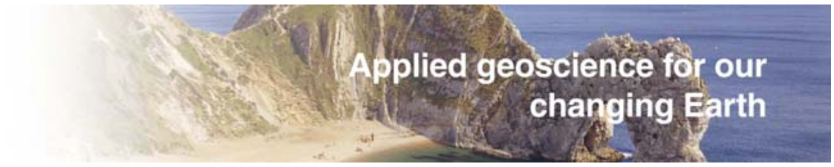
British Geological Survey
NATURAL ENVIRONMENT RESEARCH COUNCIL



Complicating factors – microbiology (and macrobiology)

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British Geological Survey

Caesium Workshop: Fukushima Recovery – understanding, modelling and managing radiocaesium decontamination
30 September to 3 October 2013

JAEA

Outline of presentation

- **Microbes and larger organisms and Cs.**
- **Microbial uptake of Cs in soils.**
- **Cs migration in deep systems.**
- **Biomineralisation.**
- **Ways forward.**

Microbes and Cs – in brief

- Cs redox chemistry simple (1 oxidation state).
- Forms:
  - Ionic bonds with oxygen-donor ligands;
  - Weak bonding with organic and inorganic ligands.
- Can be taken up into cells. Cs^+ analogous to K^+ (macro-nutrient).
- Accumulated in bacteria, cyanobacteria, algae, yeast and fungi.
- Strong radiocaesium retention attributed to microbial activity in organic rich layers of upland soils (low illite) - Persistently available for **animal uptake**. Any work?
- Strongly taken up by clays. But microbes appear to compete with Cs in batch sorption experiments.

This suggests:

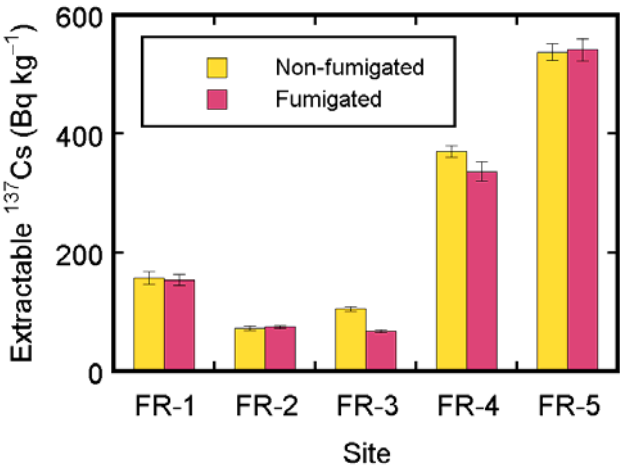
Bioremediation would be useful for Fukushima

- It could reduce potential for Cs migration in soils and rocks.
- Possibilities include:
  - Using microbes to trap radionuclides within soil/rock preventing transport to human environment (work already started for Fukushima e.g. Koarashi et al, 2012).
  - Production of biofilms/biominerals in soils and rocks blocking pathways for fluid flow and contamination migration.

Microbial uptake of Cs in soils

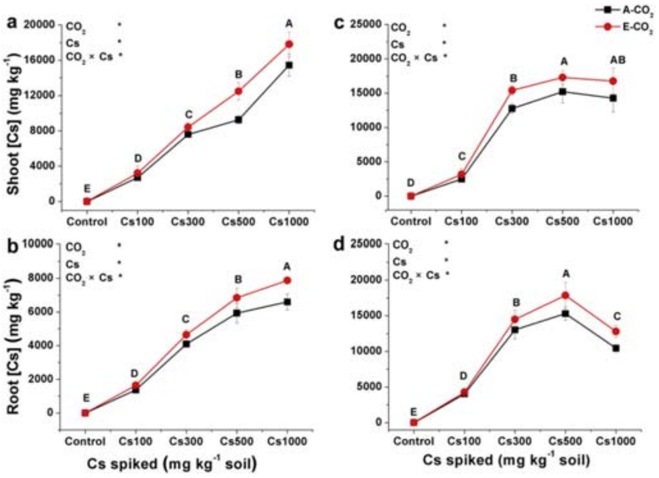
Cs retention in Fukushima forest soils Koarashi et al, 2012)

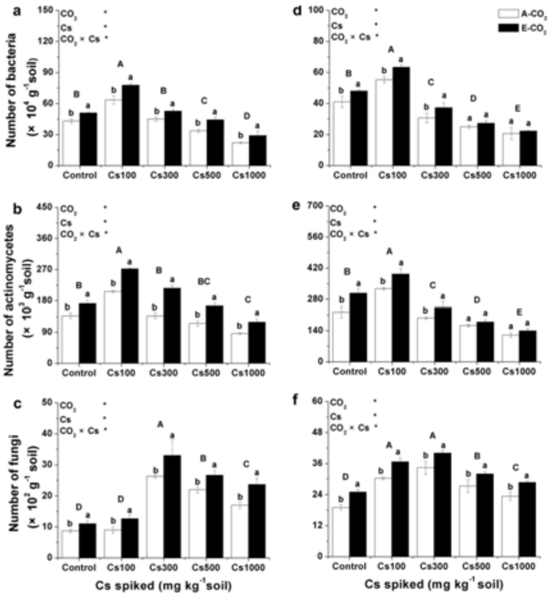
- Cs-137 extractability did not increase after microbes destroyed…
- Suggested that microbial uptake is less useful when compared to uptake onto minerals etc.
- Other types of sterilisation? Same impacts? Macrobiota?



Koarashi et al, (2012). Scientific Reports 2: 1005

Impacts of CO_2 enhancement on Cs uptake by *Phytolacca americana* (C3 species) and *Amaranthus cruentus* (C4 species)





Song et al, (2012). J Env. Rad. 112, 29-37.

CO_2 - 360 and 860 μL L^{-1}

Would need evaluation with plant/crop species at Fukushima…

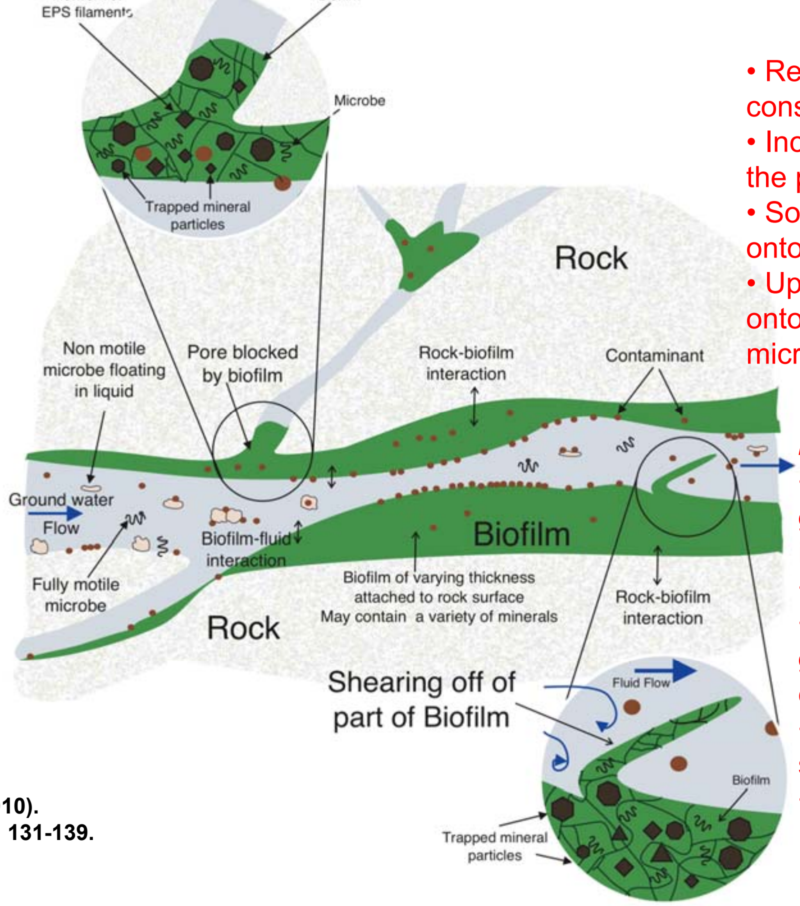
This suggests:

- Uptake of Cs onto microbes could be enhanced and coupled with phytoremediation.
- Would require a more detailed evaluation.
- Impacts of other organics.
- Experimental and field studies…

- But what about macrobiota?
- Perturbations in leaf litter and in soils is biological (microbes, ants, worms etc)...
- How is Cs uptake being included in models?

Cs migration in deep systems

Microbes and Rock Transport Processes



Direct effects

- Reduction of fluid flow by constriction of pore throats
- Increase in tortuosity of the pore network flow paths
- Sorption of contaminants onto biofilm
- Uptake of contaminants onto mobile and motile microbes

Indirect effects

- Biofilm-rock-groundwater interactions
- Redox changes
- Alteration of groundwater chemistry
- Alteration of rock surface
- Biomineralisation

Coombs et al. (2010).
Q J Eng Geol. 43: 131-139.

The impacts of biofilm processes on contaminant transport in granular and fracture systems

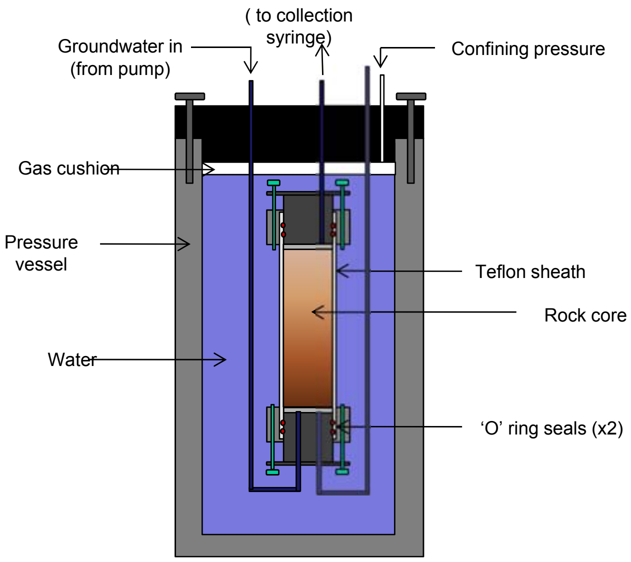
Process	Potential impacts		Likely significance	
	Granular system	Fracture system	Granular system	Fracture system
Filament breadth	2	2	2	2
Shearing	2	1	2	1
Sorption	1	1	1	1
Development of microchemical environments	1	1	1	1
Colloid formation	2	2	2	2

1 = high impact / highly significant
2 = some impact / some significance
3 = low impact / not significant

Experimental methodologies

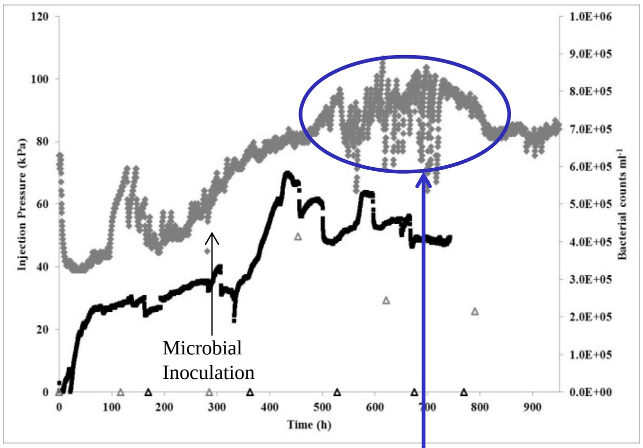
Hama et al. (2001). Clay Minerals. 36: 599-613.
Tuck et al. (2006). J. Geochem. Exp. 90: 123-133.
Coombs et al. (2008). Min. Mag. 72: 393-397.
Harrison et al. (2011). Min. Mag. 75: 2449-2466.
Wragg et al. (2012). Min. Mag. 76: 3251-3259.
Wagner et al. (2013). Env Science: Processes and Impacts. 15(8): 1501-1510.

Flow through column experiments

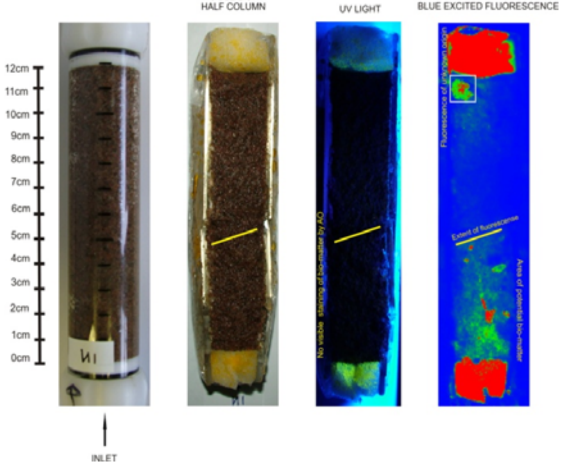


Pressurised Biological Flow Apparatus

Pressure changes – Fractured Mudstone (~39 days)



Change in flow due to cycling of biofilm formation/breakthrough of fluid

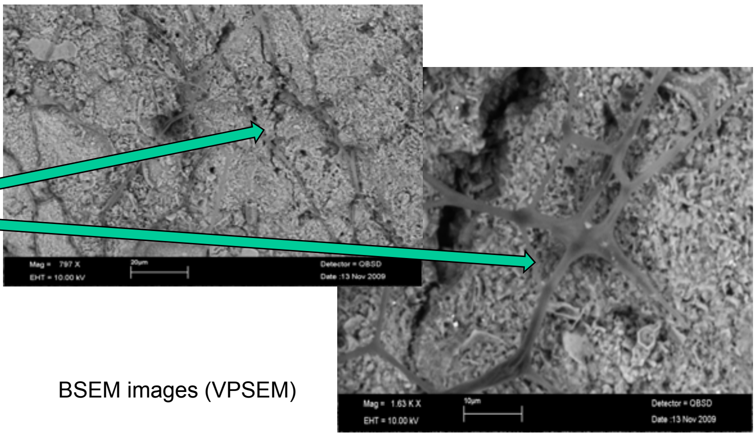


Diorite (crushed) - Analysis of core showing biofilm formation (~147 days)

Mineralogical and petrographical observations

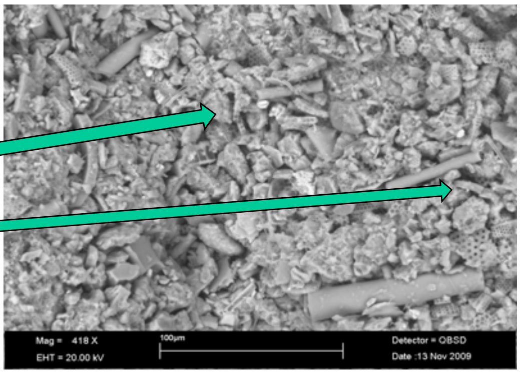
Horonobe mudstone – starting material

- Organic filaments present in channels – no cellular structure. Fungal hyphae?
- BUT no mineralogical alteration. Pyrite was fresh.
- Fracture surface comprises rock mineral particles.



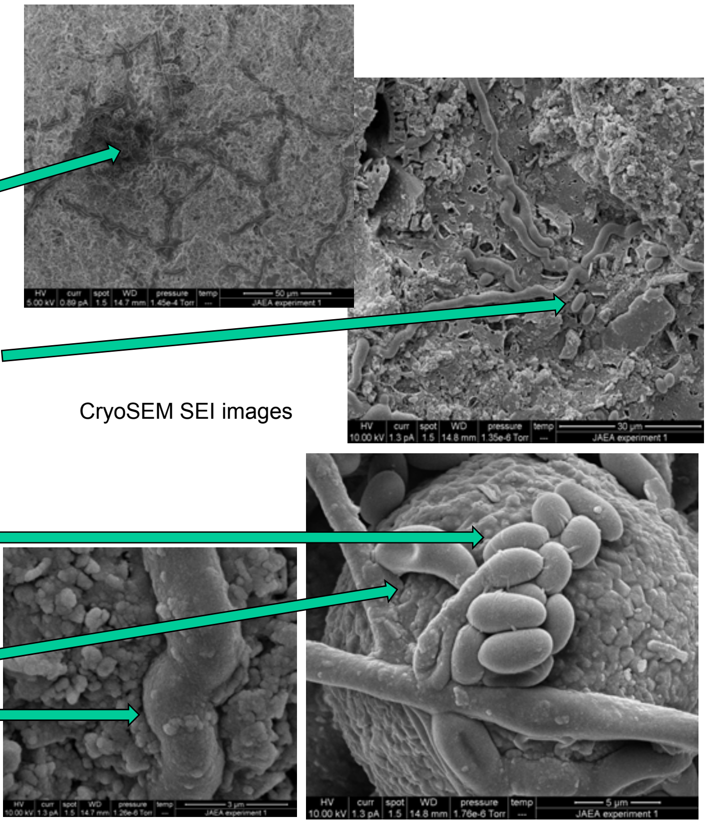
BSEM images (VPSEM)

- Siliceous diatoms
- Illitic clay and quartz
- Fine grained pyrite in pods or fine crystals



Horonobe mudstone – biotic (39 days)

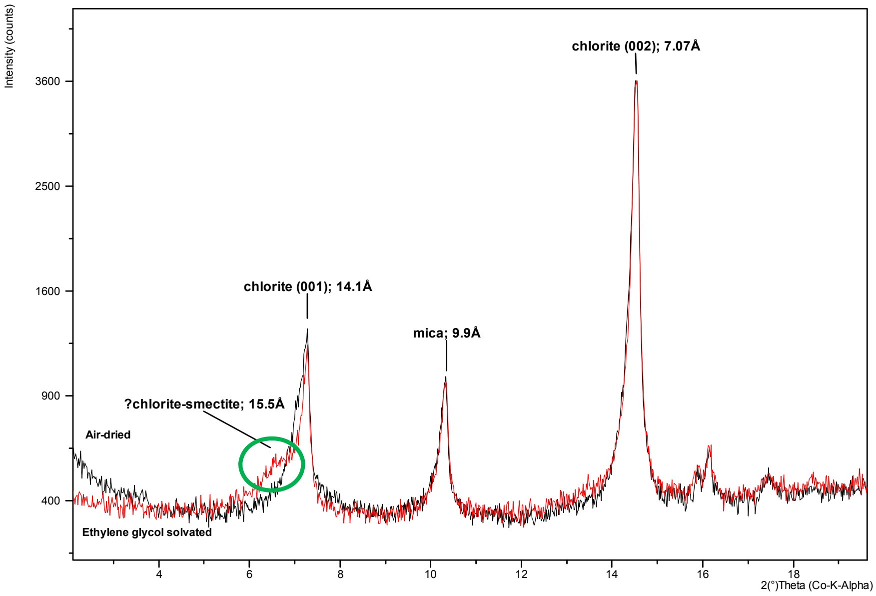
- Filaments comprise ‘strings’ of ‘rod like’ cells (same morphology as *P. denitrificans*).
- Clusters of cells also observed
- Isolated rod like cells also present – likely *P. denitrificans*
- No obvious secondary mineral alteration
- Redox sensitive mineral e.g. pyrite coated in microbial structures
- Biofilaments have caused etching and dissolution of pyrite and silicate substances



CryoSEM SEI images

XRD analyses

Clay mineralogical analysis



<u>Äspö granodiorite (<2µm):</u>

•Mixed-layer clay formation in anaerobic experiments using *P. aeruginosa* (Figure) and *Shewanella putrefaciens / Desulfovibro aespoeensis.*

•No secondary clay mineral formation in aerobic experiment, control samples and pre-experimental material.

<u>Horonobe mudstone (<2µm):</u>

•No secondary clay mineral formation observed.

The work has shown

- Microbes can form biofilms on mudstone and diorite in these experiments.
- Morphology of structures depends on e.g. hydrodynamic environment, nutrients, rock substrate, pore space.
- Diorite
  - Mobilisation and trapping of fines in diorite by microbial action;
  - Formation of 2e clay minerals enhanced by microbes (crushed material with greater surface area).
- Mudstone
  - No mobilisation of fines. No 2e clay mineral formation;
  - Pyrite dissolution.
  - Rock transport properties affected.
- **Complex biological/mineralogical interactions**

APPROACH COULD BE USED TO HELP OPTIMISE FUKUSHIMA REMEDIATION – DEEP ENVIRONMENT

Use of biomineralisation processes to reduce permeability

- Biogeochemical Application in Nuclear Decommissioning and Waste Disposal (BANDD) project
- Two main applications for biomineralisation processes:
  - The use of biomineralisation to reduce subsurface permeability
  - The use of microbial biomineralisation processes for solid-phase capture of radionuclides

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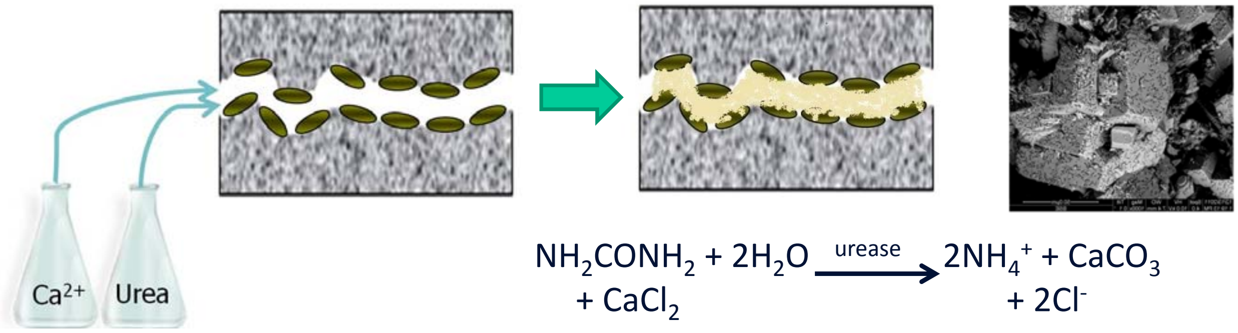
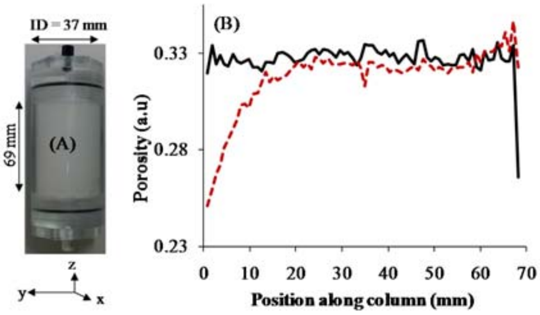
Microbial Induced Calcite Precipitation

- Stimulate bacterial calcite formation to clog fractures in rock → limit fluid flow through the fracture.
- In situ permeability reduction of the host rock → limit radionuclide migration



$$NH_2CONH_2 + 2H_2O + CaCl_2 \xrightarrow{urease} 2NH_4^+ + CaCO_3 + 2Cl^-$$

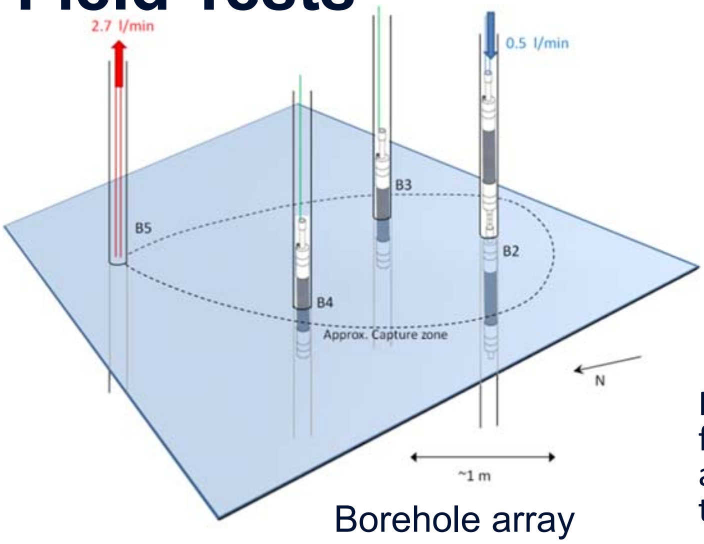
- Develop novel MRI techniques to image & quantify bioprecipitation



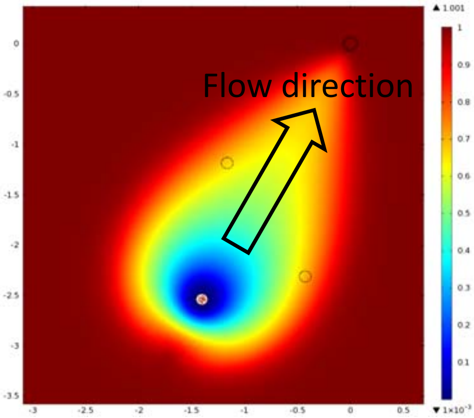
1D magnetic resonance profiles depicting the porosity along the column
t = 0 — t = 65 h ---.

Laboratory Tests

Field Tests



Borehole array



Modelled distribution of fracture transmissivity after grouting, normalised to initial value (i.e. T/T_0)

Cs, Microbes and Fukushima

- **Around Fukushima**
  - Is Cs sorbed onto minerals or onto microbes? Enhancement possibilities. What is the role of macrobiota?
- **On site**
  - Biofilm/Biomineralisation may assist here.
  - May help off-site too (groundwater contamination).

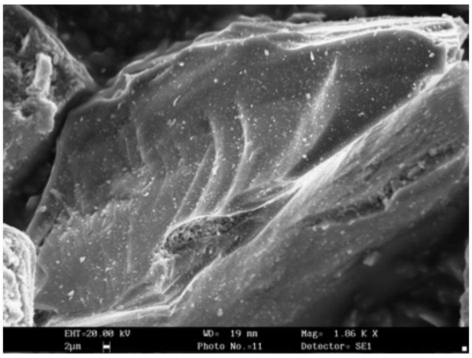
Possible way forward

- Experimental programme to assess how microbes trap Cs using Fukushima soils and rocks, types of indigenous organisms. Enhancement possibilities. Needs to consider macrobiota.
- Impacts of biofilm formation on Cs transport in shallow and deep environments at Fukushima.
- Assessment of the benefits of biomineralisation on contaminant containment.
- Will need laboratory and field studies. Computer modelling would be needed to simulate biological and chemical processes.

Rock type	Experimental system	Atmosphere	Microbial culture	Flow conditions
Crushed granodiorite 125-250 mm (Äspö, Sweden)	Short-term flow-through expts (5 days max). Synthetic groundwater.	Anaerobic	*Shewanella putrefaciens* and *Desulfovibro aespoeensis* Column experiments: ~10^{7} bacteria ml^{-1}	Flow rate of 0.5 ml hr^{-1} using peristaltic pump
	Flow-through column expts (90 days). Synthetic groundwater.	Aerobic	*Pseudomonas aeruginosa* (~10^{4} bacteria ml^{-1} in feed reservoir of 23 litre)	Fixed pressure head maintained with peristaltic pump (0.18 ml hr^{-1} to 270 ml hr^{-1})
	Flow-through column expts (147 days). Synthetic groundwater.	Anaerobic	*Pseudomonas aeruginosa* (one single 10 ml inoculation of ~10^{7} bacteria ml^{-1})	Syringe pumps (0.625 ml hr^{-1})
Intact mudstone with multiple fractures (Horonobe, Japan)	Pressurised columns (39 days). Synthetic groundwater with 0.25 g^{-1} acetate	Aerobic	*Pseudomonas denitrificans* (~10^{5} bacteria ml-^{1})	Constant fluid flow (0.3 ml hr^{-1}) under pressurised conditions

Crushed Äspö granodiorite

(125-250 mm)

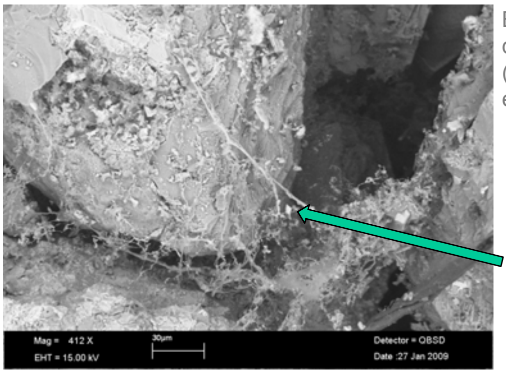


Pre-experimental
SEM image of typical crushed diorite grain (quartz) showing fracture surface with fine-grained material

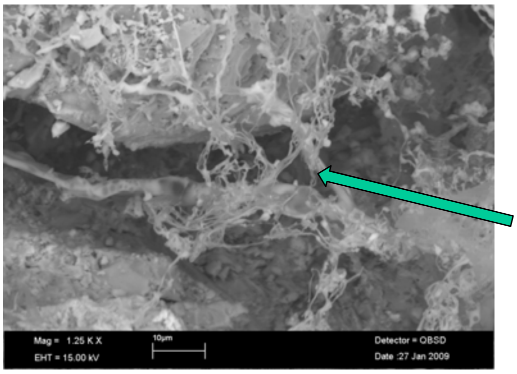
After 273 days complete biofilm coverage

Post-experimental (5 days)

BSEM images of crushed diorite from anaerobic biotic (P. *aeruginosa*) column experiments



Strands of biofilaments. Mineral debris can be seen as bright particles trapped in the organic matter.



Intergranular granodiorite pore space, spanned by the amorphous meshwork of biofilaments.