Influence of biofilms on transport of fluids in subsurface granitic environments – Some mineralogical and petrographical observations of materials from column experiments

**Coombs, P.**, West, J. M., Wagner, D., Turner, G., Noy, D. J., Milodowski, A. E., Lacinska, A., Harrison, H. and Bateman, K.

British Geological Survey (BGS), Kingsley Dunham Centre, Keyworth, Nottingham, NG12 5GG, UK.

email addresses: Coombs pcoo@bgs.ac.uk, West (jmwes@bgs.ac.uk); Wagner (dwagner@bgs.ac.uk), Turner (ght@bgs.ac.uk), Noy (djn@bgs.ac.uk), Milodowski (aem@bgs.ac.uk), Lacinska (alci@bgs.ac.uk), Harrison (hmh@bgs.ac.uk), Bateman (kba@bgs.ac.uk),

(1st level) Abstract

Landfill and radioactive waste disposal risk assessments focus on contaminant transport and are principally concerned with understanding the movement of gas, water and solutes through engineered barriers and natural groundwater systems. However, microbiological activity can impact on transport processes changing the chemical and physical characteristics of the subsurface environment. Such effects are generally caused by biofilms attached to rock surfaces. This paper will present some mineralogical and petrographical observations of materials extracted at the completion of an experimental column study which examined the influences of biofilm growth on groundwater flow through crushed diorite from the Äspö Hard Rock Underground Research Laboratory, Sweden.

(1st level) Introduction

Risk assessments for landfills and geological repositories for radioactive waste are primarily based on the precepts of contaminant transport; and are concerned with understanding the movement of gas, water and solutes through engineered barriers and natural groundwater systems, within the concept of 'Source', 'Pathway' and 'Receptor'. The emphasis on solute migration for landfill investigations is reflected in the theoretical development used during numerical simulation. However, microbes living in such environments can have an impact on transport processes (Cunningham *et al.* 1997; Keith-Loach and Livens 2002). Microbial activity in any environment is generally located on chemical or physical interfaces, usually within biofilms, and the impacts can be both physical (e.g. altering porosity) and/or chemical (e.g. changing pH, redox conditions, complexation) and may result in intracellular or extracellular mineral formation or degradation (Beveridge *et al.* 1997; Konhauser *et al.*1998; Tuck *et al.* 2006). Where biofilm growth in a host rock promotes mineral precipitation it can reduce water inflow and this can be a positive effect for limiting the transport of contaminants. Experimental work at the Äspö Underground Research Laboratory (URL) has investigated some of these chemical and physical effects in more detail in the context of the geological containment of radioactive waste in hard rock (diorite) environments. The significance of microbiological processes in the containment of radioactive waste has long been recognised (West and McKinley 2002) and, consequently, detailed evaluations of the biofilms present on the walls of the URL and on the significance of indigenous microbial populations has been a key area of work (Pedersen 1999). An *in-situ* study at the URL

examined the redox buffering of groundwater in vertical fracture-zones penetrated by recent, oxidising, meteoric water and showed that indigenous bacteria were capable of maintaining reducing conditions in the deep groundwaters (Banwart, 1995). Experimental work has also simulated the interactions of indigenous microbes with mineralogical surfaces associated with groundwater flow systems at Äspö (Hama *et al*. 2001); and ascertained that these microbes can either concentrate relevant chemical species for mineral formation in localised microenvironments or accelerate clay formation, the implications of this being that local hydrological conditions can be changed by microbial activity (Tuck *et al.* 2006). Also, biogenic mineral precipitates and trapped mineral matter are much more chemically and physically stable than a biofilm, persisting in the system long after the biofilm has decayed or been removed (Brydie *et al.* 2005). An experimental flow-through column study has now been undertaken which examined the influences of biofilm growth on groundwater flow though crushed diorite from the Äspö Hard Rock Laboratory, Sweden, particularly the significance of changes in groundwater pH. This paper describes some mineralogical and petrographical observations of extracted materials at the end of the experiments.

(1st level) Laboratory Techniques

The aim of the experiment was to evaluate how biofilms, generated by soil bacteria *Pseudomonas aeruginosa*, influenced the flow of synthetic Äspö groundwater through crushed Äspö diorite rock under two pH conditions. Details of the rock material and fluid components are given elsewhere (Hama *et al.* 2001). In brief, crushed diorite, with size-range of 250µm to 500µm, was packed into vertical borosilicate glass columns (25 mm I.D x 250mm). An artificial saline groundwater, chemically similar to one of the groundwater types from Äspö (Hama *et al.* 2001), was made up in the laboratory and sterilised through a 0.25µm filter prior to use. *P. aeruginosa*, bacteroa known for biofilm-forming properties, were prepared using methods described in Hama *et al* (2001). Two columns were set-up (one allowing changes in experimental parameters and the other as the control). Groundwater from a single reservoir containing the bacteria (10 ml inoculation containing 6.99 x $10^8$ organisms added to 23 l) was passed through both columns (initial flow rates: Column 1 = 2.33 ml $min^{-1}$; Column 2 = 0.11 ml $min^{-1}$) for a period of 726 hours. At this point, the pH of groundwater into Column 2 was changed from 7.2 to 5.2 with flow coming from a separate groundwater reservoir. The configuration of the columns at this point in the experiment is shown in Figure 1. The pH of the groundwater was changed back to 7.2 at 966 hours. The experiments ran for 2209 hours.

(1st level) Analytical Techniques

Flow rates through the column were measured using mass balance and samples of the groundwater were taken at regular intervals for microbiological and chemical analysis. At the end of the experimental period, a fluorochrome solution (acridine orange) was added to determine the location of the biofilm and bacterial numbers in the columns. Details of all analytical techniques and reagents are given elsewhere (Hama *et al.* 2001). The columns were then longitudinally sliced open by cutting lengthwise through the glass wall using a modeller's diamond saw and then slicing the contents in half using a bronze 'cheese wire'. Cut surfaces were photographed under ultraviolet (UV) illumination to show distribution of the acridine orange. In both

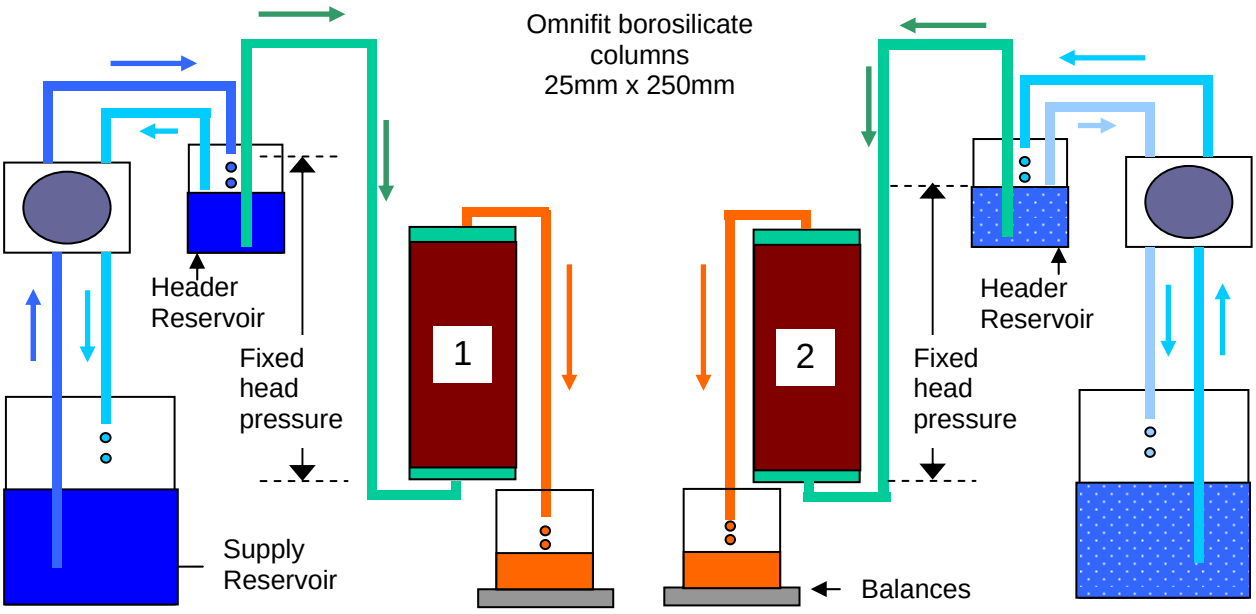


Figure 1. Diagram of the experimental setup of the dual columns after injection of *P. aeruginosa*

columns, the biofilm development and bacterial presence was limited to within 90 mm of the inlet of the column. Samples for petrographical analysis by scanning electron microscopy (SEM) were then taken along the lengths of both columns. Observations were made on: cryogenically-preserved subsamples (cryoSEM); freeze-dried subsamples; and subsamples prepared by solvent-displacement and drying with diffusive impregnation with polystyrene cement. SEM analysis was performed using a LEO 435VP variable pressure digital scanning electron microscope equipped with an Everhart-Thornley type detector for secondary electron imaging (SEI) and a KE Developments four-quadrant (4 diode-type) solid-state detector for backscattered electron imaging (BSEM). The instrument was also equipped with an Oxford Instruments CT1500 cryogenic sample handling and preparation unit directly interfaced to the SEM chamber, with a cryogenically-cooled SEM sample stage attachment. Phase/mineral identification was aided by qualitative observation of energy-dispersive X-ray spectra recorded simultaneously during SEM observation, using an Oxford Instruments INCA energy-dispersive X-ray microanalysis (EDXA) system.

(1$^{st}$ level)Results and Discussion

Changes in flow rates throughout the experiment are given in Figure 2 and show how fluid flow fluctuates considerably in the first ~700 hours. However, in both columns fluid flow drops to ~0.1 ml min$^{-1}$ from ~700 hours. Change in pH appears to have no effect on fluid flow. Petrographical analysis of the residues from both columns demonstrated that biofilm had formed in both experiments. Observations showed that biofilm and biofilament growth crossed the fluid-saturated intergranular pore spaces and trapped and bound mineral fines within pore throats, resulting in pore constriction and blockage (Fig. 3). This could at least partially account for the observed reduced permeability observed during the course of the experiment. CryoSEM observations also revealed that a non-aqueous, non-wetting organic liquid was also present within the biofilm (Fig. 4). It occurred as fine “oily” or “waxy” droplets that appear to represent a dispersed emulsion in the aqueous pore fluid, or were trapped by or attached to biofilm. It is possible that this material represents an

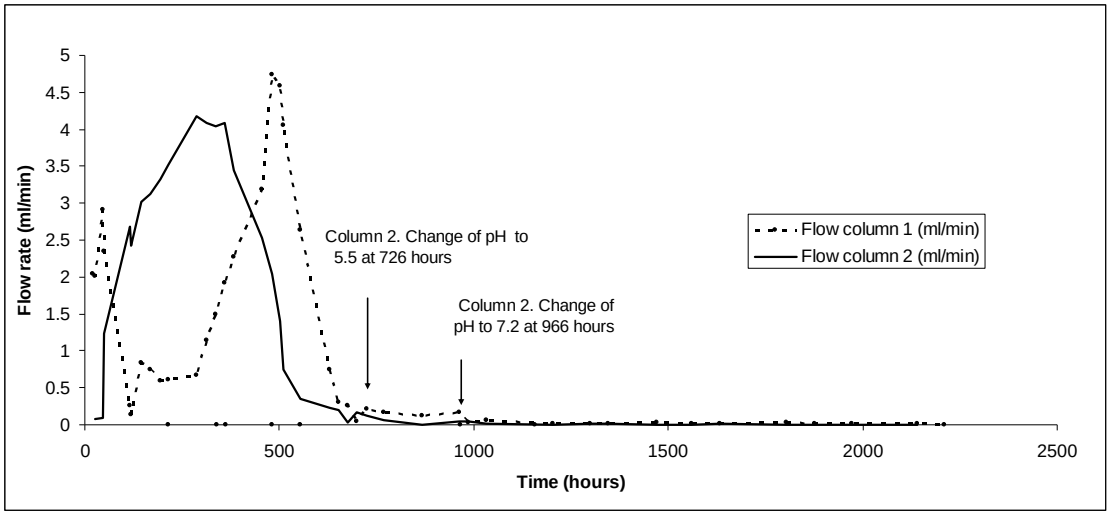


Figure 2. Flow rates through columns 1 and 2. Fluid pH was changed in column 2 as indicated.

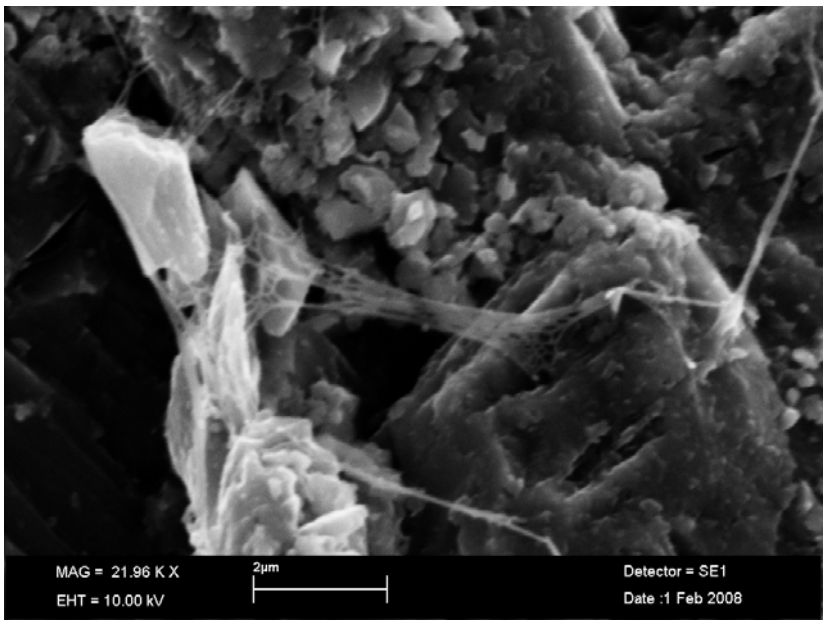


Figure 3. SEM image (SEI) showing "web-like" biofilm binding together the loose fine mineral debris within pore throats. Column 2, sample 3B.

immiscible organic liquid produced by the bacteria. This species is known to produce alginate (Hassett, 1996), which is an organic liquid that is immiscible with water. The development of biofilm appears to have been limited to within the first third of the columns' length (i.e. close to the inlet zone, where biological activity was indicated by acridine orange staining). There was little evidence of development or preservation of biofilm further along the columns. The change of pH did not appear to influence biofilm production. The different SEM preparation and analysis techniques produced markedly different preservations of the fabrics. For example, cryoSEM demonstrated the preservation of emulsion within pore spaces which was not observed in the other preparation methods. Difficulties were also encountered with each technique e.g. during cryoSEM, freezing was too slow during sample preparation and resulting ice crystal growth caused

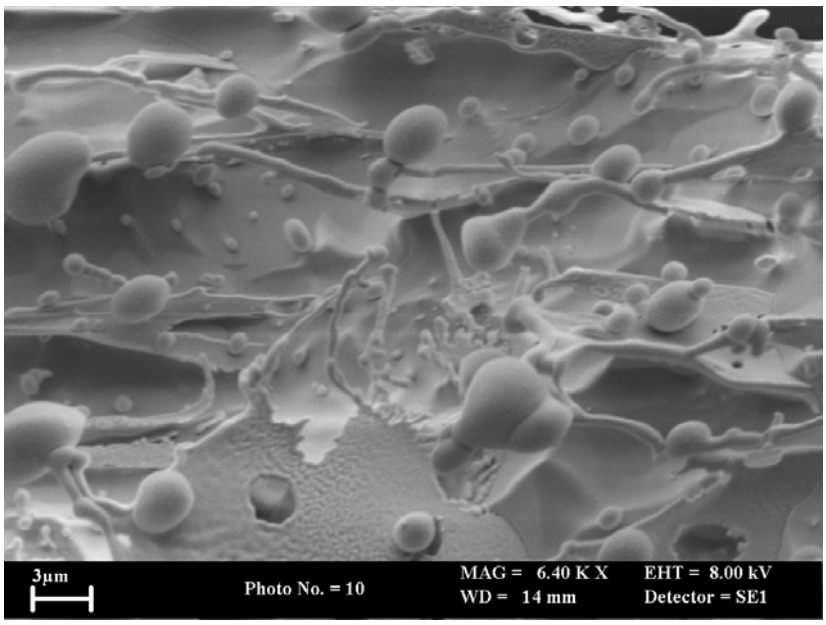


Figure 4. CryoSEM image (SEI) showing detail of organic emulsion droplets attached to, or nucleated on, biofilaments. Column 1, sample 1A.

significant fabric damage. Future cryoSEM, using small sample sizes would probably result in more rapid freezing and better sample preservation. Samples prepared by solvent-replacement drying, followed by impregnation with polystyrene solution produced a coherent sample that could be readily handled in the SEM. However, the polystyrene cement almost completely filled intergranular pore spaces thereby limiting possible observations. Nevertheless, it was possible to make observations on the upper surfaces of the grains, and this technique would appear to have some promise for future work.

## (1st level) Conclusions

These preliminary observations have shown that biofilms and biofilaments have formed in both columns and these appear to have influenced fluid flow. There was no apparent difference in fluid flow through the columns when the pH of the fluid was changed. This suggests that biofilms, once formed in dioritic environments, are robust and consequently they may have long-term effects on rock transport properties.

## (1st level) Acknowledgements

This work was funded by the BGS ‘BioTran’ and ‘MaDCap’ projects and is published with the permission of the Executive Director, BGS (NERC).

## (1st level) References

Banwart, S., 1995. The Äspö Redox Investigations in Block Scale Project summary and implications for repository assessment. Swedish Nuclear Fuel and Waste Management Co., Stockholm, Sweden. SKB Technical Report, 95-26.

Beveridge, T. J., Makin, S. A.. Kadurugamuwa, J. L. and Li, Z., 1997. Interactions between biofilms and the environment. FEMS Microbiology Reviews 20, 291-303.

Brydie, J.R., Wogelius, R.A., Merrifield, C.M., Boult, S., Gilbert, P., Allison, D., Vaughan, D.J., 2005. The μ2M project on quantifying the effects of biofilm growth on hydraulic properties of natural porous media and on sorption equilibria: an overview. In: Shaw, R.A. (Editor). Understanding the micro to macro behaviour of rock-fluid systems. Geological Society, London. Special Publication 249, 131-144.

Cunningham, A. L., Warwood, B., Sturman, P., Horrigan, K., James, G., Costerton, J. W. and Hiebert, R., 1997. Biofilm processes in porous media – practical applications. In: Amy, P.A., Haldeman, D. L. (eds), The Microbiology of the Terrestrial Deep Subsurface. CRC Lewis Publishers, 325-344.

Hama, K., Bateman, K., Coombs, P., Hards, V.L., Milodowski, A.E., West, J.M., Wetton, P.D., Yoshida, H., and Aoki, K., 2001. Influence of bacteria on rock-water interaction and clay mineral formation in subsurface granitic environments. Clay Minerals 36, 599-613.

Hassett D. J., 1996. Anaerobic production of alginate by *Pseudomonas aeruginosa*: alginate restricts diffusion of oxygen. Journal of Bacteriology 178, 7322–7325.

Keith-Roach, M. J. and Livens, F. R. (eds), 2002. Interactions of microorganisms with radionuclides. Elsevier, Oxford, UK. 400pp.

Konhauser, K. O., Fisher, Q. J., Fyfe, W.S., Longstaff, F. J. and Powell, M. A. 1998. Authigenic mineralisation and detrital clay binding by freshwater biofilms: The Brahmani River, India. Geomicrobiology Journal 15, 209-222.

Pedersen, K., 1999. Subterranean microorganisms and radioactive waste disposal in Sweden. Engineering Geology 52, 163-172.

Tuck, V. A., Edyvean, R. G.J., West, J. M., Bateman, K., Coombs, P., Milodowski, A. E. and McKervey, J. A., 2006. Biologically induced clay formation in subsurface granitic environments. Journal of Geochemical Exploration 90, 123-133.

West, J. M. and McKinley, I. G., 2002. The geomicrobiology of radioactive waste disposal. In: Bitton, G. (ed) The Encyclopaedia of Environmental Microbiology. John Wiley, New York. 227 – 249.