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Microbial impacts on ^{99m}Tc migration through sandstone under highly alkaline conditions relevant to radioactive waste disposal

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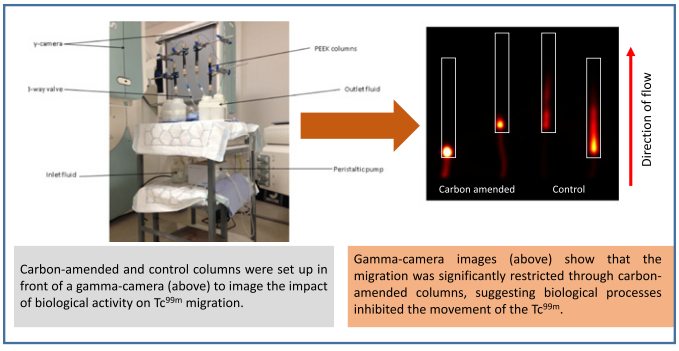
HIGHLIGHTS

- High-pH column experiments assembled representative of aspects of a GDF for ILW
- Biological processes caused restriction of Tc^{99m} migration.
- Gas generation and microbial Fe(III) reduction implicated
- H_2 oxidizers dominate column sediments.
- Implications for geodisposal of intermediate level radioactive waste

GRAPHICAL ABSTRACT



Carbon-amended and control columns were set up in front of a gamma-camera (above) to image the impact of biological activity on Tc^{99m} migration.

Gamma-camera images (above) show that the migration was significantly restricted through carbon-amended columns, suggesting biological processes inhibited the movement of the Tc^{99m}.

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ABSTRACT

Geological disposal of intermediate level radioactive waste in the UK is planned to involve the use of cementitious materials, facilitating the formation of an alkali-disturbed zone within the host rock. The biogeochemical processes that will occur in this environment, and the extent to which they will impact on radionuclide migration, are currently poorly understood. This study investigates the impact of biogeochemical processes on the mobility of the radionuclide technetium, in column experiments designed to be representative of aspects of the alkali-disturbed zone. Results indicate that microbial processes were capable of inhibiting ^{99m}Tc migration through columns, and X-ray radiography demonstrated that extensive physical changes had occurred to the material within columns where microbiological activity had been stimulated. The utilisation of organic acids under highly alkaline conditions, generating H_2 and CO_2, may represent a mechanism by which microbial processes may alter the hydraulic conductivity of a geological environment. Column sediments were dominated by obligately alkaliphilic H_2-oxidising bacteria, suggesting that the enrichment of these bacteria may have occurred as a result of H_2 generation during organic acid metabolism. The results from these experiments show that microorganisms are able to carry out a number of processes under highly alkaline conditions that could potentially impact on the properties of the host rock surrounding a geological disposal facility for intermediate level radioactive waste.

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1. Introduction

The UK's concept for the disposal of intermediate level radioactive waste (ILW) will involve a deep geological disposal facility (GDF), with numerous engineered barriers potentially containing cementitious materials, as, for example a backfill material (NDA, 2010). Movement of groundwater through a cementitious repository will cause an alkaline plume (pH ≈ 13) to develop, which will interact with components of the host rock forming an alkali-disturbed zone (ADZ). This ADZ will impact on microbial populations and processes within the host rock; these biogeochemical processes may in turn play a defining role in controlling the migration of key radionuclides through the geosphere, although it is thought that the upper pH limit for microbial activities is approaching pH 12 (Rizoulis et al., 2012). Biofilm formation, for example, may lead to changes in the physical properties of the host rock (Coombs et al., 2010), by causing a decrease in pore volume (Taylor et al., 1990) and therefore retarding radionuclide migration. Most microorganisms are capable of colonising surfaces, with several environmental cues thought to play a role in promoting biofilm formation including oxygen availability and pH (Babauta et al., 2013). Microbial cells secrete extracellular polymeric substances (EPS) comprising polysaccharides, nucleic acids, proteins and lipids which immobilise cells. The composition of the extracellular matrix is influenced by the microbial community composition, shear forces imposed on the biofilm structure, and other environmental factors (Flemming and Wingender, 2010). Microbial mineral precipitation, including microbially induced calcite formation may have similar impacts (DeJong et al., 2006, Cuthbert et al., 2012), particularly under the calcium-rich conditions that will be present in a cementitious repository. These processes may lead to alterations of pore space geometries, altering the hydraulic conductivity of the medium, and potentially resulting in a reduction in flow velocity (Hand et al., 2008).

The mechanisms by which microbial processes may impact on transport pathways in a GDF environment are wide-ranging and will depend on the local conditions. For example, processes that result in gas production, including organic matter utilisation, may cause pressure increases with the potential to damage waste containing barriers (Bonin et al., 2000). Depending on the rate at which gas generation occurs in a GDF, the consequences for fluid transport within a GDF host rock may be varied. Gas accumulation in a porous medium may lead to pore blockages (Taylor et al., 1990); the extent to which microbial gas generation occurs in a GDF host rock will depend on factors such as the availability of cellulose degradation products for microorganisms to metabolize for example. The UK's ILW inventory contains 2800 t of cellulosic materials which will degrade under the hyper-alkaline conditions generated in a GDF (NDA, 2014), although there remain uncertainties regarding the concentrations of cellulose degradation products that will be available in a GDF host rock when the pH conditions are suitable for microbial metabolism (Humphreys et al., 2010).

As well as physically blocking transport pathways, microbial metabolism may also control radionuclide movement through the geosphere via a range of other mechanisms. These include the alteration of mineral surfaces, for example, by forming biofilms that coat grain surfaces decreasing the availability of sorption sites, or by causing mineral dissolution that may impact on the ability of radionuclides to sorb to mineral surfaces (Brookshaw et al., 2012). Microbe-radionuclide interactions may also directly impact on the transport of radionuclides, via mechanisms including biosorption to ligands such as carboxyl, amine and phosphate groups associated with the cell surface, bioaccumulation within cells, and direct redox transformations e.g. bioreduction (Lloyd and Macaskie, 2002). Several Fe(III)-reducing microorganisms have demonstrated the ability to directly reduce redox active radionuclides to insoluble species, for example the reduction of U(VI), Np(V) and Tc(VII) to insoluble tetravalent forms under circum-neutral conditions (Lloyd, 2003). However, few studies have addressed microbial influences on radionuclide mobility under highly alkaline conditions representative of aspects of a GDF for ILW. The work that has been carried out investigating these high pH systems has suggested that microorganisms may play a role in immobilising some radionuclides up to a certain pH limit. As an example, Williamson et al. (2014) demonstrated that microbial U(VI) reduction occurred in microcosm experiments at pH 10.5, with most of the U(IV) formed associated with the solid phase.

Fe(II)-bearing minerals including biogenic siderite and vivianite resulting from the microbial reduction of Fe(III) may reduce radionuclides abiotically (Brookshaw et al., 2012). This mechanism has the potential to retard radionuclide migration through the geosphere, as several radionuclides including Tc are immobile in their reduced state (Lloyd et al., 2000). ^{99}Tc (a fission product of U-235 and Pu-239) is one of the UK's priority radionuclides (Walke et al., 2012) because of its long half-life (2.13×10^5 years; Zachara et al., 2007), and high mobility in its oxidised form [Tc(VII)] (Lear et al., 2010). Previous studies have investigated the mechanisms by which biogenic Fe(II) is capable of reducing Tc(VII) to Tc(IV), forming a poorly soluble precipitate (Zachara et al., 2007, McBeth et al., 2011). However, little is known about this process under the highly alkaline conditions that will be present in a GDF for ILW in the UK. A study carried out by Liu et al. (2008) demonstrated that the rate at which reductive immobilisation of Tc(VII) by Fe(II) occurs becomes slower as pH increases. Thorpe et al. (2014) found similar results when investigating Tc(VII) reduction with pre-reduced Fe(II)-bearing minerals, although a similar rate of Tc(VII) reduction was observed in microcosms at both circum-neutral pH and at pH 9, when Tc(VII) and Fe(III) reduction was concurrent.

This paper presents results of column experiments that aimed to investigate impacts of microbial processes under highly-alkaline conditions relevant to a GDF for ILW on transport in sandstone, and the resulting implications for Tc mobility. As a result of the potential microbial processes in a GDF and their subsequent impacts on transport processes discussed in the previous paragraphs, we hypothesize that in sediment columns incubated at high pH, microbial processes within these columns may inhibit the migration of radionuclides. To investigate these processes, high pH surface waters (pH ≈ 12.1) were collected from a hyper-alkaline spring which has formed at a legacy lime workings site in the Peak District, UK. This fluid was pumped through sandstone columns with and without acetate and lactate. The impact of microbial colonisation on the mobility of ^{99m}Tc was assessed using a multidisciplinary approach including geochemical, mineralogical and molecular ecological analyses, coupled with radionuclide imaging techniques.

2. Methods

2.1. Field sampling

A hyper-alkaline spring is present at Harpur Hill, Buxton (53.236082° N, −1.9171250° W), which formed as a result of percolation of rainwater though lime kiln waste (Rizoulis et al., 2012). Surface waters (pH ≈ 12.1, calcium hydroxide dominated) were collected from the hyper-alkaline spring at Harpur Hill, Buxton in sterile bottles. 1 L bottles were filled completely to ensure no headspace remained, and were stored at 4 °C for approximately two weeks.

2.2. Column experiments

Four polyether ether ketone (PEEK) columns (L = 15 cm; ID = 0.75 cm; Applied Research Europe, Berlin, Germany) were packed with approximately 10 g crushed sandstone (grain size < 500 μm). Surface waters collected from the field site were distributed between two sterile polypropylene vessels. Half of the fluid was amended with carbon (5 mM acetate, 5 mM lactate and 50 mgL^{-1} yeast extract). Columns were assembled in duplicate, with two receiving carbon-amended fluid and two receiving unamended fluid. Prior to experiment assembly, all components were sterilised by soaking in Virkon (active ingredients

include potassium peroxymonosulfate and sodium chloride) for several hours and flushed through with sterile deionised water. Pressure transmitters (Danfoss MBS3000 0-4 bar 4–20 mA transmitter, M&M controls, Manchester UK) were fitted at the inlet and outlet of all columns; pressure was logged in mA every 2 min and a calibration curve was prepared to convert mA to bar. Column experiments were carried out under anaerobic conditions (95% N_2, 5% H_2) for 247 days. A peristaltic pump was used to continuously upflow fluid through the columns at a flow rate of 100 μL h^{-1}.

Outlet fluid samples were collected every two weeks, and fluid samples were collected from the starting fluid to monitor changes in fluid chemistry and biological activity approximately every five weeks. Samples were collected and preserved accordingly.

2.3. Geochemical analyses

The pH measurements of fluid samples were collected using a Mettler Toledo pH probe which had been calibrated with pH 7 and pH 10 buffer solutions. A HANNA ORP probe was used to measure redox potential. Anions and organic acids were quantified by ion chromatography using a Dionex ICS5000 Dual Channel Ion Chromatograph. Weak acid extractable Fe(II) and total bioavailable Fe(III) was quantified spectrophotometrically as follows: sediment samples (approx. 0.1 g) were weighed and then digested in 4.9 mL 0.5 M hydrochloric acid for 1 h prior to performing a Ferrozine assay (Lovley and Phillips, 1986). Total biologically available Fe was quantified by reducing all bioavailable Fe within the sample using 6.25 M hydroxylamine hydrochloride for 1 h, and then quantified in duplicate after reaction with Ferrozine.

2.4. Cell counts

Microbial cells were enumerated in starting and outlet fluids from columns throughout the experiment. A direct counting method was applied using acridine orange staining; samples were prepared by first fixing 1 mL of sample in 10 mL 1% glutaraldehyde. A 5 mL aliquot of the fixed sample was filtered on to a 0.22 μm Millipore membrane filter and stained for two minutes with acridine orange, before rinsing with isopropyl alcohol. Slides were viewed under a Zeiss universal microscope using a Zeiss III RS epi-fluorescence head with filter set 09 (40–490 nm).

2.5. Gamma camera imaging of ^{99m}Tc migration through columns

At the end of the experiment, columns were transferred to the Nuclear Medicine department of the Manchester Royal Infirmary for ^{99m}Tc imaging. 25–40 MBq of ^{99m}Tc (as pertechnetate) was injected through one port of a three way tap attached to column inlets. N_2 sparged alkaline groundwater was then pumped through the columns at a flow rate of 6 mLh^{-1} for approximately 12 h using a peristaltic pump. Gamma camera images were taken at 15 min intervals throughout the course of the experiment using a dual-headed Symbia T6 gamma camera (Siemens Healthcare). Once imaging was complete, columns were stored for one week at 4 °C to allow decay of the ^{99m}Tc to background levels. Data were processed in Xeleris (GE Healthcare). For image analysis, the column images were divided into rectangular regions each covering one-third of the column length (inlet, mid-section and outlet). Geometric means of counts were calculated from both heads of the gamma camera to give results proportional to the radioactivity in the column, corrected for radioactive decay and normalised to account for the variation in activity injected into each of the columns.

2.6. X-ray radiography

At the end of the experiment, one carbon-amended and one control column were subjected to X-ray radiography to image the internal structure of the crushed sandstone within the packed columns. Columns were imaged using a GE Isovolt 320 kV unit (GE, Brisbane, Australia) at Intertek Non-Destructive Testing, Derby. Images were collected of the length of the columns (0°), and then the columns were rotated to image at 90°. Images were collected at a distance of 1200 mm from the focal spot in the detector head and imaging was carried out at a 120 kV–4 mA setting for 45 s.

2.7. Scanning electron microscopy

Sediment samples were collected from along the columns under anaerobic conditions and rinsed with 100% isopropyl alcohol to remove traces of the experimental fluid. For SEM imaging of sediment samples, samples were first air dried at room temperature for several hours and then transferred to an SEM stub. Samples were carbon coated before viewing using an FEI Quanta 600 ESEM.

2.8. Destructive sampling of columns

Following imaging, columns were cut into 3 cm sections by first cutting grooves to a depth of 2.5 mm into the columns using a lathe. Columns were then transferred to anaerobic conditions (95% N_2, 5% H_2), and cut all the way through using a hacksaw. The sediment at the exposed ends of the sections was removed with a sterile spatula to ensure no material was sampled that was contaminated by cutting tools.

2.9. DNA extraction, PCR amplification and sequencing

DNA was extracted from sediment samples collected from 3 cm intervals along the columns, and some key outlet fluid samples using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc.) following the manufacturer's instructions, and stored at −20 °C until analysed. A 16S rRNA gene PCR amplification was carried out on the DNA extracts to assess whether enough DNA was present to carry out 454 pyrosequencing on the samples. A pyrosequencing methodology was then applied to the DNA extracts (where PCR products were detected), targeting a 311 bp region of the 16S rRNA gene. The primer sets used were as follows: 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) (Lane, 1991) and 338R (5′-GCTGCCTCCCGTAGGAGT-3′) (Daims et al., 1999). Sequencing was carried out using a Roche 454 Life Science GS Junior system at the University of Manchester. Phylogenetic analysis was carried out using the QIIME software pipeline (Caporaso et al., 2010). Operational taxonomic units (OTUs) were picked using usearch with 97% sequence similarity (Edgar, 2010). The Ribosomal Database Project (RDP; Cole et al., 2009) was used to assign phylogeny with a minimum confidence of 80%. Representative sequences for each OTU were identified using a Blastn search.

Ribosomal Intergenic Spacer Analysis- RISA (Cardinale et al., 2004) was also carried out on DNA extracts from the outlet fluids of the column experiments to investigate population changes over time. Primer set ITSF/ITSFReub was utilised and consisted of 5′-GTCGTAACAAGGTAGCCGTA-3′ (forward primer) and 5′-GCCAAGGCATCCACC-3′ (reverse primer). PCR conditions are described in Cardinale et al. (2004). A 3% agarose gel was use to visualise RISA PCR products.

3. Results and discussion

Potential microbial impacts on the transport properties of porous media are wide ranging and are documented in numerous studies conducted at circumneutral pH. To address the limited amount of complementary data available on high pH systems, relevant to the UK's concept for geological disposal of ILW, flow-through experiments were conducted using columns containing sandstone, and an aqueous phase consisting of surface water collected from a high pH lime-kiln waste impacted field site. Control experiments containing no added carbon were compared to experimental systems supplemented with the electron donors acetate and lactate.

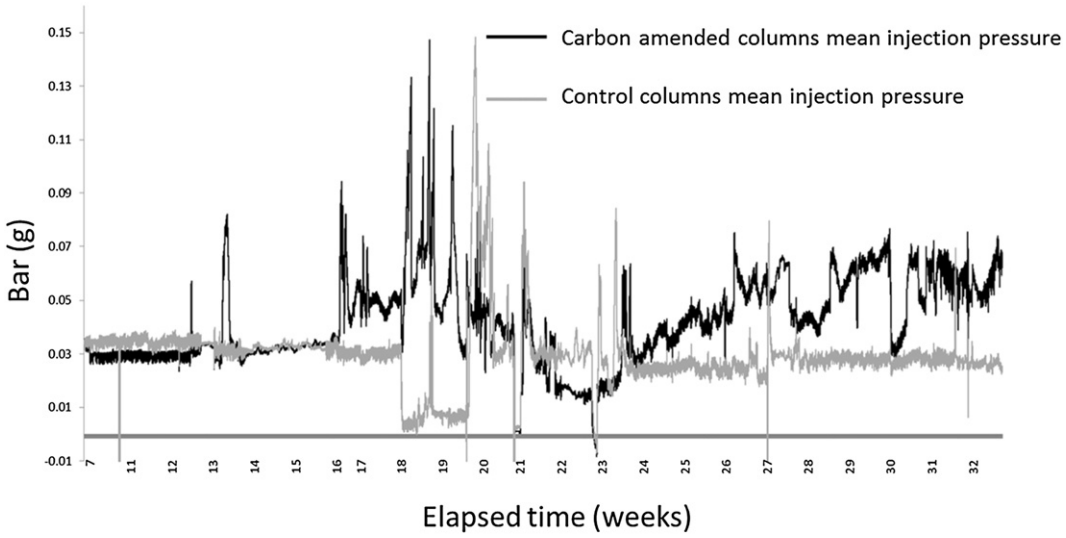

Fig. 1. Mean injection pressures of carbon amended and control columns. Mean values were calculated from duplicate values collected for each time-point.

3.1. Transport properties

During our 247 day experiments, injection pressure increases were observed in all columns at approximately 18–20 weeks (Fig. 1). After these pressure increases were observed, a decrease to pressures comparable to starting injection pressures was seen. After approximately 23 weeks, steady increases in injection pressures in the carbon-amended columns were observed, reaching almost 0.1 bar (g). Although these pressure increases are relatively small, crushed sandstone has a high porosity and permeability, and a slow flow rate (100 μL h^{-1}) was used. Consequently, these pressure increases could be of significance.

Abiotic processes that may have caused these initial increases in injection pressure could include clogging as a result of the movement of fines (Oliveira et al., 2014), or mineralogical alterations as shown in Fig. 2. Calcium silicate hydrate (CSH) phases formed a web like precipitate over quartz grains (a) and gelatinous coatings (f), while grains of potassium feldspar coated in calcite (b) and a gel like silicate hydrate were also noted. These reaction products have also been observed in other studies of hyper-alkaline experimental systems (Hodgkinson and Hughes, 1999). Hexagonal plates of kaolinite were shown to have expanded, also shown by Xie et al. (2006), and were coated with a gel like phase at the edge of the plates (c), and were also observed with a

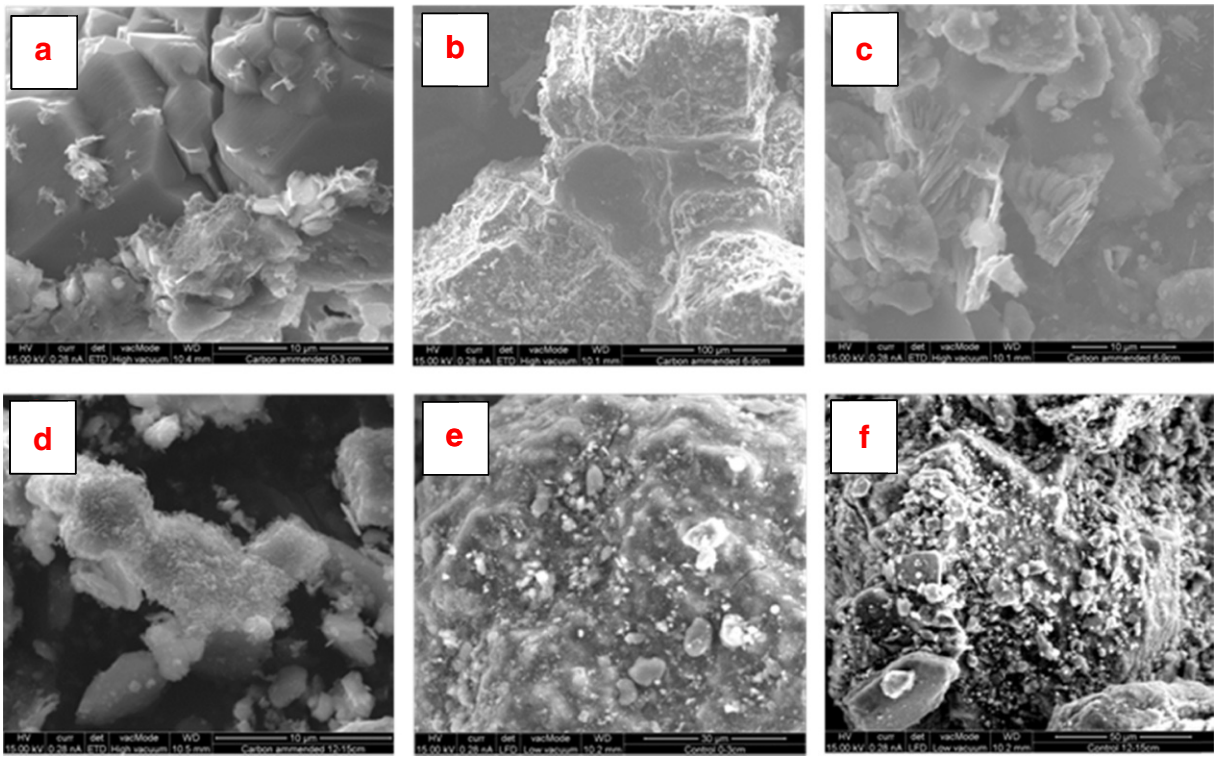

Fig. 2. Scanning electron micrographs of sediments collected from columns at the end of the experiment. (a) Carbon amended 1 column 0–3 cm, (b) carbon amended 1 column 6–9 cm, (c) carbon amended 1 column 6–9 cm (d) carbon amended 1 column 12–15 cm, (e) control 1 column 0–3 cm, (f) control 1 column 12–15 cm.

fluffy gel like silicate hydrate coating (e). Debris was shown to adhere to grain surfaces by clumping together with reaction products (g).

The decreases in pressure could perhaps be a result of processes such as the breakthrough of fines (Gravelle et al., 2011). Injection pressure increases towards the end of the carbon-amended experiments (after approximately 23 weeks) appear to be more sustained, and remained at approximately 0.1 bar (g), perhaps implying a microbial role in these increases in injection pressure. These processes could include bioclogging, although sediment samples were subjected to confocal laser scanning microscopy using nucleic acid stains to visualise live and dead cells, epifluorescence microscopy and ESEM (data not shown), and biomass levels on the sediments appeared to be very low.

At the end of the experiment, columns were subjected to additional analyses to further investigate how microbial activity had impacted on the transmissive properties of the sandstone. As biogeochemical profiles had suggested that microbial processes had been stimulated at high pH in the carbon-amended columns, the impact of these stimulated microbial communities on Tc mobility was assessed by applying a spike of ^{99m}Tc (25–40 MBq initial activity) pumped through the columns at a rate of 6 mL h^{-1} and monitoring its mobility using a gamma camera (Fig. 3).

A much faster flow rate of 6 mL h^{-1} was used to ensure enough pore volumes of high pH fluid flushed the short (6 h) half-life tracer through the columns. The use of a faster flow rate may have altered the transport properties of the crushed material, as sudden changes in fluid velocity have been shown to alter permeability of porous media (Mays and Hunt, 2005). Nevertheless, Figs. 3 and 4 demonstrate that the migration of the ^{99m}Tc occurred much more slowly through the carbon-amended columns compared to the controls.

Fig. 4 shows the location of the activity within the columns throughout the tracer test, indicating that at the end of the 12 h imaging period, the ^{99m}Tc was still retained in the carbon-amended columns; no activity was detected in the control columns after 1 h. There are a number of possible explanations for the inhibition of the ^{99m}Tc migration through the carbon-amended columns. At the end of the tracer test it was noted

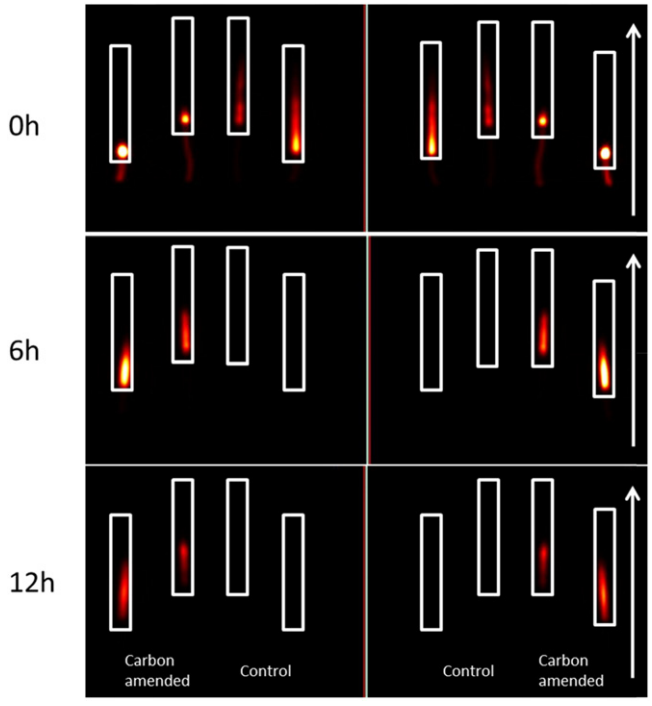

Fig. 3. Gamma-camera images of columns (outlined) at 0 h, 6 h and 12 h. Images on the left were collected with head on of the gamma camera and images on the right were collected with head two of the gamma camera. Carbon amended and control columns are labelled in each of the images, and the white arrows show the direction of flow.

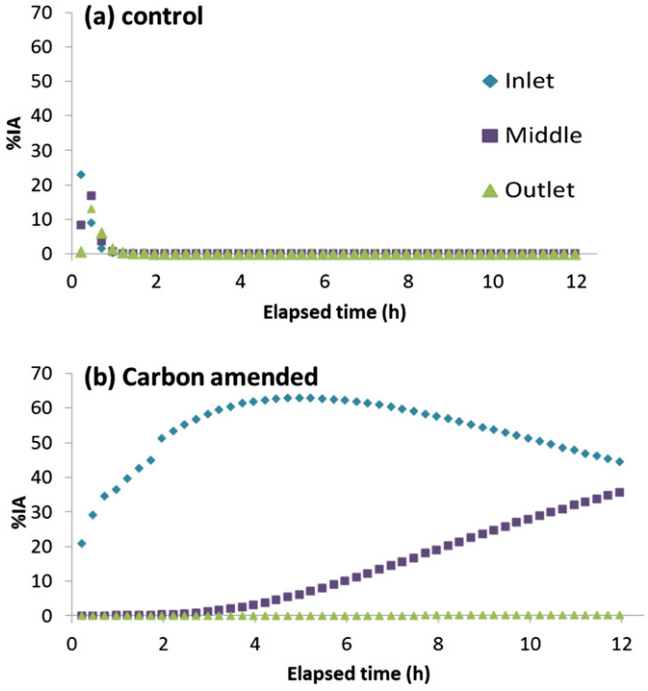

Fig. 4. Mean decay corrected counts of Tc^{99m} in (a) control columns (b) carbon amended columns. % IA: % of injected activity.

that the expected amount of fluid had travelled through the control columns (approximately 70 mL), whereas only a few mL of fluid passed through the carbon-amended columns (no leaks were detected throughout the tracer test). An increase in back pressure in the carbon-amended columns as a result of the faster flow rate, could explain the clogging effect that was observed, due to processes such as shearing of biofilm, and movement of fines towards the outlet. X-ray radiography also revealed extensive physical changes in the carbon-amended column that may have impacted the transmissive properties of the crushed sandstone. Gas generation as a result of some of these processes could be responsible for the extensive spherical voids present in carbon-amended columns. Spherical voids (presumed to be gas bubbles) were observed in both columns (Fig. 5), though to a much greater extent in the carbon-amended column. The transport of gas bubbles to the column outlet could have contributed to the clogging effect that was noted in the carbon-amended columns; previous experiments investigating the impacts of microbial gas generation on transport have shown that biogenic H_2 gas stopped fluid flow through columns (Gu et al., 1999). Biogenic gas generation has been known to lead to significant reductions in hydraulic conductivity, when entrapment of bubbles in a porous medium may occur in pore spaces, inhibiting fluid transport (Baveye et al., 2010).

The formation of gas bubbles in porous media is dependent on a number of factors, including the rate at which gases are being generated and utilised (Amos and Mayer, 2006), pressure (Hunt and Berry, 1956), and flow rate (Garstecki and Gañán-Calvo, 2005). Gas generation in sandy sediments has previously been shown to form spherical bubbles (as seen in Fig. 5), with the sand responding fluidly as a result of stresses imposed by gas build up (Bouderau et al., 2005). Gas bubbles are known to impact on the hydraulic conductivity of porous media (Amos and Mayer, 2006), and have also been shown to be able to accumulate fine particles, further impacting on hydraulic conductivity (Goldenberg et al., 1989). It is possible that the gas bubbles noted in the columns were generated near the column inlet via microbial metabolism, and were transported to the outlet end of the columns in the direction of flow as a result of the upflow experimental design. The slow flow rate used in this experiment, coupled with the large porosity of the crushed material, may have allowed gases to accumulate, leading to the

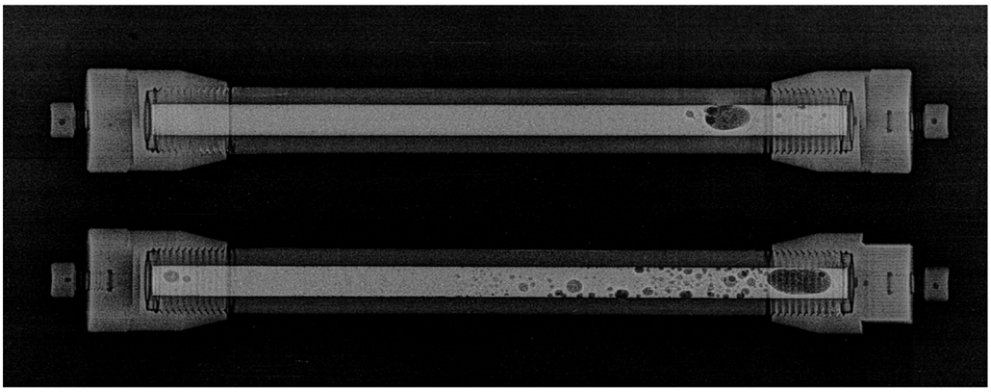

Fig. 5. X-ray radiographs of carbon amended 2 and control 2 columns. During the experiment, flow occurred from the inlet to outlet. (−) control; (+) carbon amended.

formation of bubbles. As the flow rate through the columns was slow, the presence of gas bubbles may not have been enough to impact on flow through the columns during the 8 month experiment (as no real decrease in flow rate was observed), but could have contributed to the pressure increases.

3.2. Geochemical analyses

During these experiments the starting fluid had an approximate pH of 12.2 and Eh of −12 mV. After being pumped through the columns, a significant decrease in pH was observed initially, after which the pH of the outlet fluid increased to values comparable to the starting fluids (Fig. 6). Although the pH of the starting fluids remained relatively constant throughout the experiment, variations in outlet fluid pH appeared to be closely related to variations in the rate and extent of microbial activity. Organic acid utilisation (Fig. 7) occurred to the greatest extent when the outlet fluids were pH 9–10. pH increases above these values were accompanied by a sudden decrease in organic acid utilisation. A decrease in Eh was observed in the outlet fluids of all columns compared to the starting fluid, although the rate of decrease appeared to be more rapid in the carbon-amended columns than the control columns. Generally, throughout the experiment, the redox potential was lower in the carbon-amended columns compared to the controls.

The extensive spherical voids that were present in the carbon-amended column (Fig. 5) could be a result of the generation of gases during fermentative processes. Lactate and acetate were added to the fluid (560 mg L^{-1} and 410 mg L^{-1} respectively) collected from the field site, although formate was already present, indicating the presence of organic acids at the field site. The acetate concentrations in the outlet fluids remained relatively constant throughout the carbon-amended experiments. At 4 weeks, lactate was absent from the outlet fluids of the carbon-amended columns, and at this time point there were concurrent increases in propionate, formate and butyric acids, which is consistent with metabolic gases including CO_2 and H_2 being generated within the columns. For example, butyric acid is generated from lactate via fermentation, with organisms such as *Clostridium* species known to be able to carry out this process (He et al., 2005). The H_2 gas generated during this process (Chong et al., 2009) may have stimulated the H_2-utilising bacteria present in the sediments at the end of the experiment. Interestingly *Clostridia* are also known to be able to produce propionate by fermenting lactate via the acrylate pathway or the succinate-propionate pathway; this process generates CO_2 (White, 1995). As the pH of the outlet fluids increased above 10, lactate utilisation stopped (and generally the generation of lactate breakdown products stopped as well), until 15 weeks, when gradual decreases in lactate were observed. The decrease in lactate and acetate in the outlet fluids from carbon-amended columns towards the end of the experiment, coupled with the lack of breakdown products could suggest that they were being completely oxidised to CO_2, or that fermentation products/oxidation intermediates were utilised so quickly that they were not detected in the outlet fluids. Acetate and lactate were generated within the control columns, perhaps as a result of the breakdown of organic materials present in the starting fluids, although without further analyses to investigate the compounds that acetate and lactate may have been metabolised from, it would be difficult to state the mechanisms responsible for their formation.

Other processes may have played a role in the inhibition of ^{99m}Tc mobility through carbon-amended columns, including the reductive immobilisation of Tc(VII) by Fe(II)-bearing minerals. Weak acid extractable Fe(II) concentrations in sediments collected from a carbon-

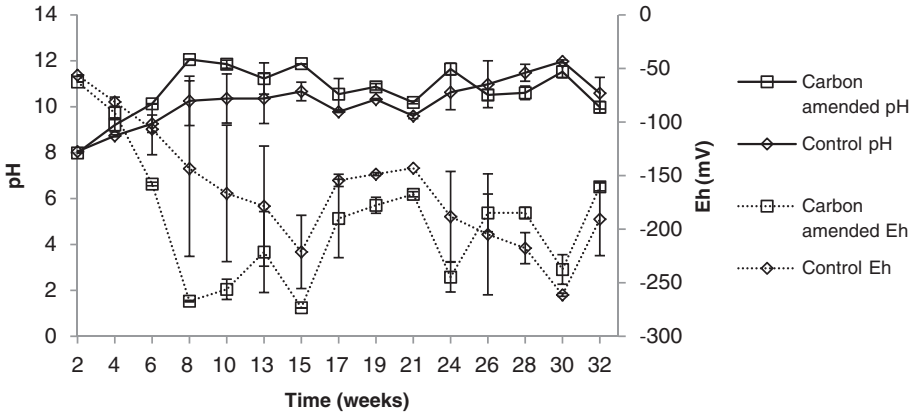

Fig. 6. pH and Eh of column outlet fluids. Error bars show standard error calculated from duplicate values.

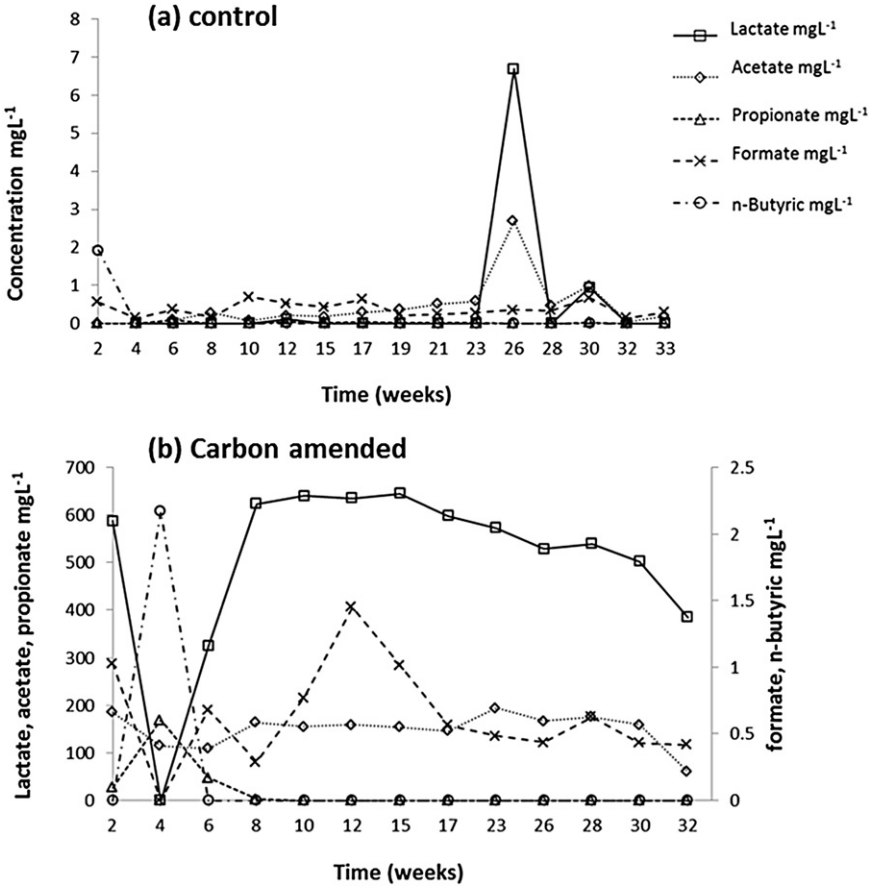

Fig. 7. Volatile fatty acid concentrations in the outlet fluids of a control column (a) and carbon (5 mM acetate and lactate) amended column (b). Data are representative of duplicate columns, with similar trends and concentrations seen in both.

amended column were higher than observed in control sediments (Fig. 8), implying the occurrence of microbial Fe(III) reduction at alkaline pH. Fe(II) concentrations in sediment samples collected over the length of the columns confirmed that Fe(III) reduction was stimulated at the carbon-amended inlet, with concentrations of approximately 4.5 mmol Fe(II) kg slurry^{-1} observed, while the concentration decreased over the length of the column. Lower concentrations of Fe(II) were observed in the control column (approximately 1.5 mmol Fe(II) kg slurry^{-1}), and variation was minimal over the length of the column.

Several studies have documented the ability of microorganisms to carry out Fe(III) reduction at high pH (Williamson et al., 2013; Pollock et al., 2007). Numerous studies investigating the biogeochemical behaviour of Tc(VII) under circum-neutral conditions have suggested reduction of Tc(VII) by Fe(II)-bearing minerals as a potential mechanism for the immobilisation of Tc in microcosm studies (McBeth et al., 2011), and in flow-through column experiments (Lear et al., 2010). Only a few studies have investigated this process under alkaline conditions; Thorpe et al. (2014) demonstrated that Tc(VII) reduction by Fe(II)-bearing minerals occurred at pH 9, whereas Williamson (2014) demonstrated the occurrence of this process at pH 10. Direct enzymatic Tc(VII) reduction by microorganisms is also known to occur under alkaline conditions; as an example Khijniak et al. (2003) described the ability of

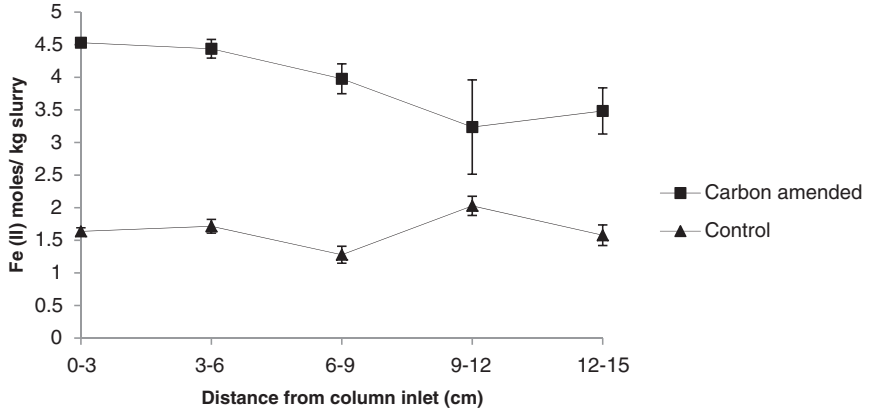

Fig. 8. Fe(II) concentration in sediments collected from 3 cm intervals along a carbon amended and control column. Error bars show standard error calculated from triplicate measurements.

soda-lake isolates to reduce Tc(VII) at pH 10. Although it is clear that microbial processes impacted on the transport of ^{99m}Tc through the crushed sandstone columns in these experiments, the precise mechanism of Tc retention clearly warrants further investigation.

3.3. Microbiological and molecular ecological analyses

Cell numbers in column starting and outlet fluids were monitored throughout the experiment, along with the molecular ecology of outlet fluids and reacted sediments to aid in understanding the spatial and temporal changes to biogeochemical processes occurring within these column systems, and to identify some of the organisms facilitating these processes.

Organic acid utilisation over the first few weeks of the experiment was extensive, and was implicated in supporting biomass growth, increases in outlet fluid bacterial community diversity and significant quantities of gases generation within the carbon-amended columns. After the decrease in cell numbers in the outlet fluids of carbon-amended columns after week 6 (Fig. 10), organic acid utilisation declined, pH increased, and the apparent diversity of the outlet fluid community decreased as demonstrated by analysing banding patterns from RISA gels (Fig. 11). Towards the end of the experiment, lactate and acetate concentrations declined, but cell numbers in the outlet fluids remained low, perhaps suggesting that most of the cells were being retained within the columns.

The bacterial communities in the outlet fluids of the column experiments varied between the control and carbon-amended columns, and also varied over time in the carbon-amended columns as demonstrated by RISA (Fig. 11). It would appear that in the starting fluids, bacterial biomass was very low, as only two very faint bands were present on the RISA gel. The community in the outlet fluid of the control column remained consistent throughout the experiment, with only one distinct band present.

The community in the outlet fluids of the carbon-amended column, however, varied over the course of the experiment, with the diversity increasing at week 6, corresponding to the time at which the highest cell counts occurred in the outlet fluids (at a time of more favourable pH for microbial metabolism). A 454-pyrosequencing approach suggested that the microbial community at this time point was dominated by an organism affiliated with *Rhizobium selenitrireducens* B1 (96% sequence similarity). Other bacteria that were dominant in the sequence library included an organism closely affiliated with *Noviherbaspirillum aurantiacum SUEMI08* (97% sequence similarity, 10.05% of the sequence library), and organisms closely affiliated with *Bacillus* spp. These bacteria that dominated the sequence library are not known to be alkaliphilic, and have been isolated from a range of non-alkaliphilic environments including volcanic mountain soil (Carro et al., 2012), Arctic marine sediments (Zhang et al., 2014), and Guaymas Basin hydrothermal sediments (Dick et al., 2006).

The bacterial community present (Fig. 12) in outlet fluids of the carbon-amended column at the end of the experiment appears to be well adapted to the highly alkaline conditions, and the sequence library was dominated by obligately alkaliphilic bacteria, as revealed by 454-pyrosequencing. The sequence library obtained from this sample was dominated (91.47% of the sequence library) by an organism closely affiliated with *Comamonadaceae bacterium* B1 (99% sequence similarity). 5.42% of the sequence library was comprised of an organism closely affiliated (99% sequence similarity) with an uncultured clone *CVCloAMPh135* belonging to the class *Clostridia*.

In addition, 454-pyrosequencing of key samples revealed spatial variations in bacterial community composition in column sediments collected at the end of the experiment. DNA was extracted from sediments collected from every 3 cm along a carbon-amended and control column, and subjected to 16S rRNA PCR; products were only obtained from DNA extracted from the inlet (0–3 cm) of the carbon-amended column, and from 0–3 cm and 6–9 cm from the control column. The DNA extracted from these samples was subsequently subjected to 454- pyrosequencing analysis (Fig. 13).

The bacterial communities present in the sediments of both the carbon-amended and control columns at the end of the experiment were dominated by a genus recently described as *Serpentinomonas* (Suzuki et al., 2014). The sequence library obtained from the carbon-amended column was dominated (80.35% of the sequence library) by a strain with 99% sequence similarity to *S. mccroryi*, as were the libraries from the control column (65.63% at 0–3 cm and 83.41% at 6–9 cm), with a strain with 99% sequence similarity to *S. raichei* also a significant component of the community (31.59% at 0–3 cm and 14.39% at 6–9 cm). These strains are obligately alkaliphilic, with optimum growth observed at pH 11, and have recently been isolated from serpentinising systems (Suzuki et al., 2014). As a result of culture-independent sequencing, Crespo-Medina et al. (2014) found that sequences with 100% identity to *S. mccroryi* dominated a community found in fluids with pH > 12 from subsurface samples from the Coast Range Ophiolite Microbial Observatory, a site of active serpentinization. The two dominant *Serpentinomonas* strains observed in column sediments in these experiments can use $CaCO_3$ as a carbon source (as well as acetate and lactate), and are capable of utilising H_2 as an electron donor; two gene clusters are present in their genomes which code for [Ni-Fe]-hydrogenases, which are thought to be of importance during autotrophic growth of the strains, enabling energy production via hydrogen oxidation (Suzuki et al., 2014). The active serpentinization at these sites generating significant quantities of H_2 and highly alkaline pore fluids, has likely selected for these *Serpentinomonas* strains, and it is possible that during the column experiments described in this study, the enrichment of these strains in column communities could perhaps be a result of H_2 generation during organic acid utilisation, or the presence of H_2 in the atmospheric composition of the anaerobic cabinet. Towards the end of the experiment, organic acid utilisation was minimal, perhaps suggesting that under the highly alkaline conditions that present in these

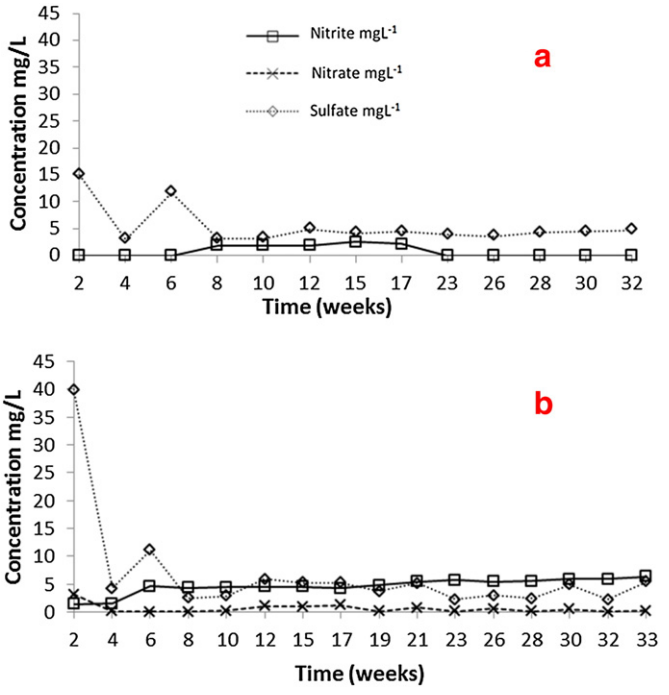

Fig. 9. Nitrite, nitrate and sulfate concentrations in the outlet fluids of carbon amended column 1 (a) and control column 1 (b). Starting concentrations of nitrate were approximately 2 mg L^{-1} in the carbon amended fluid and approximately 4 mg L^{-1} in the control fluid. No nitrite was detected in the starting carbon amended fluid; approximately 0.8 mg L^{-1} was detected in the control starting fluid. Approximately 2 mg L^{-1} sulfate was detected in both of the starting fluids. Data are representative of duplicate columns, with similar trends and concentrations seen in both.

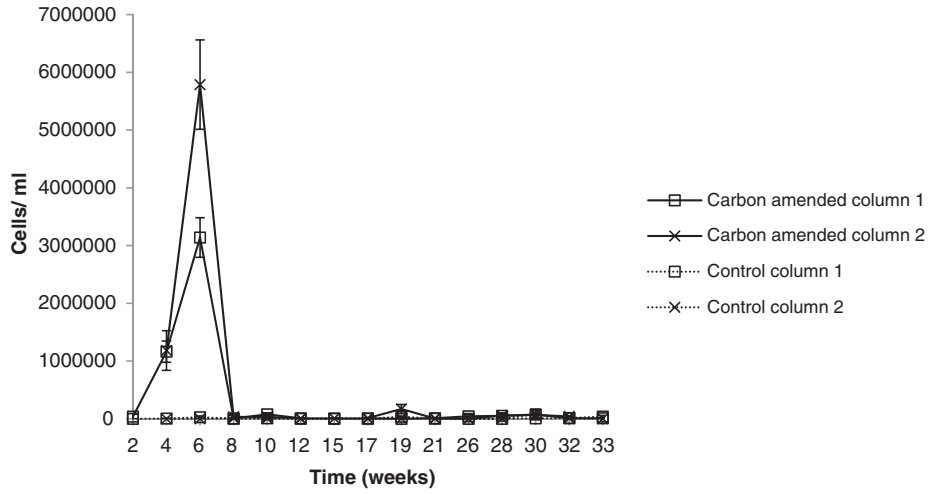

Fig. 10. Total cell numbers in column outlet fluids. Error bars show standard error calculated from 5 replicates.

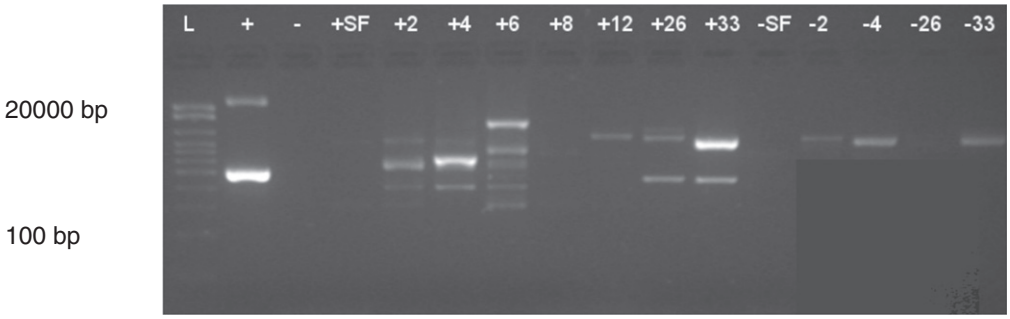

Fig. 11. Ribosomal Intergenic Spacer Analysis (RISA) PCR products from outlet fluids of column experiments. (L) ladder, (+) positive control (*Geobacter sulfureducens*), (−) negative control (sterile DIW), (+SF) carbon amended starting fluid, (+2, 4, 6, 8, 12, 26, 33) outlet fluids from carbon amended 1 column at 2, 4, 6, 8, 12, 26 and 33 weeks, (−SF) control starting fluid, (−2, 4, 26, 33) outlet fluids from control column 1 at 2, 4, 26 and 33 weeks.

experiments (apart from at the beginning when the pH was slightly lower and organic acid utilisation was extensive), autotrophic growth with H_2 as the electron donor was the dominant mechanism for bacterial growth. This process represents a potential mechanism for the prevention of over-pressurisation in a GDF that may occur as a result of H_2 generation during steel corrosion under highly alkaline conditions (NDA, 2010).

A strain with 98% sequence identity to *Alkaliphilus crotonatoxidans* was also present in the carbon-amended column (comprising 11.75% of the sequence library). This bacterium is known to dismutate crotonate (an intermediate in the oxidation of butyrate) to acetate and butyrate (Cao et al., 2003). Growth does not occur with lactate, butyrate or acetate, suggesting that during these experiments, crotonate may have been generated as a result of butyrate utilisation. Sulfate, nitrate and nitrite concentrations were also monitored in outlet fluids throughout the experiment. Initially, after the fluid was pumped through the columns, a sulfate spike was observed (much greater concentrations than the ≈2 mg L^{-1} present in the starting fluids), after which concentrations returned to values comparable to those of the starting fluids. A strain with 95% sequence similarity to the obligately alkaliphilic (pH optimum of 9.5) *Dethiobacter alkaliphilus AHT1* was present in the sediments from the carbon-amended column (comprising 3.79% of the sequence library). This organism is capable of utilising H_2 as an electron donor, and elemental sulfur and polysulfide as electron acceptors (Sorokin et al., 2008). In microcosm studies carried out with fluids from serpentinising sites amended with thiosulfate and sulfide (Crespo-Medina et al., 2014), the resulting microbial community was dominated by sequences similar to this organism, and it may have been involved in sulfur metabolism in these experiments.

Nitrate disappeared from all starting fluids in samples collected throughout the course of the experiment (Fig. 9); a nitrite build up was observed in the starting fluids (data not shown), suggesting nitrate reduction may have been occurring in the starting fluids, rather than in the columns themselves. The strain closely related to *S. mccroryi* present

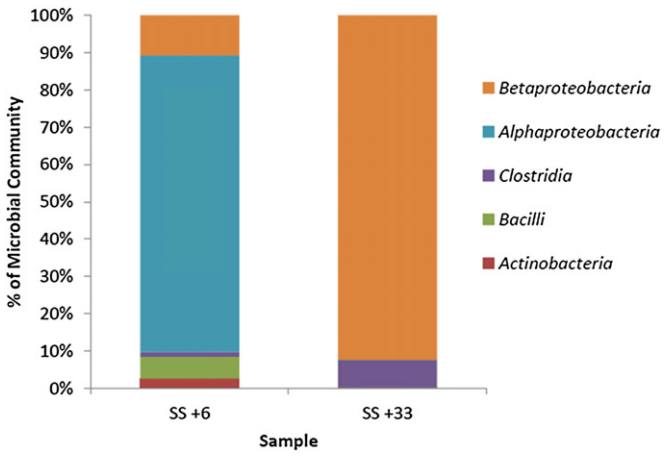

Fig. 12. Bacterial community composition (class level) of communities present in the outlet fluids of a carbon amended column after 6 weeks (+6) and after 33 weeks (+33).

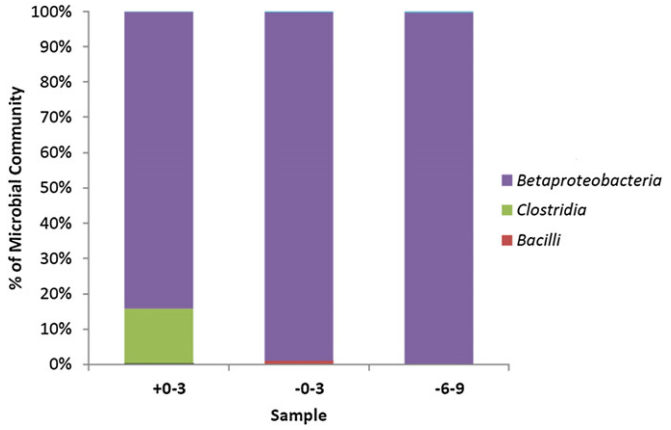

Fig. 13. Composition of bacterial communities (class level) on sediments collected from column experiments. (+0–3) sediment collected from 0 to 3 cm from the inlet of a carbon amended column, (−0–3) from 0 to 3 cm from inlet of a control column, (−6–9) from 6 to 9 cm from inlet of a control column.

in both the carbon-amended and control column sediments is capable of utilising nitrate as an electron acceptor (Suzuki et al., 2014), and may play a role in nitrate reduction within these experiments.

4. Conclusions

The results from this study indicate that microbial communities can impact on the transport properties of a porous medium under highly alkaline conditions representative of conditions that will be present in a GDF for ILW in the UK. Previous studies have demonstrated the ability of microorganisms to facilitate processes that may impact on radionuclide mobility in a GDF for ILW, for example Rizoulis et al. (2012) showed that microorganisms were capable of respiring a range of electron acceptors at pH 11. Bassil et al. (2015) also showed that microorganisms were capable of degrading ISA (a molecule that can form as a result of alkaline cellulose degradation), under highly alkaline conditions, indicating the potential for microorganisms to utilise cellulose degradation products as carbon sources in a GDF environment. Column experiments suggest that microorganisms existing under these conditions play a role in the biogeochemical cycling of Fe, nitrate, and sulfate, and are capable of metabolising organic acids under such conditions (as proxies for some of the cellulose degradation products that may be present as a result of alkaline degradation of cellulosic materials that will be present in ILW). Some of these processes are responsible for gas generation, possibly impacting on the hydraulic conductivity of these column experiments, resulting in a decrease in mobility of ^{99m}Tc through the columns (compounded by bioreduction of soluble Tc(VII) to less soluble Tc(IV)). Other processes such as bioclogging and (bio) mineral precipitation may have also played a role in controlling fluid transport. The bacterial communities observed in sediments from these column experiments were dominated by obligately alkaliphilic bacteria capable of utilising H_2 as an electron donor. Overall the results from this study underline the potential importance of microorganisms in biogeochemical and physical processes of relevance to geological disposal. Further work is required to fully understand the extent to which each of these processes contribute to the alteration of the transport properties of the sandstone in these experiment, and other host rocks in relevant geological formations.

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