stochiometry and experience with microbial urease cementation, e.g. DeJong et al. (2007), Whiffin et al. (2008).

Approximately 100 mL of a pH=7.8 solution containing 383 mM urea (reagent grade, Sigma-Aldrich), 272 mM $CaCl_2$-$2H_2O$ (laboratory grade, Alfa Aesar) was used for the first application in each acrylic tube. Subsequent applications employed approximately 50 mL of a pH=7.6 solution containing 416 mM urea and 289 mM $CaCl_2$-$2H_2O$. Solution concentrations, while variable, were formulated within a reasonably similar range as a matter of convenience. In each application, the cementation fluid was poured into the top of the acrylic tube with the bottom closed off. The cementation fluid was allowed to stand, loosely covered, in the acrylic tube for at least 24 hours and then drained out the bottom of the cylinder. The next application followed immediately after drainage was complete. Drainage was accomplished by puncturing the base of the cylinder with a 20-gauge needle. When drainage was complete, the needle was removed and the puncture was plugged with a dab of silicone. Occasionally, the needle became plugged and an additional needle was inserted through the base. The triaxial columns were filled with sand in the same manner as tube 1 and then received 2 applications (each application ≈ 250 ml) of cementation solution with 1.4g/L enzyme.

In each application of cementation fluid, the fluid was added until it rose to approximately ½-inch (12-mm) above the soil line. After 2 applications, tubes #2 and #3 were allowed to air dry for several days and then analyzed. Experimentation with tube #1 was continued for several more days as three more batches of cementation fluid were applied. The last 2 applications of cementation fluid were allowed to slowly drain through the needle in the base immediately after application rather than sit for 24 hours (drainage rate ≈10-25ml/hour). The triaxial columns were allowed to stand for at least a week after the second cementation fluid application and then drained.

After drainage was complete, the triaxial columns were moved to a triaxial testing device. After draining the specimens from the acrylic tubes and after the completion of the triaxial tests, all samples were triple washed with de-ionized water. Tubes #2 and #3 were separated in 3 layers, while tube #1 was separated into six layers (for better resolution). Each layer from the specimens in the acrylic tubes and the entire mass of the triaxial specimens were acid washed to determine $CaCO_3$ content by oven drying for 48 hours, weighing, digesting with warm 1M HCl, washing, drying, and reweighing to determine carbonate mineral content.

Several of the cemented specimens were analyzed for mineral identification using X-Ray Diffraction (XRD). Samples were ground in an agate mortar and pestle and powdered onto a standard glass slide for analysis. Scanning electron microscopy (SEM) imaging was performed on intact cemented chunks of material with an Agilent 8500 Low-Voltage SEM (LV-SEM). A LV-SEM is a field emission scanning electron microscope capable of imaging insulating materials, such as organic and biological substances without the need for a metal coating and without causing radiation damage to samples.

2.2 *Ottawa F-60 Sand*

A triaxial column was prepared using Ottawa F-60 silica sand (mean grain size 0.275 mm, coefficient of uniformity 1.74) to

investigate enzymatic ureolytic $CaCO_3$ precipitation in a finer grained material. The specimen was prepared in the same manner as described for the triaxial columns for the Ottawa 20-30 sand. The cementation fluid for the first of the two applications contained approximately 2.0 g/L enzyme, 400 mM urea (reagent grade, Sigma-Aldrich), 300 mM $CaCl_2$-$2H_2O$ (laboratory grade, BDH) at pH=7.7. The fluid for the second application contained 1 M urea-$CaCl_2$-$2H_2O$ solution at pH=7.8 without any enzyme. After the test, the triaxial specimen was washed and subject to acid digestion in the same manner as the Ottawa 20-30 triaxial specimens.

3. RESULTS

3.1 *Acrylic Tubes*

Approximately 100 ml of cementation solution was delivered per application for the first application in each acrylic tube. However, the amount of solution the tube would accept was notably reduced in subsequent applications, when less than 75 ml was typically required to fill the tubes to ≈ ½ inch (12 mm) above soil line. At the conclusion of the experiment, precipitation was visible along the entire length of tubes 1 and 2. Internally the cementation was variable, with some highly cemented zones and other zones with little to no cementation.

Tube 1 yielded mostly small, loose chunks of sand with strong effervescence upon digestion. Most of this column appeared un-cemented and exhibited unusually viscous behavior when wet. A fairly large (compared to column diameter) piece of strongly cemented sand (not breakable without tools) formed in the deepest layer of tube 1. Tube 2 had many small chunks of weakly cemented sand with strong effervescence upon digestion. Tube 3 had little to no precipitation in the top layer (i.e. this layer did not show any indication of carbonate upon acid digestion.) The deepest layer of tube 3 contained many pieces of weakly cemented sand that effervesced strongly upon digestion. The middle layer of tube 3 contained a few pieces of cemented sand that effervesced moderately upon digestion. The results from the acid washing are presented in Table 1.

Table 1. Results from *Experiment Set 1* using 20-30 Ottawa silica sand

Summary of Results					
Tube #	**Layer**	**Weight Change via Digestion**	**Amt. of $CaCO_3$ (g)**	**Total Amt. $CaCO_3$ (g)**	**Theor. Max $CaCO_3$ (g)**
1	1	11%	3.57	11.8	≈14.5
	2	3.8%	1.67		
	3	2.7%	1.73		
	4	2.1%	1.40		
	5	2.3%	1.74		
	6	2.0%	1.64		
2	1	0.76%	0.63	2.07	≈ 4.35
	2	0.65%	0.69		
	3	0.49%	0.75		
3	1	0.23%	0.31	3.57	≈ 4.35
	2	0.58%	0.63		
	3	1.7%	2.63		

The theoretical maximum $CaCO_3$ content is the stoichiometric maximum balanced on initial concentrations. The primary experimental differences between the tests are (1) the number of applications of cementation fluid and (2) the manner in which the urease was delivered. The results indicate that there is greater carbonate precipitation with increasing number of applications, as expected. The data show more precipitation in (or on) the top layer of tubes 1 and 2 but not in tube 3, as the enzyme was physically confined to the lower-third layer in tube 3 during sample preparation. In the top layer of tube 3, where no urease was mixed with the sand, carbonate precipitation was nearly undetectable. There was no visual evidence of precipitation and practically no measurable change in weight of this layer after acidification (weight change = 0.23%). In the bottom layer of tube 3, where 3 g of dry enzyme was mixed with the soil, there was a weight change of 1.7% following acid washing. The middle layer of this specimen had a minor change in weight (0.58%), possibly due to uneven distribution of the layers during preparation or splitting of the specimen or to upward migration of urease from the bottom layer.

XRD analysis, presented in Figure 1, confirms that calcite is the mineral phase present in the cemented soil chunks. LV-SEM images, presented in Figure 2, show silica (quartz) sand particles cemented with calcium carbonate and various morphological features associated with the cementation process on the silica surface.

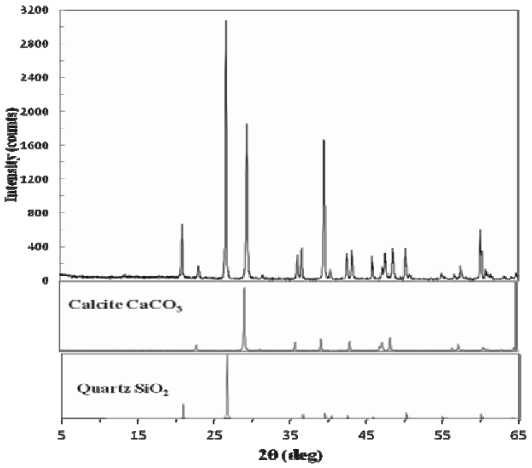

Figure 1. XRD results from cemented sand sample (top plot). Quartz & calcite standards (middle & bottom plot, respectively).

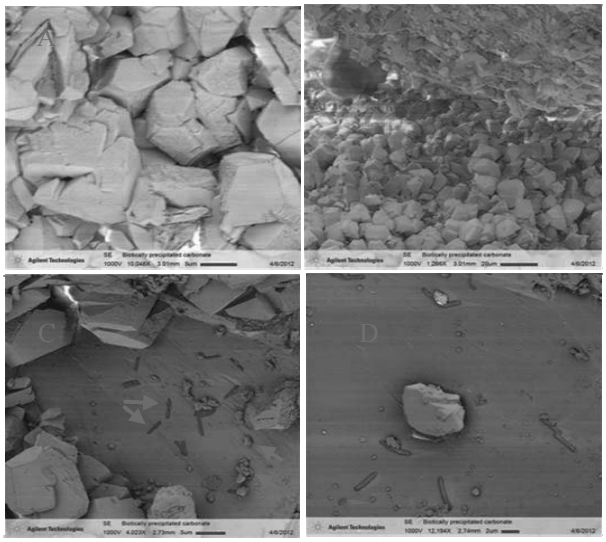

Figure 2. LV-SEM images a.) Well-grown and cementing calcite crystals; b.) Cementing calcite crystals at inter-particle contact; c.) Indention of quartz surface (blue arrows) and nucleation of calcite crystals (red arrows); d.) Calcite crystal growing on quartz surface.

3.2 Triaxial Columns

The three triaxial sand columns (2 Ottawa 20-30 sand columns and 1 Ottawa F-60 sand column) were tested in drained triaxial compression prior to acid digestion. All three columns were able to stand upright after removal of the split mold. The results

of the triaxial compression tests performed on the 20-30 Ottawa sand are presented in Figure 3 and the results for the F-60 Ottawa sand are presented in Figure 4. The carbonate cement content for one of the 20-30 silica sand columns was 2.0% $CaCO_3$ (by weight). The carbonate content of the other 20-30 Ottawa sand column could not be quantified due to unintended sample loss. The carbonate cement content for the finer grained F-60 Ottawa sand was 1.6% $CaCO_3$ (by weight). The results show substantial strength increase for all 3 sand columns tested.

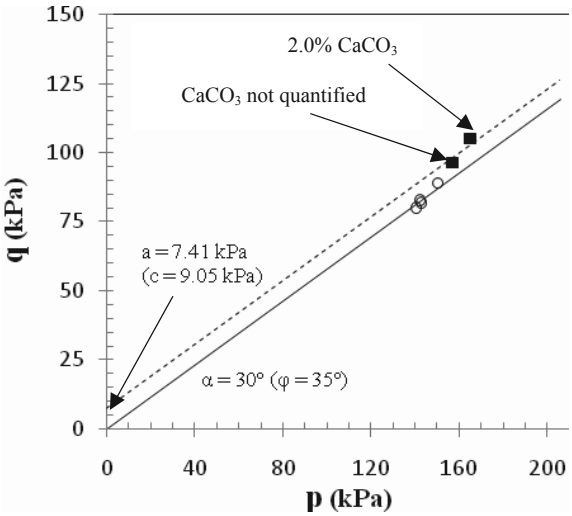

Figure 3. p-q plot failure envelopes for 20-30 silica sand: ■Cemented (D_r = 60%); ○ Uncemented (D_r = 60%)

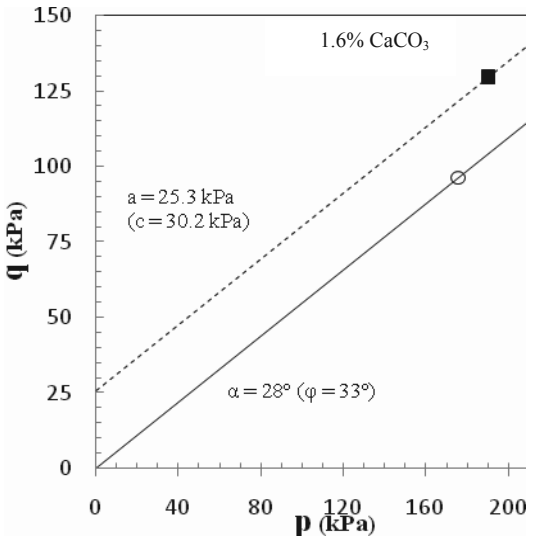

Figure 4. p-q plot failure envelopes for F-60 silica sand: ■Cemented (D_r = 35%); ○ Uncemented (D_r = 37%);

4. CONCLUSION

Sand column tests at Arizona State University have shown that agriculturally-derived urease can be used to induce calcium carbonate precipitation in sand. Sand columns were developed using Ottawa 20-30 and F-60 sand and three different preparation methods: dry pluviation followed by percolation of a calcium-urease-urea cementation solution, pluviation into a calcium-urease-urea cementation solution, and mixing the sand with urease prior to pluviation with a calcium-urea solution. Cementation was observed in all of the columns. XRD and SEM testing confirmed that calcium carbonate (specifically calcite) was the cementing agent. Acid digestion showed that increased applications yielded correspondingly greater carbonate precipitation. The quality of cementation, as determined by the effort needed to break apart cemented chunks of sand, varied depending on the sampling location within the column. Triaxial test results on cemented columns showed substantial strength increase over non-cemented columns at the same relative density.

5. REFERENCES

Baumert, K.A., Herzog, T., Pershing, J., 2005**.** Navigating the Numbers Greenhouse Gas Data & International Climate Policy *World Resources Institute*

Blakely, R.L. and Zerner, B., 1984. Jack Bean Urease: The First Nickel Enzyme. *Journal of Molecular Catalysis* 23, 263–292.

Burbank, M., Weaver, T., Lewis, R., Williams, T., Williams, B. and Crawford, R. 2012. Geotechnical Tests of Sands Following Bio-Induced Calcite Precipitation Catalysed by Indigenous Bacteria *ASCE JGGE* 132, DOI: 10.1061/(ASCE)GT.1943-5606.0000781.

Chou, C.W., Seagren, E.A., Aydilek, A.H., and Lai, M. 2011. Biocalcification of Sand through Ureolysis. *JGGE* 137, 1179–1189.

Ciurli S., Marzadori C., Benini S., Deiana S. and Gessa C., 1996. Urease from the soil bacterium Bacillus pasteurii: Immobilization on Ca-polygalacturonate. *Soil Biol. & Biochem*. 28, 811-817.

Das, N., Kayastha, A.M. and Srivastava, P.K., 2002. Purification and characterization of urease from dehusked pigeonpea (Cajanus cajan L.) seeds. *Phytochemistry* 61 (5), 513-521.

DeJong, J.T., Fritzges, M.B., and Nusslein, K. 2006. Microbially Induced Cementation to Control Sand Response to Undrained Shear. *ASCE JGGE* 132, 1381–1392.

DeJong, J.T., Mortensen, B.M., Martinez, B.C., and Nelson, D.C., 2010. Bio-mediated soil improvement. *Ecological Engineering* 197-210.

Harkes, M. P., van Paassen, L. A., Booster, J. L., Whiffin, V. S., and van Loosdrecht, M. C. M. 2010. Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement. *Ecological Engineering* 36 (2), 112–117.

Karatas, I., 2008. Microbiological Improvement of the Physical Properties of Soils. PhD. Dissertation, Department of Civil, Environmental, and Sustainable Engineering Arizona State University, Tempe, AZ.

Kavazanjian, E. and Karatas, I., 2008. Microbiological Improvement of the Physical Properties of Soil*, 6th International Conference on Case Histories in Geotech. Eng.*, Arlington, VA August 11-16.

Krogmeier, M.J., McCarty, G.W. and Bremner, J.M., 1989. Phytotoxicity of foliar-applied urea. *Proc. Natl. Acad. Sci.,* USA 86, 8189–8191.

Marzadori, C., Miletti, S., Gessa, C. and Ciurli, S., 1998. Immobilization of jack bean urease on hydroxyapatite: urease immobilization in alkaline soils. *Soil Biol. & Biochem*. 30 (12), 1485-1490.

Pettit N. M., Smith A. R. J., Freedman R. B. and Burns R. G., 1976. Soil urease: activity, stability, and kinetic properties. *Soil Biol. & Biochem*. 8, 479-484.

Srivastava, P.K. and Kayastha, A.M., 2001. Characterization of gelatin-immobilized pigeon pea urease and preparation of a new urea biosensor. *Biotechnology and Applied Biochemistry* 34, 55-62.

van Paassen, L.A., Ghose, R., van der Linden, T.J.M., van der Star, W.R.L., and van Loosdrecht, M.C., 2010. Quantifying Biomediated Ground Improvement by Ureolysis: Large-Scale Biogrout Experiment. *ASCE JGGE* 136, 1721–1728.

van Paassen, L.A., Daza, C.M., Staal, M., Sorokin, D.Y. and van Loosdrecht, M.C., 2008. In situ soil reinforcement by microbial denitrification. *1st Int. Conf. on Bio-Geo-Civil Engineering*, Netherlands, 124-133, June 23-25.

Whiffin, V.S., van Paassen, L.A., and Harkes, M.P., 2007. Microbial Carbonate Precipitation a Soil Improvement Technique. *Geomicrobiology* 24, 1-7.