

## Laboratory examination of microbial effects upon redox in a geological disposal site for radioactive waste

K. Bateman  
J. West

British Geological Survey, Keyworth, Nottingham, NG12 5GG,  
UK

K. Aoki  
H. Yoshida

Power Reactor and Nuclear Fuel Development Corporation of  
Japan

P. Coombs  
M. R. Gillespie  
P. Henney  
S. Reeder  
A. E. Milodowski

British Geological Survey, Keyworth, Nottingham, NG12 5GG,  
UK

The Power Reactor and Nuclear Fuel Development Corporation (PNC) of Japan is currently undertaking a joint research programme with the Swedish Nuclear Fuel and Waste Management Company (SKB). This programme will investigate the capability of a near-repository host rock to consume dissolved oxygen in groundwater remaining in the backfill and near-field rock following the operational phase of a nuclear waste repository. It is planned to carry out these investigations *in situ* in a single hydraulically-conductive fracture within SKB's underground research facility at the Äspö Hard Rock Laboratory, Sweden. The project objectives are described by Banwart (1995) which gives details of 'The Redox Experiment in detailed Scale' (REX) to be conducted at Äspö. The laboratory experiments described here, were aimed at simulating the conditions within the REX Experimental Block to examine the interaction of microbes with mineralogical surfaces involved with groundwater flow. The mineralogical nature of the fracture surfaces and subsequent changes to those surfaces will be important with regard to radionuclide sorption and retardation, and also with respect to buffering groundwater redox through rock-water interaction. The mineralogy of the surfaces will also be important with respect to microbial interaction in terms of redox reactions mediated by microbial activity, and in providing substrates for microbes.

### Experiments

A series of batch experiments were conducted to study rock-water interactions. These experiments were designed to study simple systems and were aimed at identifying relevant reactions both chemical

and biological. They were essentially pilot studies to aid in the development of microbial and analytical geochemical procedures, as well as gathering basic data on rock-water interactions relevant to the Äspö site. The experiments were conducted in reactors, constructed of PEEK. The experiments conducted were of either 1, 2, or 3 weeks duration, at a temperature of 30°C. All reaction vessels were sterilised by autoclaving prior to assembly, and contained 20 g of crushed Äspö Diorite and 20 ml of Äspö groundwater. Analysis of the Äspö Diorite showed it to be composed of major quartz, with minor albite, mica, orthoclase, calcite and trace amounts of chlorite and possibly titanite and magnetite.

The experiments were assembled in an anaerobic chamber ( $H_2/CO_2/N_2$  atmosphere) and contained either sulphate reducing bacteria (SRB, *Desulfovibrio asponium*), Iron reducing bacteria (IRB, *Shewanella putrefaciens*) or a mixture of both SRB and IRB, together with control experiments without any bacteria. Both SRB and IRB have been highlighted as being of particular significance in Äspö geochemistry (Pedersen, 1995). The implications of both iron and sulphate reduction are discussed in detail in an earlier study (Banwart, 1995). The bacteria were cultured in appropriate enrichment media before being added to the reaction vessels in the predetermined numbers.

On termination of the experiments the reactors were opened in an anaerobic chamber and samples of the fluids and solids removed for analysis. The fluids were characterised both chemically (major anions and cations; trace elements; redox sensitive species) and microbially (by epifluorescence microscopy (Hobbie, 1977)) before and after the experimentals.

Mineralogical characterisation of the reacted solids was also performed.

### Results and conclusions

The chemical analyses of fluid phases from the batch systems showed evidence for dissolution of primary minerals. Many of the redox sensitive species ( $\text{NH}_4/\text{NO}_3/\text{NO}_2$ ,  $\text{As}^{\text{III}}/\text{As}^{\text{V}}$ ,  $\text{Se}^{\text{IV}}/\text{Se}^{\text{VI}}$ ) were found to be below the detection limits of analytical methods used. Total S remains fairly constant (within experimental and analytical errors) in all the experiments. In the experiments using SRB there is a small amount of reduced sulphur observed which suggests that the SRB were 'active' in these experiments, although the concentrations of  $\text{HS}^-$  detected were small. No  $\text{HS}^-$  was observed in the other experiments. There appears to be little dissolution of any iron phases present in the rock used in the experiments.

Both SRB and IRB appeared to be able to grow in the batch systems albeit for a limited period due to exhaustion of nutrient and energy supplies. SRB seem to have a greater effect on groundwater chemistry than IRB with sulphide being produced. IRB appear to have no effect on groundwater chemistry in these experiments. However, when the two types of bacteria are mixed together, the IRB appear to dominate the system.

There is evidence for minor mineralogical alteration during in all of the solid residues examined. No evidence was found for the precipitation of secondary reaction products. SEM examination of grain surfaces showed that in most cases, the sand-sized fraction which represents the bulk of the solid phase in the experiments, showed little evidence of dissolution. However in all experiment 'fines', which were produced during rock crushing, did show evidence of dissolution. These 'fines' appear to change from original sharply angular

morphologies, to progressively more rounded particles in the longer duration experiments.

No difference in alteration characteristics was observed between experiments with and without bacterial inoculations. Only tentative identification of bacterial structures could be made from observations, even in experiments where high populations were reported. The biofilaments, biofilms and cellular microbial structures which were observed closely resemble those present in Pedersen *et al.* (1996).

In the experiments with IRB, the bacteria were found to be preferentially concentrated on grains with rough surfaces. Cells or strings of cells were found to be more densely aggregated within hollows and other irregularities on the surfaces of quartz or the cleavage steps in feldspar grain surfaces. This would suggest that these bacteria are encouraged by sites which enable them to attach themselves (or their biofilms) to the mineral surfaces.

Further work using flowing systems is now underway to examine in more detail the interaction of microbes with mineralogical surfaces.

### Acknowledgements

We thank PNC for funding this work. This work is published with the permission of the Director of the British Geological Survey.

### References

- Banwart, S. (Ed). (1995) *SKB Technical Report*, 95–26. Stockholm.
- Pedersen, K. and Karlsson, F. (1995) *SKB Technical Report*, 95–10. Stockholm.
- Pedersen, K., Arlinger, J., Ekendahl, S. and Hallbeck, L. (1996) *Fems Microbiology Ecology*, 19, 249–62.
- Hobbie, J.E., Daley, R.J. and Jasper, S. (1977) *Appl. Environ. Microbiology*, 33, 1225.