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

Erica MacLachlan

Gráinne El Mountassir

Rebecca J. Lunn

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

Corresponding Author:

Name: Gráinne El Mountassir

Address:

Department of Civil and Environmental Engineering,

John Anderson Building,

University of Strathclyde

107 Rottenrow,

Glasgow,

G4 0NG

Telephone: +44 141 548 3252

Fax: +44 141 553 2066

E-mail address: grainne.elmountassir@strath.ac.uk

Abstract

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 and mixing with an accelerator destabilises the suspension producing a gel. The influence of sodium, calcium and ammonium chloride accelerators on gel time, rate of gelation and shear strength of the resulting gel were investigated in this study. 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: Grout, Silica sol, Colloidal silica, Bacterial ureolysis, Gel time, Shear strength

1. Introduction

Cementitious grouts are commonly used to reduce hydraulic conductivity in fractured rock and increasingly microfine and ultrafine cements are used for penetrating fine aperture fractures. However, their penetration below 50-100µm remains limited without hydraulic jacking. For geological repositories of high level nuclear waste (HLW), fractures with apertures down to 10µm 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). Thus, there is a need for the development of alternative grouts which 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.

In this study, the direct addition of three different accelerators (sodium, calcium and ammonium chloride) was investigated and the gel time, rate of gelation and shear strength parameters 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.

2. Materials and Methods

2.1 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 the following properties: dynamic viscosity ~ 10 mPa.s, density = 1.3 g/cm³, pH = 9.5-9.8 and SiO₂ concentration = 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 (LVT DVII model) in accordance with ASTM D4016-93. 200ml of silica sol: 40ml of accelerator was used in the viscosity tests and double these volumes for the shear strength tests. Shear strength tests were carried out using a Wykeham Farrance laboratory shear vane apparatus (BS1377-7:1990). Specimens were sealed to prevent evaporation and shear strength was measured after: (i) 1 day and (ii) 7 days in an environment with a relative humidity of between 40 – 60 %. Viscosity and shear strength tests were carried out in a temperature controlled laboratory at 20 °C.

2.2 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. *S. pasteurii* were initially grown on solid Brain Heart Infusion (BHI) agar with urea (20g/l). A single colony was then transferred to liquid BHI growth medium (37g/l) again supplemented with urea (20g/l) and cultures were grown at 25°C over 24 hours. The bacterial suspensions were produced by separating the bacteria from the growth medium by centrifuging (at 8,000 rpm for 4 mins) and diluting with sterile tap water until the required optical density (OD_{600}) was achieved (measured using a UV-VIS spectrophotometer @600nm). 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 0.145M urea was prepared by mixing 20ml of 4 OD_{600} bacterial suspension and 20ml of 1.74M Urea together and then mixing with 200ml of silica sol. To give a final $OD_{600}=0.67$ the solutions were mixed as before but with 20 ml of 8 OD_{600} bacterial suspension. To achieve optical densities >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 80ml of 1 OD_{600} was re-suspended in 20ml of sterile water to give an equivalent of 4 OD_{600} . Separation of the bacteria from growth medium ensures limited further growth, while ureolysis provides the existing *S. pasteurii* with energy for metabolic processes.

2.3 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):

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

Ammonium concentrations were determined using the colorimetric Nessler method for aqueous solutions as described in 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; DeMuynck *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 ammonium concentration within the silica sol to be estimated using conductivity measurements and hence calculation of k_{urea} .

3. Results

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

3.1 Monovalent and divalent cation accelerators

Comparing Grout A (0.29M NaCl) and Grout B (0.033M $CaCl_2$) in Figure 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 mins) than for Grout A (38 mins) and the rate of gelation much higher, 767 mPa.s/min compared to 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 inter-particle collision, hence accelerating aggregation and gelation. After 1 day, Grout A and B had a shear strength of 28 kPa (Table 1), after 7 days Grout B exhibited a higher shear strength (76 kPa) compared to Grout A (51 kPa), indicating that cation valency also influences strength gain.

During visual inspection of Grout B (0.033M CaCl_2) large aggregations were noted on mixing and the resulting gel had distinguishable layers in varying shades of white. This indicates a degree of heterogeneity resulting from the direct addition of calcium chloride. It is likely that aggregations also form on the direct addition of sodium chloride; however these only became visible at higher salinity concentrations.

3.2 Monovalent cation accelerators

It is evident that Grout C (0.29M NH_4Cl) gelled much quicker than Grout A (0.29M NaCl). This is despite the fact that both accelerators have monovalent cations and had the same final concentration (Figure 2). The rate of gelation was also higher for Grout C (Table 1). After 1 day both grouts had similar shear strength, however after 7 days, Grout C (0.29M NH_4Cl) exhibited a higher shear strength of 79 kPa compared to 51 kPa for Grout A.

The observed differences in the viscosity curves and shear strengths between these two monovalent ions (Na^+ and NH_4^+) is 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), thus it 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.

3.3 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 max of 0.29M 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

(Figure 2). However, if we compare direct addition versus *in situ* production for grouts with a similar gel time, (Grout D 0.16M NH_4Cl and Grout E), Grout E had a rate of gelation 2.8 times that of Grout D (546 mPa.s/min compared to 192 mPa.s/min, Table 1) and a 25% higher 7-day shear strength than Grout D.

3.4 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 0.67OD₆₀₀ and 0.33OD₆₀₀. For the grout with the higher numbers of bacteria present (0.67OD₆₀₀) the gel time is much shorter and the rate of gelation higher than for the 0.33OD₆₀₀ grout. This can be explained by considering the production of NH_4^+ ions which is dependent on bacterial density (OD₆₀₀) (Figure 4). Ureolysis proceeds rapidly in the silica sol due to the fact that here the bacteria have been 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 (0.67OD₆₀₀) due to the higher ureolysis rate constant (k_{urea}).

4. 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 of more than 1 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 back flow (particularly if connected to a tunnel), fingering, and grout erosion (Emmelin *et al.*, 2007; Axelsson *et al.*, 2008).

Our research shows that it is possible to use bacterial ureolysis to induce the gelation of silica sol and for a given gel time 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 our 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 (Figure 5a) and multiple cells (Figure 5b) embedded within Grout E after 1 day. 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). This 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 and urea, and then pumped into the subsurface via injection points. While separation of *S. pasteurii* from the growth medium as conducted in our experiments 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, the use of this technique in soils would require consideration of the bacteria size relative to the interconnected porosity (Mitchell & Santamarina, 2005; Phadnis & Santamarina, 2011).

5. 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 D4016 (1993). Standard Test Method for Viscosity of Chemical Grouts by Brookfield Viscometer (Laboratory Method). *ASTM International*, West Conshohocken, PA, 1993.
- Axelsson, M., Gustafson, G., Fransson, Å. & Funebag, J. (2008). Design criteria for permeation grouting in hard rock at great depths. *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 8/06/2012.
- 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), *SKB Report R-05-40*, Swedish Nuclear Fuel and Waste Management Co., Stockholm Sweden.
- BS 1377-7:1990. Methods of test for soils for civil engineering purposes. Shear strength tests (total stress). *British Standards Institution*. 62 pages. ISBN: 0580182649
- DeJong, J.T., Fritzges, M.B., & Nüsslein, K. (2006). Microbial Induced Cementation to Control Sand Response to Undrained Shear. *Journal of Geotechnical and Geoenvironmental Engineering* **132**, No. 1, 1381-1392.
- DeJong, J.Y. Soga, K., Kavazanjian, E., Burns, S., van Passen, L.A., Al Qabany, A., Aydilek, A., Band, S.S., Burbnak, M., Caslake, L.F., Chen, C.Y., Chen, X., Chu, J., Ciurli, S., Esnault-Filet, A., Fauriel, S.s Hamdan, N., Hata, T., Inagaki, Y., Jeffers, S., Kuo, M., Laloui, L., Larrahondo, J., Manning, D.A.C., Martinez, B., Montoya, B.M., Nelson, D.C., Palomino, A., Renforth, P., Santamarina, J.C., Seagren, E.A., Tanyu, B., Tsearsky, M., & Weaver, T. (2013). Biogeochemical processes and geotechnical applications: progress, opportunities and challenges. *Géotechnique* **63**, No. 4, 287 -301.
- De Muynck, Willem, Leuridan, S., Van Loo, D., Verbeken, K., Cnudde, V., De Belie, N., & Verstraete, W. (2011). Influence of Pore Structure on the Effectiveness of a Biogenic Carbonate Surface Treatment for Limestone Conservation. *Applied and Environmental Microbiology* **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. *SKB Report R-07-30*, Swedish Nuclear Fuel and Waste Management Co., Stockholm Sweden.
- Funebag, J. & Axelsson, M. (2003). Hydrogeological characterisation and sealing of narrow fractures in hard rock – A case study. *RMZ-Materials and Geoenvironment* **50**, No. 1, 121–124.
- Funebag, J. (2007). Grouting of tunnel at great depths – How to meet strict demands? *Proceedings of the third workshop on low pH cements for a geological repository, Paris, June 13th-14th 2007*. ESDRED Workshop, 124-133.
- Funebag, J. & Gustafson, G. (2008). Design of grouting with silica sol in hard rock: New design criteria tested in the field, part I. *Tunnelling and Underground Space Technology* **23**, No. 1, 1-8.
- Mitchell, J. K. & Santamarina, J. C. (2005). Biological considerations in geotechnical engineering, *Journal of Geotechnical and Geoenvironmental Engineering* **131**, No. 10, 1222-1233.
- Phadnis, H. S. & Santamarina, J. C. (2011). Bacteria in sediments: pore size effects, *Géotechnique Letters* **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. *Journal of 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. *Colloids and Surfaces A: Physicochemical Engineering Aspects* **315**, No. 1-3, 23 - 30.
- Summers, L. E., Purkaple, J. D. & Allison, J. D. 1988. Laboratory evaluation of crosslinked polymer gels for water diversion, in: Stahl, G. A., Schuulz, D. N. (Eds.), *Water-soluble polymers for petroleum recovery*.

- Proceedings of a National Meeting of the ACS entitled Polymers in Enhanced Oil Recovery and the Recovery of other Natural Resources, held September 7-12, 1986, Anaheim, California, Plenum Press, New York, pp. 313-328.
- Tobler, D.J., Cuthbert, M.O., Greswell, R.B., Riley, M.S., Renshaw, J.C., Handley-Sidhu, S., & Phoenix, V.R. (2011). Comparison of rates of ureolysis between *Sporosarcina pasteurii* and an indigenous groundwater community under conditions required to precipitate large volumes of calcite. *Geochimica et Cosmochimica Acta* **75**, No. 11, 3290-3301.
- Trompette, J. L. & Meireles, M. (2003). Ion-specific effect on the gelation kinetics of concentrated colloidal silica suspensions. *Journal of colloid and interface science* **263**, No. 2, 522-527.
- Van Paassen, L.A., (2011). Bio-mediated ground improvement: From laboratory experiment to pilot applications, *ASCE GeoFrontiers 2011: Advances in Geotechnical Engineering*, 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 Biogrout experiment. *Journal of Geotechnical and Geoenvironmental Engineering* **136**, No. 12, 1721- 1728.
- Whiffin, V.S. (2004). Microbial CaCO₃ Precipitation for the Production of Biocement. *Science and Engineering School of Biological Sciences and Biotechnology. Murdoch University*. PhD thesis.
- Young, A. J., Warwick, P., Milodowski, A. E. & Read, D. (2013). Behaviour of radionuclides in the presence of super plasticiser. *Advances in Cement Research* **25**, No. 1, 32-43.

List of Figures

Figure 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. Values of t_{gel} and r_{gel} reported are averages of each triplicate.

Figure 2. Viscosity-time curves for the gelation of silica sol destabilised using different accelerators. Table 1 summarises the properties of each grout marked on Figure 2.

Figure 3: Viscosity-time curves for *in situ* destabilisation via bacterial ureolysis at two different bacterial densities: 0.33OD₆₀₀ and 0.67OD₆₀₀. Experiments were performed in triplicate.

Figure 4. Ammonium production and k_{urea} in silica sol at two different equivalent bacterial densities: 0.33OD₆₀₀ and 0.67OD₆₀₀. Initial urea concentration used for determination of k_{urea} was 1.5M. Closed and open circles are ammonium concentrations estimated from conductivity measurements.

Figure 5. Scanning Electron Microscope images of grout E obtained after 1 day, 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.

List of Tables

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

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

Grout	Accelerator	Final molarity (M) of accelerator ¹	Gel time (mins) ²	Rate of gelation (mPa.s/min) ²	Shear strength after 1 day ² (kPa)	Shear strength after 7 days ³ (kPa)
A	NaCl	0.29	38	324	28	51
B	CaCl ₂	0.033	29	767	28	76
C	NH ₄ Cl	0.29	4	1451	30	79
D	NH ₄ Cl	0.16	141	192	24	39
E	0.33OD ₆₀₀ Bacterially- induced NH ₄ ⁺	Max. 0.29 NH ₄ ⁺	155	546	21	50

308 ¹After mixing with silica sol in 5:1 ratio
309 ² Average of three specimens tested
310 ³ Single measurement

Figure 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. Values of t_{gel} and r_{gel} reported are averages of each triplicate.

Figure 2. Viscosity-time curves for the gelation of silica sol destabilised using different accelerators. Table 1 summarises the properties of each grouts marked A to E.

Figure 3: Viscosity-time curves for *in situ* destabilisation via bacterial ureolysis at two different bacterial densities: 0.33OD₆₀₀ and 0.67OD₆₀₀. Experiments were performed in triplicate.

Figure 4. Ammonium production and k_{urea} in silica sol at two different equivalent bacterial densities: 0.33OD₆₀₀ and 0.67OD₆₀₀. Initial urea concentration used for determination of k_{urea} was 1.5M. Closed and open circles are ammonium concentrations estimated from conductivity measurements.

Figure 5. Scanning Electron Microscope images of grout E obtained after 1 day, 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.