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Comparison of microbiological influences on the transport properties of intact mudstone and sandstone and its relevance to the geological disposal of radioactive waste

J. Wragg[1], H. Harrison[1,*], J. M. West[1] and H. Yoshikawa[2]

[1] British Geological Survey, Keyworth, Nottingham NG12 5GG, UK
[2] Japan Atomic Energy Agency (JAEA), Muramatsu 4-33, Tokai-mura, 319-1194 Ibaraki, Japan

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ABSTRACT

The role of the microbial activity on the transport properties of host rocks for geological repositories, particularly in the far-field, is an area of active research. This paper compares results from experiments investigating changes in transport properties caused by microbial activity in sedimentary rocks in Japan (mudstones) and sandstone (UK).

These experiments show that both *Pseudomonas denitrificans* and *Pseudomonas aeruginosa* appear to survive and thrive in pressurized flow-through column experiments which utilized host rock materials of relevance to radioactive waste disposal. Indeed, despite there being a difference in the numbers of organisms introduced into both biotic experiments, numbers appear to stabilize at ~10^5 ml^{-1} at their completion. Post experimental imaging has highlighted the distinct differences in biofilm morphology, for the chosen rock types and bacteria, with *Pseudomonas aeruginosa* derived biofilms completely covering the surface of the sandstone host and *Pseudomonas denitrificans* forming biofilament structures. Regardless of substrate host or choice of microbe, microbial activity results in measurable changes in permeability. Such activity appears to influence changes in fluid flow and suggests that the transport of radionuclides through the far-field will be complicated by the presence of microbes.

Keywords: biofilm growth, geological disposal, mudstone, *Pseudomonas*, radioactive waste, sandstone, transport properties.

Introduction

The significance of the potential impacts of microbial activity on the transport properties of host rocks for geological repositories is an area of active research. Most work has focussed on the far-field environs, in granite, (Sweden) and mudstone (Japan). The far-field is considered to be the geosphere beyond the repository. As the UK does not yet have a potential site for deep geological disposal of radioactive waste, British research programmes involving biogeochemical processes [such as the BIGRAD consortium (http://www.nerc.ac.uk) and the British Geological Survey's BioTran project (http://www.bgs.ac.uk)] are focussing on 'generic' rock types with a mineralogy that could be considered broadly similar to that of a potential host UK geology. One of the generic rocks that has been selected is a sandstone of the late Permian to Triassic Sherwood Sandstone Group, which contains quartz, feldspars, muscovite/chlorite/illite and iron oxides and is relevant to a number of potential geological disposal facility (GDF) options in the UK. This paper compares some of the results obtained from flow-through

* E-mail: hmh@bgs.ac.uk
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column experiments investigating transport processes in Japanese mudstones and Sherwood sandstone.

Geological background

Two host substrates were chosen for comparison in this study: mudstone supplied by the Japan Atomic Energy Agency (JAEA) and sandstone, supplied by the National Geoscience Data Centre at the British Geological Survey (BGS). The mudstone was obtained from Horonobe in north-west Hokkaido, Japan, and the sandstone was obtained from Lincolnshire in the UK. The Horonobe area is a host site for an underground research laboratory (URL) which studies problems associated with the deep geological disposal of radioactive waste (Harrison *et al*., 2010). The mudstone used in this experiment came from a sequence of marine sandstones, mudstones and shales deposited within the Mesozoic Tempoku Basin. The geological setting and background to this material are described more fully in previous studies by Milodowski *et al.* (2004) and Harrison *et al.* (2010, 2011). Rocks of the Sherwood Sandstone Group are important as aquifers, oil and gas reservoirs and potential reservoirs for the storage of CO_2. Based on information in Milodowski and Rushton (2008) samples from the Cleethorpes No.1 geothermal borehole were chosen for these experiments, from a depth range of 1312.26–1315.13 m.

Laboratory techniques

The aim of the study was to evaluate the influence of biofilms generated by different bacteria on groundwater flow through the selected host rock types. The bacteria and associated host rock types under study were the denitrifying soil bacteria *Pseudomonas denitrificans* with mudstone from Horonobe (Harrison *et al*., 2010, 2011) and *Pseudomonas aeruginosa* with sandstone from Lincolnshire (West *et al*., 2011). Evaluation of biofilm growth and subsequent groundwater flow in both host rocks was assessed under biotic conditions (in the presence of added bacteria) and under control conditions (where no bacteria were added) using a flow-through column methodology. Harrison *et al.* (2010, 2011) provide a full description of the host rock mineralogy and groundwater composition, sample preparation techniques and methodologies for the experimental work. No specific effort was made to ensure that the experiments were run under anoxic conditions.

Experimental bacteria

Pseudomonas denitrificans was chosen for the mudstone experiments. This decision was based on groundwater information from Horonobe (Tochigi *et al*., 2007) which showed that denitrifying bacteria were likely to be the group of organisms with the greatest activity in this rock type. *Pseudomonas aeruginosa* was used in the sandstone experiments because of its biofilm (exopolysaccharide, EPS) forming properties (Vaughan *et al*., 2001). These bacteria can also grow under aerobic conditions in the presence of nitrate (which they use as a respiratory electron acceptor) and they show resistance to high concentrations of salts. Freeze-dried cultures of *P. denitrificans* (NCIMB 9496) and *P. aeruginosa* (NCIMB 10548) were received from National Collection of Industrial, food and Marine Bacteria (NCIMB), UK. Culture preparation is detailed in Harrison *et al*. (2010, 2011) and West *et al*. (2011).

Flow-through column experiment methodology

Biotic and control experiments were carried out for both host rock types, using a flow-through column operated at a constant rate of fluid flow and under pressurized conditions. Changes in biological parameters, confining pressure and temperature were monitored throughout the experiment. The mudstone experiments were short-term pilot studies [maximum of 39 days (936 h)] using synthetic groundwater (0.18 M NaCl) as described in Harrison *et al*. (2010, 2011), whereas the experiments conducted using the sandstone were carried out over a longer time period [maximum 273 days (6552 h)] with synthetic saline groundwater (0.25 M NaCl) as previously described by West *et al*. (2011). Deep subsurface groundwater is nutrient poor (West *et al*., 2002) which result in very slow growth rates, so for practical experimental reasons both synthetic groundwaters were supplemented with sodium acetate (3.05 mM), to provide a source of organic carbon to increase bacterial growth rates. They were also sterilized by filtration though a 0.2 μm filter. The flow-through column experiments were performed using intact rock cores, with the mudstone containing naturally occurring

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longitudinal fractures and the sandstone having a porosity of ~15–20% (West *et al*., 2011). The cores were positioned vertically in a Teflon sheath with end caps allowing fluid flow through the column. The assembly was then placed in a pressure vessel. Schematics of the completed experimental rig with the pressure vessel and rock core assembly are provided by Harrison *et al*. (2010, 2011) and West *et al*. (2011). The cores were not pre-saturated with synthetic groundwater prior to the start of the experiment. The experimental parameters are summarized in Table 1.

Analyses

Microbial numbers in the fluid injected into the column apparatus and in the reacted fluids collected from the outlet of the experimental rig were evaluated using epifluorescence microscopy (Harrison *et al*., 2010, 2011; West *et al*., 2011).

The mineralogical and morphological characteristics of the original test materials and post-experimental residues were determined using a number of complimentary techniques. The techniques include X-ray diffraction (XRD) for quantitative whole rock and qualitative clay mineral analysis. Petrographic analysis was undertaken using cryogenic (cryoSEM), variable pressure (VPSEM) and environmental (ESEM) scanning electron microscopy (SEM) techniques to identify morphological characteristics and study the mineralogy of fracture surfaces. Detailed sample preparation, instrumentation and set-up information, together with sample preservation and storage information for the study are documented in detail in Harrison *et al*. (2010, 2011) and West *et al*. (2011).

Microbiological results

The numbers of organisms injected into 'biotic' experiments differed between the mudstone and sandstone columns. The sterile synthetic fluids injected into the 'biotic' column experiments were inoculated with 1.18×10^5 (standard error (SE) = 8.88×10^3) bacteria ml^{-1} of *P. denitrificans* 11 days (264 h) after the start of the experiment for the mudstone; and 2.53×10^7 (3.93×10^6 SE)) bacteria ml^{-1} *P. aeruginosa* at 38 days (911 h) after the start of the experiment for the sandstone (Table 1). The fluids were then passed through the mudstone and sandstone columns for a further 28 days (672 h) and 235

TABLE 1. Summary of column flow-through experimental conditions.

Test Material	Mudstone		Sandstone	
	Control	Biotic	Control	Biotic
Starting pressure	1250–1260 kPa	1250–1260 kPa	1250–1260 kPa	1250–1260 kPa
Pump rate	300 µl hr^{-1} (~7.2 ml day^{-1})	300 µl hr^{-1} (~7.2 ml day^{-1})	300 µl hr^{-1} (~7.2 ml day^{-1})	300 µl hr^{-1} (~7.2 ml day^{-1})
Bacterium	–	*Pseudomonas denitrificans*	–	*Pseudomonas aeruginosa*
Starting bacterial count (per ml)	–	1.18×10^5 (8.88×10^3 SE)	–	2.53×10^7 (3.93×10^6 SE)
Inoculation time	–	264 hours (11 days)	–	911 hours (38 days)
Termination time	744 hours (31 days)	936 hours (39 days)	4464 hours (186 days)	6552 hours (273 days)

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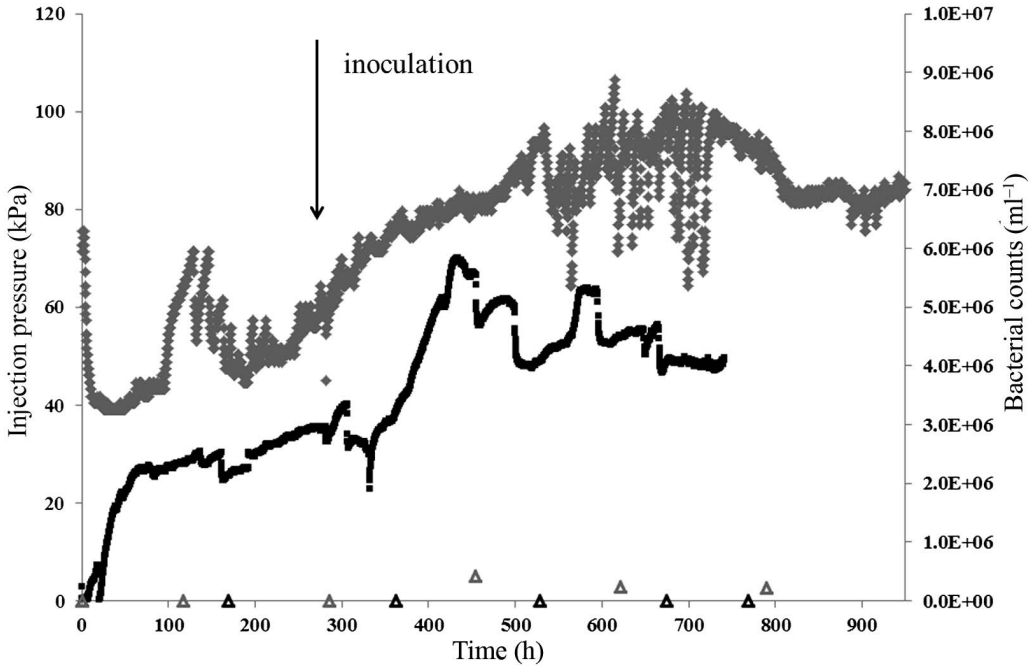

FIG. 1. Injection pressure and bacterial counts for the control and biotic mudstone column experiments. The control pressure and bacterial counts are shown by a black line and black open triangles, respectively. The biotic pressure and bacterial counts are shown by a grey line and grey open triangles, respectively.

days (5640 h), respectively. Samples of outflow fluid were collected at intervals and the bacterial count determined by epifluorescence microscopy. Figures 1 and 2 summarize bacterial numbers together with injection pressure changes for the biotic and control columns for the mudstone and sandstone respectively[1]. Figure 3 shows the injection pressure changes and bacterial counts for both rock types under control conditions. These figures demonstrate that comparatively few or no bacteria were viable in the mudstone and sandstone control experiments, for the comparable time period. A bacterial transit time of between 7 and 14 days (168–336 h) was indicated for the sandstone (Fig. 2), subsequent numbers of organisms leaving the column then fluctuated suggesting that the bacterial population exiting the column was changing throughout the experiment. The transit time for the sandstone core was similar to that observed by Harrison *et al*. (2011) for the mudstone core. Figures 1 and 2 indicate that although the two rock types were inoculated with different numbers of bacteria (approximately two orders of magnitude difference) and that the strains of bacteria, the rocks and the experimental timescales were different, the numbers of viable bacteria measured at the end of the experiments were broadly similar (4.47×10^5 bacteria ml^{-1} (7.06×10^4 SE) and 1.50×10^6 bacteria ml^{-1} (4.51×10^5 SE) for the mudstone and sandstone, respectively).

Mineralogical results and optical microscopy observations

Starting materials

Quantitative bulk XRD data for representative samples of the two host rock types is summarized in Table 2. The mudstone is composed of quartz (39.2%), albite (20.0%) and 'mica' [undifferentiated mica species possibly including muscovite, biotite, illite, illite/smectite and others] (22.1%) and minor/trace amounts of K-feldspar, 'kaolin' (one of the kaolin-like minerals including halloysite and kaolinite), chlorite, pyrite and smectite. In comparison, Table 2 shows that the sandstone

[1] N.B. The axes are different for both plots.

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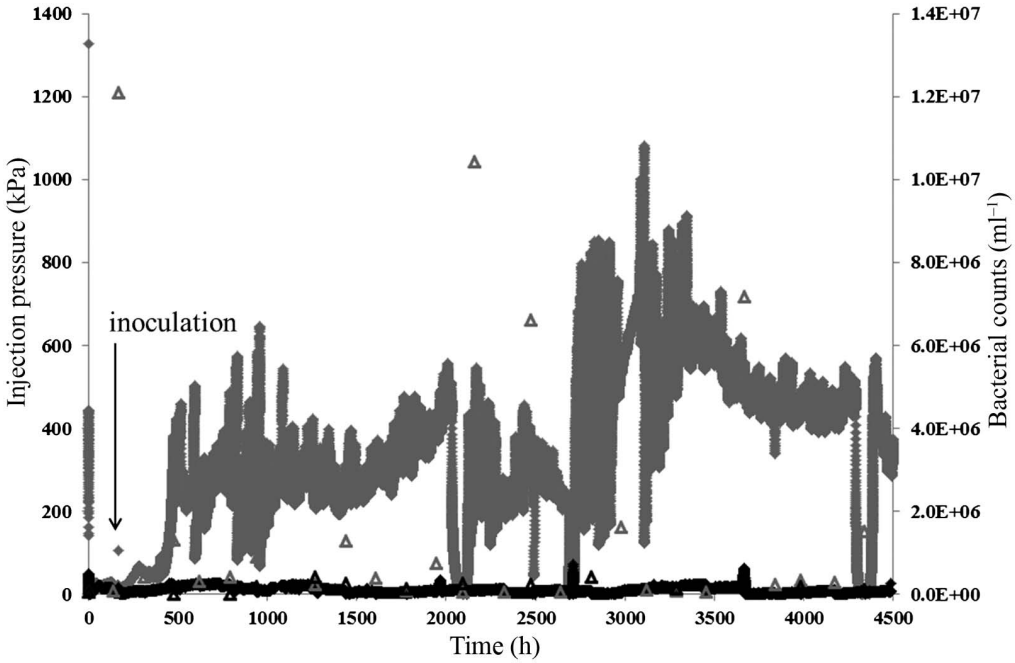

FIG. 2. Injection pressure and bacterial count data for the control and biotic sandstone column experiments. The control pressure and bacterial counts are shown by a black line and black open triangles, respectively. The biotic pressure and bacterial counts are shown by a grey line and grey open triangles, respectively.

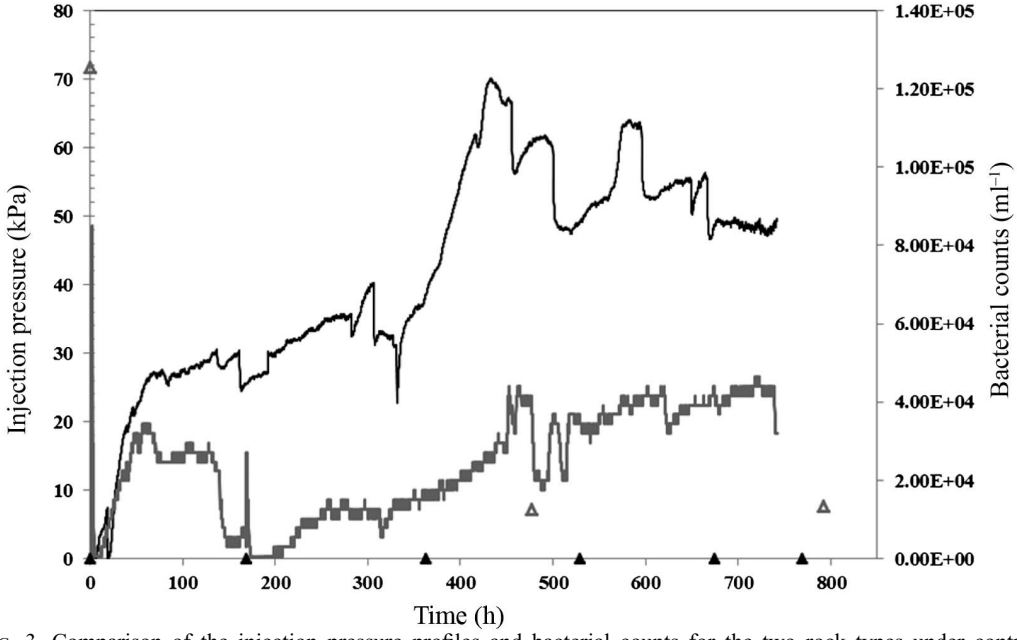

FIG. 3. Comparison of the injection pressure profiles and bacterial counts for the two rock types under control conditions. To enable comparison, only the first ~800 h after the start of data logging of sandstone experiment is shown. The mudstone pressure and bacterial counts are shown by a black line and open triangles, respectively. The sandstone pressure and bacterial counts are shown by a grey line and open triangles, respectively.

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TABLE 2. Quantitative bulk XRD analysis of representative samples of mudstone and sandstone. Crystalline mineralogy in wt.%.

Mineral	Mudstone	Sandstone
Albite	20	4.6
Chlorite	2.6	0.7
'Kaolin'	2.1	n.d.
K-feldspar	8.7	16.9
'Mica'	22.1	3
Quartz	39.2	72

The term 'mica' describes undifferentiated phyllosilicates including muscovite, biotite, illite and illite/smectite.
The term 'kaolin' includes the polymorphs of kaolinite and 'halloysite'.

contains minor/trace amounts of albite, kaolin and chlorite but is dominated by quartz (72%).

Scanning electron microscope images of the mudstone and sandstone starting materials are shown in Fig. 4*a*,*b*, respectively. Silt-sized fragments of the delicate microporous siliceous frustules of diatoms and silica-sand grade material are present in the mudstone (Fig. 4*a*). The mudstone fracture surface (Fig. 4*a*) is overgrown by a 1–2 μm diameter interconnected mesh of organic filaments. The morphology of the filaments resembles fungal hyphal structures. These features may be a result of contamination from initial drilling of the sample (Harrison *et al*., 2011).

An SEM photomicrograph of the sandstone starting material (Fig. 4*b*), shows it to be a finely laminated, clast supported, fine- to medium-grained sandstone. The laminae are moderately to well sorted and vary in thickness from 3 mm, for the darker, fine-grained and ferruginous more clay-rich laminae, to 5 mm for the cleaner sandier laminae. Most of the porosity is intergranular with a very high proportion of oversized pores in comparison to the grain size of the sandstone, indicating that their secondary origin has resulted from the dissolution of unstable detrital grains (Schmidt and McDonald, 1979). No biological features were observed.

Post experimental materials

Detailed SEM observations of the material surfaces in the control columns found minimal evidence of any biogenic structures, whereas examination of the post experimental biotic cores clearly identified biofilm formation. Fig. 5*a*,*b* illustrates examples of biofilm for the two host rocks. The biofilm observed in the biotic mudstone experiment using *P. denitrificans* appeared as bio-filaments or isolated rod-like clusters of cells, which penetrate the fractures in the material (Fig. 5*a*). Figure 5*b* depicts the biofilm produced in the biotic column experiment with the sandstone substrate using *P. aeruginosa.* In contrast to *P. denitrificans* on mudstone (Fig. 5*a*), this biofilm completely covers the surfaces of the substrate.

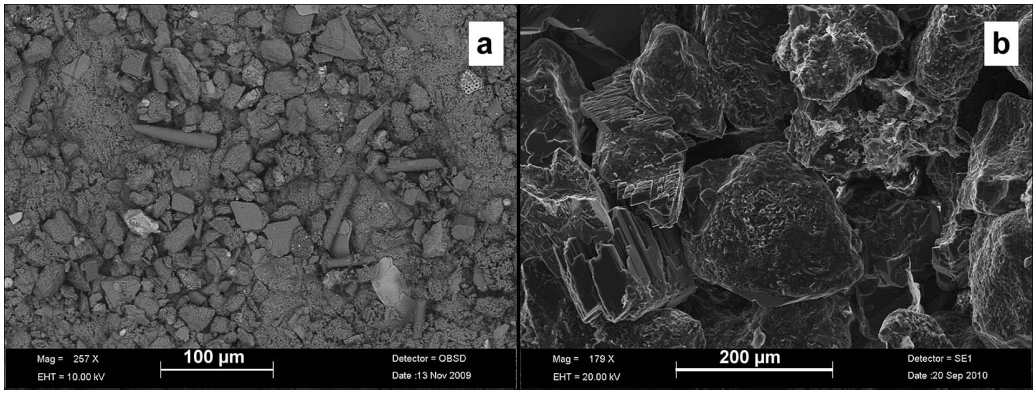

FIG. 4. (*a*) A BSEM image (from VPSEM) of mudstone starting material showing fine silt particles accumulated within the channel formed by the "ridge and furrow" lineaments on the fracture surface. (*b*) A SEM image of sandstone starting material showing euhedral authigenic K-feldspar and quartz overgrowth cements with some illite–smectite clay coating on detrital quartz grains (courtesy of A. E. Milodowski, BGS).

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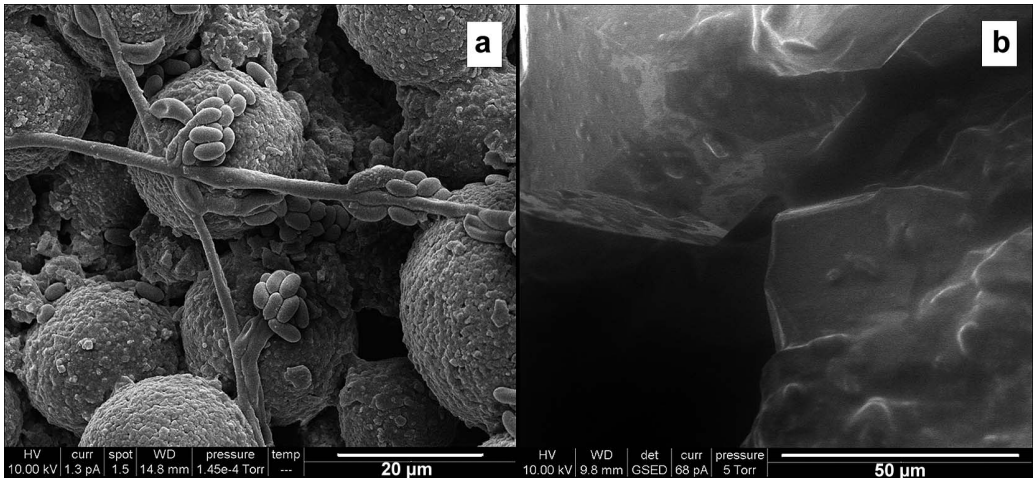

FIG. 5. (*a*) A CryoSEM SEI image showing detail of the clusters of rod-like cells associated with the biofilm resting on fresh framboidal pyrite in the reacted mudstone. High-vacuum cryoSEM, gold coated sample, FEI ESEM instrument (courtesy of A. E. Milodowski, BGS). (*b*) An ESEM SEI image of reacted sandstone showing the largely planar pore walls to a macropore covered by *P. aeruginosa* derived biofilm which is darker in its colouration and locally bridges smaller gaps (above and right of centre) (courtesy of J. Rushton, BGS).

No mineralogical evidence of oxidation of the redox-sensitive minerals was noted and no obvious changes in clay mineralogy were observed for the two rocks, suggesting that introduction of *P. denitrificans* or *P. aeruginosa* had minimal, if any effect on the clay composition and mineralogy of the two substrates.

Physical measurement results

Both biotic and control experiments for the different rock materials were performed at a constant flow rate (Table 1). Changes in injection and confining pressure were continuously logged by pressure transducers during the tests. Figures 1 and 2 show the injection pressure changes occurring in the biotic mudstone and sandstone experiments, respectively, with Fig. 3 summarizing the control experiments for the mudstone and sandstone. Each figure shows the experiment duration as time in hours along the *x* axis and the recorded pump pressure in bar along the *y* axis. A secondary axis indicates the number of viable bacteria exiting each system, as bacterial counts ml^{-1}. The sandstone experiment was undertaken for a longer period (273 days/6552 h) than the mudstone, although only data from the first 700–800 h (33 days) of the control experiment is shown in Fig. 3 and 4500 h (188 days) of the biotic experiment is shown in Fig. 2. This allows for direct comparisons of the results from both control and biotic experiments in Figs 2 and 3.

Control column experiments

Comparison of the two control experiments shows that, in general, similar trends in the pressure were observed, with an initial increase over the initial ~60 h, followed by a stabilization phase, up to ~350 h, at 30 and 15 kPa for the mudstone and sandstone, respectively. For both host rock types, steady increases in pressure were observed, which were followed by a period of stabilization [at ~600 h (25 days) in Fig. 3] and then a variation in pressure readings.

The changes observed during the initial hours of the experiment are thought to relate to the movement of water into pore spaces during the initial pressurisation of the system. During the experimental period, several changes in pressure were observed, which are considered to be a result of changes in flow geometry promoting changes in overall permeability within both the different host rock types.

Biotic column experiments

Figures 1 and 2 summarize the contrasting pressure changes between the biotic and control experi-

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ments for the mudstone and sandstone. Prior to the injection of bacteria, the specified sterile artificial groundwater was pumped through the both core assemblies for a nominal period, 264 h (0.18 M NaCl for 11 days) for the mudstone and 912 h (0.25 M NaCl for 38 days) for the sandstone (Table 1). In order to inoculate each column, the pump was stopped, causing a brief dip in pressure, the sterile water replaced with 500 ml of inoculated water and the pump restarted. Both host rock pressure profiles (Figs 1 and 2) show that a post inoculation pressure increase was observed in each test rig compared to the control experiments, with an average pressure of 84 kPa observed for the mudstone and 488 kPa for the sandstone (i.e. an order of magnitude difference between the two host rocks). This pressure difference is attributed to the physical differences between the two host rocks, which define the fluid flow through them. The mudstone was dominated by fractures because of its friable nature, one lateral and one vertical resulting in a highly permeable core and a low measured pressure within the core, whereas the sandstone was intact with a porosity of 15–20%. Despite such physical differences, the pressure profiles indicate broadly similar trends. Prior to bacterial injection, initial changes in core permeability are considered to be a result of movement of fines, blocking pore spaces and resulting in localized pressure increases, followed by breakthrough and establishment of new pathways resulting from a pressure increase by the use of a constant flow rate. The pressure increases observed post inoculation, compared to the control experiments (Fig. 3) are likely to be the result of partial blocking of pore spaces because of microbial activity. For both rock types short, but rapid, sawtooth like changes in pressure are evident over the post inoculation period. These pressure profiles are symptomatic of a dynamic system exhibiting localized intermittent changes in permeability, presumably brought about by the partial clogging of pore spaces and fractures by fines and/or biofilm followed by flushing because of an increase in localized hydraulic pressure.

Discussion

Despite the differences in (1) duration of both experiments (mudstone at 38 days/912 h or sandstone at 273 days/6552 h), (2) introduced species (*P. denitrificans* or *P. aeruginosa)* and (3) rock type (mudstone or sandstone), both column experiments showed that biofilm growth was possible. Both column experiments, showed that the bacterial species chosen are able to survive in saline pressurized conditions (West and McKinley, 2002). Moreover, although microbial inoculation numbers into the two different rock types differed by two orders of magnitude, the numbers of organisms in the outlet fluid were similar at the conclusion of both experiments. This suggests that the experimental conditions for both rock types could support approximately 10^5 organisms ml^{-1} as observed in the outlet fluids. There are likely to be more organisms associated with the biofilm itself. Substrate mineralogy and the microbes utilized in the experiments differed; it is therefore not unexpected that the resultant biofilms showed contrasting morphology. The biofilm developed on the mudstone appeared as bio-filaments whereas the biofilm formed on the sandstone completely covered the surfaces of the material. Comparison of the biotic and control experiments for the two rock types indicated that, in general, biofilm formation was not observed in the control experiments. No evidence of dissolution effects or alteration of the mudstone or sandstone starting materials was observed in either the biotic or abiotic experiments.

Fluctuations in injection pressure within the cores were detected during the biotic and control column experiments for both rock types. As noted by Harrison *et al*. (2011) small changes in the pressure profile of the control columns, related to small changes in permeability, could be the result of partial blocking of pore spaces by fines and the subsequent flushing of material as new pathways were established. Comparison of the pressure profiles of the two rock types under biotic conditions showed similar patterns. Post-inoculation injection pressure increases were observed, despite the physical differences in the materials, the experimental timescales and the order of magnitude difference in the pressure readings for the two host materials. The short but rapid sawtooth like changes in pressure are only observed under biotic conditions.

Conclusions

The comparative study has shown that both *Pseudomonas denitrificans* and *Pseudomonas aeruginosa* survive and thrive in pressurized flow-through column experiments in host rock matrices of relevance to radioactive waste disposal: diatomaceous mudstone (from Horonobe, Japan) and sandstone (from

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Lincolnshire, UK). Despite there being a difference in the numbers of organisms introduced into both biotic experiments, numbers appear to stabilize at ~10^5 ml^{-1} at their completion.

Post-experimental imaging has highlighted the distinct differences in biofilm morphology, with *Pseudomonas aeruginosa* derived biofilms completely covering the surface of the sandstone host and *Pseudomonas denitrificans* forming bio-filament structures. Neither host rock type showed evidence of post-experimental dissolution or alteration effects in comparison to the starting materials.

Regardless of substrate host or choice of microbe, microbial activity results in quantitative changes in permeability, as monitored by pressure increases specific to these column experiments. Such activity is considered to influence changes in fluid flow and suggests that the transport of radionuclides through the far-field will be complicated by the presence of microbes. Indeed, it is possible that some radioactive waste components may serve as a nutrient source for microbial activity, encouraging biofilm growth which could impact on both physical clogging and related biogeochemical changes, thus affecting radionuclide transport.

Further work is required to establish the significance of these observations to current fluid-flow modelling predictions.

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