OR/12/023 Laboratory techniques

Wragg, J, Rushton, J, Bateman, K, Green, K, Harrison, H, Wagner, D, Milodowski, A E, and West, J M. 2012. Microbial Impacts of CO2 transport in Sherwood Sandstone. *British Geological Survey Internal Report*, OR/12/023.

Experimental overview

The aim of this study was to evaluate how biofilms, generated by soil bacteria *P. aeruginosa*, influenced the flow of synthetic saline groundwater through intact Sherwood Sandstone. Two experiments, one biotic and the other an abiotic control, were carried out using BFA operated at a constant flow rate and under pressurised conditions. Changes in biological and chemical parameters were monitored throughout the experiment together with changes in confining pressure and temperature. The experiments were conducted over approximately 31 days and 89 days for the control and biotic experiments respectively, using sandstone samples taken from the Sherwood Sandstone Group from the Cleethorpes borehole (See Table 1). A saline groundwater (0.25 M as NaCl) was prepared, supplemented with sodium acetate (0.25g l^{-1}) to provide a readily available source of organic carbon to promote and sustain microbial growth, as this pilot experiment was short-term (Table 1), and sterilised by filtration though a Sartorius filter (0.2 µm). For the biotic experiment the groundwater was saturated with carbon dioxide (CO_2). Outflow fluids were collected from both cores and analysed for their major and trace element chemistry, to investigate the microbial effects on migration.

For the control experiment (*i.e.* no injection of microorganisms), the apparatus was fully assembled and filled with artificial groundwater on 4th November 2011. The system was monitored until decommissioning on the 5th December 2011 giving a total run time of 31 days.

The biotic experiment was started on 8th December 2011 and was filled with artificial groundwater. The system was monitored until December 15th 2011, when the pump was re-filled with artificial groundwater saturated with CO_2, 7 days after the start of the experiment. This was followed by injection of *P. aeruginosa* on 18th January 2012, 41 days after assembly of the experiment. Pumping continued until the March 6th 2012. The pump was stopped and the experiment was decommissioned the next day — a total of 89 days from the beginning of the experiment.

Flow-through column methodology

The flow-through column experiments were performed using intact Sherwood Sandstone rock core. Core material was positioned vertically in a Teflon sheath with stainless steel end caps allowing fluid flow through the column and the assembly was then placed in a pressure vessel. Schematics of the completed experimental rig with the pressure vessel and rock core assembly are shown in Figures 1 and 2 with photographs of the assembled apparatus in Plates 1 and 2. Once assembled, the pressure vessel was partially filled with deionised water and pressurised to 4000 kPa (40 bar). The cores were not pre-saturated with synthetic groundwater prior to the start of the experiment. The synthetic saline groundwater was used to fill the syringe pump and the flow rate was set at 300 µl h^{-1} (~7.2 ml day^{-1}). The control column was not injected with the organisms and the test was run for 31 days. The synthetic saline groundwater in the 'biotic' column was replaced by CO_2 saturated synthetic saline groundwater 7 days after column assembly and the column injected with *P. aeruginosa* after a

further 34 days. This experiment was terminated after a total of 89 days after assembly.

Pressure transducers, shown in Figure 1 as PT 1 and PT 2, were used to monitor the pressure changes within the cores while the syringe pumps controlled the flow-rate. The transducer outputs were recorded, along with actual pressure measurements on a calibrated DRUCK DPI 610 pressure calibrator and this data were subsequently used to calibrate the pumps and transducers. Fluid samples were collected by syringe at regular intervals for chemical and biological analyses.

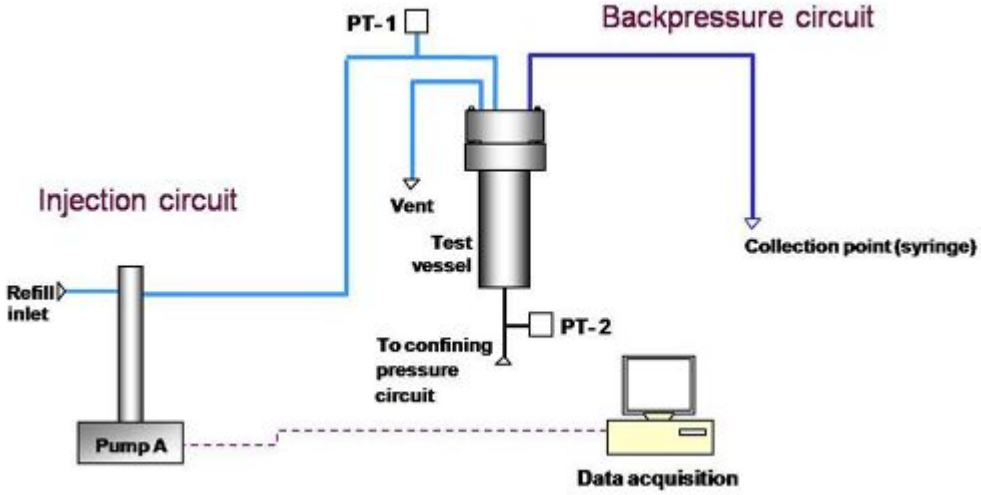

Figure 1 Schematic of column design.

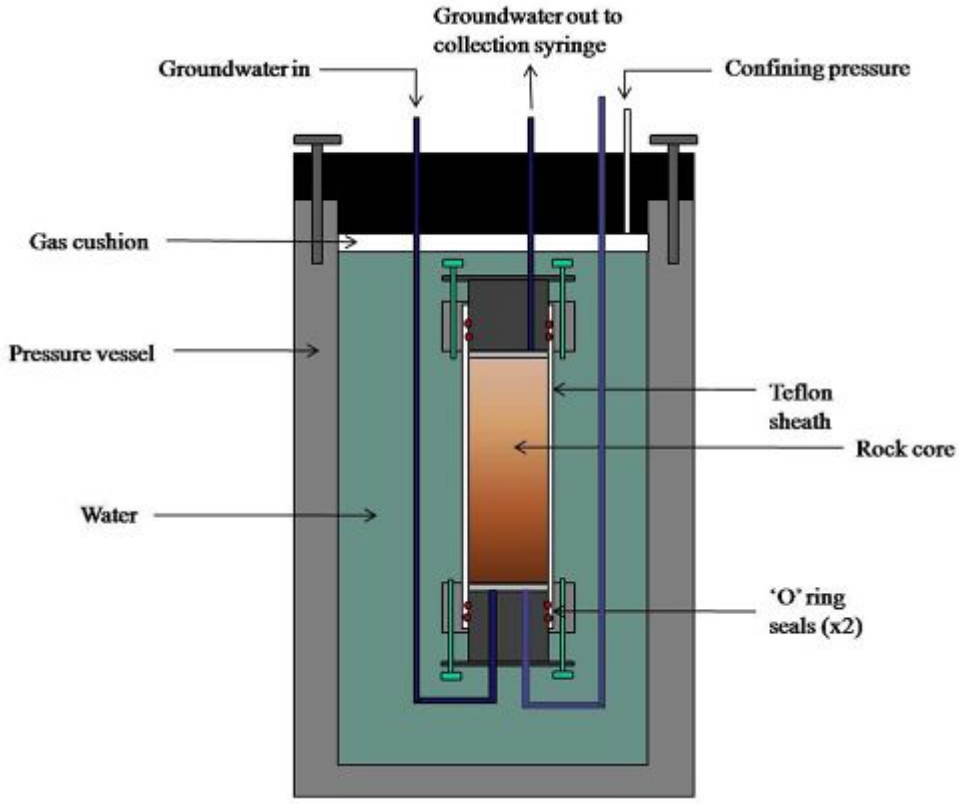

Figure 2 Schematic of pressure vessel with column containing rock core.

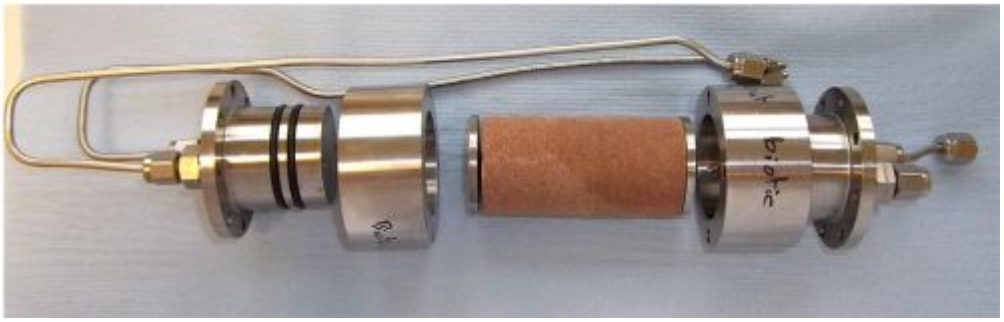

Plate 1 Photograph of column components prior to final assembly and insertion into pressure vessel.

Plate 2 Photograph of assembled apparatus showing pressure vessel and syringe pump.

Preparation of fluids

The synthetic groundwater was prepared from AR grade solid reagents. A saline (0.25M NaCl) solution was prepared by dissolving 14.6 g l^{-1} of NaCl in 18.4 ΩM water. Sodium acetate ($CH_3.COONa.3H_2O$) was added as 0.25 g l^{-1} (TOC = 22 mg l^{-1}). The fluid was then filter sterilised using a 0.2 µm filter and refrigerated until assembly of the experiment. Synthetic groundwater saturated with CO_2 was prepared by bubbling CO_2 at 1 atmosphere through the fluid for 24 h prior to

injection on to the column.

Selection and preparation of intact core material

Based on background BGS information on the Sherwood Sandstone Group (Milodowski and Rushton, 2008[1]) and previous studies as part of the BioTran project, investigating biofilm formation on samples from the Sherwood Sandstone Group (West et al, 2011[2]), a sandstones with appropriate permeability characteristics from the Cleethorpes No.1 borehole was identified for use in this study. To produce the samples, parallel plugs were longitudinally cut from the MPLP510 sample core using a 37 mm (nominal) diameter (ID) diamond-impregnated core barrel. The sample details are provided in Table 1.

Table 1 Summary details of the sandstone core samples.

Borehole	Stratigraphy	Rock Type	Sample Depth (m)	Sample Code	Experiment
Cleethorpes No. 1 Lincolnshire	Sherwood Sandstone Group (undifferentiated)	Feldspathic sandstone	1312.26-1312.41	MPLP510	Control and Biotic

Bacterial culture

P. aeruginosa was selected for its biofilm (exopolysaccharide — EPS) forming properties (Vaughan et al, 2001[3]) and has been used previously in BioTran experiments (Harrison et al, 2009[4]). It is a gram-negative rod, 0.5 to 0.8 µm wide by 1.5 to 3.0 µm in length and is a pathogen to humans. The matrix of the *P. aeruginosa* biofilm is composed of an alginate polymer of mannuronic and glucuronic acids. Its natural habitat is soil but it is also common to water and vegetation. *P. aeruginosa* is primarily aerobic but will grow under anaerobic conditions in the presence of nitrate which it can use as a respiratory electron acceptor; it is also resistant to high concentrations of salts. In this respect, it is a suitable strain for the experiment as synthetic saline groundwater is being utilised.

P. aeruginosa (NCIMB 10548) was received in a freeze dried state and resuscitated by adding 0.5 ml of sterile Nutrient broth (OXOID). This suspension was then sub-cultured onto agar slopes (OXOID CM3) and into a 50 ml flask of sterile nutrient broth. The slopes were refrigerated to maintain a stock culture for future experiments. The flask was placed on an orbital shaker, and incubated overnight at 36ºC, to encourage microbial growth. After 24 h the actively growing culture was then further inoculated into 500 ml flasks of sterile nutrient broth to achieve a large volume of bacteria. The culture was then transferred to 35 ml sterile tubes and centrifuged at 4600 rpm for 20 minutes. The supernatant was aseptically removed and the volume replaced with sterile artificial saline groundwater. The tubes were remixed and the centrifugation process repeated four times until all traces of culture media were ‘washed’ from the bacteria. Decreasing volumes of synthetic groundwater were added at each stage to concentrate the bacteria. The resulting fluid was then added to approximately 500 ml of synthetic groundwater, this produced sufficient volume to fill the syringe pump. A 1 ml sample of each suspended culture was removed by sterile pipette and preserved in gluteraldehyde fixative solution (Jass and Lappin-Scott, 1992[5]) prior to microscopic examination. The total number of bacteria inoculated was then determined by direct counting using epifluorescence microscopy (Hobbie et al, 1977[6]; Jass and Lappin-Scott, 1992[5]).

Decomissioning of experiments

The control experiment was terminated on 5th December 2011 with decommissioning of the biotic experiment on 6th March 2012. Plate 3 shows the decommissioning procedure. Plates 4 and 5 show the intact control and biotic columns prior to opening for mineralogical analyses, the direction of

flow is indicated by arrows.

Plate 3 Example of column assembly removal from pressure vessel.

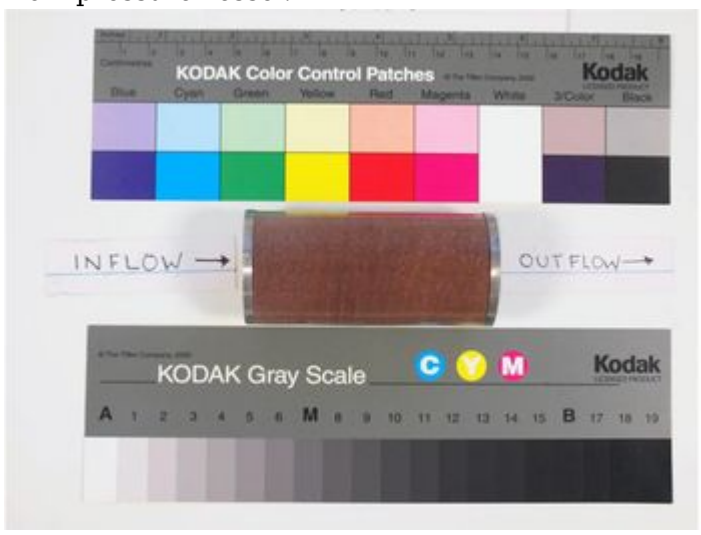

Plate 4 Control column after removal from pressure vessel and end pieces. Arrows indicate direction of fluid flow during the experiment.

Plate 5 Biotic column after removal from pressure vessel. Arrows indicate direction of fluid flow during the experiment.

References

1. ↑ MILODOWSKI, A E, and RUSHTON, J C. 2008. Mineralogical and porosity characterisation of potential aquifer and seal units for carbon capture and storage methodologies for the CASSEM project. *British Geological Survey Research Report, CR/08/153*.
2. ↑ WEST, J M, MCKINLEY, I G, PALUMBO-ROE, B, and ROCHELLE, C A. 2011. Potential impact of CO_2 storage on subsurface microbial ecosystems and implication for groundwater quality. *Energy Procedia*, 4, 3163–3170. doi:10.1016/j.egypro.2011.02.231.
3. ↑ VAUGHAN, D J, WOGELIUS, R, BOULT, S, and MERRIFIELD, C. 2001. Quantifying the effects of biofilm growth on hydraulic properties and on sorption equilibrium microscopic to macroscopic measurements. *Progress summary 02/01–11/01 Williamson Research Centre, University of Manchester*.
4. ↑ HARRISON, H, WEST, J M, BATEMAN, K, CAVE, M, COOMBS, P, HARRINGTON, J, LACINSKA, A, MILODOWSKI, A E, TURNER, G H, and WAGNER, D. 2009 Microbial effects on transport processes (BioTran) — Anaerobic flow- through Experiments using crushed Diorite and Pseudomonas aeruginosa (April 2008–March 2009). *British Geological Survey Open Report OR/09/033*.
5. ↑ [5.0] [5.1] JASS, J, and LAPPIN-SCOTT, H M. 1992. Practical course on biofilm formation using the modified Robbins Device. University of Exeter.
6. ↑ HOBBIE, J E, DALEY, R J, and JASPER, S. 1977. Use of nucleopore filters for counting bacteria by fluorescent microscopy. *Applied and Environmental Microbiology*, 33, 1225–1228.

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