Iron- and calcium-based biogrouts for porous soils

❶ **Volodymyr Ivanov** PhD
School of Civil and Environmental Engineering, Nanyang Technological University, Singapore and (currently) Visiting Professor, Department of Civil, Construction & Environmental Engineering, Iowa State University, Ames, IA, USA

❷ **Jian Chu** PhD
School of Civil and Environmental Engineering, Nanyang Technological

University, Singapore and (currently) Professor and James M. Hoover Chair for Geotechnical Engineering, Department of Civil, Construction & Environmental Engineering, Iowa State University, Ames, IA, USA

❸ **Viktor Stabnikov** PhD
School of Civil and Environmental Engineering, Nanyang Technological University, Singapore and (currently) Associate Professor, Department of Biotechnology, National University of Food Technologies, Kiev, Ukraine

Chemical grouts are often used to reduce the hydraulic conductivity of soil for seepage control purposes. However, chemical grouts can be expensive and environmentally unfriendly. Therefore, two new biogrouts or microbial-based grouting materials were developed. The first was a calcium-based biogrout, which contained urease-producing bacteria, calcium chloride and urea for the crystallisation of calcite due to enzymatic hydrolysis of urea. The second was an iron-based biogrout, which consisted of urease-producing bacteria, ferric chelate, and urea for the precipitation of ferric hydroxide due to enzymatic hydrolysis of urea. Both biogrouts reduced the hydraulic conductivity of loose clean sand from 5×10^{-5} to $1 \cdot 4 \times 10^{-7}$ m/s when the precipitated metal content was at $1 \cdot 5\%$ w/w or lower. The unconfined compressive strength of the treated specimens also increased. However, at a low metal content, the amount of improvement was still much less than that obtained by chemical grouting. Therefore, suitable geotechnical applications of the calcium-based and iron-based biogrouts at a precipitated metal content of $1 \cdot 5\%$ w/w or less would be mainly the bioclogging of porous soils for the sealing of aquaculture or wastewater treatment ponds and landfill sites, and for plugging of the piping in dams and retaining walls in sandy soil.

1.   Introduction

Grouting of porous soil using chemical solutions or cement suspensions is often used in geotechnical engineering to increase the shear strength and reduce the permeability of the soil (Karol, 2003). However, the viscosity of chemical or cement grouts are often too high to be used for very fine sand or fine grained soil. Some of the chemical grouts are sometimes expensive or environmentally unfriendly. Grouting can be performed by impregnating repeatedly mixed solutions of either calcium chloride ($CaCl_2$) and sodium carbonate (Na_2CO_3), or calcium chloride and sodium sulfate (Na_2SO_4), or calcium chloride and disodium phosphate (Na_2HPO_4), or ferrous chloride ($FeCl_3$) and sodium hydroxide (NaOH) (Bird and Putman, 2008). However, the chemical reaction between these components is too fast and production of insoluble compounds occurs quickly. Therefore, the depth of penetration, size of the crystals and mechanical strength of the binding between the soil particles could not be

properly controlled to achieve the intended improvement in either the hydraulic or the mechanical properties of the soil.

These disadvantages of chemical grouting can be overcome by adopting the so-called biogrouting method, a new approach based on the microbially induced calcium carbonate precipitation (MICP) process. In this approach, the enzymatic reaction of urea (carbamide) hydrolysis is used to control the pH and precipitation of calcium carbonate according to the following reaction:

1.
$$(NH_2)_2CO + 2 \cdot H_2O + CaCl_2 \rightarrow CaCO_3\downarrow + 2 \cdot NH_4^+ + 2 \cdot Cl^-$$
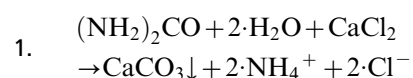
This reaction is catalysed by enzyme urease produced by different micro-organisms. The urease activity controls the rate of calcite precipitation or crystallisation inside the pores of the soil due to an increase of pH and release of carbonate ions.

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In general, there are several mechanisms that control the biogrouting for soil or rock: (1) filling the voids and channels in soil with inorganic compounds generated by the activity of micro-organisms; (2) filling the pores and channels in soil with microbial biomass and polysaccharides; (3) binding the particles with inorganic compounds, mediated by microorganisms; (4) binding the particles with microbial biomass and polysaccharides; and (5) forming salt bridges between the soil particles and colloids mediated by micro-organisms (Ivanov, 2010; Ivanov and Chu, 2008). The biogrout fills the voids, channels or fissures in soil or rock through microbial activity or by the microbial products.

A common approach for biogrouting is to use the MICP processes. Several researchers have proposed the use of active, urease-producing bacteria for the precipitation of calcium carbonate and clogging and strengthening of sandy soil (Bachmeier *et al.*, 2002; Chu *et al.*, 2009, 2011; Ferris and Stehmeier, 1992; Frankel and Bazylinski, 2003; Gollapudi *et al.*, 1995; Harkes *et al.*, 2010; Nemati and Voordouw, 2003; Nemati *et al.*, 2005; Stocks-Fischer *et al.*, 1999; Whiffin *et al.*, 2007; Yasuhara *et al.*, 2011). Not only bacteria, but also enzyme urease can be used for production of calcite in situ (Nemati and Voordouw, 2003; Nemati *et al.*, 2005; Sarda *et al.*, 2009; Yasuhara *et al.*, 2011). Calcium-based biogrout could be effective in many geotechnical applications (Al-Thawadi, 2011; DeJong *et al.*, 2010; Ivanov and Chu, 2008; Mitchell and Santamarina, 2005) and was tested in the field (van Paassen and van Loosdrecht, 2010; van Paassen *et al.*, 2009, 2010).

There are only a few known data available on the effects of the content of precipitated calcium in a porous medium on the hydraulic permeability of that medium (Whiffin *et al.*, 2007; Yasuhara *et al.*, 2011). The biological agents used in these studies were different. Bacterial cells were used in Whiffin *et al.* (2007) and enzyme urease in Yasuhara *et al.* (2011). To the authors' knowledge there have been no data published on the use of precipitated iron hydroxide for the reduction of permeability of soil. In the present study a new method to use iron-based biogrout for bioclogging is introduced. Iron hydroxide was selected as the clogging compound because hundreds of thousands of tons of iron salts are produced annually. Another reason for the selection of iron hydroxide was that natural sandstone often originates from sand impregnated with ferric oxides or hydroxides (Pettijohn *et al.*, 1987). The results of the present study on the use of calcium-based and iron-based biogrouts for the reduction of permeability of soil are presented. A comparison of the clogging effect of calcium- and iron-based biogrouts on sand is also made.

2. Materials and methods
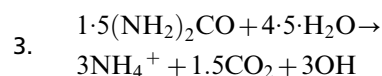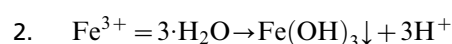
2.1 Materials used
A standard sand ASTM C778 was used for the experiments. This rounded grain silica sand had a mean grain size of 0·42 mm. The specific gravity was 2·65.

An alkalophilic strain of urease-producing bacteria (UPB) *Bacillus* sp. VS1 isolated from a tropical soil was used in this research. It is a spore-forming, Gram-positive, rod-shaped bacteria cultivated on the medium with tryptic soya broth, urea and nutrients as described earlier (Chu *et al.*, 2012; Stabnikov *et al.*, 2011). The suspension of these bacteria with a biomass concentration of 8 g of dry biomass per litre was used to initiate biocementation of sand.

The calcium-based biogrout included the following components: (1) suspension of biomass of *Bacillus* sp. VS1; (2) solution containing 82·5 g/l (final concentration was 0·75 mol/l) calcium chloride and 90 g/l (final concentration was 1·5 mol/l) of urea.

The iron-based biogrout included the following components: (1) suspension of biomass of *Bacillus* sp. VS1; (2) solution containing 36·7 g/l (final concentration was 0·1 mol/l) of ethylenediamine-tetra-acetic acid (EDTA) ferric monosodium (BDH Laboratory Supplies, England), pH 4·8, and 9 g/l (final concentration of 0·15 mol/l) of urea, with the addition of NaOH (final concentration of 4·5 mmol/l) to adjust the pH to pH 6·0 before grouting. This solution of ferric ions chelated with EDTA was not hydrolysed at pH 6·0. Ferric hydroxide was intensively precipitated from the solution when its pH was increased above 6·5.

The addition of urease-producing bacteria and urea was necessary to maintain pH above the neutral value because hydrolysis of ferric ions was accompanied by acidification of the solution, which could be neutralised due to enzymatic hydrolysis of urea according to the following equations:

2. $$Fe^{3+} = 3 \cdot H_2O \rightarrow Fe(OH)_3 \downarrow + 3H^+$$

3. $$1 \cdot 5(NH_2)_2CO + 4 \cdot 5 \cdot H_2O \rightarrow 3NH_4^+ + 1.5CO_2 + 3OH$$

2.2 Grouting
Specimens of grouted sand were prepared in the laboratory by injection of biogrouts into sand. For calcium-based biogrout, 25 ml of bacterial suspension was injected from the bottom to the top of the 50 ml syringes filled with 50 ml of sand. The inner diameter of the syringe was 27 mm. Sand was incubated for 2 h with bacterial suspension for adsorption of cells and enzymes and then the suspension was drained off by gravity. The sand was washed with 25 ml of distilled water to remove the remaining cultural liquid. After the water was drained out, 25 ml of solution, which contained 82·5 g/l (0·75 mol/l) calcium chloride and 90 g/l (1·5 mol/l) of urea, was injected from the bottom to the top of the syringes. After incubation for 24 h for crystallisation of calcite, the remaining solution was drained off by gravity.

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For iron-based biogrout, 25 ml of bacterial suspension were injected from the bottom to the top of the 50 ml syringes filled with 50 ml of sand. Sand was incubated for 2 h for adsorption of cells and enzymes on sand and then the suspension was drained off by gravity. The sand was washed with 25 ml of distilled water to remove the remaining culture liquid. After the water was drained away, 25 ml of solution containing 36·7 g/l (final concentration of 0·1 mol/l) of EDTA ferric monosodium and 9 g/l (final concentration of 0·15 mol/l) of urea, with the addition of sodium hydroxide (final concentration was 4·5 mmol/l) were injected from the bottom to the top of the 50 ml syringes filled with sand, incubated for 24 h for hydrolysis of ferrous salt and urea. The remaining solution was drained off by gravity.

The bottoms of the plastic syringes were cut out at the end of treatment and the specimens were pushed out of the syringe carefully using the plunger. To increase the quantity of the calcium or iron precipitated, the biogrout components were injected repeatedly for several more rounds. The experiments were made in triplicate to check the repeatability and quantify the changes to the sand properties statistically.

2.3 Measurements and microscopy
Before the sand was taken out of the syringe, a permeability test was carried out by applying a constant head difference of 0·5 m to the 185 mm long sample in a downward direction. After the permeability test, the wet sample was removed from the syringe for an unconfined compression test. Scanning electron microscopy (SEM) using a Leica Stereoscan 420 and light microscopy using an Olympus SZx9 stereomicroscope were performed. Tests for the determination of calcium concentration and the urease activity of the centrifuged bacterial suspension by measuring the changes of conductivity were also performed using the procedure described in Chu *et al.* (2012) and Stabnikov *et al.* (2012). The urease activity was also measured by the production rate of ammonia using the Nessler method (APHA, 1999). The concentrations of iron in the solution before and after treatment of the sand were also determined using an inductively coupled plasma–optical emission spectrophotometer (ICP–OES) Perkin Elmer (APHA, 1999).

3. Results

3.1 The permeability of sand treated with biogrouts
The permeability of the treated sand (k) is plotted aginst the metal content in Figure 1 for sand treated with both calcium-based and iron-based biogrouts. The permeability of the untreated sand was of the order of $5·1 \times 10^{-4}$ m/s. For sand treated with calcium- or iron-based grout, the permeability decreased as a function of the precipitated metal content. When the precipitated calcium content was 1·24% w/w, the permeability of the treated sand became $1·6 \times 10^{-7}$ m/s. The permeability of the sand treated with the iron-based biogrout dropped to $2·7 \times 10^{-6}$ m/s at a precipitated iron content (F, % w/w) of 0·35%. This was a sharp

drop. However, the subsequent reduction in permeability was slow. The permeability at $F = 1·4\%$ was $1·8 \times 10^{-7}$ m/s.

After treatment with the calcium-based biogrout, the pH in the sand during the biogrouting process increased from 6·0 to 8·7 due to microbial hydrolysis of urea. The pH of sand treated with the iron-based biogrout during bioclogging increased from 6·0 to 9·1 due to microbial hydrolysis of urea.

Figure 1 has demonstrated that both the calcium-based and iron-based biogrouts could reduce the permeability of sand to the order of magnitude of 10^{-7} m/s. However, the rate of reduction was different for the two processes. This was probably due to the difference in the precipitation processes and the precipitates. The images of biogrouted sand samples (Figure 2) show that the calcium-based biogrout produced white amorphous or crystallised calcium carbonate, whereas the iron-based biogrout produced brown precipitate without visible crystals.

3.2 The strength of sand treated with biogrouts
The unconfined compressive strengths of the sand treated with the two types of biogrout (S) are plotted against the precipitated metal content (% w/w) in Figure 3. The curve for the calcium-based biogrout (C, %) was fitted by the following equations (Figure 3):

4. $$S = 14·352C^2 - 0·716C \quad \text{kPa}$$

The curve for iron-based biogrout (F, %) was fitted by:

5. $$S = 13·714F^2 + 36·985F \quad \text{kPa}$$
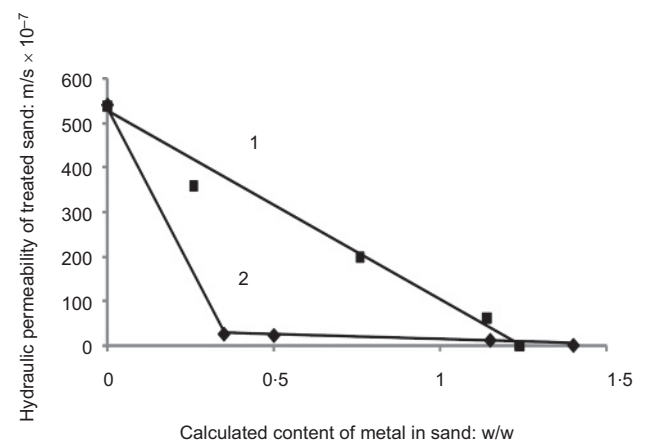
Figure 1. Effect of metal content in the treated sand on hydraulic permeability of sand: Curve 1, bioclogging using calcium-based biogrout; curve 2, bioclogging using iron-based biogrout
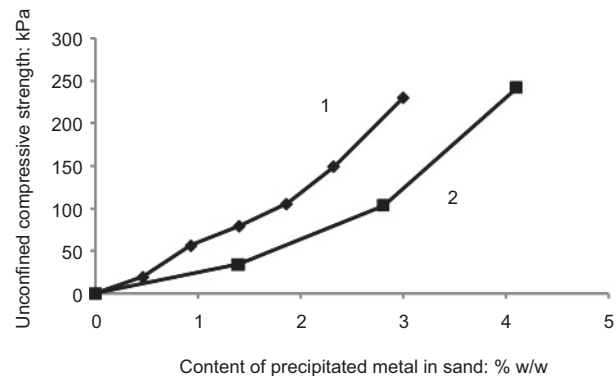
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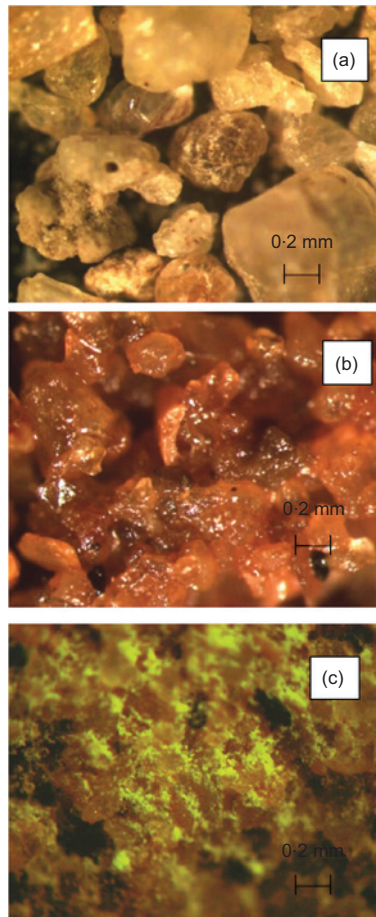
Figure 2. Micrographs of untreated sand (a) and sand treated with iron-based biogrout (b) or calcium-based biogrout (c)

Figure 3. Unconfined compressive strength of wet grouted sand: Curve 1, sand treated with iron-based biogrout; curve 2, sand treated with calcium-based biogrout

As shown in Figure 3, the strength of the wet biocemented sand at a precipitated metal content below 1·5% was low and the value increased to 230 to 240 kPa when the precipitated metal content was above 3%. This value was lower than the strength value obtained from sand treated with cement or other chemical grout. Therefore, suitable geotechnical applications of calcium-based and iron-based biogrouts at a precipitated metal content of less than 1·5% would be bioclogging to reduce the permeability of the porous soil rather than biocementation to its strength.

4. Discussion

In comparison with conventional chemical or cement grouting techniques, biogrouting may be a less expensive and more environmentally friendly alternative. It is also less demanding for construction machinery as the viscosity of the biogrouts are much lower and the particles are much smaller (Ivanov and Chu, 2008; van Paassen et al., 2009). Therefore, both calcium-based and iron-based biogrouts can be used for the sealing of aquaculture ponds, construction of the algal biofuel production

ponds, wastewater treatment ponds and landfill sites. Other potential applications may include plugging of the piping in dams and retaining walls, construction of subsurface impermeable barriers and immobilisation of soil contaminants (DeJong et al., 2010; Ivanov and Chu, 2008).
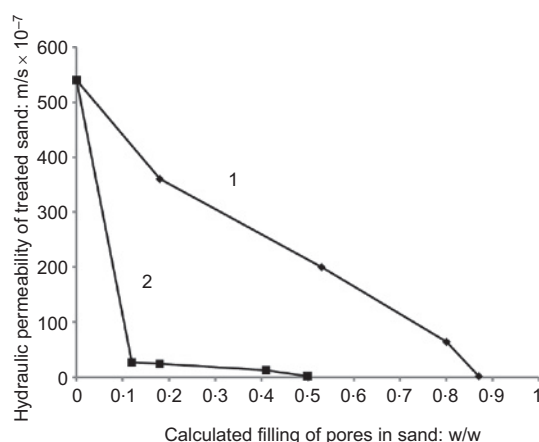
For calcium-based biogrout, a few model-scale tests or field trials have been carried out. One example is the bio-mediated improvement of soil supporting a shallow footing foundation where the settlement induced by loading of the footing was decreased by five times at a footing stress of about 30 kPa (DeJong et al., 2010). Another large-scale test with a volume of up to 100 m^3, has been carried out at the Technical University of Delft (van Paassen and van Loosdrecht, 2010; van Paassen et al., 2009, 2010). These model tests have demonstrated the technical feasibility of using calcium-based biogrout for ground improvement. The tests of Whiffin et al. (2007) and van Paassen et al. (2009, 2010) have shown that significant strength gain was only achieved when the precipitation of the calcium content was above 3·5%. The precipitated calcium content in the present experiments was lower than this value as the aim of the present study was to reduce the permeability of the soil only.

It is known that the ferric hydroxides produced by the hydrolysis of ferric salts can form different kinds of particles and colloids (Meyer et al., 2000). The ferric hydroxide precipitated in sand from the iron-based biogrout looked like gel but not like an amorphous precipitate or crystals (Figure 2). Probably, such a gel-like material has better clogging properties than the crystalloid precipitate produced from calcium-based biogrout.

It should be pointed out that only a small percentage of the void volume was filled with calcium carbonate or ferric hydroxide after bioclogging. The calculations from the present experimental data were based on a sand porosity of 40%, with the

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specific gravity of calcium carbonate $= 2 \cdot 7$ and the specific gravity of ferrous hydroxide $= 3 \cdot 4$. The results of the calculations demonstrated that even a filling of $0 \cdot 5$ to $0 \cdot 9\%$ of the void volume in sand was sufficient to reduce the permeability of sand to $1 \cdot 4 \times 10^{-7}$ m/s (Figure 4). This confirmeds that bioclogging was performed mainly in the vicinity of the particle–particle contacts or in the channels between the sand particles as has been shown by other researchers (DeJong *et al.*, 2010).

The major problem with iron-based biogrout using iron reagents is that most of the available ferric chloride or sulfate cannot be used because the solutions of these salts have a pH value of about 2. This pH cannot be increased to 6 or 7 because of the fast hydrolysis of these salts with the formation of insoluble ferric hydroxide at circumneutral pH. Therefore, ferric ions must be chelated with the chemicals ensuring stability of ferric chelates at circumneutral pH. In the present experiments with iron-based biogrout, EDTA, which is a potent chelator from the group of aminopolycarboxylates, was used. However, if this is not economical, there are many other ferric chelators that may be chosen from the group of aminopolycarboxylates such as nitrilotriacetic acid, diethylenetriaminepentaacetic acid and ethylenediamine-disuccinate (Nowack, 2002), or from the group of phosphonates such as dimethyl methylphosphonate, amino tris(methylene phosphonic acid), and many others (Nowack and Van Briesen, 2005). The worldwide production of aminopolycarboxylates and phosphonates is several hundred thousand tons, so these chemicals are readily available for the production of iron-based biogrout. Another way to diminish the cost of iron chelates for biogrouting could be the production of the solution of dissolved ferrous chelates at neutral pH using cheap iron ore, organic waste materials, and iron-reducing bacteria (Ivanov *et al.*, 2009).

5. Conclusion

In the present study, a relatively new approach to use a microbial-mediated process for grouting was investigated and a new biogrouting material, the iron-based biogrout, was introduced. The use of both calcium-based and iron-based biogrouts to reduce the permeability of sand from an order of 10^{-4} m/s to $1 \cdot 4 \times 10^{-7}$ m/s was demonstrated. This reduction in permeability was achieved when the precipitated metal content was as low as $1 \cdot 5\%$ w/w or when only 1% of the void volume in sand was filled with the precipitated compounds.

Figure 4. Reduction of hydraulic permeability of sand as result of bioclogging of the sand pores using calcium-based (1) or iron-based (2) biogrouts

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