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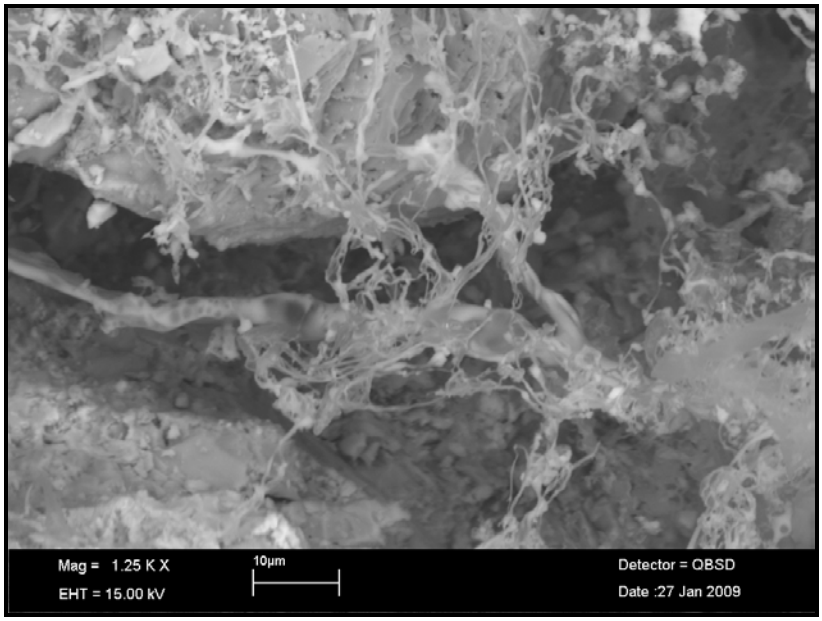
Microbial effects on transport processes (BioTran) - Anaerobic flow-through Experiments using crushed Diorite and *Pseudomonas aeruginosa* (April 2008-March 2009)

Radioactive Waste Programme

Research Report OR/09/33



BRITISH GEOLOGICAL SURVEY

RADIOACTIVE WASTE TEAM

OPEN REPORT OR/09/33

Microbial effects on transport processes (BioTran) - Anaerobic flow-through Experiments using crushed Diorite and *Pseudomonas aeruginosa* (April 2008-March 2009)

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Keywords

Biofilm; Äspö diorite; Anaerobic; Column experiments; *Pseudomonas aeruginosa*.

Front cover

BSEM image showing an intergranular pore space, spanned by the amorphous meshwork of biofilaments

Bibliographical reference

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Foreword

As part of the Radioactive Waste Team work, the BioTran project is examining microbial effects on transport processes. This report details progress on the experimental programme, which aims to address some of the knowledge gaps relating to the influences of biofilms on transport of fluids through crushed rock materials. The experimental work described in this report uses crushed diorite and *Pseudomonas aeruginosa* in anaerobic flow-through systems.

Acknowledgements

Our thanks to SKB for the diorite from the Äspö Underground Research laboratory, Sweden.

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Summary

Risk assessments for landfills and geological repositories for radioactive waste are primarily based on the precepts of contaminant transport; and are concerned with understanding the movement of gas, wastes and solutes through engineered barriers and natural groundwater systems, within the concept of 'Source', 'Pathway', and 'Receptor'. The emphasis on solute migration for landfill investigations is reflected in the theoretical development used during numerical simulation. However, microbes living in such environments can have an impact on transport processes (Bateman *et al.*, 2006; Chapelle 2000; Cunningham *et al.*, 1997; Fredrickson *et al.*, 1989; Keith-Loach and Livens 2002; West and Chilton 1997). Microbial activity in any environment is generally located on chemical or physical interfaces, usually within biofilms, and the impacts can be both physical (e.g. altering porosity) and/or chemical (e.g. changing pH, redox conditions) and may result in intracellular or extracellular mineral formation or degradation (Beveridge *et al*., 1997; Ehrlich 1999; Konhauser *et al*., 1998; Milodowski *et al*., 1990; Tuck *et al.*, 2006). These processes could all impact on fluid flow through fractures and porous media by, for example, blocking of constrictions in fracture flow pathways and pore throats.

The BioTran project was initiated to examine the effects of microbes on transport processes, especially in the context of contaminant properties of host rocks. An understanding of these microbial processes will also be relevant to other areas such as bioremediation of contaminated land, borehole and reservoir clogging and enhanced oil recovery. More broadly, these processes impinge on aquifer recharge, pathogen survival, and ultimately on groundwater protection. To date, the project has comprehensively reviewed the available literature and developed methodologies for experimental studies to provide information and data for existing transport models (Bateman *et al.*, 2006; Coombs *et al.*, 2008; Wagner *et al.*, 2007).

The BioTran project has focussed on materials from the Äspö Underground Research Laboratory (URL) in the context of the geological containment of radioactive waste in hard rock (diorite) environments (Banwart *et al.*, 1995). Early experimental work simulated the interactions of indigenous microbes with mineralogical surfaces associated with groundwater flow systems at Äspö (Hama *et al.*, 2001); and ascertained that these microbes can either concentrate relevant chemical species for mineral formation in localised microenvironments or accelerate clay formation, the implications of this being that local hydrological conditions can be changed by microbial activity (Tuck *et al.*, 2006).). It has also been shown that minerals precipitated biogenically are chemically and physically very stable and persist in the system long after the original biofilm has decayed or been removed (Brydie *et al.*, 2005).

As a result of these studies, the BioTran project undertook a pilot study to examine the influences of biofilm growth by growing the bacterium *Pseudomonas aeruginosa* aerobically on groundwater flowing though crushed diorite taken from the Äspö Hard Rock Laboratory, Sweden (West *et al.*, 2008). The results of this study demonstrated that biofilms could be grown in both flow cells and flow-through columns within a few days on a diorite substrate through which artificial groundwater containing minimal nutrients was passed. Also, biofilm development could be successfully imaged in several ways, including by visible and UV light microscopy and cryogenic scanning electron microscopy (cryoSEM).

The flow-through column experiment has now been repeated in this current study but under anaerobic conditions over an extended experimental period of 147 days (3524 hours). The experiment showed that:

- *P. aeruginosa* biofilms can be grown anaerobically using diorite and synthetic Äspö groundwater as a growth medium, although the rate of growth is significantly slower.

The respiratory metabolism of *P. aeruginosa* is essentially aerobic; it is however known to grow in the absence of oxygen if nitrate is available as a respiratory electron acceptor. The Äspö groundwater used in this column experiment had negligible nitrate, the fact that growth was observed under anaerobic conditions demonstrates the metabolic versatility and minimal nutritional requirements of the *P. aeruginosa* strain;

- Numbers of *P. aeruginosa* decreased over the duration of the experiment but were able to survive up to 2684 hours;
- CryoSEM observations of the experimental column material showed that biofilm was formed during the course of the experiment;
- Biofilm only developed in the first 0 cm to 4 cm of the column as demonstrated by direct observations and by petrographic analysis of the experimental column at cessation of the investigation;
- Observations of bacteria-free control columns were vitally important as the experimental column material revealed many features that could easily have been superficially identified as being of biological origin;
- The presence of porewater salt caused considerable ambiguity in the identification of biofilaments and biofilm because the salt structures often closely resembled amorphous features anticipated for biofilm development filaments;
- No change in pH was observed during the course of the experiment;
- The growth of biofilm in the anaerobic environment was insufficient to effect pressure and fluid flow through either column;
- The fluid chemistry data shows a rapid reduction in both Mn and Ba concentrations despite no appreciable levels of Mn or Ba being detectable in the groundwater. It was therefore assumed that Mn and Ba ions were present in the inoculant or had originated from the column itself.

Future investigations will move away from dioritic sand-packed columns and focus on biofilm growth within complete sandstone rock cores under representative in-situ conditions of pressure and groundwater chemistry.

1. Introduction

1.1 BACKGROUND

Risk assessments for landfills and geological repositories for radioactive waste are primarily based on the precepts of contaminant transport; and are concerned with understanding the movement of gas, wastes and solutes through engineered barriers and natural groundwater systems, within the concept of 'Source', 'Pathway', and 'Receptor'. The emphasis on solute migration for landfill investigations is reflected in the theoretical development used during numerical simulation. However, microbes living in such environments can have an impact on transport processes (Bateman *et al.***,** 2006; Chapelle 2000; Cunningham *et al.*,1997; Fredrickson *et al.*, 1989; Keith-Loach and Livens 2002; West and Chilton 1997). Microbial activity in any environment is generally located on chemical or physical interfaces, usually within biofilms, and the impacts can be both physical (e.g. altering porosity) and/or chemical (e.g. changing pH, redox conditions) and may result in intracellular or extracellular mineral formation or degradation (Beveridge *et al*., 1997; Ehrlich 1999; Konhauser *et al*., 1998; Milodowski *et al*., 1990; Tuck *et al.*, 2006). These processes could all impact on fluid flow through fractures and porous media by, for example, blocking of constrictions in fracture flow pathways and pore throats.

The BioTran project was initiated to examine the effects of microbes on transport processes, especially in the context of contaminant properties of host rocks. An understanding of these microbial processes will also be relevant to other areas such as bioremediation of contaminated land, borehole and reservoir clogging and enhanced oil recovery. More broadly, these processes impinge on aquifer recharge, pathogen survival, and ultimately on groundwater protection. To date, the project has comprehensively reviewed the available literature and developed methodologies for experimental studies to provide information and data for existing transport models (Bateman *et al.*, 2006; Coombs *et al.*, 2008; Wagner *et al.*, 2007).

1.2 THE CONTEXT OF THE EXPERIMENTAL WORK

The experimental work in BioTran project has focussed on materials from the Äspö Underground Research Laboratory (URL) in the context of the geological containment of radioactive waste in hard rock (diorite) environments. The significance of microbiological processes in the containment of radioactive waste has long been recognised (West and McKinley, 2002) and, consequently, detailed evaluations of the biofilms present on the walls of the URL and on the significance of indigenous microbial populations has been a key area of work (Pedersen 1999). An *in-situ* study at the URL examined the redox buffering of groundwater in vertical fracture-zones penetrated by recent, oxidising, meteoric water and showed that indigenous bacteria were capable of maintaining reducing conditions in the deep groundwaters (Banwart, 1995). Experimental work has also simulated the interactions of indigenous microbes with mineralogical surfaces associated with groundwater flow systems at Äspö (Hama *et al.*, 2001); and ascertained that these microbes can either concentrate relevant chemical species for mineral formation in localised microenvironments or accelerate clay formation, the implications of this being that local hydrological conditions can be changed by microbial activity (Tuck *et al.*, 2006). It has also been shown that minerals precipitated biogenically are chemically and physically very stable and persist in the system long after the original biofilm has decayed or been removed (Brydie *et al.*, 2005).

As a result of these studies, the BioTran project has undertaken pilot studies to examine the influences of biofilm growth on groundwater flow though crushed diorite from the Äspö Hard Rock Laboratory, Sweden.

Early experiments focussed on developing methodologies to grow biofilms aerobically in the laboratory and observe their development (West *et al*., 2008). This was accomplished by means of a closed system of polytetrafluoroethylene (PTFE) tubing connecting reservoirs of sterile synthetic groundwater to either a column or a flow cell. Circulation of the groundwater was by means of a peristaltic pump. The effects of biofilm growth on fluid flow using an open system of PTFE tubing connecting a header vessel to a pair of columns packed with crushed Äspö diorite were also studied (West *et al*., 2008). Here, the peristaltic pump was only used to maintain a constant level of groundwater in the header vessel, rather than circulating the fluid directly through the columns. This arrangement was adopted to eliminate the pulsed flow effect of the peristaltic pump from the initial experiment. The header vessel system provided a fixed pressure, so changes in flow rate were proportional to permeability. Both columns were inoculated with a biofilm forming bacterium, *Pseudomonas aeruginosa* but the first column was kept as a dedicated control whereas conditions in the second column were changed, i.e. the pH of the groundwater was modified and latterly, phage specific to *P. aeruginosa* was introduced. Flow rates through the columns were measured by mass balance and samples of the groundwater taken at regular intervals for chemical analysis. The changes in flow rate and hydraulic conductivity during the course of the experiment were complex and differed significantly between the two columns. However, the maximum flow rate through both columns of approximately 4.5 ml min^{-1} was achieved between 250 and 500 hours following inoculation; hydraulic conductivity decreased at similar rates in both columns from around 500 hours to a point at around 1000 hours where no flow occurred. Changes in fluid chemistry were less apparent. Changes to groundwater pH and the addition of a bacteriophage specific to *P.aeruginosa* in the second column did not produce an observable change in flow rate.

The aim of the 2008-2009 experimental programme was to perform an experiment similar in design to the aerobic experiment but under anaerobic conditions, in order to mimic a post-closure repository environment when reducing conditions have been established. The objectives of the experiment aimed to;

- Evaluate the impact of biofilm on transport properties with time;
- Estimate the effects of biofilms on mineralogical and geochemical parameters during the experiment;
- Determine the fate of the microbes during the experiment.

2 Experimental Design

The experiment was conducted in an anaerobic environment in order to control atmospheric conditions. In the previous aerobic study (West *et al*., 2008) constant head reservoirs were used to supply artificial groundwater to columns containing crushed Äspö diorite under a fixed pressure. Flow rates through the columns were measured by mass balance and samples of the groundwater taken at regular intervals for chemical analysis. For this anaerobic experiment, constant head reservoirs were replaced by syringe pumps. The syringe pumps were programmed to supply artificial groundwater at a constant flow rate through the packed diorite columns, thereby providing more control over the experiment. Additionally, because of limited space in the anaerobic chamber, it was necessary to physically reduce the scale of the experiment. The columns used were also shorter and the system supplying the groundwater to the columns was

modified, but the composition of the artificial groundwater, the rock type, type of bacterium and dual column setup remained unchanged.

A schematic of the experimental setup for the anaerobic experiment is shown in Figure 1.

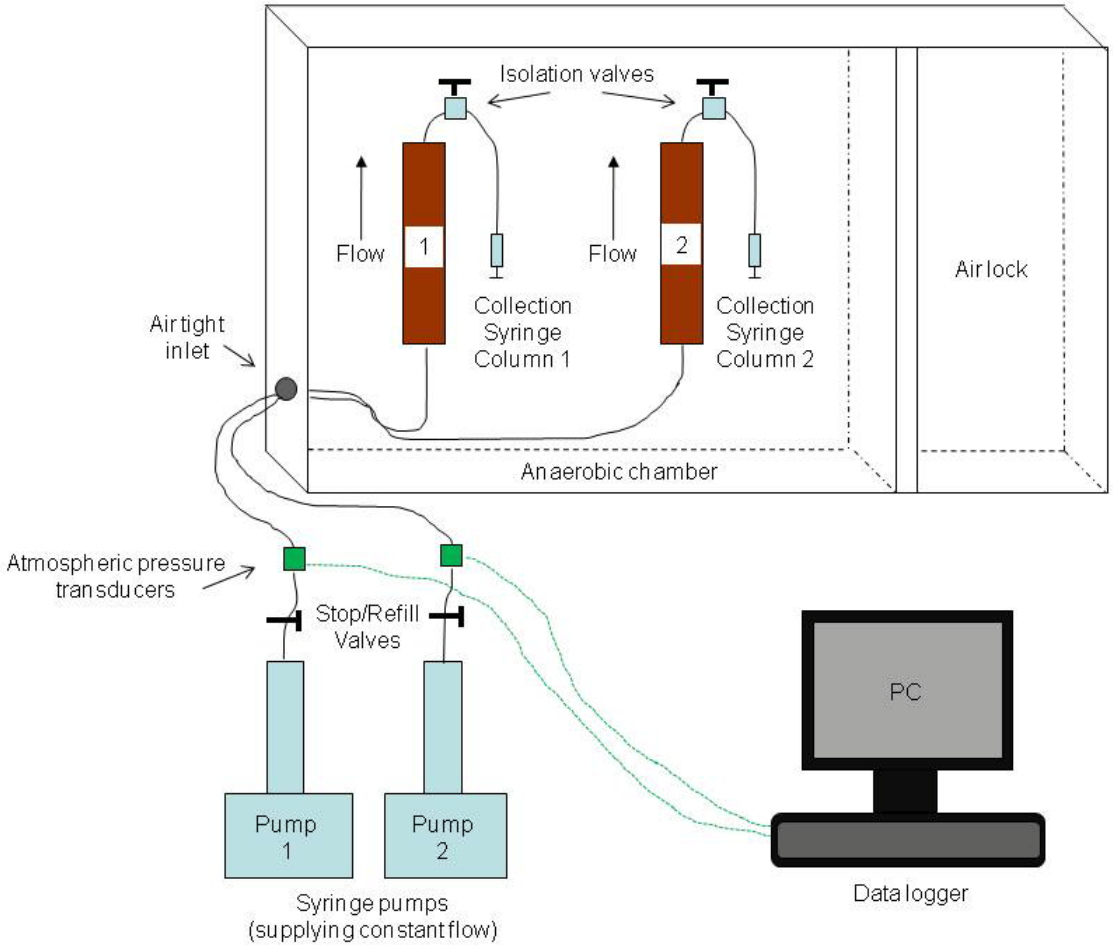


Figure 1. Schematic of the anaerobic experiment

3 Apparatus

Omnifit precision liquid chromatography glass columns capable of operating up to 150 psi were purchased from Kinesis (part no.006RG-25-15). The columns used in the earlier aerobic experiments were 250 mm long. In this anaerobic study, the columns were only 150 mm long, since space was restricted within the anaerobic cabinet.

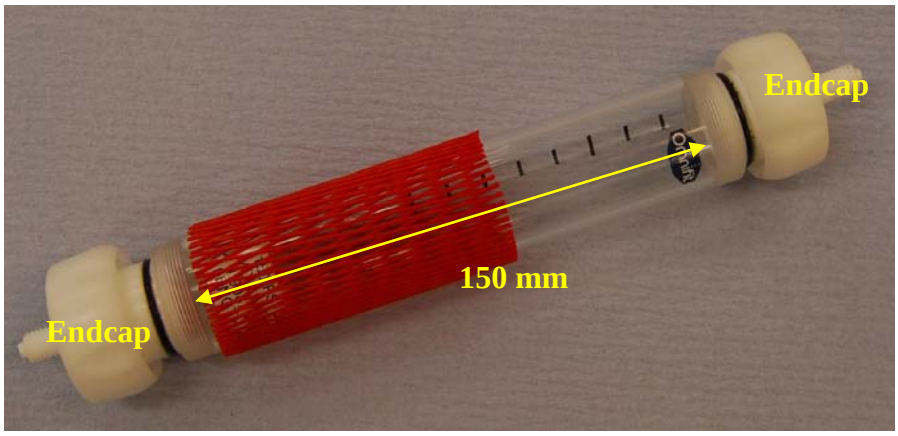


Plate 1. Image showing the type of Omnifit column used in the experiment.

The columns were supplied with PTFE fixed end pieces; each had polyethylene (PE) frits and 0.625 cm. endcaps to which 1.5875 mm. O.D. PTFE tubing was attached. The tubing used to connect the columns to the syringe pumps was made of PTFE with a 1.5875 mm O.D. and 0.50 mm I.D. An image of the type of column used in the experiment is shown in Plate 1.

The experiment was set up in an automatic controlled environmental anaerobic chamber as shown in Plate 2. Large items of equipment entered the atmospherically controlled chamber via the airlock and the operator accessed the experiment directly via the glove ports.

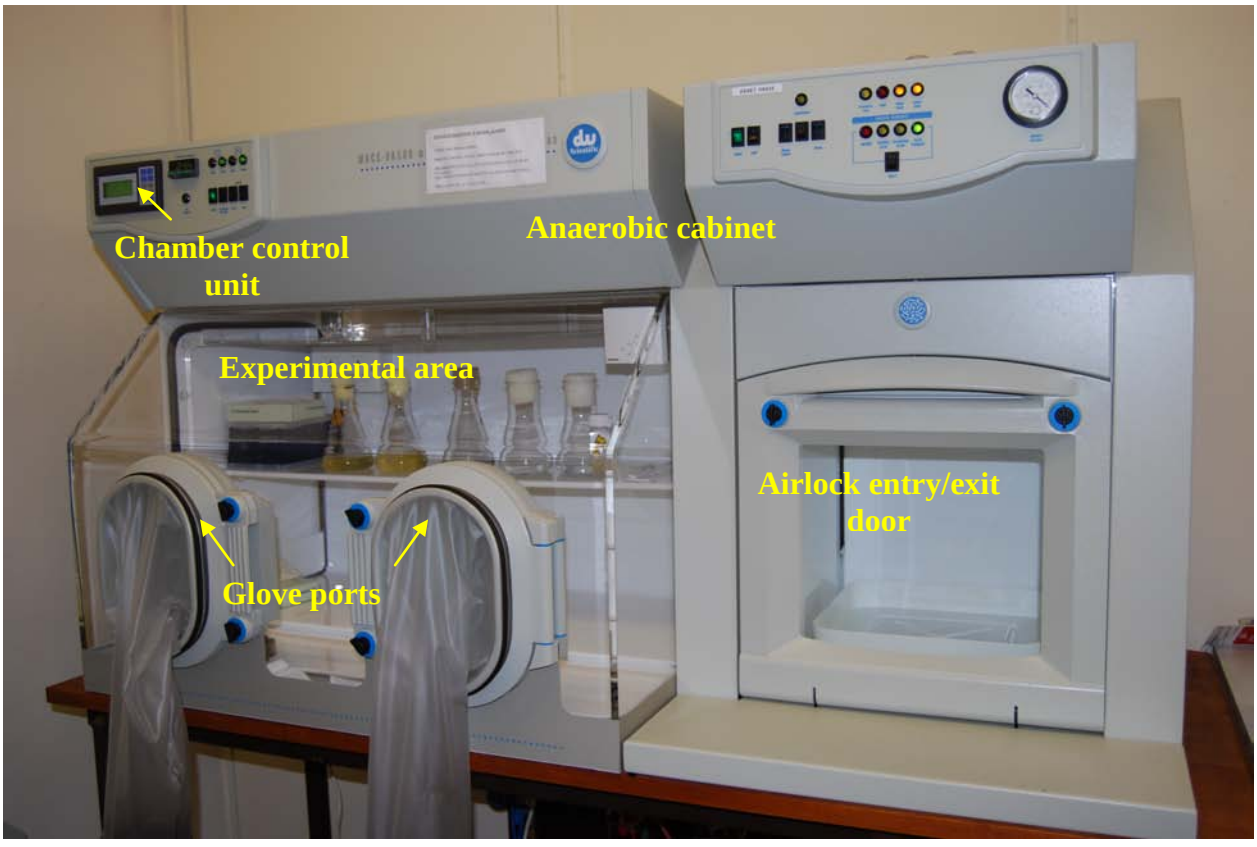


Plate 2. The anaerobic chamber; the experimental area is on the left of the picture and an entry/exit airlock for equipment is on the right.

4 Materials and Methods

4.1 ÄSPÖ SYNTHETIC GROUNDWATER

A synthetic groundwater based on the composition of Äspö borehole water (ID KA1755A) (Hama *et al*., 2001) was prepared and the pH adjusted to 7.2 with 0.1 M HCl as shown in Table 1. The synthetic groundwater was filter sterilised through a 0.2 µm VacuCap 90 filter unit, (Gelman Sciences Prod 4622). The flow of groundwater into the columns was maintained at a constant 0.625 ml hr^{-1} using ISCO syringe pumps of 500 ml capacity. Changes in pressure within the system were logged continuously.

Table 1. Composition of synthetic groundwater

Synthetic groundwater	(g l^{-1})
$NaH_2PO_4.2H_2O$	0.0002
$NaHCO_3$	0.028
KCl	0.023
$SrCl_2.6H_2O$	0.243
$MgSO_2.7H_2O$	0.508
$CaSO_4.2H_2O$	0.835
$CaCl_2.2H_2O$	15.245
NaCl	7.403
Concentration	**(mg l^{-1})**
Ca^{2+}	4351
Mg^{2+}	50
Na^{+}	2920
K^{+}	12
Cl^{-}	11919
Sr^{2+}	80
HPO_4^{-}	0.1
HCO_3^{-}	20
SO_4^{2-}	664
Total S	222

4.2 ÄSPÖ DIORITE SAND

Äspö diorite sand was prepared from whole rock. It was first crushed, then jaw-split by a manual rock-splitter equipped with hardened chromium steel jaws. The fragmented material was then passed twice through a laboratory jaw-crusher, equipped with hardened chromium steel

jaws, to produce a material with particle sizes ranging from 250 to 500 µm, but which was still representative of the whole rock. At each stage of the preparation, fines were formed due to the substantial differences in friability of the minerals within the diorite. Jaw crushing alone produced particles of differing size, some of which were already <150 µm. To reduce this effect, fine material was discarded after jaw crushing and a multi-stage milling regime was then carried out on the remaining broken rock fragments with each mill run lasting a minute or less. After jaw crushing, the fragments were milled in a hardened chrome steel Tema mill and sieved through mesh sieves. After each milling stage, the sample was sieved through 500 µm and 250 µm sieves. Material with a particle size of <250 µm was discarded while material coarser than 500 µm was re-milled. All the sieve fractions ranging from 250 µm to 500µm were then combined to produce the final material. Microscopic examination of the selected material showed inhomogeneity in terms of particle size and mineral composition (Plate 3). The columns were packed with this selected material under gravity and gently compacted by tapping the sides of the column.

Plate 3. Digital image taken at 10X magnification illustrating the heterogeneity of the diorite grains, the particles shown range between 250 µm and 500 µm in size.

4.3 BACTERIAL CULTURE

As in previous experiments, *Pseudomonas aeruginosa* was selected for its biofilm (exopolysaccharide - EPS) forming properties (Vaughan *et al*., 2001). 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 of 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 this anaerobic experiment as the Äspö groundwater has high sodium chloride levels.

4.3.1 Preparation of bacterial culture

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 subcultured 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 hours 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 Äspö groundwater. The tubes were remixed and the centrifugation process repeated four times until traces of culture media were 'washed' from the bacteria.

4.4 EPIFLUORESCENCE MICROSCOPY

Epifluorescence microscopy uses a short wavelength transmission source to fluoresce a sample stained with the nucleic acid selective cationic fluorochrome. In the experiments, the fluorescent stain, Acridine Orange, or N,N,N',N'-tetramethylacridine3,6-diamine ($C_{17}H_{19}N_3$), was used to determine total cell counts (Jass and Lappin-Scott, 1992); and to assess the extent of biofilm growth in the flowcell and columns. Acridine Orange is capable of permeating cells and interacts with DNA and RNA by intercalation or electrostatic attractions. When the fluorescent stain interacts with DNA, which is spectrally similar to fluorescein, the excitation maximum is at 502 nm and the emission maximum at 525 nm (green) while RNA interactions shift the excitation maximum to 460 nm (blue) and then emission maximum to 650 nm (red). Thus, it is possible to determine if cells are metabolically active as they appear red; or inactive when they appear green.

4.4.1 Preparation of gluteraldehyde fixative

Gluteraldehyde fixative was used prior to staining with Acridine Orange for epi-fluorescence microscopy. A solution of cacodylic acid was prepared by dissolving 8 g of acid in 500 ml of demineralised water and adjusting the pH to 7.4 if required with filter sterilised solutions of 1M sodium hydroxide or 1M hydrochloric acid. The solution was filter sterilised through a 0.45 µm Millipore filter. A 25% gluteraldehyde solution was diluted with the cacodylate buffer to make a 0.5% gluteraldehyde solution.

4.4.2 Preparation of Acridine Orange

Acridine Orange was dissolved in a mixed potassium monobasic/dibasic phosphate buffer. The potassium phosphate dibasic buffer solution was prepared by dissolving 4.35 g of AnalaR© grade potassium *ortho*phosphate (K_2HPO_4) in 500 ml of demineralised water and sterilising through a 0.45 µm Millipore filter. The potassium phosphate monobasic buffer solution was prepared by dissolving 3.40 g of AnalaR© grade potassium dihydrogen *ortho*phosphate (KH_2PO_4) in 500 ml of demineralised water and sterilising through a 0.45 µm Millipore filter. To prepare the Acridine Orange stain, 42 ml of potassium phosphate dibasic buffer solution was combined with 8 ml of potassium phosphate monobasic buffer solution, the pH was adjusted to pH to 7.5 with the monobasic solution and 5mg of Acridine Orange stain was added. The Acridine Orange stain was syringe filter sterilised through a 0.45 µm Millipore filter.

4.4.3 Procedure for epi-fluorescence microscopy

Bacterial cells were fixed by pipetting 1 ml of the synthetic groundwater to 10 ml of gluteraldehyde fixative. A pre-wetted black Nucleopore membrane was transferred to the bottom of a Millipore filter apparatus with sterilised forceps, ensuring that the surface of the membrane was in contact with the filter base before clamping on the chimney and drawing vacuum. A 5 ml aliquot of sample of the fixed bacterial solution was pipetted into the chimney of the filter apparatus and vacuum drawn until the sample was filtered. The vacuum was then released. A 1 ml aliquot of the Acridine Orange stain was dispensed into the filter apparatus, with minimal disturbance of the bacteria on the filter and allowed to stain for two minutes before removing the

staining solution under vacuum. Whilst maintaining the vacuum, 1.5 ml of isopropyl alcohol was then carefully added to 'destain' the sample. The vacuum was run for a short period to dry the filter before releasing the vacuum. The membrane was transferred using sterilised forceps to a dry filter paper in a small petri dish and allowed to dry for at least 20 minutes. The dry stained membrane was then placed onto a glass microscope slide and a drop of immersion oil was placed onto the membrane before covering with a cover slip. The number of stained bacterial cells on each slide was counted using a Zeiss microscope fitted with a fluorescence lamp (The excitation wavelength of Acridine Orange is between 450-490 nm). Using a gridded eyepiece and a 10x lens the filter membrane was brought into focus. The magnification was increased to a 100x oil immersion lens to count the numbers of bacteria per gridded area. Twenty fields of view were counted to give mean numbers of bacteria and standard deviations were calculated.

4.5 FLUID CHEMISTRY METHODS

Fluid chemistry of the groundwater was determined using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Ion Chromatography (IC) and Flow-Injection Visible Spectometry. The determination of major and trace cations in aqueous samples was performed on a Varian Vista ICP-AES using the procedure described in BGS Technical Procedure AGN 2.3.5 Issue 8.0 A Dionex DX-600 Ion Chromatograph was used to determine the anion concentration; this procedure is detailed in BGS Technical Procedure AGN 2.3.6 Issue 11.0. The determination of reduced iron (Fe^{2+}) was also considered to be important as the bacterium is known to sequester iron during growth. An automated flow-injection visible spectroscopic method was therefore used to determine Fe^{2+}; this method again used the Dionex DX-600 Ion Chromatograph according to BGS Technical Procedure AGN 2.3.11 Issue 4.0.

4.6 POROSITY DETERMINATION

At the start of the experiment the porosity of each column was determined by weight using the following equations. Equation 1 calculates the volume of the solid material within the packed column giving a value for the volume of solids by assuming the minerals have the same density as quartz (2.64 g m l^{-1}). Equation 2 calculates the volume of fluid equivalent to the pore volume in ml, assuming the density of the synthetic groundwater is 1.0 g m l^{-1} and the column is fully water saturated. Equation 3 calculates the porosity of the columns as a percentage of the total volume.

$$\text{Volume of solid (ml)} = \frac{\text{mass of dry packed column (g)} - \text{mass of empty column (g)}}{2.64}$$

Equation 1. Calculation of the volume of solid material in the columns

$$\text{Volume of fluid (ml)} = \frac{\text{mass of wet packed column (g)} - \text{mass of dry packed column (g)}}{1.0}$$

Equation 2. Calculation of the volume of fluid within the columns

$$\text{Porosity (\%)} = \frac{\text{volume of fluid (ml)}}{\text{volume of fluid (ml)} + \text{volume of solid (ml)}} \times 100$$

Equation 3. Calculation of column porosity as a percentage of the total volume

To calculate porosity, the columns were weighed empty, after packing with dry diorite sand and weighed again after saturation with synthetic groundwater. Using the equations given above, the porosity of columns was calculated.

4.7 LASER-STIMULATED SCANNING FLUORESCENCE IMAGING

The technique of Laser-Stimulated Scanning Fluorescence Imaging (LSSFI) was performed at the end of the experiment to determine the extent of biofilm growth. Solid diorite material was carefully removed from the column and scanned using an Amersham Biosciences STORM™ 860 variable mode laser scanning system, equipped with both red (635 nm) and blue (450 nm) laser diodes for red- and blue-excited fluorescence, with long-pass filters[1] to allow recording of fluorescence emissions with wavelengths >650 nm and >520 nm, respectively. Fluorescence images were scanned and recorded at a spatial resolution of 100 µm per pixel. The images can be displayed as grey level or false-colour images where the grey level scale or colour can be set to reflect the intensity of fluorescence. The fluorescence emission of the Acridine Orange fluorochrome is at approximately 530 nm (Cowden and Curtis, 1976), which fluoresces under blue-excitation (Booth *et al*., 1988).

4.8 CRYOGENIC SCANNING ELECTRON MICROSCOPY

Examination of the solid diorite material at cessation of the column experiments were made on cryogenically-preserved subsamples and examined by cryogenic Scanning Electron Microscopy (cryoSEM). CryoSEM analysis was performed using a LEO 435VP variable pressure digital scanning electron microscope. The SEM instrument was equipped with a KE-Developments four-quadrant (4 diode-type) solid-state detector for backscattered electron imaging (BSEM). The instrument was also equipped with an Oxford Instruments CT1500 cryogenic sample handling and preparation unit. This was coupled directly to the SEM chamber via an airlock, giving access to a cryogenically-cooled SEM sample stage. Phase/mineral identification was aided by qualitative observation of energy-dispersive X-ray spectra recorded simultaneously during SEM observation, using an Oxford Instruments INCA energy-dispersive X-ray microanalysis (EDXA) system.

The brass collet and cup containing the subsample were attached to the cryoSEM sample holder and the subsamples were then rapidly pre-frozen by immersing in melting liquid nitrogen-solid nitrogen slush. The purpose of rapid pre-freezing was to produce an ice glass rather than coarse ice crystals that might cause microstructural deformation within the sample. Using the brass collet and cup sample holder enabled an undisturbed fracture surface to be obtained for cryoSEM imaging. This was achieved by breaking off the frozen collet, filled with the uppermost part of the frozen subsample, thus leaving a fresh face in the cup. The entire process was completed under vacuum in the cryo preparation chamber by using a purpose built chisel incorporated in the unit (Plate 4).

The brass cup was then transferred via an airlock into the SEM and mounted on a cryogenically-cooled sample stage (at -170°C).

Once inside the SEM, the sample stage was slowly warmed to between -80 and -60°C in order to ablate the frozen interstitial pore water under vacuum. This process allowed the intergranular pore fabrics to be revealed. The rate of ablation was controlled by constant observation under the SEM so as to just reveal the delicate intergranular and grain-coating features and not cause or

[1] An optical filter that transmits light of wavelengths longer than a specified cutoff. The filter rejects light with wavelengths that are shorter than the cutoff.

minimise any collapse of the fabric by ablating the ice too quickly. When the desired "development" of the sample surface was achieved, the stage was rapidly re-cooled to -170 °C. This stopped further ablation of ice from the sample. The samples were then observed under the SEM using BSEM imaging under variable pressure/low vacuum (0.4 torr). This allowed the frozen samples to be observed in the SEM without the need for coating with an electrically-conductive material such as gold or carbon (required for conventional high vacuum SEM analysis).

Images obtained using cryoSEM enabled a direct comparison to be made between control column material and experimental column subsamples. The aim of cryoSEM imaging was two-fold:

- Any organic material present in the experimental column could be better distinguished from any 'organic like', non-organic materials present in the crushed diorite prior to inoculation;
- Any artefacts of sample preparation, particularly in relation to salt crystallisation would be revealed.

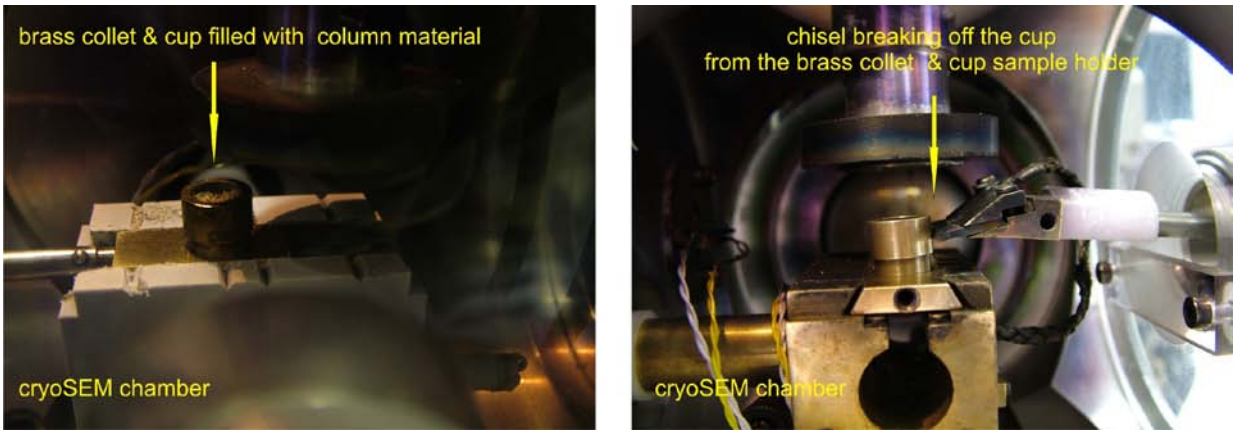


Plate 4. Photograph showing the brass collet & cup filled with subsample, placed in the cryoSEM chamber, prior to cryoSEM analysis.

5 Experimental Procedure

5.1 EXPERIMENTAL SETUP

The experimental setup of the anaerobic experiment is shown in Plate 5. Due to the limited amount of space available within the anaerobic chamber, the syringe pumps were setup outside the chamber and the tubing connecting the pumps to the columns entered the chamber via an air tight port. Stop/Fill values positioned at the outflow of the syringe pumps served two purposes, to allow the pumps to be refilled during the experiment and as an entry port for the inoculum.

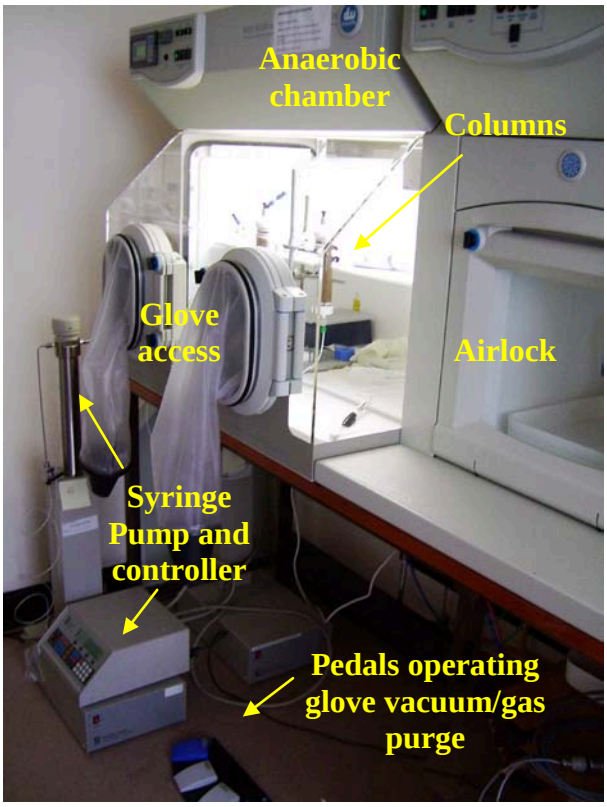


Plate 5. The experimental setup of the anaerobic cabinet.

The method of seeding the diorite columns with bacteria differed from that used previously in the aerobic experiment; only a single dose of bacteria was introduced into the columns via the Stop/Fill value as opposed to seeding a large floor-standing reservoir which continuously propagated the columns with bacteria. A 10 ml aliquot of inoculant of *Pseudomonas* containing 1.28×10^{-7} bacteria ml^{-1} was suspended in sterile artificial groundwater and then injected into the system. A sample of artificial groundwater was taken at the start of the experiment and after each refill of the pumps for reference purposes. A concise diary was kept to log times of fluid chemistry and microbiological sampling and to note any important observations in the status of the experiment. The gas mix in the chamber was 90% nitrogen, 5% carbon dioxide and 5% hydrogen; the temperature was maintained at room temperature (25°C). The columns within the environmental cabinet are shown in close-up in Plate 6 and Plate 7.

5.2 SAMPLING FOR MICROBIOLOGICAL ANALYSIS AND FLUID CHEMISTRY

At regular intervals, samples of the fluid from the column outflow were retained for biological and chemical analysis. Preservation methods were applied depending on the analysis required and the samples were refrigerated at between 1 and 8°C for analysis as a single batch at the conclusion of the experiment.

Sterile collection syringes were pre-weighed prior to use and transferred into the chamber via the glove ports. Once inside the chamber, the outflow of the column was halted briefly by isolating the columns from the syringes using the isolation valves. Although the pressure within the system was continuously logged by the data logger software, the pressure in each column system was noted prior to sampling since exchanging the sampling syringes, and stopping the pumps in order to refill them, effected the pressure within the system. The new sterile syringes replaced

those filled by the column outflow after which the isolation value was re-opened, (Plate 8 and Plate 9). The filled syringes were removed from the chamber via the airlock and re-weighed. Using the mass of the syringe before and after filling, and the time duration between sampling, the flow rate in ml hr^{-1} was calculated.

The samples of fluid collected from the column outflow were stabilised as required, given a unique identifier and stored at between 1 and 8°C to prevent degradation. At the start of the experiment, samples of column fluid were taken daily (Monday to Friday). The flow rate of the syringe pumps was set to 0.625 ml hr^{-1} (15 ml day^{-1}), limiting the sample volume available for multiple analyses.

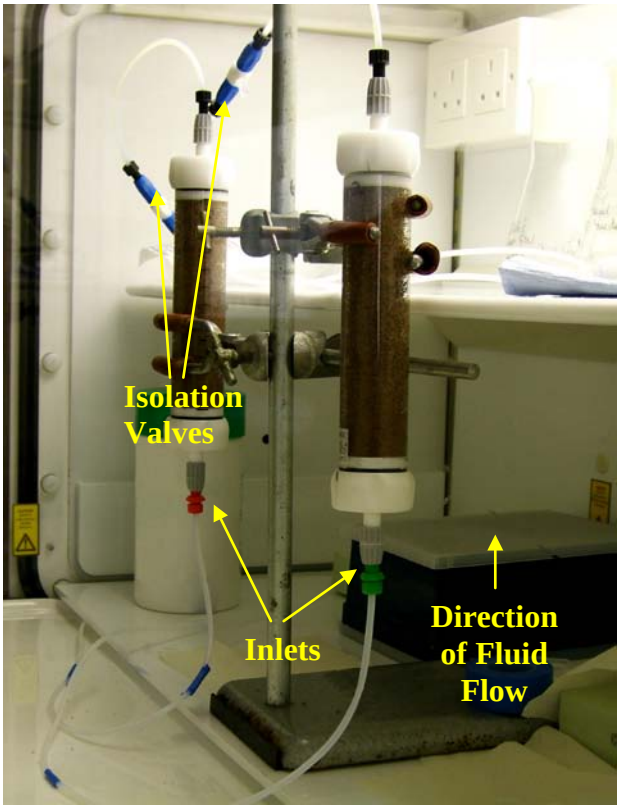


Plate 6. The columns packed with diorite sand positioned within the anaerobic chamber. The artificial groundwater is pumped in at the base of the column and exits at the top.

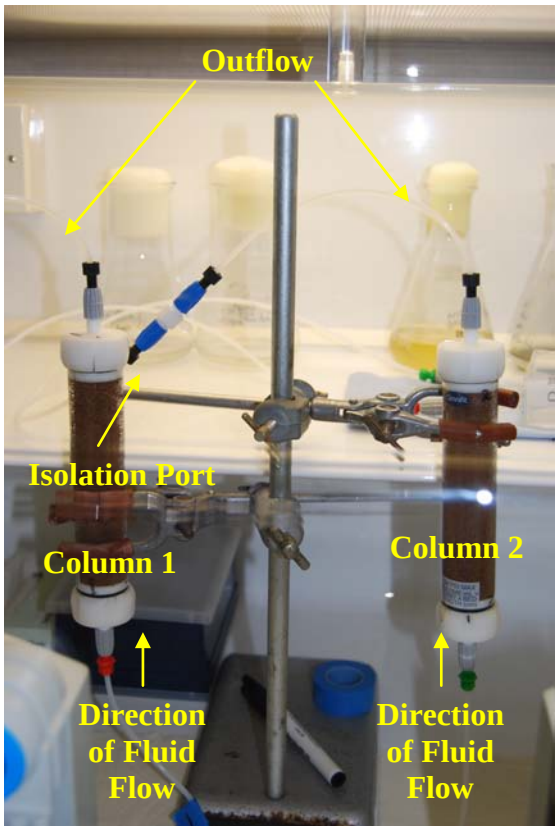


Plate 7. The positions of Column 1 and Column 2 within the anaerobic cabinet and one of the fluid isolation ports on the column outlet. The direction of fluid flow through the column is also indicated.

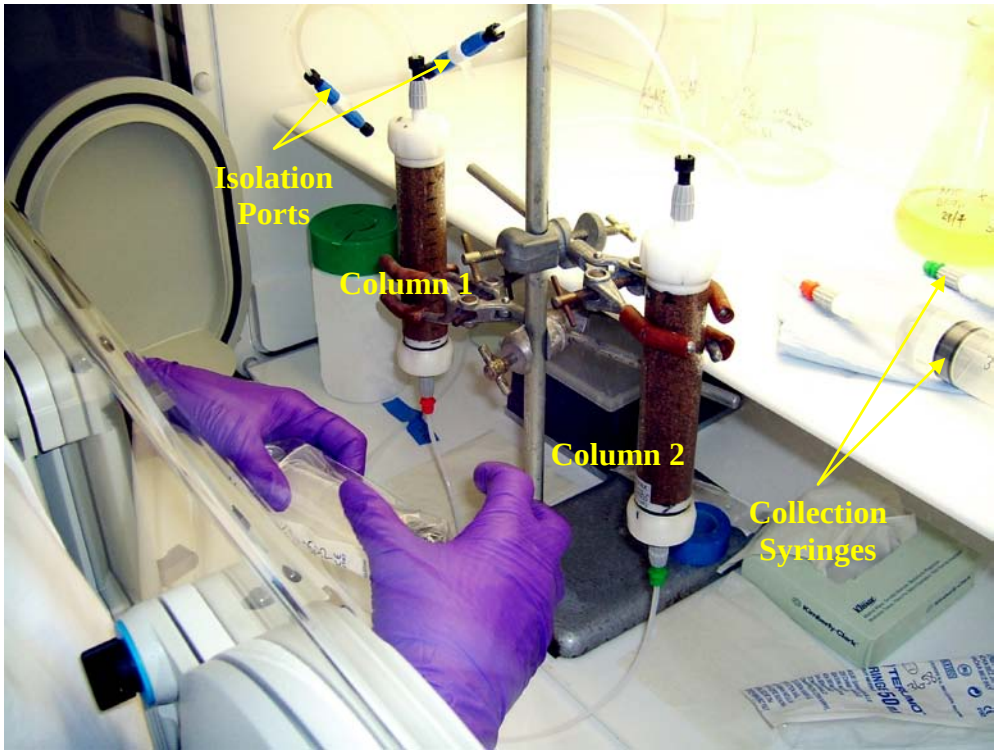


Plate 8. Syringe changing process. Fluid entered the columns at the base and flowed out of the top where isolation valves were fitted. The valves were closed prior to replacement of the syringes and re-opened once sampling was complete.

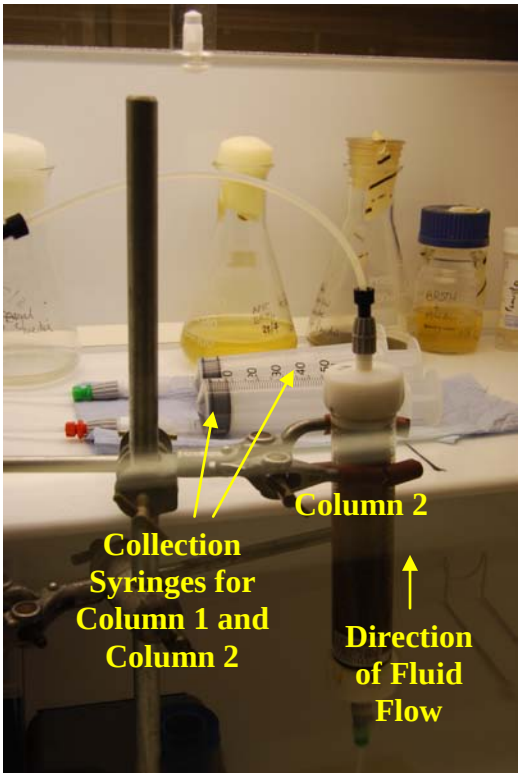


Plate 9. Column 2 is shown with newly fitted syringes for both Column 1 and Column 2 in readiness for fluid outflow collection.

Plate 10. Subsamples of column fluid were dispensed into sample tubes inside a laminar flow cabinet. This image shows the procedure of filtering the groundwater through a Millipore filter to remove bacteria.

Subsamples of column fluid were dispensed within a laminar flow cabinet to limit the possibility of microbial contamination of the column fluid. For each sample of column fluid collected by syringe, the following procedure was adopted:

1. A 1 ml aliquot of raw (unfiltered) column fluid was added to 10 ml of gluteraldehyde fixative in a Sterilin vial in preparation for microbial counts and stored between 1 and 8°C.
2. The remaining raw (unfiltered) column fluid was then passed through a 0.2 µm Sterilin Millipore filter in order to sterilise the sample by removing any viable pathogenic bacteria.
3. 1 ml of column fluid (filter sterilised as in stage 2), was preserved with 1% v/v with respect to concentrated Aristar © grade nitric acid for ICP-AES analysis and stored between 1 and 8°C.
4. A sample of column fluid (filter sterilised as in stage 2), was retained for reduced iron analysis by preserving to a final concentration of 0.1% 2,2'–dipyridyl solution immediately after collection. This sample was stored between 1 and 8°C prior to analysis.
5. One ml of column fluid (filter sterilised as in stage 2) was retained for IC analysis. This sample did not require preservation other than storage between 1 and 8°C prior to analysis.
6. The remainder of the filtered sterile fluid (prepared in stage 2), was used for the analysis pH, this test was carried out as soon as possible after sample collection. Plate 10 shows a collection syringe filled with column fluid attached to the Sterilin Millipore filter; here the column fluid is being filter sterilised for pH analysis.

5.3 SAMPLING OF EXPERIMENTAL COLUMNS AND BIOLOGICAL STAINING PROCEDURE

Following cessation of the column experiment, one of the duplicate columns was longitudinally sliced open. This was achieved by first cutting lengthwise through the glass wall of the column, using a small hand-held diamond modelling saw (Plate 11). The packed crushed diorite filling the column was then sliced in half by drawing a bronze cheese-wire along the length of the column, through the cut glass walls (Plate 12). One half of the experimental column was then sprayed with Acridine Orange solution to stain for the presence of any biofilm and bacteria in the column (Plate 13). Any microbial materials stained by Acridine Orange would fluoresce red or green under ultraviolet (UV) light. The cut surface of Column 1 was photographed in normal visible light, under ultraviolet (UV) illumination and in addition, a digital laser-stimulated scanning fluorescence imaging (LSSFI) was also used to image the distribution of Acridine Orange exposed within the whole cut surface of the column (Section 6.6). Subsamples of solid material were taken from the centre and edge of the exposed unstained half of the column along its length using a sterile spatula and preserved in gluteraldehyde fixative to determine bacterial distribution (Section 6.4).

In order to evaluate the distribution of organic matter and biofilm development Column 1 was sampled at 1 cm intervals along its entire length (12 cm) to produce 13 sub-samples for examination. These subsamples were then examined by cryogenic scanning electron microscopy (cryoSEM) (Section 6.8). A single subsample constituted a representative volume of the

undisturbed material, present within each centimetre of the column. Subsamples were then carefully extracted by using a 7 mm diameter acetate tube (Plate 14-a).

Plate 11. Longitudinally cutting open BioTran Column 1 using a hand-held diamond saw. A portable vacuum dust extraction unit was used to control the glass dust and any biological hazard.

Plate 12. Crushed diorite fill within Column 1 was sliced longitudinally by drawing a bronze cheese wire though the cut walls of the column to produce two halves.

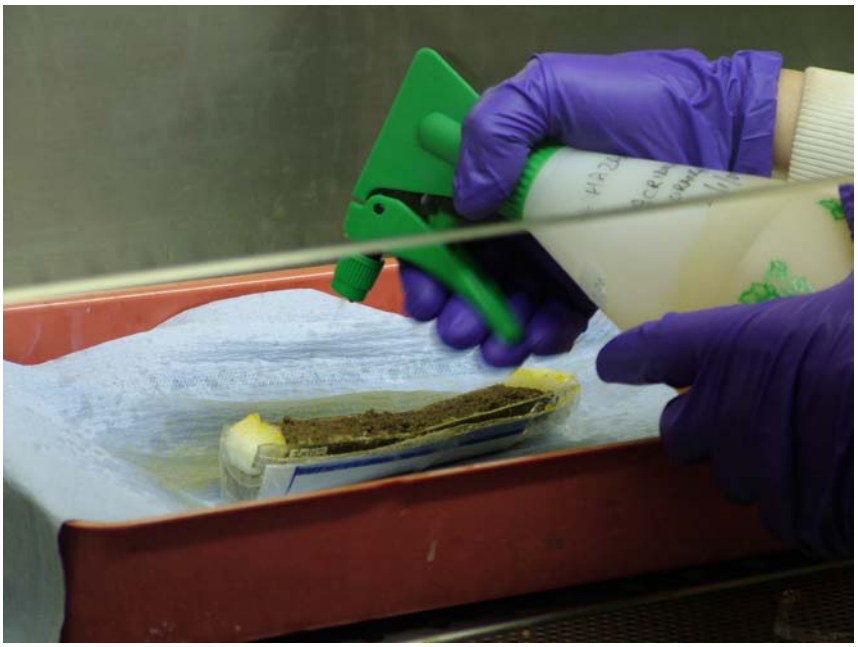


Plate 13. Photograph showing one half of Column 1 being sprayed with Acridine Orange stain.

A single subsample from the experimental column, derived from 0-1 cm was examined unwashed. This was carried out in order to determine the extent of artefacts due to salt crystallisation. The formation of NaCl crystals in the subsamples could produce artefacts that obscure the original texture of the sample and along with ice crystal formation may contribute to disturbing or destroying the delicate structure of any organic fabric present. The unwashed subsample was found to be substantially damaged by the saline fluid and therefore, the remaining twelve subsamples were washed with seven drops of tap water. Seven drops were chosen so as not to completely destroy the intergranular fabric whilst attempting to replace the briny synthetic Äspö fluid in the diorite pore spaces with water. This was achieved by allowing the fluid to percolate through the sample and brass collet and cup, where it was absorbed by the glass-fibre filter paper as shown in Plate 14-b. The control column subsamples were washed in the same manner, as indicated in Appendix 2.

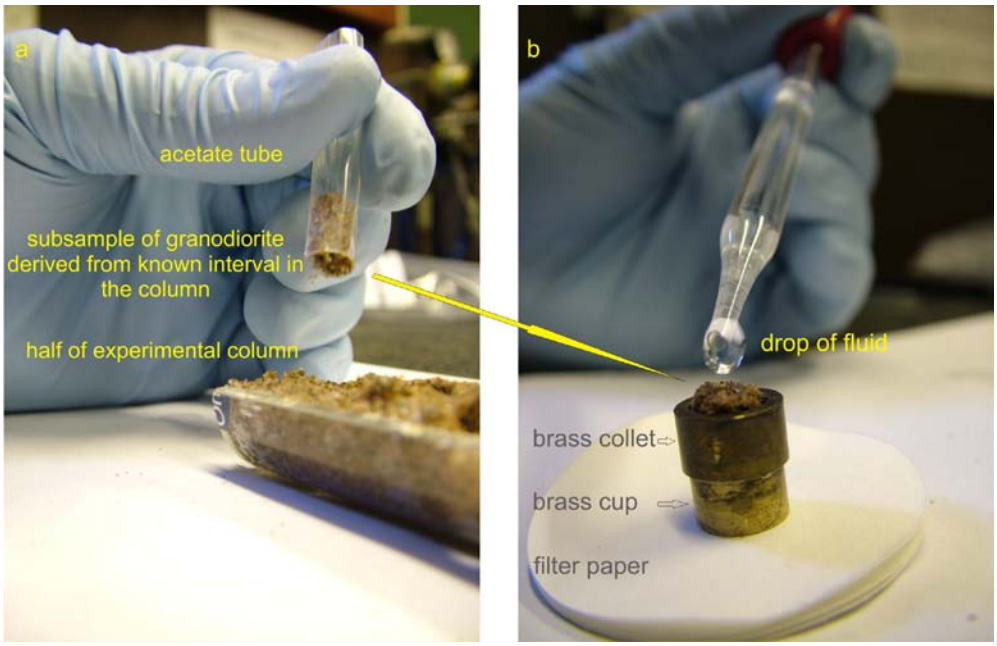


Plate 14. Photographs showing: a-sampling the experimental column using an acetate tube; b-washing subsample with drops of fluid.

5.4 PREPARATION OF CONTROL MATERIAL

Previously, in the aerobic study (West *et al.*, 2008), both columns were inoculated with *P.aeruginosa* with Column 1 being used as a control whilst Column 2 was subjected to changes in pH and the introduction of bacteriophage. At the outset of this latest work, the aim was to perform an experiment similar in design to the aerobic experiment but in an anaerobic environment. However, during the course of this anaerobic study, it became apparent that the rate of bacterial growth was retarded considerably by the lack of oxygen and little, if any, biofilm was visible in either column. This was not unexpected as *P.aeruginosa* is primarily an aerobic bacterium and while it can grow anaerobically, the low nutrient levels in the synthetic Äspö groundwater limit the bacterium's ability to utilise nitrate as a respiratory electron acceptor. The slow growth rate caused the original experimental plan to be revised since adjustment of groundwater pH/infection by bacteriophage in one of the columns was not considered to be viable. Consequently, since both the anaerobic columns had been inoculated, this left the experiment without a bacteria-free column which could be used as a control. In order to produce control material for use as a reference for comparison with post experimental material, three sterile 'mini' control columns were prepared containing crushed diorite, immersed in either tap water or synthetic Äspö groundwater with no bacteria present.

Crushed diorite 'mini' columns were made by modifying 3 ml disposable plastic pipettes, sterilised by flushing with 100 % ethanol. All subsequent column preparation work was carried out in a laminar flow cabinet in order to ensure aseptic conditions. Each pipette was then cut to size with a sterile scalpel by firstly removing two thirds of the filling bulb at the top. This provided a convenient filling 'funnel'. Then, the column was cut to size by removal of the first 2 ml of tube. Each empty column was then plugged at one end with approximately 5 mm of pipette filter in order to prevent diorite spillage whilst enabling fluid percolation. The columns were then placed in Sterilin® 28 ml Universal sample bottles and filled by spatula with diorite, which had been previously sterilised under ultra-violet light. Each column was tapped lightly during filling in order to pack the grains as closely as possible. Tap water and synthetic Äspö groundwater were syringe filtered using 2 µm filters. Two of the columns were then filled using sterile syringes with the filtered synthetic Äspö groundwater and the remaining one, with the

filtered tap water. Each column was allowed to drain into the sample bottle during filling until the fluid level in the bottle equalled that of the top of the diorite in the column, thus ensuring that each column was fully saturated. The sample bottles were then sealed with screw caps and the columns left at room temperature for three days in the laminar flow cabinet until they could be sampled for cryogenic scanning electron microscopy (cryoSEM) shown in Plate 15.

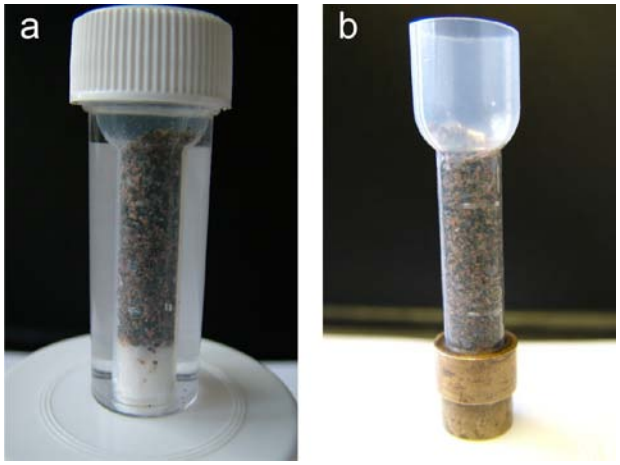


Plate 15. Photographs showing a closely packed diorite 'mini' column, (a)-fully saturated with fluid within a sterile sample bottle and (b)-removed, prior to cryoSEM analysis

5.5 SAMPLING OF CONTROL MATERIAL

A subsample was obtained from each of the three separate 'mini'columns. The first two had been submerged in sterile, synthetic Äspö groundwater. The third subsample had been submerged in sterile tap water. In order to extract a subsample from a column, the column was removed from the fluid saturated sample bottle. A scalpel was then used to cut off the pipette filter tip to allow a plug of column material to be carefully pushed into a brass collet and cup (8 mm diameter and 12 mm height) for cryoSEM preparation (Plate 16). The cup had a 4 mm hole in the base in order to allow fluid to percolate through the sample. Each of the subsamples were then examined by cryogenic scanning electron microscopy (cryoSEM) (Section 6.7).

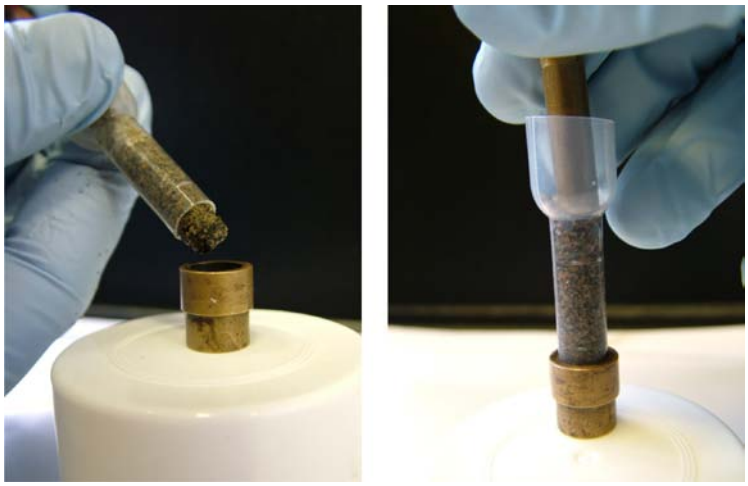


Plate 16. Photograph showing sampling of control column material.

6 Results

6.1 POROSITY

Initial calculations showed good agreement between the columns with Column 1 having a porosity of 43.3% and Column 2 a porosity of 43.1%. The porosity calculations appear in full in Appendix 1.

6.2 FLOW RATE AND PRESSURE

Examination of the flow rates through the columns during the course of the experiment showed no significant trend. This was due to the introduction of syringe pumps which maintained a constant flow rate into the columns and the slow growth of the bacteria under anaerobic conditions. A few outliers were noted but these were due to pump refills or leaking columns. The pressure within the system was continuously logged throughout the course of the experiment. Fluctuations in pressure were observed; these coincided with sampling times and were particularly noticeable during weekend periods. Early on the experiment, the root cause was found to be due to the type of syringe being used for sample collection. At the start of the experiment, samples were taken daily (Monday to Friday), so 15 ml syringes were attached to the column outflow. On Fridays, 50 ml syringes replaced the 15 ml syringes to accommodate the additional outflow volume expected over the weekend period. However, altering the syringe volume was found to cause a change in back pressure which was recorded by the pressure logger because the pressure required to fill a 50 ml syringe was greater than that to fill a 15 ml syringe. In order to reduce this effect, the sampling interval was changed to alternate days (Monday, Wednesday and Friday) with 50 ml syringes being used exclusively. An annotated graph of the pressure recorded by the data logger is shown in Figure 2.

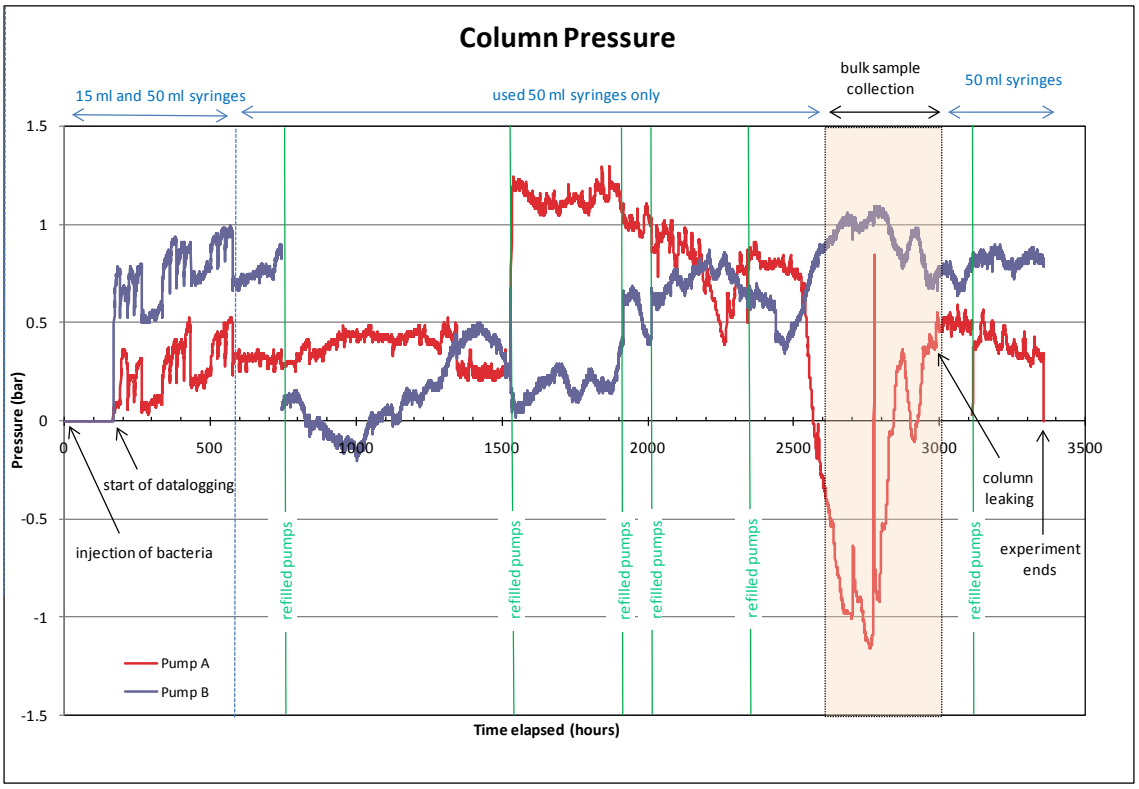


Figure 2. Pressure data recorded by the data logger for Pump A (Column 2, red) and Pump B (Column 1, blue).

The dotted vertical blue line on Figure 2 shows the point at which 50ml syringes were used exclusively (approx 500 hours). Before this time, the short-term pressure variations were caused entirely by the differences in back pressure from using both 15 ml and 50 ml syringes during sample collection. Figure 2 also shows annotations where the pumps were stopped for refilling as indicated by solid vertical green lines. The drop in pressure observed for pump B (Column 1) between 3020 and 3118 hours took place over the Christmas period when bulk samples were collected with a consequent reduction in back pressure. Pump A (Column 2) seemed relatively unaffected, suggesting that this column was less susceptible to back pressure changes.

6.3 MICROBIOLOGY OF COLUMN FLUIDS

The results (means) for total bacterial counts of water samples collected from the outflow of Column 1 and Column 2 using epi-fluorescence microscopy are given in Table 2 and shown graphically in Figure 3.

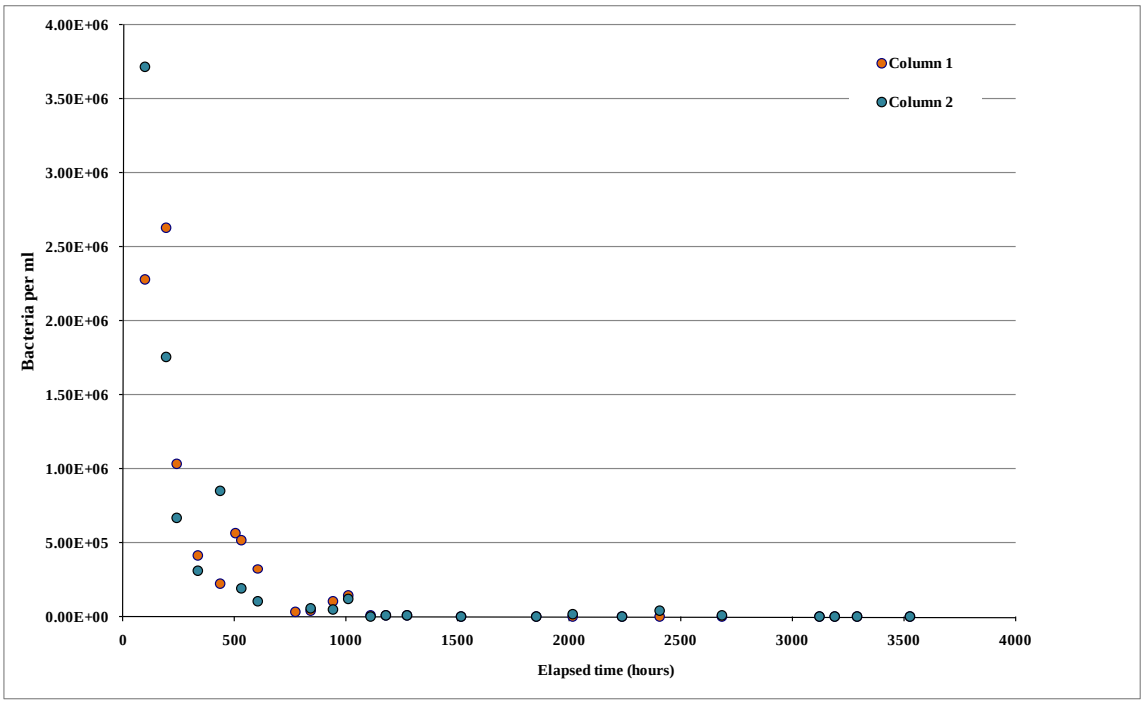


Figure 3. Mean total counts of bacteria by epi-fluorescence microscopy from experimental fluids collected from Column 1 and Column 2.

The method of inoculating the columns with bacteria differed from that used previously in the aerobic experiment; only a single dose of bacteria was introduced into the columns via the Stop/Refill value as opposed to seeding a large floor-standing reservoir which continuously propagated the columns with bacteria. 10 ml of synthetic groundwater containing $1.28x10^7$ ml^{-1} of *P. aeruginosa* was injected into the column inlet valve of each column at the start of the experiment (time 0*). Results of the bacterial counts of the experimental fluids show a rapid decrease in number in both columns after 192 hours. In Column 1 mean bacterial numbers decreased from $2.63x10^6$ bacteria ml^{-1} at 192 hours to $1.03x10^6$ bacteria ml^{-1} at 240 hours and continued to decrease to a mean of $3.69x10^4$ bacteria ml^{-1} at 838 hours. Bacterial numbers then increased to means of $1.02x10^5$ bacteria ml^{-1} at 938 hours and $1.41x10^5$ bacteria ml^{-1} at 1009 hours. Between 1104 hours and 1850 hours mean numbers remained fairly constant between $1.17x10^3$ bacteria ml^{-1} and $7.75x10^3$ bacteria ml^{-1}. No bacteria were detected in the fluid from Column 1 at 2014 hours, 2232 hours, 3118 hours or at end of the experiment at 3189 hours.

Bacterial counts for Column 2 also showed a similar trend. Mean bacterial numbers decreased from $3.72x10^6$ bacteria ml^{-1} at 96 hours to $3.06x10^5$ bacteria ml^{-1} at 334 hours. There was a small increase in mean numbers at 434 hours to $8.48x10^5$ bacteria per ml which then decreased to $4.71x10^4$ bacteria ml^{-1} at 938 hours. Between 1104 hours and 1850 hours mean numbers remained fairly constant between $1.57x10^3$ bacteria ml^{-1} and $5.43x10^3$ bacteria ml^{-1}. In contrast to column 1, a mean of $1.41x10^4$ bacteria ml^{-1} were detected at 2014 hours. No bacteria were detected in the fluid from Column 2 at 2232 hours, 3118 hours or at end of the experiment at 3189 hours.

Table 2. Mean total bacterial counts by epi-fluorescence microscopy of water samples collected after flowing through Column 1 and Column 2.

Time since injection (h)	Column 1 Bacteria ml^{-1}	Standard error	Column 2 Bacteria ml^{-1}	Standard error
0*				
96	$2.28x10^6$	$9.57x10^5$	$3.71x10^6$	$1.33x10^5$

Time since injection (h)	Column 1 Bacteria ml^{-1}	Standard error	Column 2 Bacteria ml^{-1}	Standard error
192	$2.63x10^{6}$	$9.88x10^{5}$	$1.75x10^{6}$	$7.12x10^{5}$
240	$1.03x10^{6}$	$3.00x10^{5}$	$6.68x10^{5}$	$3.30x10^{4}$
334	$4.16x10^{5}$	$2.84x10^{4}$	$3.06x10^{5}$	$2.71x10^{4}$
434	$2.20x10^{5}$	$2.01x10^{4}$	$8.48x10^{5}$	$2.12x10^{4}$
501	$5.65\ x10^{5}$	$1.85x10^{5}$		
526	$5.18x10^{5}$	$3.63x10^{4}$	$1.89x10^{5}$	$1.89x10^{4}$
600	$3.22x10^{5}$	$2.97x10^{4}$	$1.02x10^{5}$	$9.11x10^{4}$
768	$3.14\ x10^{4}$	$5.50x10^{3}$		
838	$3.69x10^{4}$	$2.28x10^{3}$	$5.50x10^{4}$	$9.11x10^{4}$
938	$1.02x10^{5}$	$1.66x10^{4}$	$4.71x10^{4}$	$1.18x10^{4}$
1009	$1.41x10^{5}$	$3.13x10^{4}$	$1.18x10^{5}$	$1.45x10^{4}$
1104	$7.85x10^{3}$	$1.55x10^{3}$	$3.14x10^{3}$	$1.10x10^{3}$
1173	$7.07x10^{3}$	$1.45x10^{3}$	$5.49x10^{3}$	$1.20x10^{3}$
1270	$5.50x10^{3}$	$1.24x10^{3}$	$5.40x10^{3}$	$1.02x10^{3}$
1513	$1.18x10^{3}$	$6.70x10^{2}$	$1.57x10^{3}$	$8.60x10^{2}$
1850	$3.46x10^{3}$	$9.80x10^{2}$	$3.14x10^{3}$	$8.80x10^{2}$
2014	$0.00x10^{0}$		$1.41x10^{4}$	$2.71x10^{3}$
2232	$0.00x10^{0}$		$0.00x10^{0}$	
2402	$3.93x10^{3}$	$5.50x10^{2}$	$3.69x10^{4}$	$2.16x10^{3}$
2684	$3.30x10^{3}$	$3.10x10^{2}$	$4.80x10^{3}$	$4.40x10^{2}$
3118	$0.00x10^{0}$		$0.00x10^{0}$	
3189	$0.00x10^{0}$		$0.00x10^{0}$	

6.4 MICROBIOLOGY OF SOLID COLUMN MATERIAL

In order to determine bacterial distribution, subsamples of solid material were taken from the centre and edge of the exposed unstained surface along the length of the sectioned column using a sterile spatula and preserved in gluteraldehyde fixative (Section 5.3). Only column 1 was examined as both columns had undergone the same experimental conditions. The weight of the preserved sample was calculated in order to determine an accurate number of bacteria g^{-1} of sample once the count had been made. The mean results are shown in Table 3 and graphically in Figure 4.

Table 3. Results of total count of bacteria by epi-fluorescence microscopy of Äspö diorite taken from Column 1 at the end of the experiment

Distance from column inlet (cm)	Centre of column Bacteria g^{-1}	Standard error	Edge of column Bacteria g^{-1}	Standard error
0-1	$8.48x10^{5}$	$4.11x10^{5}$	$2.30x10^{6}$	$2.07x10^{6}$
1-2	$3.08x10^{6}$	$1.31x10^{5}$	$7.85x10^{5}$	$3.93x10^{5}$
2-3	$1.71x10^{6}$	$9.06x10^{5}$	$4.62x10^{5}$	$2.77x10^{5}$

3-4	$6.56x10^5$	$2.11x10^6$	$5.94x10^5$	$3.30x10^5$
4-5	$3.27x10^5$	$3.02x10^5$	$3.34x10^5$	$3.83x10^4$
5-6	$9.61x10^4$	$1.60x10^4$	$0.00x10^0$	
7-8	$1.54x10^5$	$1.84x10^4$	$1.08x10^5$	$1.56x10^5$
8-9	$9.67x10^5$	$9.51x10^4$	$5.95x10^5$	$5.12\ 10^5$
9-10	$1.87x10^5$	$2.25x10^4$	$9.43x10^4$	$1.71x10^4$
10-11	$2.75x10^5$	$3.65x10^4$	$3.17x10^5$	$3.12x10^4$
11-12	$4.55x10^5$	$2.20x10^4$	$1.77x10^6$	$5.66x10^5$

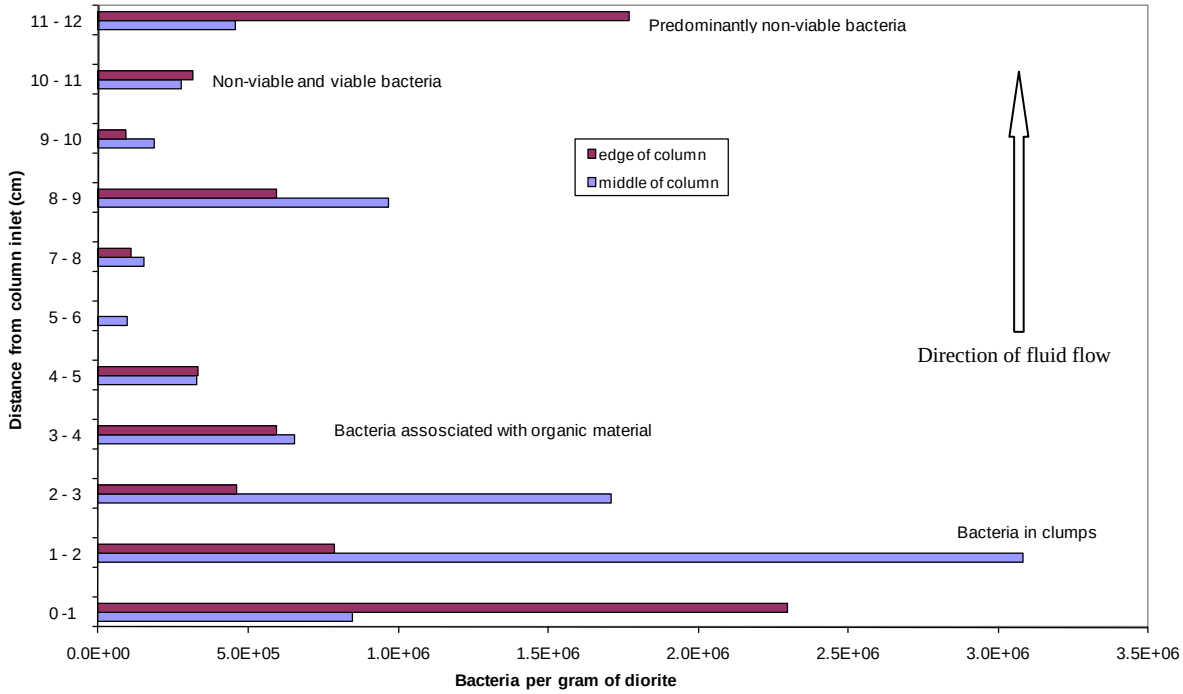


Figure 4. Mean total count of bacteria by epi-fluorescence microscopy sampled from Column 1 Äspö diorite at the end of the experiment.

Bacterial numbers were greatest at the inlet end of the column. At 0 cm to 3 cm the mean bacterial count ranged from $2.29x10^6$ bacteria g^{-1} to $4.62x10^5$ bacteria g^{-1} at the outer edge of the column. The highest numbers occurred in the centre of the column, ranging from $8.48x10^5$ bacteria g^{-1} to $3.08x10^6$ bacteria g^{-1}. Under the microscope the bacteria appeared to be aggregated in groups rather than as individual cells. At 3 cm to 4 cm the groups of bacteria also appeared to be associated with organic material, possibly exopolysaccharides (EPS) from biofilm formation. The mean number of bacteria gradually decrease along the length of the column to $9.61x10^4$ bacteria g^{-1} at 5 cm to 6 cm (middle) with none detected at the outer edge at this point. There was a small increase in mean numbers of $9.66x10^5$ bacteria g^{-1} (middle) and $5.95x10^5$ bacteria g^{-1} (edge) at 8 cm to 9 cm. At the end of the column, at 11 cm to 12 cm there were $1.76x10^6$ bacteria g^{-1} at the edge of the column but most of these appeared to be non-viable bacteria. It appears that they had collected at the outlet end of the column which is also confirmed by the UV image of distribution as shown in Plate 17.

6.5 FLUID CHEMISTRY

The results of the fluid chemistry analysis for Column 1 and Column 2 are tabulated in Appendix 3. No significant changes were observed in pH of the outflow from Column 1 and Column 2 during the course of the experiment. The starting pH of Column 1 was 7.24 and ranged between 6.01 and 7.18. The pH of Column 2 was similar; the starting pH was 7.20 and ranged between 6.14 and 7.18.

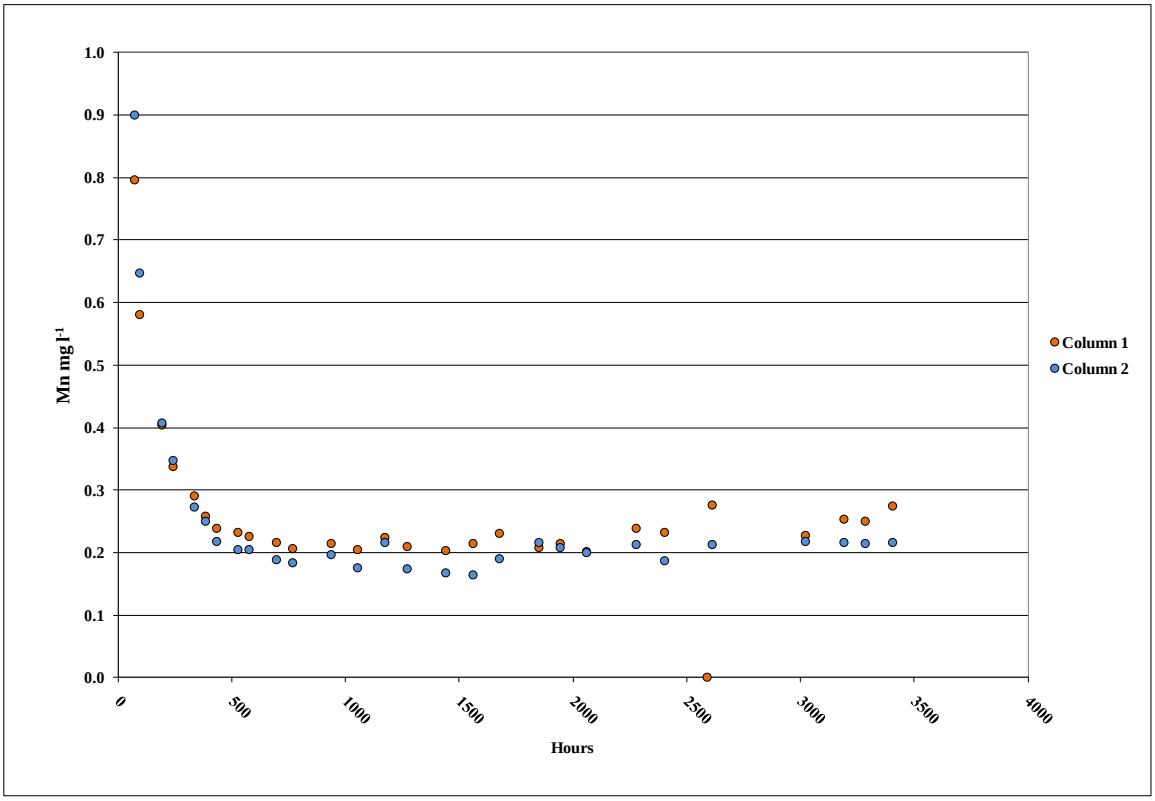


Figure 5. Plot of Mn concentration (mgl^{-1}) against experiment duration time (hours)

Plots of concentration of analytes in the column outflow against time showed no obvious trends for the majority of analytes. However, a correlation was found between manganese and barium with both columns showing a similar trend. Maximum concentrations of both Mn and Ba were found at the start of the experiment and the concentrations of both analytes decreasing rapidly in the first 500 hours. The Mn concentration appears to stabilise after 500 hours, but this was probably due to the concentration approaching the limit of detection (0.25 mg l^{-1}). The Ba concentration continues to fall after 500 hours, albeit at a slower rate, until the limit of detection is reached (0.05 mg l^{-1}).

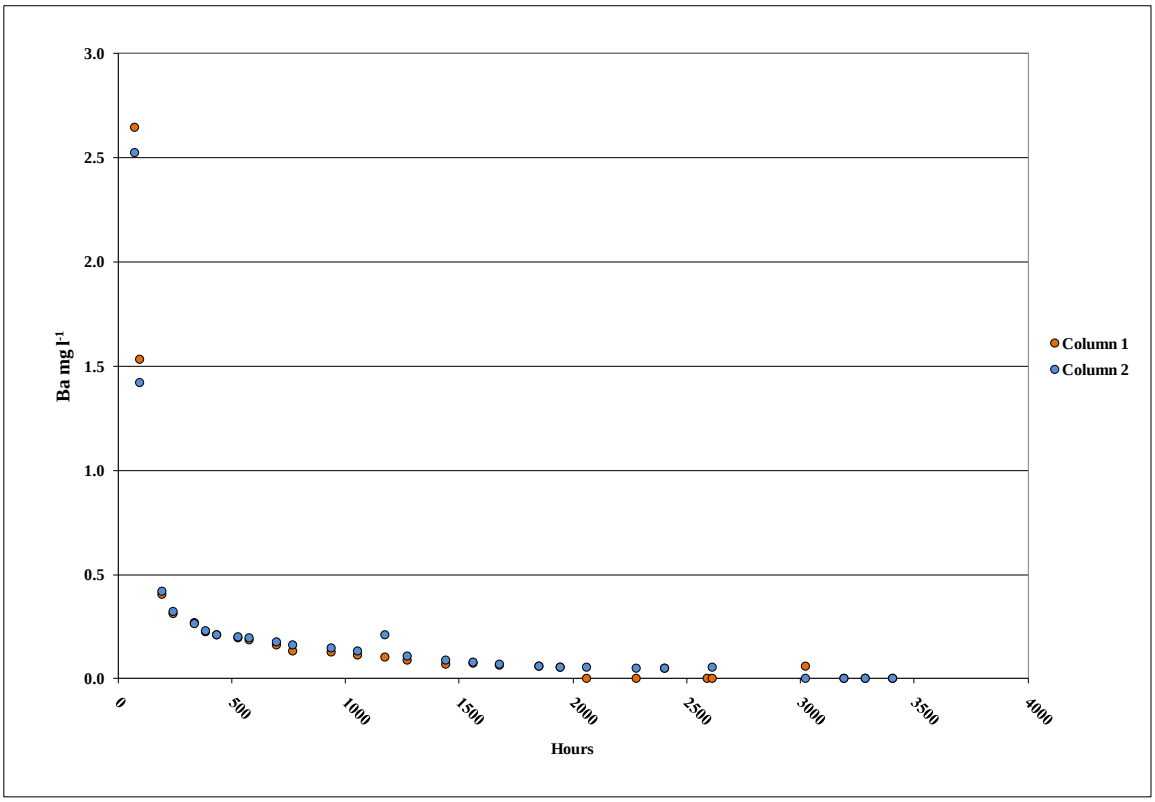


Figure 6. Plot of Ba concentration (mgl^{-1}) against experiment duration time (hours)

6.6 LASER-STIMULATED SCANNING FLUORESCENCE IMAGING OF EXPERIMENTAL COLUMNS

Laser-stimulated scanning fluorescence imaging (LSSFI) was carried out of the freshly-cut surface of the experimental column, after staining the surface with Acridine Orange (Plate 13). Only the blue excited image (Plate 17) gave any indication of where potential biological material was present along the column. Regions of intense orange indicated the highest level of response with lime green and dark blue representing decreasing intensity; royal blue depicts background levels where no biological material was found. It should be noted from this image, that the intense orange areas at either end of the column were caused by an accumulation of Acridine Orange in the foam plastic supporting the diorite and does not indicate the accumulation of bacteria or the formation of biofilm.

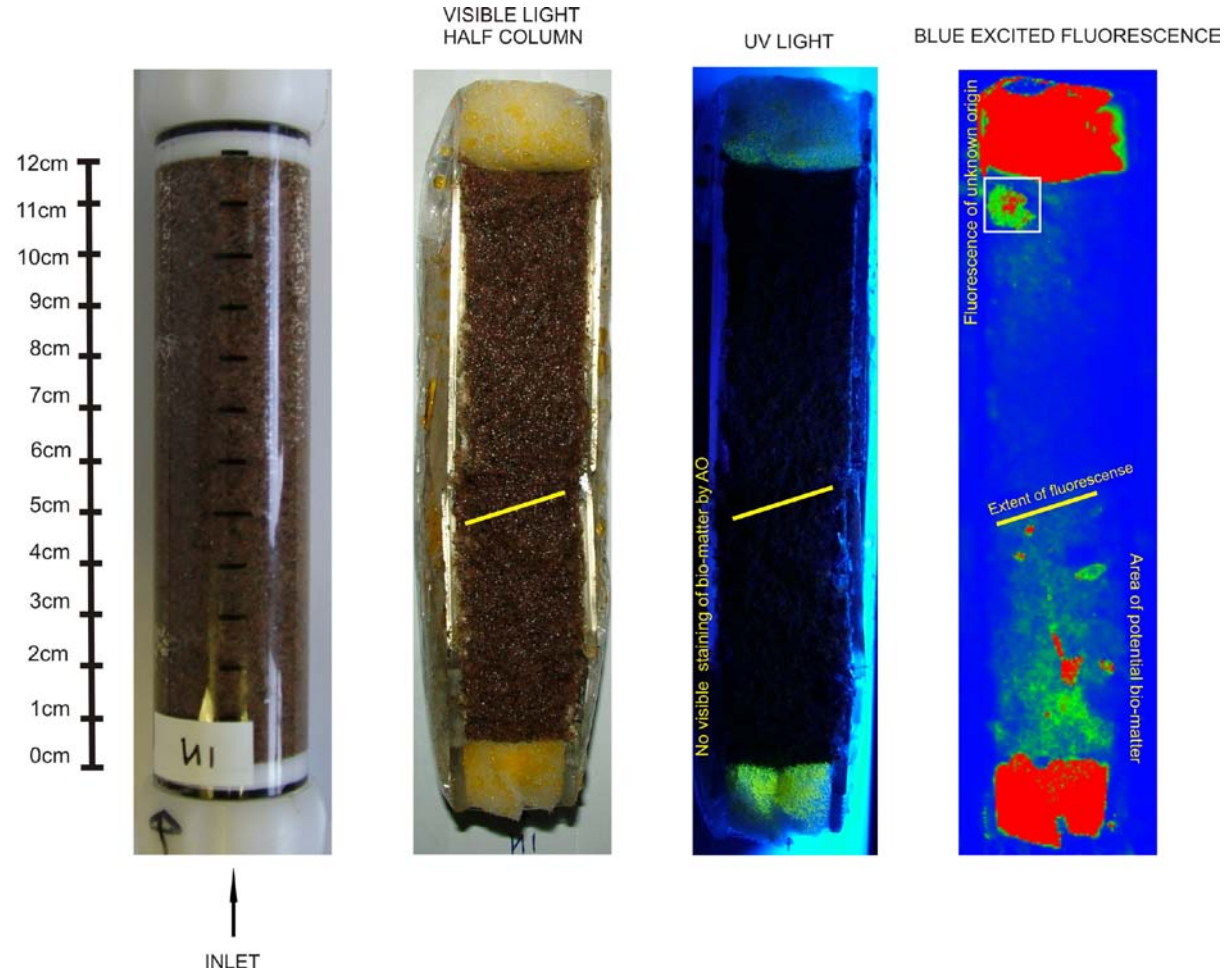


Plate 17. Visual images of the Acridine Orange stained half of Column 1 obtained in visible light, UV light and a blue excited fluorescence scan.

6.7 CRYOGENIC SCANNING ELECTRON MICROSCOPY OF CONTROL COLUMN MATERIAL

The subsample obtained from the crushed diorite saturated and washed in tap water revealed that there was no salt contamination present after ice ablation, as expected. The grain sizes were fairly uniform (Plate 18). Fines were also seen to be present on the surfaces of the coarser mineral grains. These may have been derived from a number of sources:

- The starting material may have been inadequately cleaned to remove the fines;

- The fines may have been produced during packing of the column by abrasion of grains.

The fine materials were lightly scattered onto grains and also in the pore spaces between them. Occasionally larger deposits of fine material were found to aggregate within pores and bridge pore throats (Plate 19).

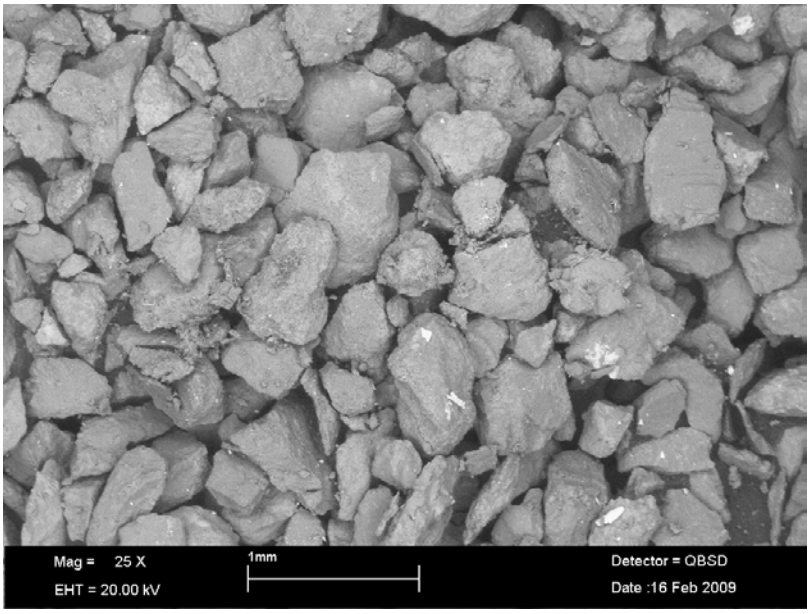


Plate 18. BSEM image showing the crushed diorite saturated and washed in tap water.

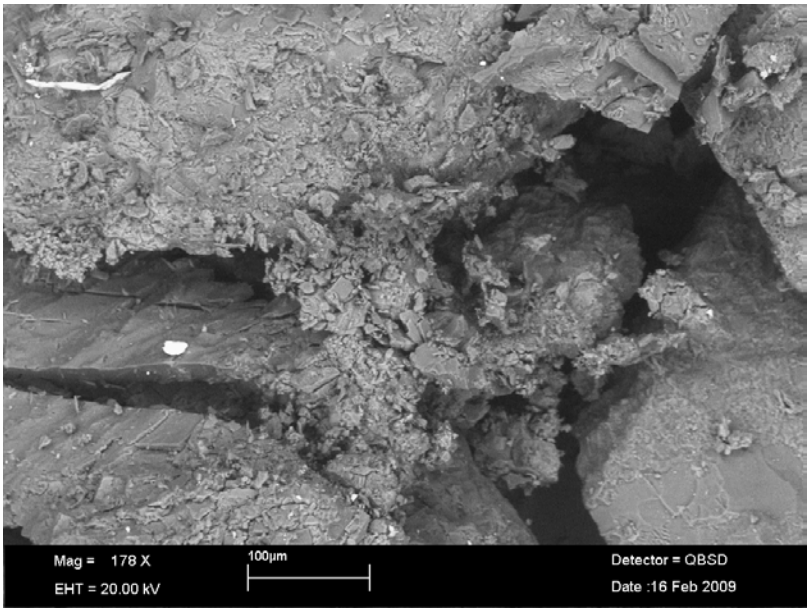


Plate 19. BSEM image showing a large aggregation of fines bridging an intergranular pore space.

The subsample obtained from the crushed diorite saturated in synthetic Äspö groundwater and washed in tap water revealed that salt contamination, although not immediately obvious at low magnification, was present (Plate 20). This demonstrated that washing the subsample in tap water was insufficient in removing all of the salt present in the synthetic Äspö groundwater, prior to freezing the subsample. Salt crystals (maximum size 100 µm) were found to bridge some intergranular pore spaces. These occurred as either fine films with fine rock material

trapped within them (Plate 21), or as 'filament-like' strands (Plate 22). The EDXA analysis revealed the presence of salt (NaCl), within the strands (Plate 22).

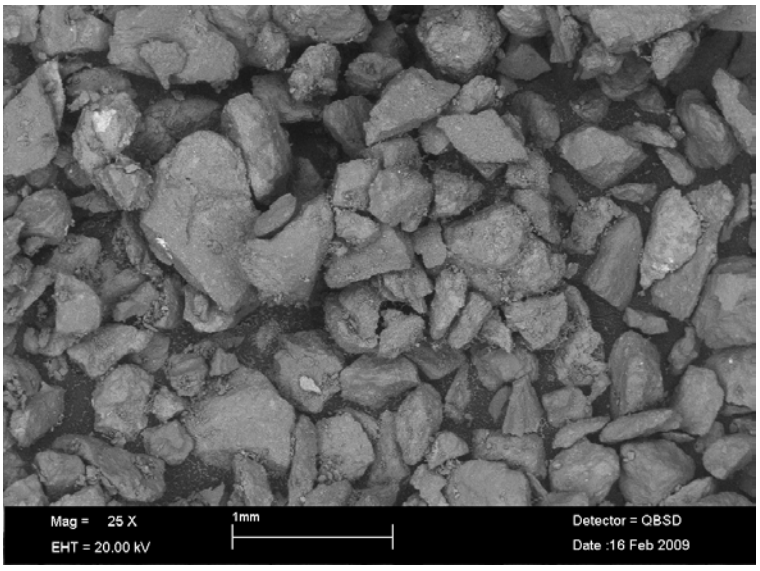


Plate 20. BSEM image showing crushed diorite saturated in synthetic groundwater and washed in tap water.

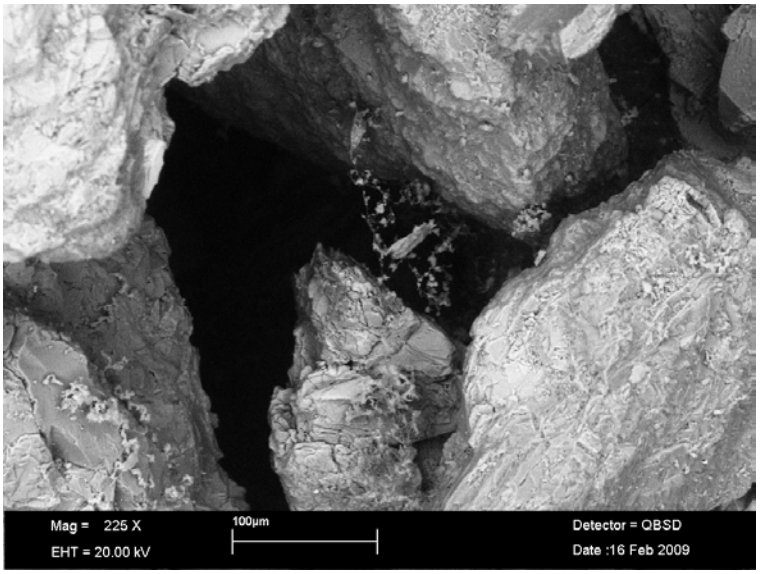
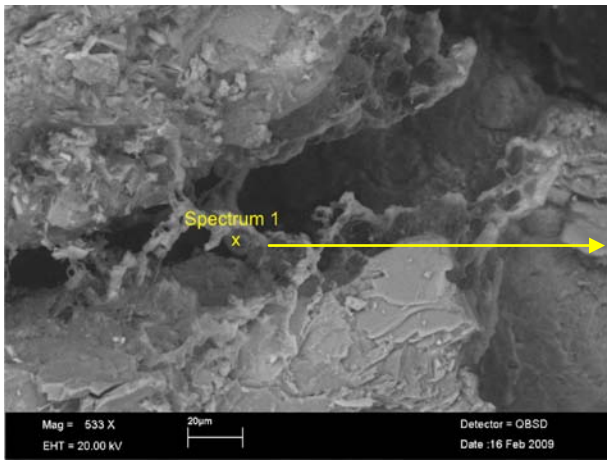


Plate 21. BSEM image showing fine rock material trapped in fine films of salt crystals, bridging an intergranular pore space.



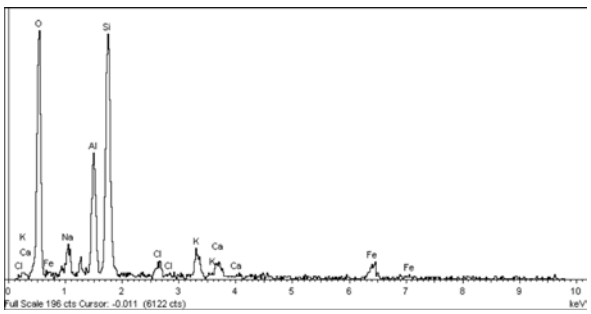


Plate 22. BSEM image of filamentous salt crystal growth bridging intergranular pore throat and, EDXA showing the presence of salt as indicated.

The crushed diorite, saturated and washed in synthetic groundwater, exhibited salt contamination, as expected. As seen previously (Plate 20), this was not evident in the low magnification image (Plate 23). Closer examination revealed much larger salt crystals (up to 400 µm in length), encrusting several grains. Parallel sheets or dendritic crystals of the order of < 5 µm in thickness were formed within the sample as a result of crystallisation of salt along the crystal boundaries of coarse dendritic ice crystals that formed within the wet sample because rapid freezing (ideally required for cryoSEM) could not be achieved with these samples (Plate 24).

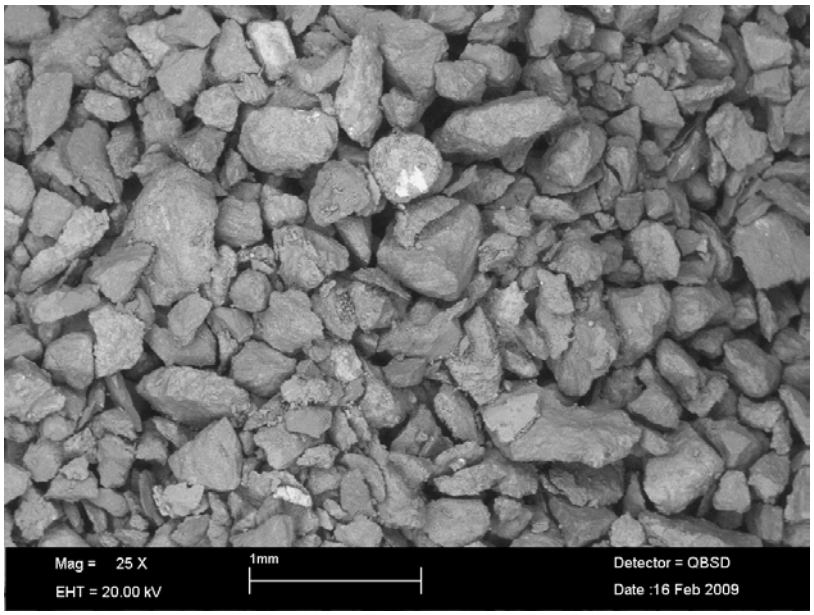


Plate 23. BSEM image showing crushed diorite saturated and washed in synthetic groundwater.

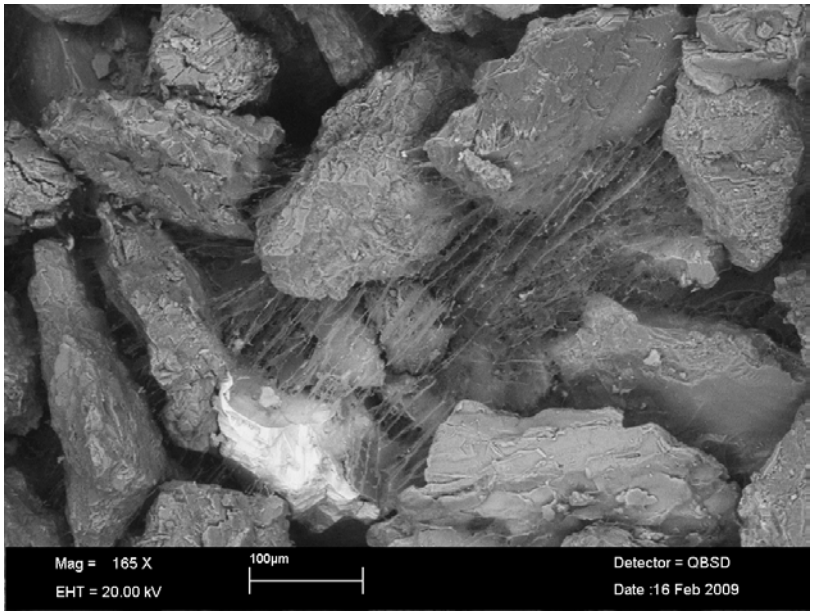


Plate 24. BSEM image showing parallel salt dendrite crystal sheets spanning several grains and intergranular pore spaces.

6.8 CRYOGENIC SCANNING ELECTRON MICROSCOPY OF EXPERIMENTAL COLUMN MATERIAL

The unwashed subsample was taken from 0-1 cm along the experimental column exhibited major artefacts due to the presence of salt in the synthetic Äspö groundwater. As a result of rapid freezing, salt crystallisation was extensive across the whole subsample surface. This process occurred along ice boundaries, occasionally revealing geometric patterns, as shown in Plate 25. This crystallisation served to mask or destroy any evidence of surface biological material.

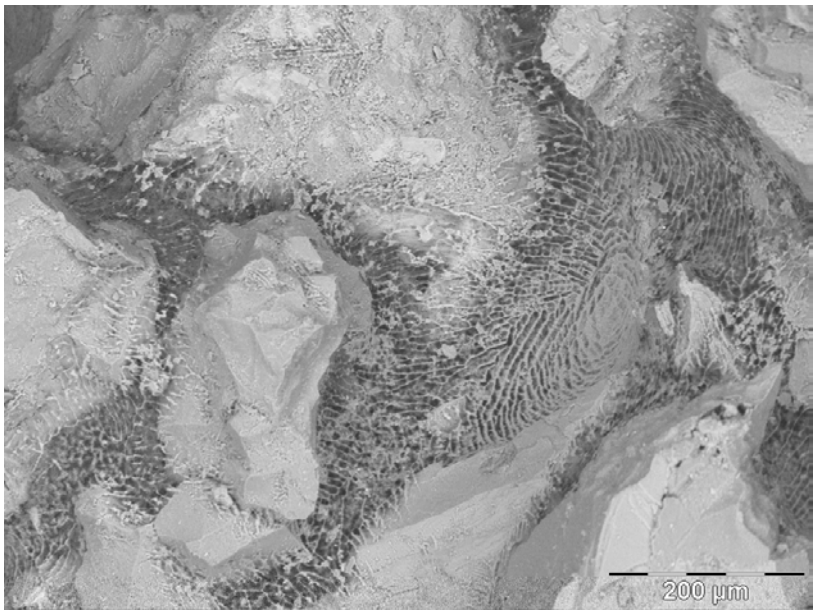


Plate 25. BSEM image showing an area of unwashed subsample, taken from 0-1cm along the experimental column. Dendritic salt crystallisation due to insufficiently rapid freezing was evident.

A diverse range of structures was seen throughout the experimental column. In many of the subsamples, similar structures were revealed. Therefore, in order to avoid repetition, only those images obtained from subsamples that best represented this structural diversity were described in detail.

The remaining 12 subsamples from the experimental column were washed. Despite the attempt to remove all of the salinity from the fluid surrounding the grains in the subsamples it became evident that some salt was still present. Only the subsamples obtained from 0-4 cm showed any evidence of the presence of biological material. This was extremely sparsely distributed within the subsamples. At 0-1 cm along the column a filamentous meshwork consisting of strands from 1-5 µm in thickness were observed to span intergranular pore spaces and appeared to be attached to grain surfaces. Within these strands, fine mineral debris were trapped and served to partially block the pore throats (Plate 26, Plate 27 and Plate 28). These appeared to be substantially different to the freezing pattern and ice formation, described above (Plate 25). Filamentous strands were amorphous, not revealing any clear geometrical patterns (Plate 28). An accumulation of mineral debris was also found to be present on small numbers of grains. These appeared to be engulfed by organic matter (Plate 29). Mineral fines were also found, spanning the intergranular pore spaces, thus partially blocking them. However, these appear to be entrapped within diaphanous salt structures (Plate 30, Plate 38). Structures such as these were commonly found throughout the entire column (Plate 35). The majority of the salt structures appeared as sub-parallel diaphanous sheets, either spanning grains or the intergranular pore spaces. These were formed on the expanding ice glass boundaries, during rapid freezing. As the ice glass grew, some of the fluid containing NaCl, mineral debris and organic matter (if present)

was pushed towards the ice glass boundaries, resulting in the formation of sub-parallel edges, as shown on Plate 31 and Plate 32. Locally, dense salt structures were seen to partially bridge and block the intergranular pore throats (Plate 36, Plate 37). The thickness of these structures provided a suitable surface for obtaining the EDXA spectrum (Plate 37), which clearly indicates the presence of NaCl. Certain salt structures displayed morphologies which may be attributed to the development of salt supported on films or filaments of organic material. This can be seen in Plate 33 and Plate 34; their appearance closely resembled the amorphous, filamentous strands shown in Plate 26, Plate 27 and Plate 28. Rarely, secondary, moldic porosity revealed more evidence that mineral fines were trapped in diaphanous salt structures. Plate 39 shows how fines and salt aggregate preserved the shape of a precursor mineral grain.

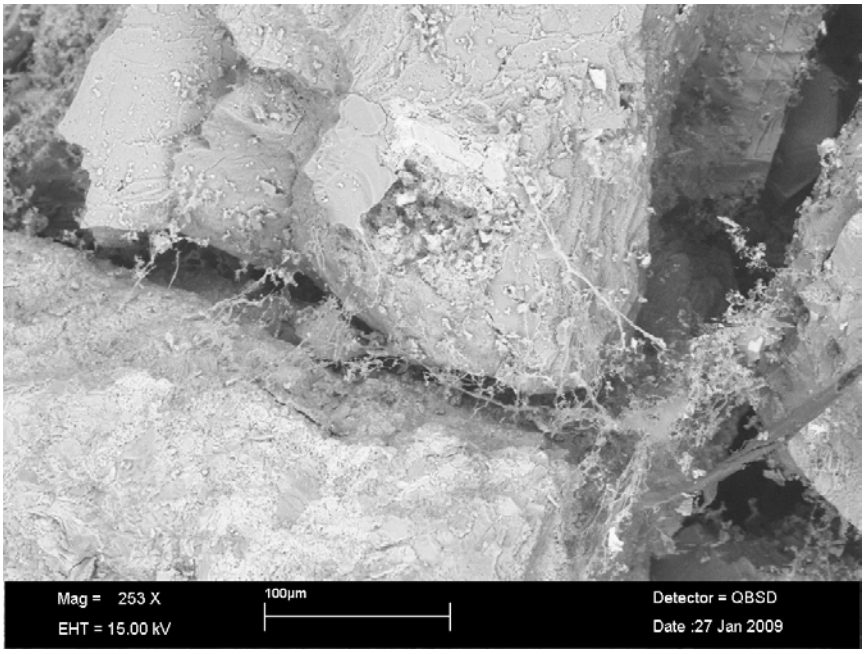


Plate 26. BSEM image (0-1 cm subsample), showing an area of washed subsample revealing a biofilament meshwork spanning grains.

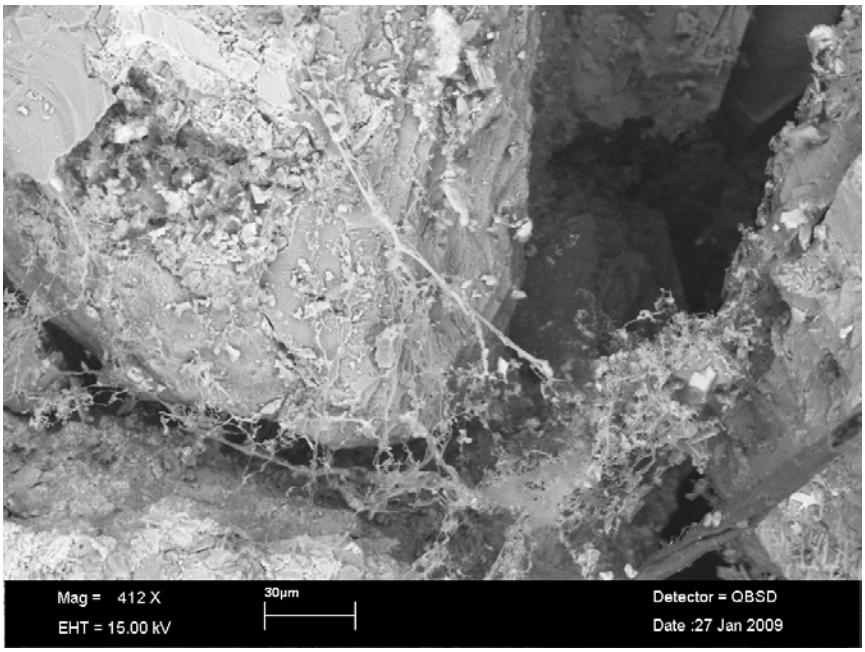


Plate 27. BSEM image (0-1 cm subsample), showing the biofilaments revealed in Plate 26, at higher magnification. Mineral debris can be seen as bright particles trapped in the organic matter.

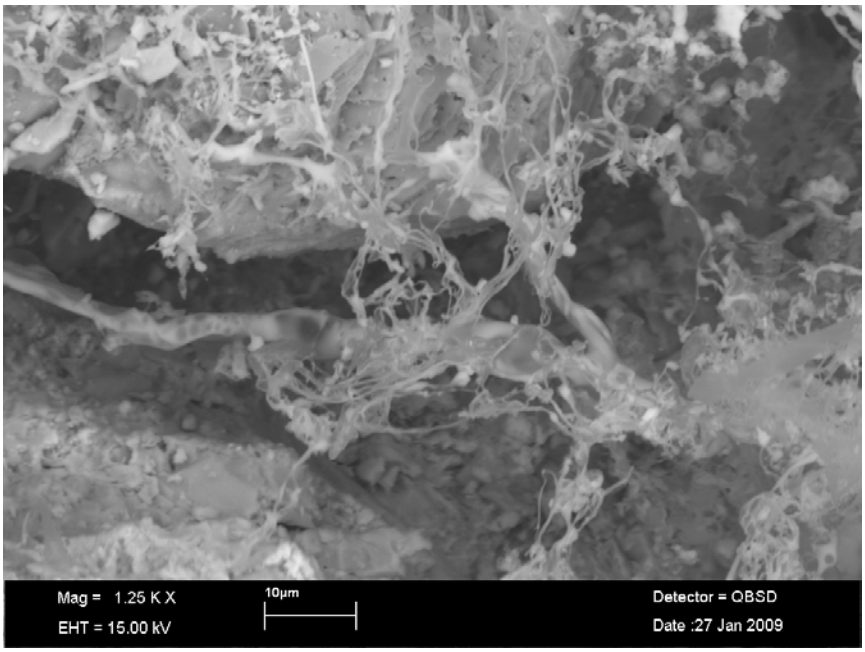


Plate 28. High magnification BSEM image (0-1 cm subsample), showing an intergranular pore space, spanned by the amorphous meshwork of biofilaments revealed in Plate 26.

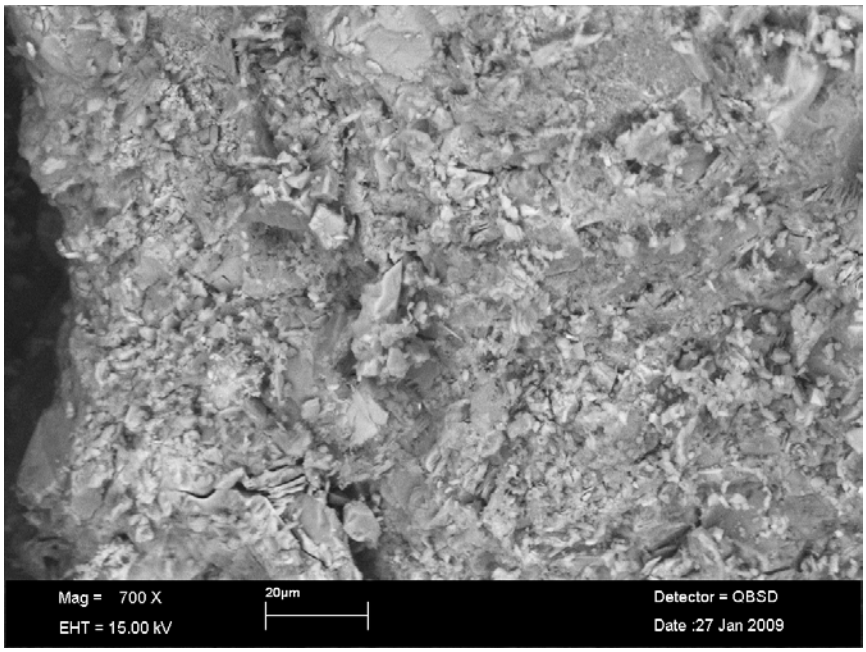


Plate 29. BSEM image (0-1 cm subsample), showing mineral debris engulfed in organic material.

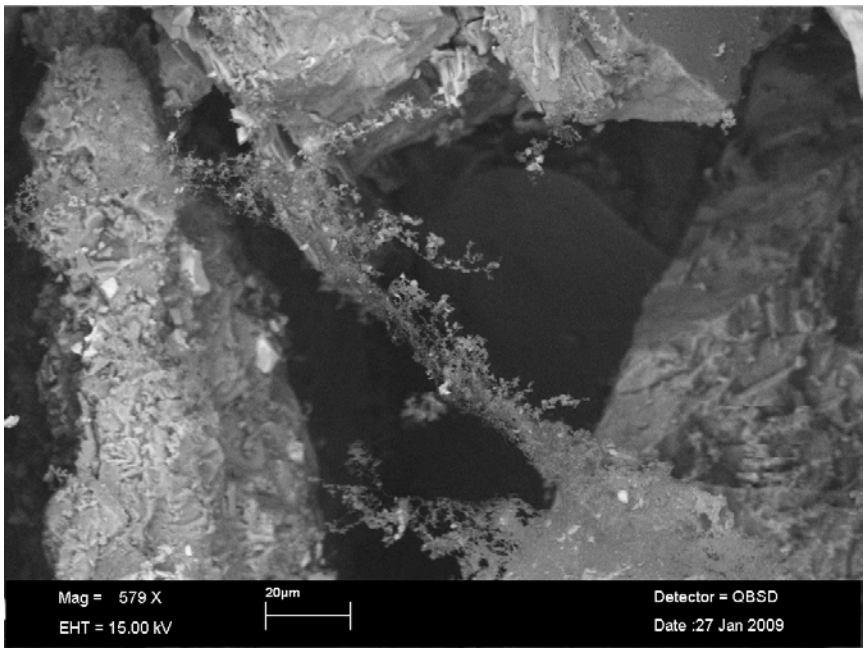


Plate 30. BSEM image (0-1 cm subsample), showing filamentous or dendritic films of NaCl crystals and entrapped mineral fines spanning intergranular pore spaces.

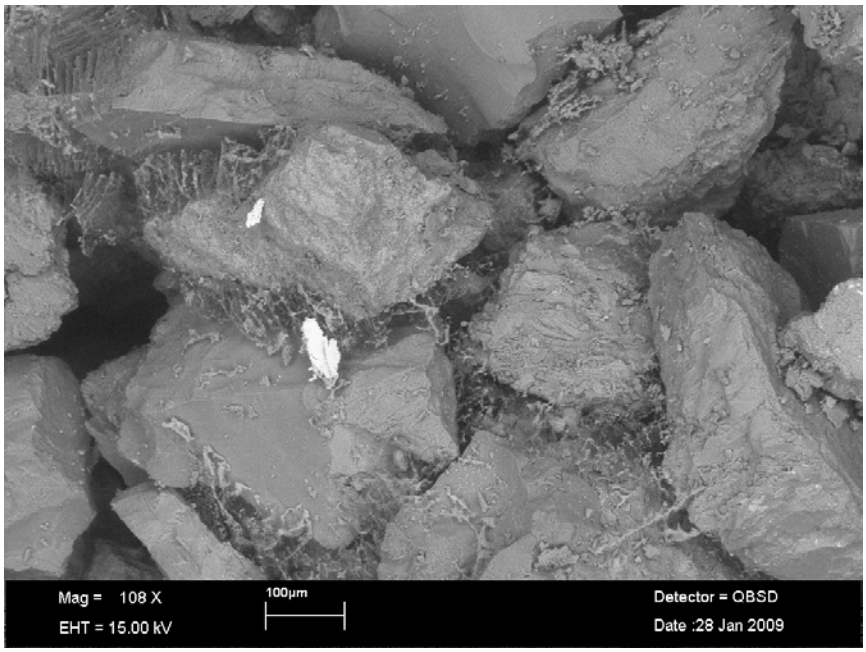


Plate 31. BSEM image (2-3 cm subsample), showing sub-parallel, thin sheets of salt formed along ice boundaries, bridging intergranular pores.

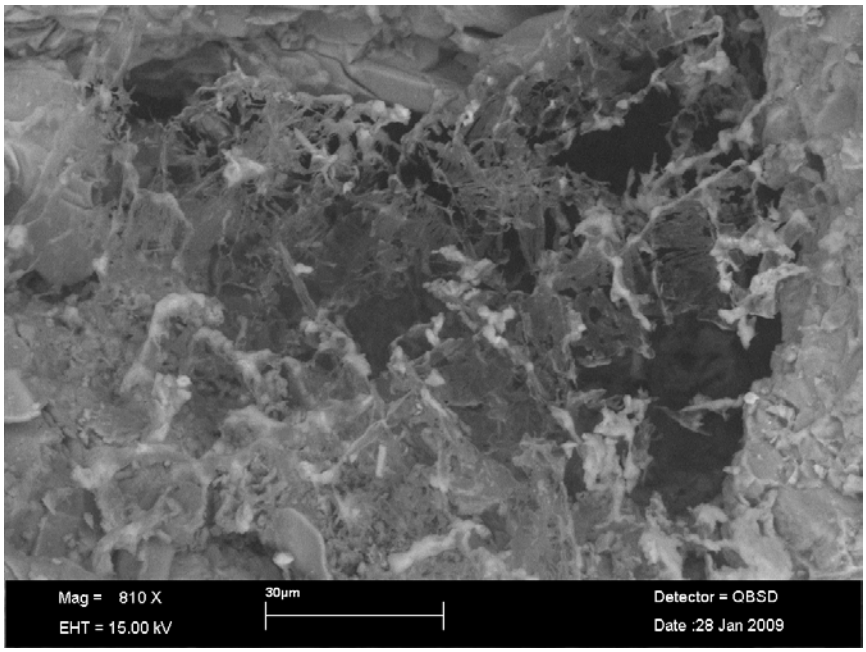


Plate 32. BSEM image (2-3 cm subsample), showing sub-parallel, thin sheets of salt formed along ice boundaries in detail.

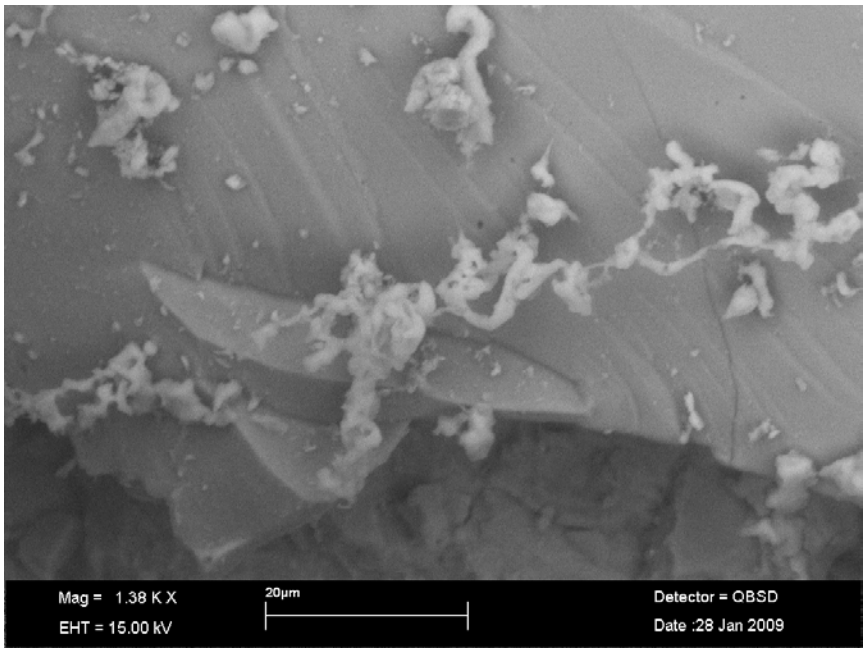


Plate 33. BSEM image (2-3 cm subsample). Salt/organic structures. This unusual morphology may reflect the presence of both salt and organic material during formation. Their appearance closely resembles the amorphous, filamentous strands in Plate 26 -Plate 28.

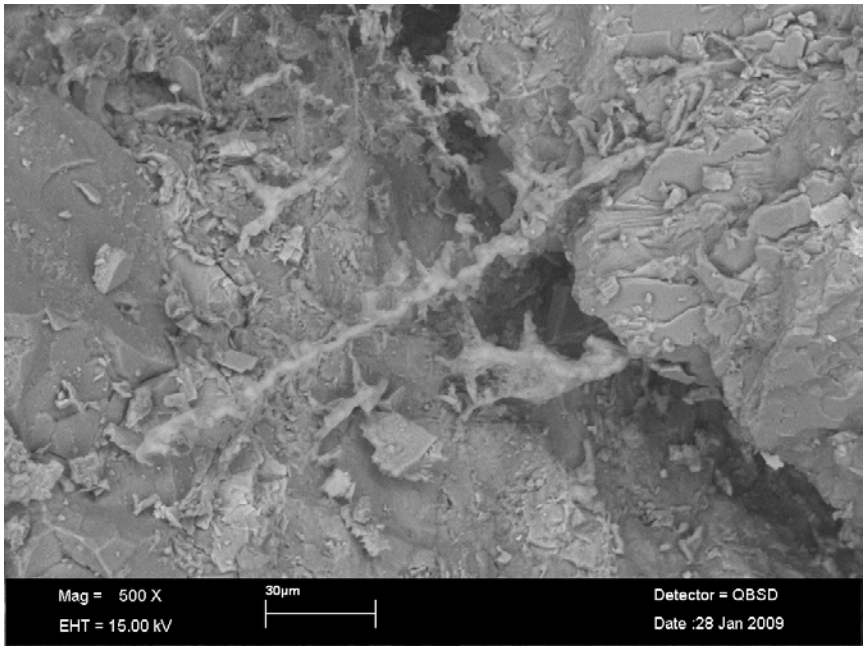


Plate 34. BSEM image (2-3 cm subsample). Salt/organic structures. This unusual morphology may reflect the presence of both salt and organic material during formation. Their appearance closely resembles the amorphous, filamentous strands in Plate 26 -Plate 28.

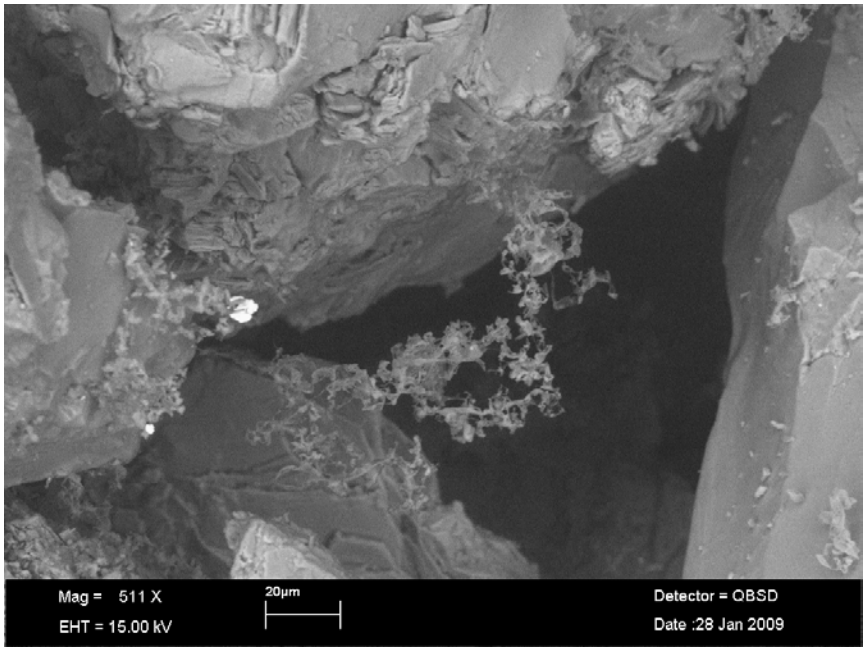


Plate 35. BSEM image (3-4 cm subsample), showing another example of diaphanous salt crystals and entrapped mineral fines spanning an intergranular pore space.

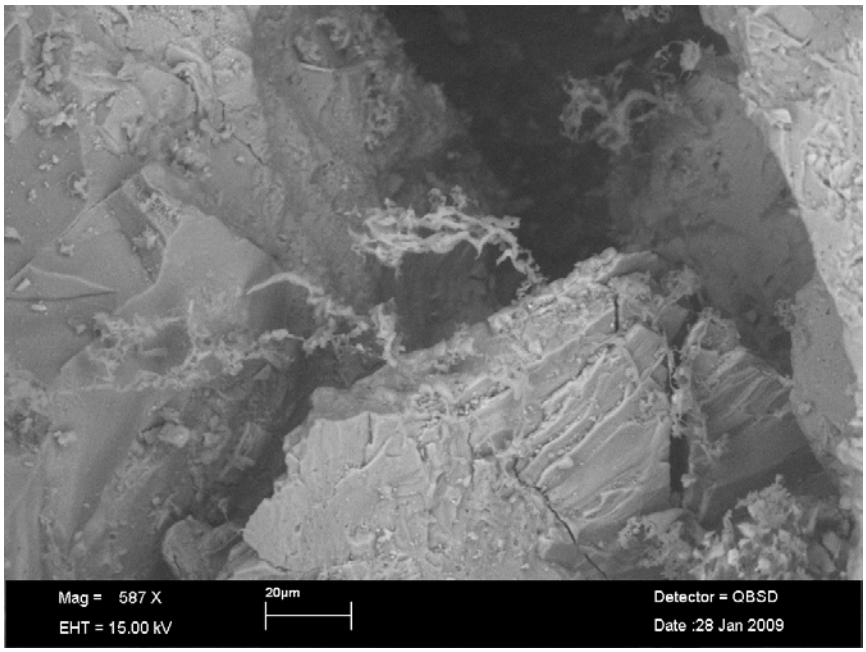


Plate 36. BSEM image (3-4 cm subsample) showing another example of salt/organic structures. Their unusual morphology may reflect the presence of both salt and organic material during formation. Their appearance closely resembles the amorphous, filamentous strands in Plate 26 -Plate 28.

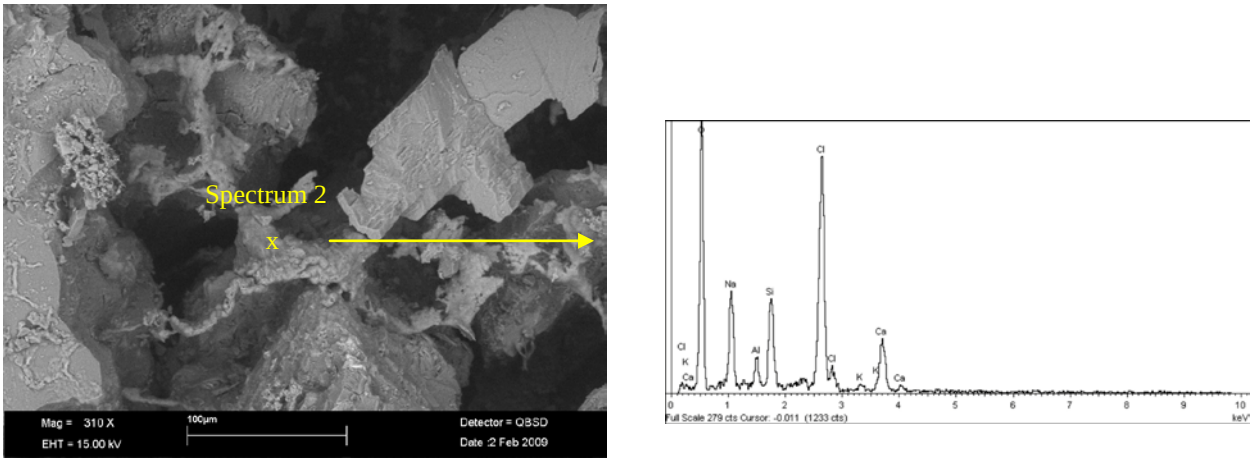


Plate 37. BSEM image (5-6 cm subsample), showing dense salt structures partially bridging and blocking intergranular pore spaces. The EDXA spectrum was obtained from the most prominent surface and clearly reveals the presence of NaCl.

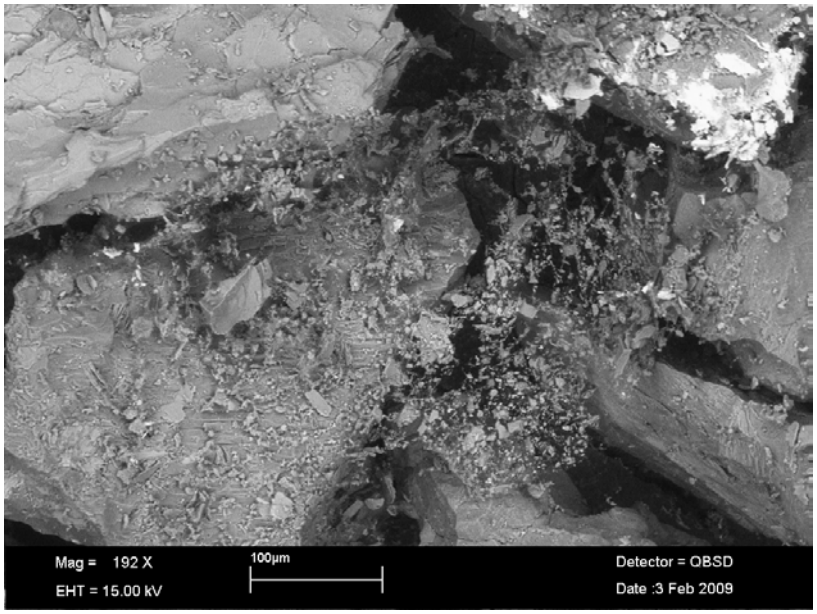


Plate 38. BSEM image (10-11 cm subsample) showing the aggregation of mineral debris accumulated in an intergranular pore throat, bonded by diaphanous salt structures.

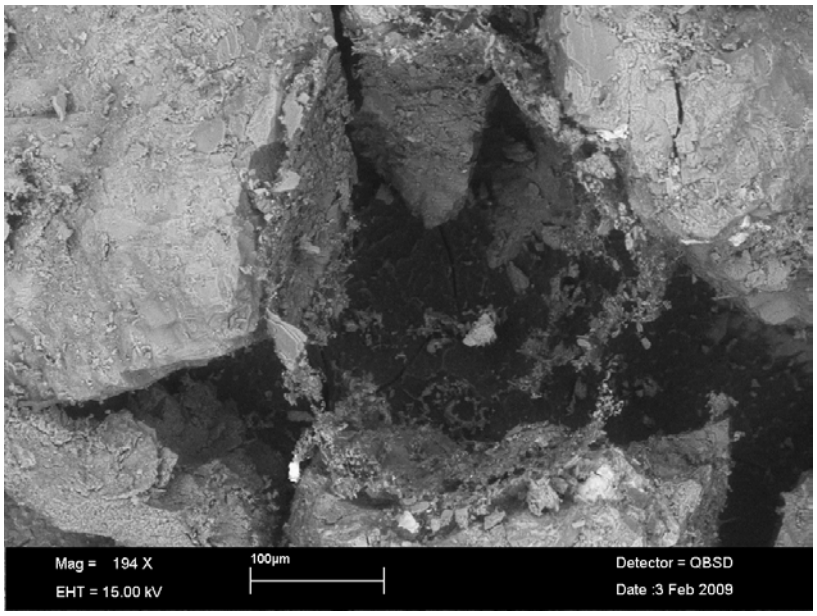


Plate 39. BSEM image (11-12 cm subsample). Moldic porosity revealed by mineral debris trapped in diaphanous salt structures accumulated around the precursor grain.

7 Discussion

7.1 FLOW RATE AND PRESSURE

Flow rates were maintained throughout the 3524 hours of the experiment and any impacts by developing biofilms would be reflected in changes in the pressure gradients ie the pressure would increase in order to maintain the flow through the columns. Changes in the pressure within the system were observed throughout the experiment although many were attributable to

experimental procedures. Nevertheless, there appears to be an overall increase in pressure in Column 2 (Figure 2) throughout the duration of the experiment. It is not possible to determine from these experiments the exact cause of this pressure increase, but it may be a result of drift in the pressure transducer recordings throughout the experiment or it could be attributable to the development of the biofilm. This observation can be compared to the results from the aerobic experiment (West *et al.*, 2008). Although the method for determining changes in transport properties in the aerobic experiments was different in that flow rates only were monitored with no control on pressure, flow virtually ceased at about 1000 hours. This can be compared to pressure recordings for Column 2 (Figure 2) which appears to be rising at around 1000 hours suggesting that biofilm production may again be starting to impact on transport properties. However, it is not possible to be more definitive about this observation without undertaking a further long-term experiment.

7.2 FLUID CHEMISTRY

A self modelling mixture resolution algorithm (SMMR), (Cave *et al.*, 2004; Cave *et al.*, 2008) was applied to the composition of the SMMR solutions obtained from each of the two columns, to see if they could be interpreted as a mixture of components arising from different inputs from reactions between the groundwater and the column material. The methodology was carried out using the chemical determinands where the majority of the data was above detection limit (Ca, Mg, Na, Cl, SO_4, NO_3, Reduced S, Si, Ba, Sr, Mn, Total Fe, Reduced Fe, Ni, and Zn). Where there were gaps in the data, where measurements had not been made, values were estimated using an imputation methodology (Schneider, 2001). The SMMR method identified four separate components; Figure 7 shows the evolution of these components for the two columns over the time of the experiment.

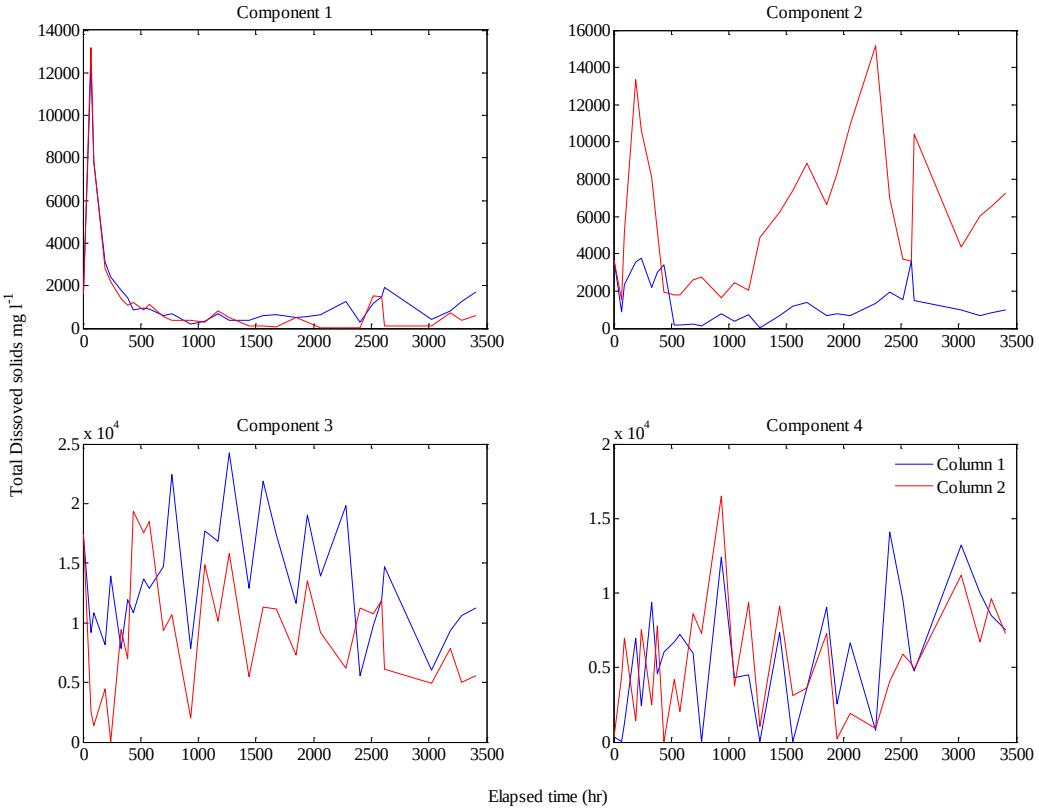


Figure 7. Evolution of the four chemical components over the time of the experiment

The major element composition of the four components was very similar, the difference in chemistry between them was mainly shown in the trace determinands (NO_3, total S, Si, Ba, Sr, Mn, Total Fe, Reduced Fe, Ni, Zn). Figure 8 shows these differences in the form of a bar plot.

Component 1 shows very repeatable behaviour between the two columns showing a sharp increase at the beginning of the experiment falling away over time (Figure 7). Figure 8 shows that component 1 is characterised by high Ba and Mn compared to the other fluids.

Component 2 has a fairly random evolution profile which is not repeatable between the columns (Figure 7) and is characterised by a high concentration of Ni, NO_3 and Sr (Figure 8)

Component 3 shows similar evolution profiles in both columns with a gentle increase and decrease over the experimental period (Figure 7). Figure 8 shows that nitrate is higher in this component compared to the others.

Component 4 does not show a well defined evolution profile in either column but there is possibly some correlation between them. Component 4 is characterised by much higher total sulphur content than the other components.

There is some evidence of different chemical reactions taking place during the experiment although further work would be required to confirm this.

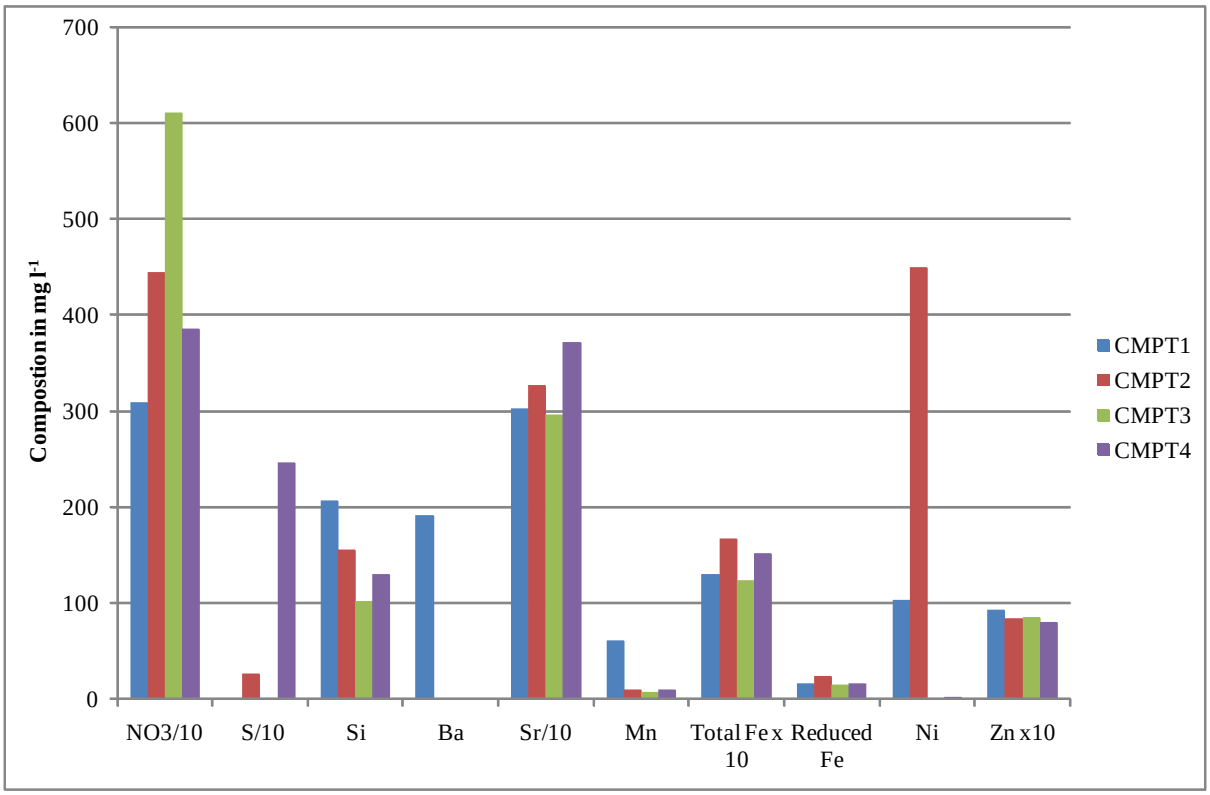


Figure 8. Composition of the trace determinands in the column fluids

7.3 MICROBIOLOGICAL OBSERVATIONS

Microbial analyses of the fluids showed a similar trend in both the inoculated columns. After injection of the bacterial culture, *P. aeruginosa* numbers of bacteria steadily decreased in both columns until 838 hours. The initial high number of bacteria 2.27 x 10^6 bacteria ml^{-1} in Column 1 and 3.71 x 10^6 bacteria ml^{-1} in Column 2 was as a result of the injection of the culture which was inoculated at 1.28 x 10^7 bacteria ml^{-1}. As the culture was added as a single inoculant, there was some loss of bacterial population due to the suspended culture flowing through the column.

Viable bacteria were detected up until 2402 hours duration showing they were able to survive within the anaerobic conditions of the Äspö diorite. During this time it appears that bacteria were mobilised from pore spaces or biofilm surfaces as shown by their presence in the outlet fluids.

Although no significant changes in flow rate were noted, it appeared that visual changes were occurring within the pore spaces in the first few centimetres of the column. Similar observations had been made in the previous aerobic experiment (West *et al*., 2008), that were associated with biofilm formation. Observations under the microscope had also shown clumps of organic material, particularly at 3 cm to 4 cm which may have been EPS associated with biofilm formation. Distribution of bacteria in the Äspö diorite (Figure 4) within Column 1 also shows bacteria concentrated in the first few centimetres of the column. This suggests they were able to survive and form biofilms by utilising nutrients flowing into the column (Plate 26, Plate 27 and Plate 28). The end of the column also had a large population of non-viable bacteria at the edge of the column. It is likely that they collected here causing some sort of plugging effect and were not carried through the outlet of the column.

7.4 MINERALOGICAL OBSERVATIONS

CryoSEM observations of the experimental column material showed that biofilm was formed during the course of the experiment. Additional cryoSEM observation of control column subsamples allowed a direct comparison to be made between the starting material and any changes present in the post-experimental material. The control columns revealed many features that were also present along the experimental column and that could easily have been superficially identified as being of biological origin. Most importantly, the presence of salt caused considerable ambiguity in the identification of biofilaments and biofilm because the salt structures often closely resembled amorphous, fine films and filaments anticipated for biofilm development filaments. The morphology of some of these structures could represent fine salt crystals supported on substrate that may be an organic filament or film.

Mineral fines were found to be abundant, not only in the experimental column but also within the control material columns, showing that these were already present in the starting material. Every precaution was taken to try to produce very rapid sample freezing, and thereby preventing the disruption of the pore fabrics as a result of the growth of coarse ice crystals. Nevertheless, the freezing achieved with the size of samples used was still too slow to prevent the formation of coarse ice crystal dendrites. Therefore, this caused some sample disruption, and consequently, only remnants of biofilm and biofilaments were seen. The SEM petrographical observations indicated that the evidence for biofilm formation was largely limited to the first 4 cm distance along the experimental column, and this is consistent with the fluorescence imaging of the Acridine Orange distribution within the intact column before sampling for petrographic and biological analysis (Plate 17). No evidence of the presence of biological material was found at the outflow end of the column (as the image also indicated). Further work is needed to ascertain whether or not this is a reliable technique for imaging this type of Acridine Orange stained biological material. The paucity of biological material observed may also be attributable to the washing process carried out during subsample preparation in order to remove salt from the intergranular fluid. Biological material may have been washed out during this process.

Clear evidence of the development of any biological material within the column was seen near the fluid inlet and mainly confined to the first 1 cm. In the next 3 cm, structures were revealed that may have been biological or part-biological in origin; although there is some ambiguity due to the presence of salt. There was little evidence of development or preservation of biofilm further along the column. The likely cause was despite *Pseudomonas aeruginosa* ability to withstand anaerobic conditions; colony growth was significantly inhibited by the absence of oxygen.

The remnants of biofilm and biofilaments were characterized by thin, <10 µm in diameter, long up to 200 µm filamentous structures, that were found to partially block and constrict the intergranular pore throats by being unevenly spread between grains and by trapping mineral fines. This potentially restricted the transport of fluids through the rock, thus reducing its permeability. Biofilm was also seen to be draped over mineral grains. This possibly resulted from collapse during sample preparation. Attempts to obtain an EDXA spectrum of the organic structures revealed that not only the analysed matter was beam sensitive but also it contained some amounts of C, which was likely to be of organic origin.

8 Conclusions

This study evaluated the effects of biofilms on fluid flow through crushed Äspö diorite in an anaerobic environment over a period of 3524 hours. Experimental data and observations showed that:

- *P. aeruginosa* biofilms can be grown anaerobically using Äspö diorite and synthetic groundwater as a growth medium, although the rate of growth is significantly slower than shown previously under aerobic conditions;
- Numbers of *P. aeruginosa* decreased over the duration of the experiment but were able to survive up to 2684 hours;
- CryoSEM observations of the experimental column material showed that biofilm was formed during the course of the experiment;
- Biofilm only developed in the first 0 to 4 cm of the column as shown by direct observations and by microscopic analysis of the experimental column on completion of the experiment;
- Observations of bacteria-free control columns were vitally important as the experimental column material revealed many features that could easily have been superficially identified as being of biological origin;
- The presence of porewater salt caused considerable ambiguity in the identification of biofilaments and biofilm because the salt structures often closely resembled amorphous features anticipated for biofilm development filaments;
- No change in pH was observed during the course of the experiment;
- Increasing pressure in one of the columns suggested that biofilm formation may have occurred. However, experimental procedures and transducer ‘drift’ may account for these observations.
- Fluid chemistry data shows a rapid reduction in both Mn and Ba from the time of injection of the bacterial culture to nominal values at around 400 hours. Since no appreciable levels of Mn or Ba were detectable in the groundwater, it was assumed that Mn and Ba were present in the inoculant or had originated from the column itself. The strong correlation between Mn and Ba was not unexpected; this phenomenon was observed during the mineralogical studies of manganese oxyhydrides, (Milodowski *et al*., 1999). In this study, Ba and other large cations (Na, K and Ca) were found to co-precipitate with, and be fixed within the crystal structure of manganese oxyhydrides.
- There is some evidence of different chemical reactions taking place during the experiment although confirmation of these findings would require further work.

The previous aerobic flow-through column experiment (West *et al*., 2008) showed that numbers of *P. aeruginosa* not only survived but were capable of forming a substantial biofilm in a low nutrient environment. In this anaerobic study, biofilm did form at the column inlet despite the total bacterial counts decreasing over time and viable bacteria were detected close to the column outlet. Taken together, the aerobic and anaerobic experiments described in this report and by West *et al.* (2008) show that, for crushed diorite, biofilm formation will be prevalent under aerobic conditions and that transport properties are greatly altered with fluid flow almost completely stopped after 1000 hours. Biofilm production also occurred under anaerobic conditions although transport properties did not seem to be greatly influenced.

These results have implications for the excavation and evolution of radioactive waste repositories, particularly in the excavation damaged zone (EDZ). Broadly, during construction and repository operation, many introduced and indigenous microbes in the excavated zone will take advantage of the introduction of O_2, contaminants and other nutrient sources in the fractured material and begin to form biofilms. The formation of biofilms will impact on geochemical, mineralogical and transport processes as shown by these experiments. In particular, hydraulic conductivity will be reduced particularly during the open and immediate post-closure periods. After closure, conditions will gradually become anoxic but organisms, such as *P. aeruginosa* which can use other alternative electron acceptors (e.g nitrate), will continue to form biofilms. The anaerobic experiment described in this report suggests that the impact of these biofilms on transport properties will be very small. Nevertheless, in the context of an evolving repository, biofilms will already have formed in the EDZ area and subsequent anaerobic biofilm formation will probably only consolidate their impacts on transport properties and geochemical and mineralogical processes. The duration of these impacts is difficult to define without understanding the long-term survival of biofilms in deep subsurface environments which is, to date, unknown. Additionally, biofilm formation will depend on the availability of nutrients and energy sources which will be specific to a particular geological setting and repository concept. Consequently, the impact of biofilm formation is site specific and would need to be evaluated as part of a site characterisation study.

The potential impacts of biofilms on the geological disposal or storage of other wastes is now being recognised (e.g. Takase et al., 2008). For example, in the geological storage of carbon dioxide, CO_2 could potentially act as an oxidant in a reducing environment which could be utilised by microbes within a biofilm. Such a biofilm could significantly impact on both geochemical, mineralogical and transport properties which have not, to date, been evaluated. Nevertheless, once again, the extent of potential impacts from such a biofilm would be heavily dependent on the available nutrients and also the amount of energy available to the microorganism from the utilised redox reactions.

8.1 FUTURE WORK

Previous work has used crushed diorite to mimic conditions in the EDZ of a repository. Future BioTran work (2009-2010) will focus on biofilm growth within intact porous rock, specifically Sherwood Sandstone, which can be considered a basement rock in the context of radioactive waste disposal. It is also a potential storage reservoir for the storage of carbon dioxide. Experiments will be run under representative *in-situ* conditions of pressure and groundwater chemistry.

Appendix 1

Porosity Calculations

	Column 1	Column 2	Unit
Column length	119	120	(mm)
Empty column weight, A	151.69	153.23	(g)
Dry packed weight, B	236.95	240.64	(g)
Wet weight packed, W	261.51	265.60	(g)
Wt of solid, (B-A)	85.26	87.41	(g)
Wt of fluid (≡ Pore Vol)	24.56	24.96	(g)
Wt of solids	32.17	32.98	(g)
Porosity	43.3	43.1	(%)

Appendix 2

Summary of sub sampling of experimental columns and control material for cryo-SEM

Subsample type	**Depth along column from inlet (cm)**	**Washed Tap water**	**Washed synthetic groundwater**	**Unwashed**
Control in groundwater	N/A	**X**		
Control in groundwater	N/A		**X**	
Control in Tap water	N/A	**X**		
Experimental	0-1			**X**
Experimental	0-1	**X**		
Experimental	1-2	**X**		
Experimental	2-3	**X**		
Experimental	3-4	**X**		
Experimental	4-5	**X**		
Experimental	5-6	**X**		
Experimental	6-7	**X**		
Experimental	7-8	**X**		
Experimental	8-9	**X**		
Experimental	9-10	**X**		
Experimental	10-11	**X**		
Experimental	11-12	**X**		

Appendix 3

Fluid Chemistry Data, Column 1

LIMS Code	Sample Code	Sample Description	Date/Time	Total Elapsed Time hr	Time Interval hr	Pressure bar	Sample Vol ml	Flow Rate mg l^{-1}	pH units	Br^- mg l^{-1}	NO_2^- mg l^{-1}	HPO_4^{2-} mg l^{-1}	F^- mg l^{-1}	Ba mg l^{-1}	Sr mg l^{-1}	Mn mg l^{-1}
12051-0060	Start GW	Starting GW	02/09/2008 14:00	0.00	0.00	0.00			7.24	<6.00	<4.00	<20.0	<2.00	<0.050	72.0	<0.050
12051-0001	1/1	Column 1/1	05/09/2008 14:00	72.00	72.00	0.50	13.86	0.193	7.10	<4.00	<2.00	<20.0	<2.00	2.65	70.1	0.796
12051-0002	1/2	Column 1/2	06/09/2008 14:00	96.00	24.00	0.70	14.00	0.583	6.36	<4.00	<2.00	<20.0	<2.00	1.53	69.0	0.581
12051-0003	1/4	Column 1/4	10/09/2008 13:45	191.75	27.25	0.50	12.54	0.460	6.54	<4.00	<2.00	<20.0	<2.00	0.406	70.3	0.405
12051-0004	1/6	Column 1/6	12/09/2008 14:00	240.00	23.50	0.60	14.61	0.622	6.58	<4.00	<2.00	<20.0	<2.00	0.314	69.7	0.338
12051-0005	1/8	Column 1/8	16/09/2008 11:45	333.75	69.75	0.60	45.69	0.655	6.57	<4.00	<2.00	<20.0	<2.00	0.271	72.6	0.290
12051-0006	1/10	Column 1/10	18/09/2012 14:00	384.00	24.00	0.70	15.03	0.626	6.63	<4.00	<2.00	<20.0	<2.00	0.227	69.0	0.258
12051-0007	1/12	Column 1/12	20/09/2008 15:30	433.50	24.00	0.90	15.63	0.651	6.42	<4.00	<2.00	<20.0	<2.00	0.213	68.6	0.239
12051-0008	1/14	Column 1/14	24/09/2008 12:00	526.00	24.50	0.80	14.97	0.611	6.42	<4.00	<2.00	<20.0	<2.00	0.199	69.7	0.232
12051-0009	1/16	Column 1/16	26/09/2008 13:30	575.50	25.00	1.00	15.41	0.616	6.39	<4.00	<2.00	<20.0	<2.00	0.187	69.9	0.226
12051-0010	1/19	Column 1/19	01/10/2012 11:45	693.75	48.25	0.80	14.76	0.306	6.48	n.s	n.s	n.s	n.s	0.161	69.9	0.216
12051-0011	1/22	Column 1/22	04/10/2008 14:15	768.25	26.00		7.73	0.297	6.36	<6.00	<4.00	<20.0	<2.00	0.133	68.1	0.207
12051-0012	1/25	Column 1/25	11/10/2008 16:00	938.00	49.00	0.00	29.97	0.612	6.25	<4.00	<2.00	<20.0	<2.00	0.130	71.7	0.215
12051-0013	1/27	Column 1/27	16/10/2008 12:00	1054.00	45.00	-0.20	28.01	0.622	6.17	<4.00	<2.00	<20.0	<2.00	0.114	71.4	0.204
12051-0014	1/29	Column 1/29	21/10/2008 11:30	1173.50	69.00	0.10	43.58	0.632	6.25	<4.00	<2.00	<20.0	<2.00	0.104	71.3	0.224
12051-0015	1/31	Column 1/31	25/10/2008 12:00	1270.00	46.00	0.10	29.12	0.633	6.28	<6.00	<4.00	<20.0	<2.00	0.090	71.4	0.210
12051-0016	1/34	Column 1/34	01/11/2008 14:15	1440.25	47.25	0.40	32.69	0.692	6.17	<4.00	<2.00	<20.0	<2.00	0.069	69.7	0.204
12051-0017	1/36	Column 1/36	06/11/2008 15:15	1561.25	48.00	0.30	26.59	0.554	6.21	<4.00	<2.00	<20.0	<2.00	0.074	71.9	0.215
12051-0018	1/38	Column 1/38	11/11/2008 11:45	1677.75	69.50	0.20	43.55	0.627	6.01	<4.00	<2.00	<20.0	<2.00	0.065	73.0	0.231
12051-0019	1/41	Column 1/41	18/11/2008 16:00	1850.00	74.00	0.20	46.42	0.627	6.38	<4.00	<2.00	<20.0	<2.00	0.062	70.9	0.209
12051-0020	1/43	Column 1/43	22/11/2008 11:45	1941.75	45.00	0.20	28.45	0.632	6.34	<4.00	<2.00	<20.0	<2.00	0.055	70.9	0.214
12051-0021	1/45	Column 1/45	27/11/2008 10:00	2060.00	46.25	0.60	29.26	0.633	6.43	<4.00	<2.00	<20.0	<2.00	<0.050	70.8	0.202
12051-0022	1/48	Column 1/48	06/12/2008 10:30	2276.50	44.00	0.60	25.92	0.589	6.36	<4.00	<2.00	<20.0	<2.00	<0.050	71.5	0.239
12051-0023	1/50	Column 1/50	11/12/2008 16:00	2402.00	52.50	0.80	33.14	0.631	6.52	<4.00	<2.00	<20.0	<2.00	0.050	72.2	0.232
12051-0024	1/52	Column 1/52	16/12/2008 11:00	2517.00	69.00	0.80	43.10	0.625	6.36	<4.00	<2.00	<20.0	<2.00	<0.050	73.3	0.246
12051-0059	Dec GW	Pump Refill	19/12/2008 12:00	2590.00	25.00				7.18	<6.00	<4.00	<20.0	<2.00	<0.050	72.4	<0.050
12051-0025	1/54	Column 1/54	20/12/2008 11:00	2613.00	23.00	0.60	22.58	0.982	6.35	<4.00	<2.00	<20.0	<2.00	<0.050	69.7	0.276
12051-0026	1/56	Column 1/56	06/01/2009 10:00	3020.00	336.00	1.00	192.96	0.574	6.42	<4.00	<2.00	<20.0	<2.00	0.063	71.3	0.227
12051-0027	1/59	Column 1/59	13/01/2009 11:00	3189.00	71.00	0.80	44.94	0.633	6.47	<4.00	<2.00	<20.0	<2.00	<0.050	71.4	0.253
12051-0028	1/61	Column 1/61	17/01/2009 12:00	3286.00	49.00	0.80	30.41	0.621	6.48	<4.00	<2.00	<20.0	<2.00	<0.050	71.2	0.250
12051-0029	1/63	Column 1/63	22/01/2009 11:00	3405.00	47.00	0.80	28.61	0.609	6.32	<4.00	<2.00	<20.0	<2.00	<0.050	70.7	0.274

Fluid Chemistry Data, Column 1

LIMS Code	Sample Code	Sample Description	Date/Time	Ca^{2+} mg l^{-1}	Mg^{2+} mg l^{-1}	Na^{+} mg l^{-1}	K^{+} mg l^{-1}	Cl^{-} mg l^{-1}	SO_4^{2-} mg l^{-1}	NO_3^{-} mg l^{-1}	Cation Total meq l^{-1}	Anion Total meq l^{-1}	Balance %
12051-0060	Start GW	Starting GW	02/09/2008 14:00	4410	50.0	2664	<12.5	14891	595	11.7	341.70	432.66	-11.75
12051-0001	1/1	Column 1/1	05/09/2008 14:00	4386	52.5	2601	37.8	14864	559	6.05	338.94	431.05	-11.96
12051-0002	1/2	Column 1/2	06/09/2008 14:00	4233	48.5	2621	20.0	14731	544	5.70	331.32	426.99	-12.62
12051-0003	1/4	Column 1/4	10/09/2008 13:45	4214	49.4	2609	13.4	14207	529	6.80	329.75	411.89	-11.07
12051-0004	1/6	Column 1/6	12/09/2008 14:00	4296	48.7	2594	<12.5	14614	550	193	332.83	426.84	-12.37
12051-0005	1/8	Column 1/8	16/09/2008 11:45	4301	50.5	2703	<12.5	14489	523	13.3	338.03	419.84	-10.79
12051-0006	1/10	Column 1/10	18/09/2012 14:00	4105	48.0	2573	<12.5	13738	529	5.77	322.31	398.66	-10.59
12051-0007	1/12	Column 1/12	20/09/2008 15:30	4223	47.9	2547	<12.5	13871	516	5.20	327.02	402.12	-10.30
12051-0008	1/14	Column 1/14	24/09/2008 12:00	4252	48.5	2592	<12.5	13922	527	12.4	330.52	403.89	-9.99
12051-0009	1/16	Column 1/16	26/09/2008 13:30	4283	48.8	2582	<12.5	13874	525	6.02	331.65	402.39	-9.64
12051-0010	1/19	Column 1/19	01/10/2012 11:45	4245	48.6	2609	<12.5	n.s	n.s	n.s	330.92	ns	n/a
12051-0011	1/22	Column 1/22	04/10/2008 14:15	4158	47.3	2538	<12.5	17439	699	<6.00	323.34	506.50	-22.07
12051-0012	1/25	Column 1/25	11/10/2008 16:00	4298	49.8	2677	<12.5	13321	494	8.43	336.68	386.22	-6.85
12051-0013	1/27	Column 1/27	16/10/2008 12:00	4406	50.0	2635	<12.5	14282	561	17.8	340.24	414.86	-9.88
12051-0014	1/29	Column 1/29	21/10/2008 11:30	4274	49.6	2622	<12.5	14661	558	7.89	333.03	425.33	-12.17
12051-0015	1/31	Column 1/31	25/10/2008 12:00	4298	49.8	2648	<12.5	19661	791	<6.00	335.38	571.11	-26.00
12051-0016	1/34	Column 1/34	01/11/2008 14:15	4198	48.4	2591	<12.5	13634	516	9.01	327.78	395.48	-9.36
12051-0017	1/36	Column 1/36	06/11/2008 15:15	4342	50.1	2671	<12.5	16284	616	7.44	338.62	472.30	-16.49
12051-0018	1/38	Column 1/38	11/11/2008 11:45	4209	50.7	2705	<12.5	14857	577	8.73	333.57	431.25	-12.77
12051-0019	1/41	Column 1/41	18/11/2008 16:00	4292	49.3	2615	<12.5	14107	520	<4.00	333.63	408.78	-10.12
12051-0020	1/43	Column 1/43	22/11/2008 11:45	4255	49.3	2619	<12.5	14748	571	9.30	331.92	428.07	-12.65
12051-0021	1/45	Column 1/45	27/11/2008 10:00	4284	48.8	2634	<12.5	14037	532	9.41	334.01	407.20	-9.87
12051-0022	1/48	Column 1/48	06/12/2008 10:30	4378	49.2	2661	<12.5	14730	584	22.4	339.92	428.06	-11.48
12051-0023	1/50	Column 1/50	11/12/2008 16:00	4233	50.2	2665	<12.5	13485	494	28.9	332.94	391.17	-8.04
12051-0024	1/52	Column 1/52	16/12/2008 11:00	4306	51.0	2710	<12.5	14136	538	27.8	338.62	410.42	-9.58
12051-0059	Dec GW	Pump Refill	19/12/2008 12:00	4378	50.3	2698	<12.5	14038	562	15.5	341.61	407.95	-8.85
12051-0025	1/54	Column 1/54	20/12/2008 11:00	4134	48.4	2578	<12.5	13842	537	7.86	324.02	401.78	-10.71
12051-0026	1/56	Column 1/56	06/01/2009 10:00	4264	49.4	2657	<12.5	13558	493	190	334.06	395.80	-8.46
12051-0027	1/59	Column 1/59	13/01/2009 11:00	4314	49.1	2665	<12.5	13598	514	15.3	336.88	394.56	-7.89
12051-0028	1/61	Column 1/61	17/01/2009 12:00	4307	48.9	2653	<12.5	13781	520	26.7	336.02	400.02	-8.70
12051-0029	1/63	Column 1/63	22/01/2009 11:00	4235	48.8	2626	<12.5	13899	530	56.6	331.19	404.04	-9.91

Fluid Chemistry Data, Column 1

LIMS Code	Sample Code	Sample Description	Date/Time	Total P mg l^{-1}	Total S mg l^{-1}	Si mg l^{-1}	SiO_2 mg l^{-1}	Reduced Fe mg l^{-1}	Oxidised Fe mg l^{-1}	Al mg l^{-1}	Co mg l^{-1}	Ni mg l^{-1}
12051-0060	Start GW	Starting GW	02/09/2008 14:00	<0.250	200	<1.88	<4.01	<0.250	<0.250	<0.250	<0.050	<0.125
12051-0001	1/1	Column 1/1	05/09/2008 14:00	<0.250	178	3.10	6.63	<0.250	<0.250	<0.250	<0.050	<0.125
12051-0002	1/2	Column 1/2	06/09/2008 14:00	<0.250	185	3.30	7.07	<0.250	<0.250	<0.250	<0.050	<0.125
12051-0003	1/4	Column 1/4	10/09/2008 13:45	<0.250	195	3.38	7.23	<0.250	<0.250	<0.250	<0.050	<0.125
12051-0004	1/6	Column 1/6	12/09/2008 14:00	<0.250	190	3.22	6.88	<0.250	<0.250	<0.250	<0.050	<0.125
12051-0005	1/8	Column 1/8	16/09/2008 11:45	<0.250	199	3.01	6.45	0.251	0.005	<0.250	<0.050	<0.125
12051-0006	1/10	Column 1/10	18/09/2012 14:00	<0.250	188	2.76	5.90	0.252	n/a	<0.250	<0.050	<0.125
12051-0007	1/12	Column 1/12	20/09/2008 15:30	<0.250	188	2.31	4.94	0.438	n/a	<0.250	<0.050	<0.125
12051-0008	1/14	Column 1/14	24/09/2008 12:00	<0.250	192	2.40	5.14	0.392	n/a	<0.250	<0.050	0.133
12051-0009	1/16	Column 1/16	26/09/2008 13:30	<0.250	193	2.39	5.12	0.300	n/a	<0.250	<0.050	0.166
12051-0010	1/19	Column 1/19	01/10/2012 11:45	<0.250	192	2.17	4.64	0.321	n/a	<0.250	<0.050	0.237
12051-0011	1/22	Column 1/22	04/10/2008 14:15	<0.250	187	2.40	5.12	0.335	n/a	<0.250	<0.050	0.269
12051-0012	1/25	Column 1/25	11/10/2008 16:00	<0.250	196	2.17	4.65	0.568	n/a	<0.250	<0.050	0.167
12051-0013	1/27	Column 1/27	16/10/2008 12:00	<0.250	198	2.20	4.71	0.400	n/a	<0.250	<0.050	0.150
12051-0014	1/29	Column 1/29	21/10/2008 11:30	<0.250	197	<1.88	<4.01	0.424	n/a	<0.250	<0.050	0.193
12051-0015	1/31	Column 1/31	25/10/2008 12:00	<0.250	197	2.32	4.97	0.335	n/a	<0.250	<0.050	0.262
12051-0016	1/34	Column 1/34	01/11/2008 14:15	<0.250	190	2.41	5.16	0.331	n/a	<0.250	<0.050	0.375
12051-0017	1/36	Column 1/36	06/11/2008 15:15	<0.250	196	2.62	5.61	0.387	n/a	<0.250	<0.050	0.502
12051-0018	1/38	Column 1/38	11/11/2008 11:45	<0.250	201	2.73	5.83	0.324	0.025	<0.250	<0.050	0.591
12051-0019	1/41	Column 1/41	18/11/2008 16:00	0.570	196	2.82	6.04	0.368	n/a	<0.250	<0.050	0.203
12051-0020	1/43	Column 1/43	22/11/2008 11:45	<0.250	196	<1.88	<4.01	0.375	n/a	<0.250	<0.050	0.245
12051-0021	1/45	Column 1/45	27/11/2008 10:00	<0.250	194	2.73	5.84	0.327	n/a	<0.250	<0.050	0.344
12051-0022	1/48	Column 1/48	06/12/2008 10:30	<0.250	196	2.62	5.60	0.380	n/a	<0.250	<0.050	0.636
12051-0023	1/50	Column 1/50	11/12/2008 16:00	<0.250	200	2.82	6.03	0.513	n/a	<0.250	<0.050	0.595
12051-0024	1/52	Column 1/52	16/12/2008 11:00	<0.250	204	2.91	6.22	0.347	n/a	<0.250	<0.050	0.754
12051-0059	Dec GW	Pump Refill	19/12/2008 12:00	0.259	201	<1.88	<4.01	<0.250	<0.250	<0.250	<0.050	<0.125
12051-0025	1/54	Column 1/54	20/12/2008 11:00	<0.250	192	<1.88	<4.01	0.275	0.018	<0.250	<0.050	1.02
12051-0026	1/56	Column 1/56	06/01/2009 10:00	<0.250	198	<1.88	<4.01	0.308	n/a	<0.250	<0.050	0.561
12051-0027	1/59	Column 1/59	13/01/2009 11:00	<0.250	197	2.25	4.82	0.274	n/a	<0.250	<0.050	0.632
12051-0028	1/61	Column 1/61	17/01/2009 12:00	<0.250	195	<1.88	<4.01	0.298	n/a	<0.250	<0.050	0.670
12051-0029	1/63	Column 1/63	22/01/2009 11:00	<0.250	196	<1.88	<4.01	<2.50	n/a	<0.250	<0.050	0.741

Fluid Chemistry Data, Column 1

LIMS Code	Sample Code	Sample Description	Date/Time	Cu mg l^{-1}	Zn mg l^{-1}	Cr mg l^{-1}	Mo mg l^{-1}	Cd mg l^{-1}	Pb mg l^{-1}	V mg l^{-1}	Li mg l^{-1}	B mg l^{-1}	As mg l^{-1}	Se mg l^{-1}
12051-0060	Start GW	Starting GW	02/09/2008 14:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0001	1/1	Column 1/1	05/09/2008 14:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0002	1/2	Column 1/2	06/09/2008 14:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	0.607	<0.375
12051-0003	1/4	Column 1/4	10/09/2008 13:45	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0004	1/6	Column 1/6	12/09/2008 14:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0005	1/8	Column 1/8	16/09/2008 11:45	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0006	1/10	Column 1/10	18/09/2012 14:00	<0.125	0.159	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0007	1/12	Column 1/12	20/09/2008 15:30	<0.125	0.144	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0008	1/14	Column 1/14	24/09/2008 12:00	<0.125	0.165	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0009	1/16	Column 1/16	26/09/2008 13:30	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0010	1/19	Column 1/19	01/10/2012 11:45	<0.125	0.134	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0011	1/22	Column 1/22	04/10/2008 14:15	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0012	1/25	Column 1/25	11/10/2008 16:00	<0.125	0.127	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0013	1/27	Column 1/27	16/10/2008 12:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0014	1/29	Column 1/29	21/10/2008 11:30	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0015	1/31	Column 1/31	25/10/2008 12:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0016	1/34	Column 1/34	01/11/2008 14:15	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0017	1/36	Column 1/36	06/11/2008 15:15	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0018	1/38	Column 1/38	11/11/2008 11:45	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0019	1/41	Column 1/41	18/11/2008 16:00	<0.125	0.159	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0020	1/43	Column 1/43	22/11/2008 11:45	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0021	1/45	Column 1/45	27/11/2008 10:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0022	1/48	Column 1/48	06/12/2008 10:30	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0023	1/50	Column 1/50	11/12/2008 16:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0024	1/52	Column 1/52	16/12/2008 11:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0059	Dec GW	Pump Refill	19/12/2008 12:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0025	1/54	Column 1/54	20/12/2008 11:00	<0.125	0.359	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0026	1/56	Column 1/56	06/01/2009 10:00	<0.125	0.139	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0027	1/59	Column 1/59	13/01/2009 11:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	3.81	<0.375
12051-0028	1/61	Column 1/61	17/01/2009 12:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0029	1/63	Column 1/63	22/01/2009 11:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	0.588	<0.375

Fluid Chemistry Data, Column 2

LIMS Code	Sample Code	Sample Description	Date/Time	Total Elapsed Time	Time Interval	Pressure	Sample Vol	Flow Rate	pH	Br^-	NO_2^-	HPO_4^{2-}	F^-	Ba	Sr	Mn
				hr	hr	bar	ml	mg l^{-1}	units	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}
12051-0060	Start GW	Starting GW	02/09/2008 14:00	0.00	0.00	0.00			7.20	<6.00	<4.00	<20.0	<2.00	<0.050	72.0	<0.050
12051-0030	2/1	Column 2/1	05/09/2008 14:00	72.00	72.00	-0.20	13.23	0.184	6.62	n.s	n.s	n.s	n.s	2.52	67.8	0.901
12051-0031	2/2	Column 2/2	06/09/2008 14:00	96.00	24.00	0.20	14.90	0.621	6.37	<4.00	<2.00	<20.0	<2.00	1.42	71.4	0.648
12051-0032	2/4	Column 2/4	10/09/2008 13:45	191.75	27.25	0.1	17.21	0.632	6.50	<4.00	<2.00	<20.0	<2.00	0.419	69.5	0.407
12051-0033	2/6	Column 2/6	12/09/2008 14:00	240.00	23.50	0.2	15.21	0.647	6.54	<4.00	<2.00	<20.0	<2.00	0.323	69.5	0.347
12051-0034	2/8	Column 2/8	16/09/2008 11:45	333.75	69.75	0.1	44.73	0.641	6.52	<4.00	<2.00	<20.0	<2.00	0.263	68.6	0.273
12051-0035	2/10	Column 2/10	18/09/2012 14:00	384.00	24.00	0.2	15.02	0.626	6.51	<4.00	<2.00	<20.0	<2.00	0.231	71.0	0.251
12051-0036	2/12	Column 2/12	20/09/2008 15:30	433.50	24.00	0.5	15.26	0.636	6.33	<4.00	<2.00	<20.0	<2.00	0.210	67.3	0.218
12051-0037	2/14	Column 2/14	24/09/2008 12:00	526.00	24.50	0.3	14.94	0.610	6.52	<4.00	<2.00	<20.0	<2.00	0.201	67.3	0.205
12051-0038	2/16	Column 2/16	26/09/2008 13:30	575.50	25.00	0.5	15.58	0.623	6.41	<4.00	<2.00	<20.0	<2.00	0.198	67.8	0.205
12051-0039	2/19	Column 2/19	01/10/2012 11:45	693.75	48.25	0.3	15.27	0.316	6.57	<4.00	<2.00	<20.0	<2.00	0.180	69.1	0.188
12051-0040	2/22	Column 2/22	04/10/2008 14:15	768.25	26.00		7.36	0.283	6.33	<4.00	<2.00	<20.0	<2.00	0.161	69.1	0.184
12051-0041	2/25	Column 2/25	11/10/2008 16:00	938.00	49.00	0.3	31.18	0.636	6.36	<6.00	<4.00	<20.0	<2.00	0.147	73.8	0.196
12051-0042	2/27	Column 2/27	16/10/2008 12:00	1054.00	45.00	0.5	28.17	0.626	6.19	<6.00	<4.00	<20.0	<2.00	0.135	71.0	0.175
12051-0043	2/29	Column 2/29	21/10/2008 11:30	1173.50	69.00	0.4	43.80	0.635	6.31	<6.00	<4.00	<20.0	<2.00	0.209	77.7	0.217
12051-0044	2/31	Column 2/31	25/10/2008 12:00	1270.00	46.00	0.4	28.78	0.626	6.39	<6.00	<4.00	<20.0	<2.00	0.110	71.0	0.174
12051-0045	2/34	Column 2/34	01/11/2008 14:15	1440.25	47.25	0.3	30.15	0.638	6.40	<6.00	<4.00	<20.0	<2.00	0.089	71.7	0.167
12051-0046	2/36	Column 2/36	06/11/2008 15:15	1561.25	48.00	0.3	25.84	0.538	6.38	<6.00	<4.00	<20.0	<2.00	0.079	71.5	0.164
12051-0047	2/38	Column 2/38	11/11/2008 11:45	1677.75	69.50	1.1	43.22	0.622	6.14	<6.00	<4.00	<20.0	<2.00	0.071	72.5	0.191
12051-0048	2/41	Column 2/41	18/11/2008 16:00	1850.00	74.00	1.3	46.58	0.629	6.41	<6.00	<4.00	<20.0	<2.00	0.060	71.7	0.216
12051-0049	2/43	Column 2/43	22/11/2008 11:45	1941.75	45.00	1.2	28.30	0.629	6.40	<6.00	<4.00	<20.0	<2.00	0.058	69.2	0.209
12051-0050	2/45	Column 2/45	27/11/2008 10:00	2060.00	46.25	1.0	29.28	0.633	6.47	<6.00	<4.00	<20.0	<2.00	0.055	71.9	0.200
12051-0051	2/48	Column 2/48	06/12/2008 10:30	2276.50	44.00	1.0	28.33	0.644	6.34	<6.00	<4.00	<20.0	<2.00	0.052	71.9	0.213
12051-0052	2/50	Column 2/50	11/12/2008 16:00	2402.00	52.50	0.9	33.24	0.633	6.55	<6.00	<4.00	<20.0	<2.00	0.053	71.5	0.188
12051-0053	2/52	Column 2/52	16/12/2008 11:00	2517.00	69.00	0.5	43.60	0.632	6.37	<6.00	<4.00	<20.0	<2.00	ns	ns	ns
12051-0059	Dec GW	Pump Refill	19/12/2008 12:00	2590.00	25.00				7.18	<6.00	<4.00	<20.0	<2.00	<0.050	72.4	<0.050
12051-0054	2/54	Column 2/54	20/12/2008 11:00	2613.00	23.00	0.9	31.78	1.382	6.41	ns	ns	ns	ns	0.054	71.8	0.213
12051-0055	2/56	Column 2/56	06/01/2009 10:00	3020.00	336.00	-1.1	215.43	0.641	6.46	<6.00	<4.00	<20.0	<2.00	<0.050	72.8	0.217
12051-0056	2/59	Column 2/59	13/01/2009 11:00	3189.00	71.00	0.3	44.69	0.629	6.38	<6.00	<4.00	<20.0	<2.00	<0.050	70.7	0.217
12051-0057	2/61	Column 2/61	17/01/2009 12:00	3286.00	49.00	0.5	30.12	0.615	6.49	<6.00	<4.00	<20.0	<2.00	<0.050	72.1	0.214
12051-0058	2/63	Column 2/63	22/01/2009 11:00	3405.00	47.00	0.5	33.89	0.721	6.41	<6.00	<4.00	<20.0	<2.00	<0.050	70.6	0.216

Fluid Chemistry Data, Column 2

LIMS Code	Sample Code	Sample Description	Date/Time	Total Elapsed Time	Time Interval	Pressure	Sample Vol	Flow Rate	pH	Br^-	NO_2^-	HPO_4^{2-}	F^-	Ba	Sr	Mn
				hr	hr	bar	ml	mg l^{-1}	units	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}
12051-0060	Start GW	Starting GW	02/09/2008 14:00	0.00	0.00	0.00			7.20	<6.00	<4.00	<20.0	<2.00	<0.050	72.0	<0.050
12051-0030	2/1	Column 2/1	05/09/2008 14:00	72.00	72.00	-0.20	13.23	0.184	6.62	n.s	n.s	n.s	n.s	2.52	67.8	0.901
12051-0031	2/2	Column 2/2	06/09/2008 14:00	96.00	24.00	0.20	14.90	0.621	6.37	<4.00	<2.00	<20.0	<2.00	1.42	71.4	0.648
12051-0032	2/4	Column 2/4	10/09/2008 13:45	191.75	27.25	0.1	17.21	0.632	6.50	<4.00	<2.00	<20.0	<2.00	0.419	69.5	0.407
12051-0033	2/6	Column 2/6	12/09/2008 14:00	240.00	23.50	0.2	15.21	0.647	6.54	<4.00	<2.00	<20.0	<2.00	0.323	69.5	0.347
12051-0034	2/8	Column 2/8	16/09/2008 11:45	333.75	69.75	0.1	44.73	0.641	6.52	<4.00	<2.00	<20.0	<2.00	0.263	68.6	0.273
12051-0035	2/10	Column 2/10	18/09/2012 14:00	384.00	24.00	0.2	15.02	0.626	6.51	<4.00	<2.00	<20.0	<2.00	0.231	71.0	0.251
12051-0036	2/12	Column 2/12	20/09/2008 15:30	433.50	24.00	0.5	15.26	0.636	6.33	<4.00	<2.00	<20.0	<2.00	0.210	67.3	0.218
12051-0037	2/14	Column 2/14	24/09/2008 12:00	526.00	24.50	0.3	14.94	0.610	6.52	<4.00	<2.00	<20.0	<2.00	0.201	67.3	0.205
12051-0038	2/16	Column 2/16	26/09/2008 13:30	575.50	25.00	0.5	15.58	0.623	6.41	<4.00	<2.00	<20.0	<2.00	0.198	67.8	0.205
12051-0039	2/19	Column 2/19	01/10/2012 11:45	693.75	48.25	0.3	15.27	0.316	6.57	<4.00	<2.00	<20.0	<2.00	0.180	69.1	0.188
12051-0040	2/22	Column 2/22	04/10/2008 14:15	768.25	26.00		7.36	0.283	6.33	<4.00	<2.00	<20.0	<2.00	0.161	69.1	0.184
12051-0041	2/25	Column 2/25	11/10/2008 16:00	938.00	49.00	0.3	31.18	0.636	6.36	<6.00	<4.00	<20.0	<2.00	0.147	73.8	0.196
12051-0042	2/27	Column 2/27	16/10/2008 12:00	1054.00	45.00	0.5	28.17	0.626	6.19	<6.00	<4.00	<20.0	<2.00	0.135	71.0	0.175
12051-0043	2/29	Column 2/29	21/10/2008 11:30	1173.50	69.00	0.4	43.80	0.635	6.31	<6.00	<4.00	<20.0	<2.00	0.209	77.7	0.217
12051-0044	2/31	Column 2/31	25/10/2008 12:00	1270.00	46.00	0.4	28.78	0.626	6.39	<6.00	<4.00	<20.0	<2.00	0.110	71.0	0.174
12051-0045	2/34	Column 2/34	01/11/2008 14:15	1440.25	47.25	0.3	30.15	0.638	6.40	<6.00	<4.00	<20.0	<2.00	0.089	71.7	0.167
12051-0046	2/36	Column 2/36	06/11/2008 15:15	1561.25	48.00	0.3	25.84	0.538	6.38	<6.00	<4.00	<20.0	<2.00	0.079	71.5	0.164
12051-0047	2/38	Column 2/38	11/11/2008 11:45	1677.75	69.50	1.1	43.22	0.622	6.14	<6.00	<4.00	<20.0	<2.00	0.071	72.5	0.191
12051-0048	2/41	Column 2/41	18/11/2008 16:00	1850.00	74.00	1.3	46.58	0.629	6.41	<6.00	<4.00	<20.0	<2.00	0.060	71.7	0.216
12051-0049	2/43	Column 2/43	22/11/2008 11:45	1941.75	45.00	1.2	28.30	0.629	6.40	<6.00	<4.00	<20.0	<2.00	0.058	69.2	0.209
12051-0050	2/45	Column 2/45	27/11/2008 10:00	2060.00	46.25	1.0	29.28	0.633	6.47	<6.00	<4.00	<20.0	<2.00	0.055	71.9	0.200
12051-0051	2/48	Column 2/48	06/12/2008 10:30	2276.50	44.00	1.0	28.33	0.644	6.34	<6.00	<4.00	<20.0	<2.00	0.052	71.9	0.213
12051-0052	2/50	Column 2/50	11/12/2008 16:00	2402.00	52.50	0.9	33.24	0.633	6.55	<6.00	<4.00	<20.0	<2.00	0.053	71.5	0.188
12051-0053	2/52	Column 2/52	16/12/2008 11:00	2517.00	69.00	0.5	43.60	0.632	6.37	<6.00	<4.00	<20.0	<2.00	ns	ns	ns
12051-0059	Dec GW	Pump Refill	19/12/2008 12:00	2590.00	25.00				7.18	<6.00	<4.00	<20.0	<2.00	<0.050	72.4	<0.050
12051-0054	2/54	Column 2/54	20/12/2008 11:00	2613.00	23.00	0.9	31.78	1.382	6.41	ns	ns	ns	ns	0.054	71.8	0.213
12051-0055	2/56	Column 2/56	06/01/2009 10:00	3020.00	336.00	-1.1	215.43	0.641	6.46	<6.00	<4.00	<20.0	<2.00	<0.050	72.8	0.217
12051-0056	2/59	Column 2/59	13/01/2009 11:00	3189.00	71.00	0.3	44.69	0.629	6.38	<6.00	<4.00	<20.0	<2.00	<0.050	70.7	0.217
12051-0057	2/61	Column 2/61	17/01/2009 12:00	3286.00	49.00	0.5	30.12	0.615	6.49	<6.00	<4.00	<20.0	<2.00	<0.050	72.1	0.214
12051-0058	2/63	Column 2/63	22/01/2009 11:00	3405.00	47.00	0.5	33.89	0.721	6.41	<6.00	<4.00	<20.0	<2.00	<0.050	70.6	0.216

Fluid Chemistry Data, Column 2

LIMS Code	Sample Code	Sample Description	Date/Time	Ca^{2+} mg l^{-1}	Mg^{2+} mg l^{-1}	Na^{+} mg l^{-1}	K^{+} mg l^{-1}	Cl^{-} mg l^{-1}	SO_4^{2-} mg l^{-1}	NO_3^{-} mg l^{-1}	Cation Total meq l^{-1}	Anion Total meq l^{-1}	Balance %
12051-0060	Start GW	Starting GW	02/09/2008 14:00	4410	50.0	2664	<12.5	14891	595	11.7	341.70	432.66	-11.75
12051-0030	2/1	Column 2/1	05/09/2008 14:00	4192	48.6	2495	27.5	n.s	n.s	n.s	323.97	ns	n/a
12051-0031	2/2	Column 2/2	06/09/2008 14:00	4176	50.4	2661	20.5	14382	542	26.3	330.67	417.44	-11.60
12051-0032	2/4	Column 2/4	10/09/2008 13:45	4051	48.6	2555	13.7	14548	554	<4.00	319.23	421.92	-13.85
12051-0033	2/6	Column 2/6	12/09/2008 14:00	4089	48.4	2574	<12.5	13350	507	21.2	321.55	387.51	-9.30
12051-0034	2/8	Column 2/8	16/09/2008 11:45	4126	47.7	2526	<12.5	14013	537	160	321.28	409.07	-12.02
12051-0035	2/10	Column 2/10	18/09/2012 14:00	4258	49.5	2600	<12.5	13925	523	20.1	331.27	404.03	-9.90
12051-0036	2/12	Column 2/12	20/09/2008 15:30	4010	47.1	2469	<12.5	16706	651	17.9	312.93	485.12	-21.58
12051-0037	2/14	Column 2/14	24/09/2008 12:00	4076	47.0	2481	18.3	13685	529	2367	317.18	435.26	-15.69
12051-0038	2/16	Column 2/16	26/09/2008 13:30	4101	47.5	2517	22.5	14116	540	96.5	320.18	411.01	-12.42
12051-0039	2/19	Column 2/19	01/10/2012 11:45	4241	48.2	2552	<12.5	13523	503	8.60	328.15	392.08	-8.88
12051-0040	2/22	Column 2/22	04/10/2008 14:15	4110	48.2	2543	<12.5	13524	513	32.9	321.25	392.71	-10.01
12051-0041	2/25	Column 2/25	11/10/2008 16:00	4600	51.4	2709	<12.5	13162	489	<6.00	353.31	381.49	-3.84
12051-0042	2/27	Column 2/27	16/10/2008 12:00	4150	49.4	2600	<12.5	14217	559	18.5	325.91	413.00	-11.79
12051-0043	2/29	Column 2/29	21/10/2008 11:30	4501	52.3	2841	<12.5	14433	558	8.06	354.28	418.92	-8.36
12051-0044	2/31	Column 2/31	25/10/2008 12:00	4330	49.2	2625	<12.5	14752	576	26.9	335.95	428.60	-12.12
12051-0045	2/34	Column 2/34	01/11/2008 14:15	4196	49.8	2637	<12.5	13291	521	127	329.81	387.84	-8.09
12051-0046	2/36	Column 2/36	06/11/2008 15:15	4351	49.9	2636	<12.5	14632	565	22.6	337.53	424.89	-11.46
12051-0047	2/38	Column 2/38	11/11/2008 11:45	4288	50.5	2658	<12.5	14784	570	220	335.40	432.48	-12.64
12051-0048	2/41	Column 2/41	18/11/2008 16:00	4200	49.9	2659	<12.5	13984	534	7.35	331.03	405.73	-10.14
12051-0049	2/43	Column 2/43	22/11/2008 11:45	4172	47.9	2565	<12.5	14312	560	16.1	325.33	415.65	-12.19
12051-0050	2/45	Column 2/45	27/11/2008 10:00	4343	49.7	2657	<12.5	14521	569	15.2	338.07	421.73	-11.01
12051-0051	2/48	Column 2/48	06/12/2008 10:30	4428	49.8	2660	<12.5	15042	574	14.4	342.43	436.52	-12.08
12051-0052	2/50	Column 2/50	11/12/2008 16:00	4335	49.8	2641	<12.5	14565	552	43.9	336.96	423.10	-11.33
12051-0053	2/52	Column 2/52	16/12/2008 11:00	ns	ns	ns	ns	14121	535	<6.00	ns	409.49	n/a
12051-0059	Dec GW	Pump Refill	19/12/2008 12:00	4378	50.3	2698	<12.5	14038	562	15.5	341.61	407.95	-8.85
12051-0054	2/54	Column 2/54	20/12/2008 11:00	4360	49.8	2646	<12.5	ns	ns	ns	338.43	ns	n/a
12051-0055	2/56	Column 2/56	06/01/2009 10:00	4337	50.2	2688	<12.5	13486	529	16.1	339.14	391.70	-7.19
12051-0056	2/59	Column 2/59	13/01/2009 11:00	4277	49.0	2598	<12.5	13639	533	<6.00	332.09	395.87	-8.76
12051-0057	2/61	Column 2/61	17/01/2009 12:00	4482	49.8	2656	<12.5	13485	521	371	344.94	397.24	-7.05
12051-0058	2/63	Column 2/63	22/01/2009 11:00	4212	48.9	2621	<12.5	13518	523	9.19	329.82	392.36	-8.66

Fluid Chemistry Data, Column 2

LIMS Code	Sample Code	Sample Description	Date/Time	Total P mg l^{-1}	Total S mg l^{-1}	Si mg l^{-1}	SiO_2 mg l^{-1}	Reduced Fe mg l^{-1}	Oxidised Fe mg l^{-1}	Al mg l^{-1}	Co mg l^{-1}	Ni mg l^{-1}
12051-0060	Start GW	Starting GW	02/09/2008 14:00	<0.250	200	<1.88	<4.01	<0.250	<0.250	<0.250	<0.050	<0.125
12051-0030	2/1	Column 2/1	05/09/2008 14:00	<0.250	189	3.73	7.97	<0.250	<0.250	<0.250	<0.050	2.06
12051-0031	2/2	Column 2/2	06/09/2008 14:00	0.319	200	3.81	8.14	<0.250	<0.250	<0.250	<0.050	3.11
12051-0032	2/4	Column 2/4	10/09/2008 13:45	<0.250	192	3.41	7.30	0.586	n/a	<0.250	<0.050	6.06
12051-0033	2/6	Column 2/6	12/09/2008 14:00	0.396	191	3.46	7.40	<0.250	<0.250	<0.250	<0.050	5.13
12051-0034	2/8	Column 2/8	16/09/2008 11:45	<0.250	188	2.79	5.96	<0.250	<0.250	<0.250	<0.050	3.86
12051-0035	2/10	Column 2/10	18/09/2012 14:00	<0.250	195	2.76	5.90	<0.250	<0.250	<0.250	<0.050	2.53
12051-0036	2/12	Column 2/12	20/09/2008 15:30	<0.250	187	2.89	6.19	<0.250	<0.250	<0.250	<0.050	0.875
12051-0037	2/14	Column 2/14	24/09/2008 12:00	0.274	187	2.69	5.75	0.289	n/a	<0.250	<0.050	0.956
12051-0038	2/16	Column 2/16	26/09/2008 13:30	0.341	187	2.86	6.12	<0.250	n/a	<0.25	<0.050	1.05
12051-0039	2/19	Column 2/19	01/10/2012 11:45	<0.250	190	2.64	5.65	<0.250	<0.250	<0.250	<0.050	1.23
12051-0040	2/22	Column 2/22	04/10/2008 14:15	<0.250	190	2.41	5.15	<0.250	<0.250	<0.250	<0.050	1.31
12051-0041	2/25	Column 2/25	11/10/2008 16:00	<0.250	205	2.81	6.01	0.262	n/a	<0.250	<0.050	0.991
12051-0042	2/27	Column 2/27	16/10/2008 12:00	<0.250	197	2.18	4.67	0.279	n/a	<0.250	<0.050	1.43
12051-0043	2/29	Column 2/29	21/10/2008 11:30	<0.250	210	2.86	6.11	0.312	n/a	<0.250	<0.050	1.13
12051-0044	2/31	Column 2/31	25/10/2008 12:00	<0.250	196	2.89	6.19	0.322	n/a	<0.250	<0.050	2.35
12051-0045	2/34	Column 2/34	01/11/2008 14:15	<0.250	198	2.85	6.10	0.325	n/a	<0.250	<0.050	2.97
12051-0046	2/36	Column 2/36	06/11/2008 15:15	<0.250	198	2.90	6.20	0.340	n/a	<0.250	<0.050	3.49
12051-0047	2/38	Column 2/38	11/11/2008 11:45	<0.250	202	3.09	6.62	0.736	n/a	<0.250	<0.050	3.48
12051-0048	2/41	Column 2/41	18/11/2008 16:00	<0.250	198	3.24	6.93	0.401	n/a	<0.250	<0.050	2.87
12051-0049	2/43	Column 2/43	22/11/2008 11:45	<0.250	189	2.80	5.99	0.427	n/a	<0.250	<0.050	3.64
12051-0050	2/45	Column 2/45	27/11/2008 10:00	<0.250	198	2.73	5.83	0.401	n/a	<0.250	<0.050	5.07
12051-0051	2/48	Column 2/48	06/12/2008 10:30	<0.250	198	2.75	5.88	0.421	n/a	<0.250	<0.050	7.06
12051-0052	2/50	Column 2/50	11/12/2008 16:00	<0.250	196	2.37	5.06	0.447	n/a	<0.250	<0.050	3.18
12051-0053	2/52	Column 2/52	16/12/2008 11:00	ns	ns	ns	ns	0.435	n/a	ns	ns	ns
12051-0059	Dec GW	Pump Refill	19/12/2008 12:00	0.259	201	<1.88	<4.01	<0.250	<0.250	<0.250	<0.050	<0.125
12051-0054	2/54	Column 2/54	20/12/2008 11:00	<0.250	197	<1.88	<4.01	0.357	n/a	<0.250	<0.050	4.90
12051-0055	2/56	Column 2/56	06/01/2009 10:00	<0.250	206	2.28	4.87	0.335	n/a	<0.250	<0.050	2.30
12051-0056	2/59	Column 2/59	13/01/2009 11:00	<0.250	196	2.76	5.92	0.341	n/a	<0.250	<0.050	2.93
12051-0057	2/61	Column 2/61	17/01/2009 12:00	<0.250	200	<1.88	<4.01	0.362	n/a	<0.250	<0.050	3.11
12051-0058	2/63	Column 2/63	22/01/2009 11:00	<0.250	195	<1.88	<4.01	0.287	n/a	<0.250	<0.050	3.60

Fluid Chemistry Data, Column 2

LIMS Code	Sample Code	Sample Description	Date/Time	Cu mg l^{-1}	Zn mg l^{-1}	Cr mg l^{-1}	Mo mg l^{-1}	Cd mg l^{-1}	Pb mg l^{-1}	V mg l^{-1}	Li mg l^{-1}	B mg l^{-1}	As mg l^{-1}	Se mg l^{-1}
12051-0060	Start GW	Starting GW	02/09/2008 14:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0030	2/1	Column 2/1	05/09/2008 14:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0031	2/2	Column 2/2	06/09/2008 14:00	<0.125	0.145	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	11.0	<0.375
12051-0032	2/4	Column 2/4	10/09/2008 13:45	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0033	2/6	Column 2/6	12/09/2008 14:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	7.61	<0.375
12051-0034	2/8	Column 2/8	16/09/2008 11:45	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0035	2/10	Column 2/10	18/09/2012 14:00	<0.125	0.145	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0036	2/12	Column 2/12	20/09/2008 15:30	<0.125	0.131	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0037	2/14	Column 2/14	24/09/2008 12:00	<0.125	0.217	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0038	2/16	Column 2/16	26/09/2008 13:30	<0.125	0.404	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0039	2/19	Column 2/19	01/10/2012 11:45	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0040	2/22	Column 2/22	04/10/2008 14:15	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0041	2/25	Column 2/25	11/10/2008 16:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0042	2/27	Column 2/27	16/10/2008 12:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0043	2/29	Column 2/29	21/10/2008 11:30	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0044	2/31	Column 2/31	25/10/2008 12:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0045	2/34	Column 2/34	01/11/2008 14:15	<0.125	0.178	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0046	2/36	Column 2/36	06/11/2008 15:15	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0047	2/38	Column 2/38	11/11/2008 11:45	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0048	2/41	Column 2/41	18/11/2008 16:00	<0.125	0.136	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0049	2/43	Column 2/43	22/11/2008 11:45	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0050	2/45	Column 2/45	27/11/2008 10:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0051	2/48	Column 2/48	06/12/2008 10:30	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0052	2/50	Column 2/50	11/12/2008 16:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	0.716	<0.375
12051-0053	2/52	Column 2/52	16/12/2008 11:00	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
12051-0059	Dec GW	Pump Refill	19/12/2008 12:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0054	2/54	Column 2/54	20/12/2008 11:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0055	2/56	Column 2/56	06/01/2009 10:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0056	2/59	Column 2/59	13/01/2009 11:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	4.39	<0.375
12051-0057	2/61	Column 2/61	17/01/2009 12:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	<0.375	<0.375
12051-0058	2/63	Column 2/63	22/01/2009 11:00	<0.125	<0.125	<0.050	<0.375	<0.050	<0.250	<0.250	<0.625	<0.625	0.565	<0.375

Fe(II): Due to low sample volume, samples were analysed without correction for natural colour.

Fe(II): Limited sample volume available for analysis; this is reflected in the detection limit quoted.

ns denotes insufficient samples for analysis

Glossary

CryoSEM	Cryogenic Scanning Electron Microscopy
BSEM	Backscattered Scanning Electron Microscopy
EDXA	Energy-Dispersive X-ray Microanalysis
SEI	Secondary Electron Imaging/image
SEM	Scanning Electron Microscopy
UV	UltraViolet
VP	Variable Pressure (cf. VPSEM)
VPSEM	Variable Pressure SEM. SEM using low vacuum operation.

References

British Geological Survey holds most of the references listed below, and copies may be obtained via the library service subject to copyright legislation (contact libuser@bgs.ac.uk for details). The library catalogue is available at: http://geolib.bgs.ac.uk.

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