

Use of bacterial ureolysis for improved gelation of silica sol in rock grouting

E. MACLACHLAN*, G. EL MOUNTASSIR* and R. J. LUNN*

Low pH silica-based grouts suitable for penetrating fine-aperture fractures are increasingly being developed for use in engineering applications. Silica sol has an initial low viscosity; mixing with an accelerator destabilises the suspension, producing a gel. The reported study investigated the influence of sodium, calcium and ammonium chloride accelerators on gel time, rate of gelation and shear strength of the resulting gel. For the first time, the potential use of bacterial ureolysis as an accelerator for the destabilisation of silica sol was also explored. This study demonstrates that bacterial ureolysis can be used to control the gelation of silica sol. The rate of ureolysis increases with increasing bacterial density, resulting in faster gel times and higher rates of gelation. In addition, for grouts with similar gel times, using bacterial ureolysis to induce destabilisation results in a higher rate of gelation, a higher final shear strength and a more uniform gel than direct addition of the corresponding chemical accelerator. These results suggest that bacterial ureolysis could potentially be used in rock grouting to achieve long gel times and hence greater penetration, while also maintaining sufficiently rapid gelation to minimise issues related to fingering and erosion of the fresh grout.

KEYWORDS: grouting; laboratory tests

ICE Publishing: all rights reserved

INTRODUCTION

Cementitious grouts are commonly used to reduce hydraulic conductivity in fractured rock, and microfine and ultrafine cements are increasingly being used for penetrating fine-aperture fractures. However, their penetration below 50–100 μm remains limited without hydraulic jacking. For geological repositories of high-level nuclear waste (HLW), fractures with apertures down to 10 μm will need to be sealed to meet acceptable leakage rates (Funehag, 2007). High pH cement grouts (pH 12–13) will not be used in the vicinity of HLW repositories due to the adverse impact of high pH leachate on the behaviour of bentonite (Bodén & Sievänen, 2005). There are also concerns that superplasticisers, used to improve the flow of cement grouts, may reduce radionuclide sorption in repository environments (Young *et al.*, 2013). There is thus a need for the development of alternative grouts that can penetrate fine-aperture fractures.

A low-viscosity grout comprising silica sol and an accelerator has been proposed (Funehag & Axelsson, 2003; Funehag & Gustafson, 2008). The accelerator, a saline solution, destabilises the suspension of nanometre-sized silica particles, resulting in a gel. The distance the grout can penetrate is governed by the gel time (and initial viscosity) (Funehag & Gustafson, 2008). In water-filled fractures, it is desirable that the grout gains strength quickly to reduce fingering of the grout front and to minimise its erosion by ingressing water. Hence, the gel time, rate of gelation and shear strength evolution are

critical properties for the design of a successful grouting campaign.

This study investigated the direct addition of three different accelerators (sodium, calcium and ammonium chloride) and the gel time, rate of gelation and shear strength parameters were determined. The potential use of bacterial ureolysis as an accelerator for the in situ destabilisation (i.e. cations produced within the silica sol) was also explored for the first time.

MATERIALS AND METHODS

Silica sol and gel properties

MP320 colloidal silica (Meyco BASF), which is sold with a sodium chloride accelerator was used in this study. The silica sol has a dynamic viscosity of ~ 10 mPa·s, a density of 1.3 g/cm³, pH of 9.5–9.8 and SiO₂ concentration of 40% (BASF, 2009).

Silica sol was mixed with accelerators in a 5 : 1 ratio. The increase in dynamic viscosity that occurs as gelling proceeds was measured using a Brookfield digital viscometer (model LVT DVII) in accordance with ASTM D4016 (ASTM, 1993). In the viscosity tests, 200 ml of silica sol to 40 ml of accelerator was used; double these volumes were used for the shear strength tests, which were carried out using a Wykeham Farrance laboratory shear vane apparatus (BSI, 1990). Specimens were sealed to prevent evaporation and the shear strength was measured after 1 d and 7 d in an environment with a relative humidity of 40–60%. Viscosity and shear strength tests were carried out in a temperature-controlled laboratory at 20°C.

Bacterial suspension

Bacterial ureolysis relies on the urease enzyme of bacteria to hydrolyse urea to ammonium ions. In this study, *Sporosarcina pasteurii* (strain ATCC 11859), a common ureolytic soil bacterium was used. The *S. pasteurii* were

Manuscript received 30 August 2013; first decision 9 October 2013; accepted 18 October 2013.

Published online at www.geotechniqueletters.com on 12 November 2013.

*Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow, UK

initially grown on solid brain heart infusion (BHI) agar with urea (20 g/l). A single colony was then transferred to liquid BHI growth medium (37 g/l), again supplemented with urea (20 g/l), and cultures were grown at 25°C for 24 h. The bacterial suspensions were produced by separating the bacteria from the growth medium by centrifuging (at 8000 rpm for 4 min) and diluting with sterile tap water until the required optical density (OD_{600}) was achieved (measured using a UV-VIS spectrophotometer at 600 nm). OD_{600} is commonly used as a method of ensuring that similar numbers of bacteria are present in prepared solutions.

Equal volumes of the bacterial suspension and urea were mixed together prior to mixing with silica sol in a 5 : 1 ratio. The accelerator of bacterially induced ammonium ions with a final $OD_{600} = 0.33$ and 0.145 M urea was prepared by mixing 20 ml of $4OD_{600}$ bacterial suspension and 20 ml of 1.74 M urea together and then mixing with 200 ml of silica sol. To give a final OD_{600} of 0.67, the solutions were mixed as before but with 20 ml of $8OD_{600}$ bacterial suspension. To achieve optical densities greater than 1, larger volumes of lower concentration suspensions were centrifuged, and the supernatant re-suspended in smaller volumes of sterile water (e.g. the supernatant of 80 ml of $1OD_{600}$ was re-suspended in 20 ml of sterile water to give an equivalent of $4OD_{600}$). Separation of the bacteria from the growth medium ensured limited further growth, while ureolysis provided the existing *S. pasteurii* with energy for metabolic processes.

Determination of ureolysis rate

The ureolysis rate constant k_{urea} was calculated for aqueous solutions from Equation 1 using experimental measurements of ammonium concentration over time $[NH_4^+]_t$, where $[urea]_0$ is the initial urea concentration (Tobler *et al.*, 2011)

$$[NH_4^+]_t = 2[urea]_0(1 - e^{-k_{urea}t}) \quad (1)$$

Ammonium concentrations were determined using the colorimetric Nessler method for aqueous solutions as described by Tobler *et al.* (2011). This method could not be used to determine the ammonium concentration in silica sol, due to its opalescence. However, since the hydrolysis of

urea produces ionic products from a non-ionic substrate it is possible to measure conductivity (Whiffin, 2004; De Muynck *et al.*, 2011). For both aqueous bacterial suspensions (OD_{600} of 0.33 and 0.67), the linear relationship between conductivity and ammonium concentrations (determined using the Nessler method) was derived (R^2 values of 0.95 and 0.98), enabling the ammonium concentration within the silica sol to be estimated using conductivity measurements and hence calculation of k_{urea} .

RESULTS

The gelation properties of the silica sol can be controlled by varying the type and concentration of the accelerator (e.g. Funehag & Axelsson, 2003). This results in changes not only to the gel time t_{gel} but also to the rate of gelation r_{gel} (defined in Fig. 1). For example, Fig. 1 illustrates that decreasing the concentration of NaCl accelerator from 0.363 M to 0.255 M increases the gel time from 17 to 120 min, with a corresponding decrease in the rate of gelation from 322 to 241 mPa·s/min. At lower concentrations, the onset of gelation is also more gradual.

Monovalent and divalent cation accelerators

Comparing grout A (0.29 M NaCl) and grout B (0.033 M $CaCl_2$) in Fig. 2, it is apparent that despite the final concentration of $CaCl_2$ being almost an order of magnitude less than that of NaCl, the gel time was slightly shorter for grout B (29 min) than for grout A (38 min) and the rate of gelation was much higher (767 mPa·s/min compared with 324 mPa·s/min). This is because the divalent calcium ion is more effective at compressing the diffuse double layer around the silica particle (Savarmand *et al.*, 2003) than the monovalent sodium ion. This reduces electrostatic repulsion between silica particles, increasing the likelihood of interparticle collisions, hence accelerating aggregation and gelation. After 1 d, grouts A and B had a shear strength of 28 kPa (Table 1); after 7 d, grout B exhibited a higher shear strength (76 kPa) than grout A (51 kPa), indicating that cation valency also influences strength gain.

Visual inspection of grout B (0.033 M $CaCl_2$) revealed large aggregations on mixing and the resulting gel had distinguishable layers in varying shades of white. This

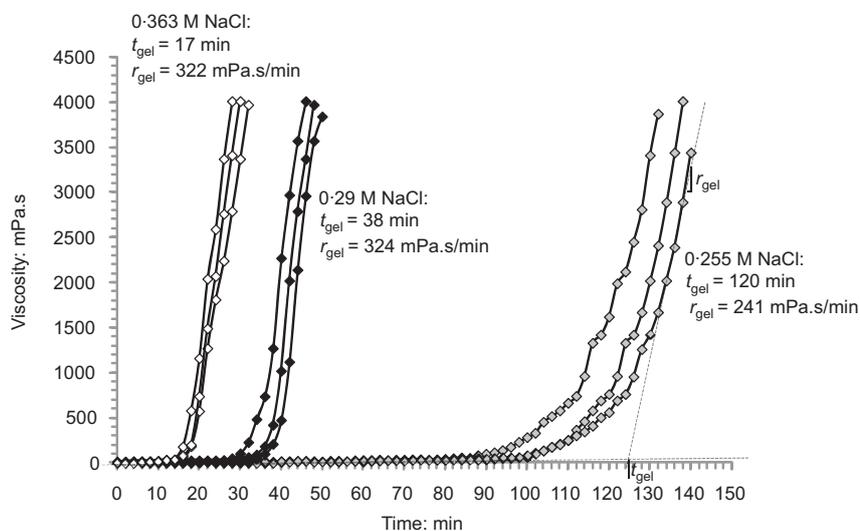


Fig. 1. Viscosity–time curves for the gelation of silica sol destabilised using sodium chloride accelerators. For each concentration, the experiment was performed in triplicate. Gel time (t_{gel}) is defined as the intercept of the extrapolations from the two straight line portions of the viscosity–times curves (Summers *et al.*, 1988) and the rate of gelation (r_{gel}) as the slope of the curve as marked on the figure. The reported values of t_{gel} and r_{gel} are averages of each triplicate

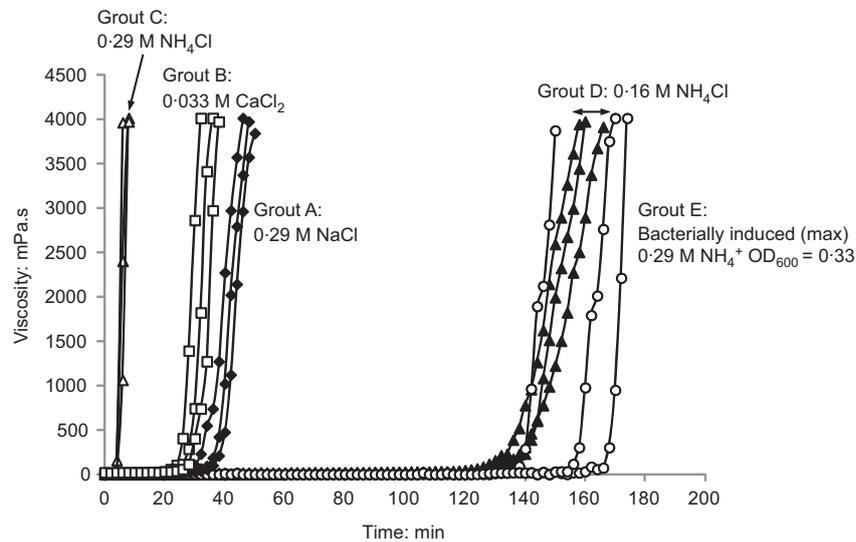


Fig. 2. Viscosity–time curves for the gelation of silica sol destabilised using different accelerators. Table 1 summarises the properties of grouts A to E

indicates a degree of heterogeneity resulting from the direct addition of calcium chloride. It is likely that aggregations also formed on the direct addition of sodium chloride, but these only became visible at higher salinity concentrations.

Monovalent cation accelerators

It is evident that grout C (0.29 M NH_4Cl) gelled much more quickly than grout A (0.29 M NaCl). This is despite the fact that both accelerators had monovalent cations and had the same final concentration (Fig. 2). The rate of gelation was also higher for grout C (Table 1). After 1 d, both grouts had similar shear strength; however, after 7 d, grout C exhibited a higher shear strength of 79 kPa compared with 51 kPa for grout A.

The observed differences in the viscosity curves and shear strengths between these two monovalent ions (Na^+ and NH_4^+) are due to ion specificity. Na^+ , due to its small size, has a high charge density and exhibits a strong interaction with water molecules (kosmotropic), resulting in a large hydration sphere (Trompette & Meireles, 2003). By comparison, NH_4^+ is a large ion with a low charge density and exhibits weaker interactions with water molecules (chaotropic); it thus has a smaller hydration sphere than Na^+ . As a result, NH_4^+ ions can adsorb closer to the silica particle surface, which may explain the higher shear strength observed. Consistent with our results, Trompette & Meireles (2003) found that chaotropic ions

are more efficient at destabilising silica sol than kosmotropic ions.

Bacterially induced ammonium ions (in situ) compared with direct addition of ammonium chloride

Comparing the direct addition of ammonium (grout C, 0.29 M NH_4Cl) with the production of NH_4^+ ions in situ (i.e. within the silica sol) by bacterial ureolysis (grout E, maximum of 0.29 M NH_4^+), it is clear that for the same (maximum) concentration of NH_4^+ ions, grout E had a longer gel time and a lower rate of gelation than grout C (Fig. 2). However, comparing direct addition versus in situ production for grouts with a similar gel time (grout D (0.16 M NH_4Cl) and grout E), grout E had a rate of gelation 2.8 times that of grout D (546 mPa·s/min compared with 192 mPa·s/min, Table 1) and a 25% higher 7-day shear strength than grout D.

Influence of bacterial density on silica sol gelation

Figure 3 compares the viscosity evolution of silica sol destabilised using two different bacterial densities, equivalent to 0.67 OD_{600} and 0.33 OD_{600} . For the grout with a higher number of bacteria (0.67 OD_{600}), the gel time is much shorter and the rate of gelation higher than for the 0.33 OD_{600} grout. This can be explained by considering the production of NH_4^+ ions, which is dependent on bacterial

Table 1. Silica gel properties for different grout and accelerator combinations as shown in Figure 2

Grout	Accelerator	Final molarity of accelerator ^a : M	Gel time ^b : min	Rate of gelation ^b : mPa·s/min	Shear strength: kPa	
					After 1 d ^b	After 7 d ^c
A	NaCl	0.290	38	324	28	51
B	CaCl ₂	0.033	29	767	28	76
C	NH ₄ Cl	0.290	4	1451	30	79
D	NH ₄ Cl	0.160	141	192	24	39
E	0.33 OD_{600} bacterially induced NH_4^+	Max. 0.29 NH_4^+	155	546	21	50

^aAfter mixing with silica sol in 5 : 1 ratio

^bAverage of three specimens tested

^cSingle measurement

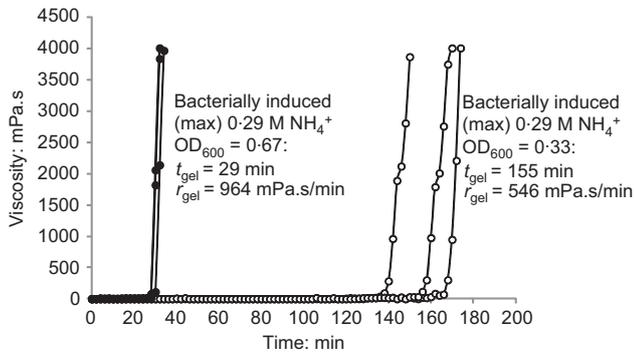


Fig. 3. Viscosity-time curves for in situ destabilisation via bacterial ureolysis at two different bacterial densities ($0.33OD_{600}$ and $0.67OD_{600}$); experiments were performed in triplicate

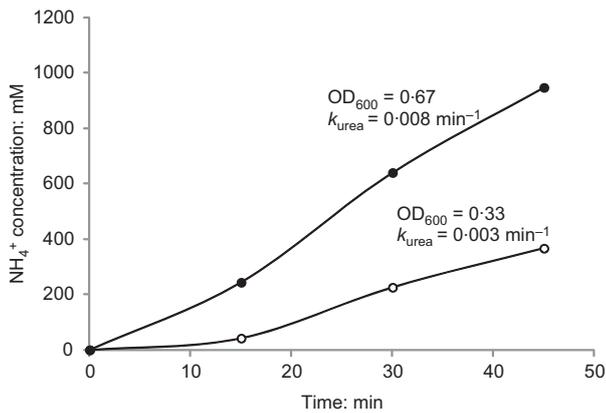


Fig. 4. Ammonium production and k_{urea} in silica sol at two different equivalent bacterial densities ($0.33OD_{600}$ and $0.67OD_{600}$). The initial urea concentration used for determination of k_{urea} was 1.5 M . Closed and open circles are ammonium concentrations estimated from conductivity measurements

density (OD_{600}) (Fig. 4). Ureolysis proceeds rapidly in the silica sol due to the fact that, here, the bacteria were grown in media containing urea; only a small initial lag can be observed. Figure 4 shows that NH_4^+ ions are produced before gelling begins in both bacterial grouts, indicating that a critical ion concentration is required for gelation and that this is reached much sooner in the higher bacterial

density grout ($0.67OD_{600}$) due to the higher ureolysis rate constant k_{urea} .

DISCUSSION

In order to use silica sol to grout fine-aperture fractures in hard rock, the gel time should enable sufficient penetration during the initial period of low viscosity and allow for mixing and pumping; workability times longer than an hour are recommended (Bodén & Sievänen, 2005). Once the gel time has been reached and pumping stopped, gelation should proceed rapidly so that there is a rapid strength gain. Reducing accelerator concentrations to control gel time compromises the gel rate and the rate of strength gain. This can result in backflow (particularly if connected to a tunnel), fingering and grout erosion (Emmelin *et al.*, 2007; Axelsson *et al.*, 2008).

This research shows that it is possible to use bacterial ureolysis to induce the gelation of silica sol and, for a given gel time, this results in a higher rate of gelation and a 25% higher final shear strength than can be achieved with the corresponding chemical accelerator (i.e. NH_4^+ ions). Furthermore, as observed in the experiments, chemical accelerators can result in heterogeneous gel formation due to the non-uniform distribution of ions during aggregation (Schantz Zackrisson *et al.*, 2008). No aggregations or clumping were observed in any of the bacterially induced gels. Figure 5 shows scanning electron microscope (SEM) images of a single cell (Fig. 5(a)) and multiple cells (Fig. 5(b)) embedded within grout E after 1 d. The relatively even distribution of bacteria engenders a more uniform distribution of ammonium cations, since production occurs in situ, resulting in a more uniform gel structure.

The consideration of biological processes in geotechnical engineering has gained increasing interest in recent years (Mitchell & Santamarina, 2005; DeJong *et al.*, 2013), with much attention focused on microbially induced calcite precipitation (DeJong *et al.*, 2006; Van Paassen *et al.*, 2010; Al Qabany & Soga, 2013). The current study demonstrates, for the first time, the potential use of microorganisms as a means of controlling the gel time of a low-viscosity grout – an entirely new avenue of research in geotechnical engineering.

Field application will require cultivation of *S. pasteurii* on a large scale, which can be carried out by biotechnology companies (Van Paassen, 2011). It is envisaged that the bacterial suspension would be first mixed with the silica sol

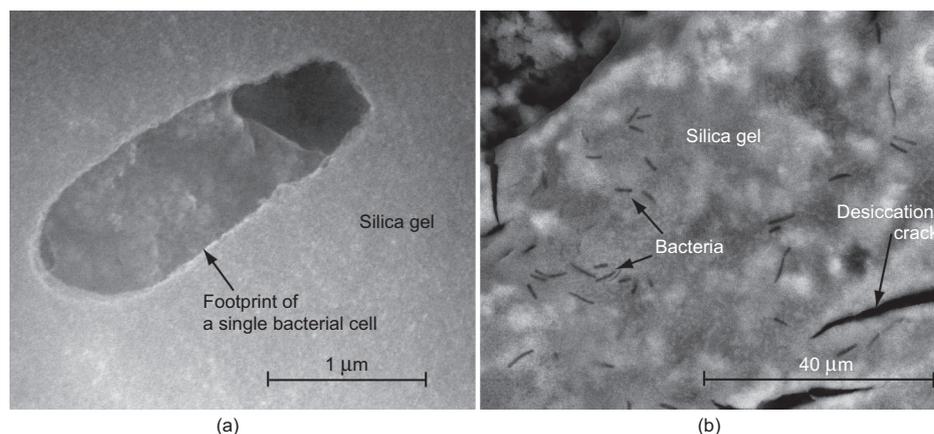


Fig. 5. SEM images of grout E obtained after 1 d, showing that (a) the bacteria remain in the form of individual cells and (b) they are relatively evenly distributed, indicating that the production of ammonium ions would be well distributed throughout the silica sol

and urea, and then pumped into the subsurface via injection points. While separation of *S. pasteurii* from the growth medium as conducted in this work may not be entirely necessary, the advantage is that the stimulation and growth of indigenous microorganisms can be avoided by not introducing additional nutrients into the subsurface. Finally, use of this technique in soils would require consideration of the bacteria size relative to the interconnected porosity (Mitchell & Santamarina, 2005; Phadnis & Santamarina, 2011).

CONCLUSIONS

This study has shown that bacterial ureolysis can be used to control the production of ammonium ions and thus the gelation of silica sol. The rate of ureolysis increases with increasing bacterial density, resulting in faster gel times and higher rates of gelation. For a specified gel time (i.e. penetration distance), the use of bacterial ureolysis leads to a higher rate of gelation, a higher final shear strength and a more uniform gel structure than direct addition of the corresponding chemical accelerator. These results suggest that bacterial ureolysis could potentially be used in rock grouting to achieve long gel times and hence greater penetration, while also maintaining sufficiently rapid gelation to minimise issues related to fingering and erosion of fresh grout.

Acknowledgements

This work was funded by a UK EPSRC research grant (EP/G063699/1) and an EPSRC doctoral studentship. The authors also thank Prof. Zoe Shipton for reviewing an early draft of the paper.

REFERENCES

- Al Qabany, A. & Soga, K. (2013). Effect of chemical treatment used in MICP on engineering properties of cemented soils. *Géotechnique* **63**, No. 4, 331–339.
- ASTM (1993). ASTM D4016: Standard test method for viscosity of chemical grouts by Brookfield viscometer (laboratory method). West Conshohocken, PA: ASTM International.
- Axelsson, M., Gustafson, G., Fransson, Å. & Funehag, J. (2008). Design criteria for permeation grouting in hard rock at great depths. *Proc. World Tunnel Congress 2008 Underground Facilities for Better Environment and Safety, Agra, India*. New Delhi: Central Board of Irrigation & Power, vol. 1, pp. 510–520.
- BASF (2009). Meyco MP320 Solvent-free, low viscosity, hydrophilic grout for rock injection and consolidation of sand and silt strata. See http://www.meyco.basf.com/en/meyco_solutions/TDS/Injection/mineralgrouts/MP320/Documents/TDS_MEYCO_MP_320.pdf (accessed 08/06/2012).
- Bodén, A. & Sievänen, U. (2005). *Low-pH injection grout for deep repositories. Summary report from a co-operation project between NUMO (Japan), Posiva (Finland) and SKB (Sweden)*. Stockholm: Swedish Nuclear Fuel and Waste Management Co., SKB report R-05-40.
- BSI (1990). BS 1377-7:1990: Methods of test for soils for civil engineering purposes. Shear strength tests (total stress). London: BSI.
- DeJong, J. T., Fritzes, M. B. & Nüsslein, K. (2006). Microbial induced cementation to control sand response to undrained shear. *J. Geotech. Geoenviron. Engng* **132**, No. 1, 1381–1392.
- DeJong, J. Y., Soga, K., Kavazanjian, E. *et al.* (2013). Biogeochemical processes and geotechnical applications: progress, opportunities and challenges. *Géotechnique* **63**, No. 4, 287–301.
- De Muynck, W., Leuridan, S., Van Loo, D. *et al.* (2011). Influence of pore structure on the effectiveness of a biogenic carbonate surface treatment for limestone conservation. *Appl. Environ. Microbiol.* **77**, No. 19, 6808–6820.
- Emmelin, A., Brantberger, M., Eriksson, M., Gustafson, G. & Stille, H. (2007). Rock grouting: current competence and development for the final repository. Stockholm: Swedish Nuclear Fuel and Waste Management Co., SKB report R-07-30.
- Funehag, J. (2007). Grouting of tunnel at great depths – how to meet strict demands? *Proc. 3rd Workshop on Low pH Cements for a Geological Repository, Paris*, 124–133.
- Funehag, J. & Axelsson, M. (2003). Hydrogeological characterisation and sealing of narrow fractures in hard rock – a case study. *RMZ Mater. Geoenviron.* **50**, No. 1, 121–124.
- Funehag, J. & Gustafson, G. (2008). Design of grouting with silica sol in hard rock: new design criteria tested in the field, part I. *Tunnell. Underground Space Technol.* **23**, No. 1, 1–8.
- Mitchell, J. K. & Santamarina, J. C. (2005). Biological considerations in geotechnical engineering. *J. Geotech. Geoenviron. Engng* **131**, No. 10, 1222–1233.
- Phadnis, H. S. & Santamarina, J. C. (2011). Bacteria in sediments: pore size effects. *Géotech. Lett.* **1**, No. 4, 91–93.
- Savarmand, S., Carreau, P. J., Bertrand, F., Vidal, D. J.-E. & Moan, M. (2003). Rheological properties of concentrated aqueous silica suspensions: effects of pH and ions content. *J. Rheology* **47**, No. 5, 1133–1149.
- Schantz Zackrisson, A., Skov Pedersen, J. & Bergenholtz, J. (2008). A small-angle x-ray scattering study of aggregation and gelation of colloidal silica. *Coll. Surf. A: Physicochem. Engng Asp.* **315**, No. 1–3, 23–30.
- Summers, L. E., Purkale, J. D. & Allison, J. D. (1988). Laboratory evaluation of crosslinked polymer gels for water diversion. In *Water-soluble Polymers for Petroleum Recovery. Proc. National ACS Meeting Polymers in Enhanced Oil Recovery and the Recovery of Other Natural Resources, Anaheim, CA* (Stahl, G. A. & Schulz D. N. (eds). New York: Plenum, pp. 313–328.
- Tobler, D. J., Cuthbert, M. O., Greswell, R. B. *et al.* (2011). Comparison of rates of ureolysis between *Sporosarcina pasteurii* and an indigenous groundwater community under conditions required to precipitate large volumes of calcite. *Geochim. Cosmochim. Acta* **75**, No. 11, 3290–3301.
- Trompette, J. L. & Meireles, M. (2003). Ion-specific effect on the gelation kinetics of concentrated colloidal silica suspensions. *J. Colloid Interf. Sci.* **263**, No. 2, 522–527.
- Van Paassen, L. A. (2011). Bio-mediated ground improvement: From laboratory experiment to pilot applications. *Proc. ASCE GeoFrontiers 2011: Advances in Geotechnical Engineering*. Reston, VA: ASCE, Geotechnical special publication 211, pp. 4099–4108.
- Van Paassen, L. A., Ghose, R., van der Linden, T. J. M., van der Star, W. R. L. & van Loosdrecht, M. C. (2010). Quantifying bio-mediated ground improvement by ureolysis: a large scale bioground experiment. *J. Geotech. Geoenviron. Engng* **136**, No. 12, 1721–1728.
- Whiffin, V. S. (2004). *Microbial CaCO₃ precipitation for the production of biocement*. PhD thesis, Murdoch University, Murdoch, WA.
- Young, A. J., Warwick, P., Milodowski, A. E. & Read, D. (2013). Behaviour of radionuclides in the presence of super plasticiser. *Adv. Cement Res.* **25**, No. 1, 32–43.

WHAT DO YOU THINK?

To discuss this paper, please email up to 500 words to the editor at journals@ice.org.uk. Your contribution will be forwarded to the author(s) for a reply and, if considered appropriate by the editorial panel, will be published as a discussion.