



Mineralogical Society

# RESEARCH IN PROGRESS MEETING 27-28TH JUNE 2019

Venue: Department of Natural Sciences,  
Manchester Metropolitan University, Oxford  
Road, Manchester M1 5GD

Meeting schedule  
and abstract booklet

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## **VENUE DETAILS**

Talks will be held in lecture theatre T0.03 in [John Dalton Central Tower building](#) on the All Saints Campus of Manchester Metropolitan University. This is a large-capacity lecture theatre with tiered seating, multiple screens and has disabled access. The theatre is immediately to the right hand side of the reception desk. Please enter the building through the Chester Street entrance, as shown on the [map](#).

## **TRAVEL**

[How to get here!](#)

## **PARKING**

The travel link above details locations for nearby car parks. Please note that if you park in an MMU visitor car park without a visitor parking permit, you will likely be fined, even if you have paid for a parking ticket. The car park is independently operated. If you do need to park in the car park please let us know well in advance of your arrival so that we may request a visitor permit for you.

## **CONTACT**

For queries relating to the venue please email [laura.c.kelly@mmu.ac.uk](mailto:laura.c.kelly@mmu.ac.uk)

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**Thursday 27<sup>th</sup> June**

- 12:00**     **Registration and Lunch**
- 13:20**     *Welcome*
- 13:30**     **Richard Wormald** (University of Huddersfield)  
*Calcium carbonate as a potential substrate for hydrogenotrophic methanogenesis at high pH*
- 13:45**     **Dawn Buchanan** (University of Manchester)  
*Investigating microbial reduction of cobalt-doped asbolane using Scanning X-ray Microscopy (SXM)*
- 14:00**     **Gianni Vettese** (University of Manchester)  
*Investigating the role of uranium(V) during microbially mediated reduction of uranium(VI)*
- 14:15**     **Erin Corbett** (University of Strathclyde)  
*Microbial and chemical variation in stormwater and SUDS output*
- 14:30**     **Simon Rout** (University of Huddersfield)  
*Microbial impacts on complexant driven radionuclide transport*
- 14:45**     **Ronald Turner** (University of Strathclyde)  
*Biogenic hydroxyapatite Cement Coatings – Synthesis and Applications*
- 15:00**     *Coffee and Posters*
- 15:30**     **Keynote: Joanne Santini** (University College London)  
*Pseudorhizobium banfieldii sp. str. NT-26: a subterranean bacterium isolated from a goldmine that lives on arsenic*
- 16:15**     **Ke-Qing Xiao** (University of Leeds)  
*Cryptic methane cycling in marine surface sediment*
- 16:30**     **Philippa Loftus** (Edge Hill University)  
*Biomineralisation-targeted genomics of the genus Bacillus: Implications for biogenic CaCO<sub>3</sub> production and biotechnology*
- 16:45**     **Casey Bryce** (University of Tübingen)  
*Microbial iron cycling during permafrost thaw in a sub-Arctic peatland*
- 17:00**     **Rose Jones** (Bigelow Laboratory for Ocean Sciences)  
*Searching for EET-microbes from the crustal deep biosphere of North Pond, Mid-Atlantic Ridge, using cathodic poised potential experiments*
- 17:15**     **Day 1 close**

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## Friday 28<sup>th</sup> June

- 9:30**      *Coffee*
- 10:00**      **Mario Toubes-Rodrigo** (Open University)  
*Methanogenesis in sulphate-rich environments and its importance for Mars exploration*
- 10:15**      **Sarah Jones** (University College London)  
*Metagenomic and metatranscriptomic sequencing provides new insights into differential expression of S and Fe metabolism genes during bioleaching*
- 10:30**      **Lisa Cliffe** (University of Manchester)  
*Persistent microorganisms in shale gas wells: What the frac is down there?*
- 10:45**      **Keynote: Arwyn Edwards** (Aberystwyth University)  
*Defrosting arctic microbiology*
- 11:30**      *Coffee and Posters*
- 12:00**      **James Byrne** (University of Tübingen)  
*Influence of microbial biomass on magnetic mineral formation in the environment*
- 12:15**      **Naji Bassil** (University of Manchester)  
*Microbial degradation of cellulose under hyperalkaline conditions; implications for radwaste geodisposal*
- 12:30**      **Emma Ransom Jones** (University of Huddersfield)  
*Understanding the lifecycle of the Tiyeni deep-bed farming system to improve food security in Malawi*
- 12:45**      **Laura Kelly** (Manchester Metropolitan University)  
*Microbial diversity of subterranean mineral-associated communities at an abandoned copper mine - Mynydd Parys, Anglesey, Wales*
- 13:00**      **Laura Newsome** (University of Exeter)  
*The geomicrobiology of metal-rich birnessite cave crusts and implications for their formation*
- 13:15**      **Sharon Ruiz Lopez** (University of Manchester)  
*Metagenomic analysis of open-air and indoor spent fuel storage ponds at Sellafield, UK*
- 13:30**      **Natalie Byrd** (University of Manchester)  
*The biogeochemical fate of polycarboxylic organic decontamination agents in high pH, low level radioactive waste disposal*
- 13:45**      **Meeting close & prizes**

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**ABSTRACTS FOR ORAL PRESENTATIONS**

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## CALCIUM CARBONATE AS A POTENTIAL SUBSTRATE FOR HYDROGENOTROPHIC METHANOGENESIS AT HIGH PH

Richard Wormald\* & Paul Humphreys

Dept. of Biological and Geographical Sciences, University of Huddersfield

\* [Richard.Wormald2@hud.ac.uk](mailto:Richard.Wormald2@hud.ac.uk)

One potential design for a geological disposal facility (GDF) for intermediate level radioactive waste (ILW) involves the use of a cement based grout (Nirex Reference Vault Backfill (NRVB)) which will establish a highly alkaline environment for extended time periods. Methane generation could impact the long-term safety of a GDF by influencing gas pressures, potentially leading to  $^{14}\text{CH}_4$  returning to the biosphere. The removal of  $\text{CO}_2$  within the near-field through alkaline carbonation reactions could render hydrogenotrophic methanogenesis inaccessible within a GDF, however highly insoluble calcium carbonates (calcite and aragonite) are expected to be present.

The ability of alkaliphilic methanogens to utilise calcium carbonate as a carbon source for hydrogenotrophic methanogenesis at high pH was investigated. Sub-cultures from alkaline (pH 10.0) methanogenic microcosms were supplied with various forms of calcium carbonates and  $\text{H}_2$  as the sole carbon and energy source. The consumption of hydrogen and generation of methane was analysed in these cultures and the methanogen community was described using 16S rRNA gene sequencing. CLSM and FISH was employed to visualise the archaeal community and the formation of biofilm on the calcite surfaces.

Calcite-amended microcosms actively consumed hydrogen and generated methane in the absence of gaseous  $\text{CO}_2$ . The rate of methanogenesis was impacted by the surface area of the mineral, with highest rates observed in microcosms supplied with NRVB. The methanogen community was dominated by hydrogenotrophic members of the *Methanobacterium* and *Methanoculleus* genera, with a lower abundance of the metabolically diverse genus *Methanosarcina* also present. CLSM revealed the presence of biofilm materials on the calcite surfaces, where both eubacteria and archaea were detected using FISH.

This data suggests that highly insoluble calcium carbonate is able to support hydrogenotrophic methanogenesis at pH 10.0, which could have a significant impact on gas generation within the near-field of an ILW-GDF.

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## INVESTIGATING MICROBIAL REDUCTION OF COBALT-DOPED ASBOLANE USING SCANNING X-RAY MICROSCOPY (SXM)

D.M. Buchanan<sup>1\*</sup>, J.R Lloyd<sup>1</sup>, B. Kaulich<sup>2</sup>, L. Newsome<sup>1</sup>, D. S. Mulroy<sup>1</sup>, G. Van Der Laan<sup>2</sup>, A. N' Diaye<sup>3</sup> & V.S. Coker<sup>1</sup>

<sup>1</sup>Williamson Research Centre for Molecular Environmental Science, School Earth & Environmental Sciences, University of Manchester, Oxford Road, M13 9PL, UK

\* [dawn.buchanan@postgrad.manchester.ac.uk](mailto:dawn.buchanan@postgrad.manchester.ac.uk)

<sup>2</sup>Diamond Light Source, Harwell Science and Innovation Campus, Fermi Ave, Didcot OX11 0DE, UK

<sup>3</sup>The Advanced Light Source, 6 Cyclotron Rd, Berkeley, CA 94720, USA

Due to its central role in rechargeable batteries, cobalt has been identified as a critical commodity. The limited number of reserves and increasing demand has driven the need to understand the natural biogeochemical cycling of cobalt and to find potential new resources. Ferromanganese nodules and lateritic deposits can contain economic amounts of cobalt within the structure of manganese minerals such as asbolane  $[(\text{Ni},\text{Co})_x\text{Mn}^{4+}(\text{O},\text{OH})_4 \cdot n\text{H}_2\text{O}]$ . Oxidized manganese is a potential terminal electron acceptor for dissimilatory microbial reduction when coupled to the oxidation of organic matter and the solubilisation and reincorporation of cobalt could occur during manganese reduction of cobalt-manganese mineral phases. An understanding of the complex processes taking place at the cell-mineral interface have long evaded scientists, hindering the development of novel metal extraction techniques. Synchrotron-based scanning X-ray microscopy (SXM) is used to investigate the metal speciation, and organic compounds at the cell-mineral interface at the nanoscale, providing information on the electron transfer processes occurring, increasing our understanding of these complex biogeochemical processes.

In this study, *Geobacter sulfurreducens* is used to enzymatically reduce synthetic cobalt-doped asbolane emulating the natural conditions present during biogenic reduction of cobalt-manganese-oxides. The release of cobalt to solution was observed and transmission electron microscopy was used to demonstrate changes in mineral morphology at key time points. Bulk X-ray Absorption Spectroscopy (XAS) at the Mn  $L_{2,3}$ -edge indicate reduction of Mn(III) to Mn(II), with stable proportions of Mn(IV) while, nanoscale XAS combined with imaging using SXM, suggest reduction from Mn(IV) to Mn(II) *via* an Mn(III) intermediate, when in close proximity to cells. Thus far, cobalt  $L_{2,3}$ -edge XAS does not suggest significant changes in cobalt speciation from the initial dominate Co(II) phase. These results show the complexity of the cell-mineral interactions and the effect they can ultimately have on the cycling and extraction of cobalt from these complex enriched environments.

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## INVESTIGATING THE ROLE OF URANIUM(V) DURING MICROBIALLY MEDIATED REDUCTION OF URANIUM(VI)

Gianni F. Vettese<sup>1\*</sup>, Kath Morris<sup>1</sup>, Louise Natrajan<sup>2</sup>, Sam Shaw<sup>1</sup>, Tonya Vitova<sup>3</sup>, Joerg Rothe<sup>3</sup>, and Jon Lloyd<sup>1</sup>.

<sup>1</sup>Williamson Research Centre for Molecular Environmental Science and Research Centre for Radwaste Disposal, School of Earth and Environmental Science, The University of Manchester, Manchester, UK

<sup>2</sup>School of Chemistry, The University of Manchester, Manchester, UK

<sup>3</sup>Institute for Nuclear Waste Disposal (INE), Karlsruhe Institute of Technology, Germany

\* [Gianni.vettese@postgrad.manchester.ac.uk](mailto:Gianni.vettese@postgrad.manchester.ac.uk)

The addition of simple organic electron donors such as acetate has been shown to stimulate the microbial reduction of soluble U(VI) to insoluble U(IV), often immobilising the radionuclide in contaminated sediments. Although this “bioremediation” process has been reported widely, the mechanisms underpinning this redox chemistry are not fully understood. It is believed that the bioreduction of U(VI) by model Fe(III)-reducing bacteria such as *Geobacter* spp. and *Shewanella* spp. proceeds via a pentavalent U(V) intermediate and that transient U(V) will be found during bioreduction, prior to its disproportionation which leads to the formation of poorly soluble U(IV) biominerals.

Previous work has demonstrated a single-electron transfer to U(VI) species forming intermediate U(V) states in *Geobacter sulfurreducens* (Jones *et al.* 2015; Renshaw *et al.* 2005). We are focusing on this transformation in the model organism *S. oneidensis* MR1, and are applying a range of techniques to explore the role of U(V) in the reduction process.

Here, we provide an overview of our experimental data for uranyl(VI) bioreduction with *Shewanella oneidensis* MR1 using a suite of spectroscopic measurements, including luminescence, Raman and X-ray absorption spectroscopy.

### References

- Jones, D.L. *et al.* (2015) *Chem. Sci.* **6**(9), 5133–38.  
Renshaw, J.C. *et al.* (2005). *Environ. Sci. Technol.* **39**(15), 5657–60.

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## **MICROBIAL AND CHEMICAL VARIATION IN STORMWATER AND SUDS OUTPUT**

**E Corbett\***, V Phoenix

Department of Civil & Environmental Engineering, University of Strathclyde, Glasgow, G1 1XJ, UK.

\* [erin.corbett@strath.ac.uk](mailto:erin.corbett@strath.ac.uk)

Sustainable Urban Drainage Systems (SUDS) provide in situ treatment of stormwater, preventing the discharge of pollution to the environment while avoiding the costs associated with dedicated water treatment facilities. SUDS clean water via a number of mechanisms, including the action of the microorganisms within them. Remarkably little is known about the composition of this microbial community or how this composition changes depending on SUDS structure and environmental conditions. This project aims to investigate variation in microbial community structure within SUDS, and determine how this variation affects water treatment.

Analysis of stormwater from 5 sites around Glasgow, Scotland and 4 sites in Florianópolis, Brazil was carried out in order to gain an understanding of how the chemical and microbial content of stormwater varies within an urban area, as this variation could affect the design and maintenance needs of the SUDS. Chemical analyses performed included ICP-OES, ion chromatography, and total suspended solids determination. Microbial community analysis will be carried out via amplification and sequencing of the 16S rRNA gene, followed by bioinformatics analysis.

The variation in water treatment effectiveness and microbial community structure in 4 rain gardens in Glasgow (differing only in soil mixture composition) will also be investigated over the next several months, by testing the input and output water using the analyses described above.

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## MICROBIAL IMPACTS ON COMPLEXANT DRIVEN RADIONUCLIDE TRANSPORT

Simon P. Rout\*, Kim A. Patel & Paul N. Humphreys

Department of Biological and Geographical Sciences, School of Applied Sciences, University of Huddersfield, Queensgate Campus, Huddersfield, HD1 3DH

\* [S.Rout@hud.ac.uk](mailto:S.Rout@hud.ac.uk)

One potential disposal concept for the UK's ILW legacy is a deep geological disposal facility with a cementitious backfill. Entry of water into such a facility will result in the generation of an anoxic, high pH environment which is expected to facilitate radionuclide retention through sorption and precipitation. Cellulosic materials within the waste are expected to undergo hydrolysis under these conditions to form isosaccharinic acids (ISA's), which are known to complex a number of radioelements potentially enhancing their solubility. ISA's are also biodegradable, meaning that any microorganisms present within the system may influence radioelement release and retention.

Within the present study, a flow based system employing a column of Ottawa sand has been used to investigate the impact of microbiology upon ISA enhanced radionuclide transport, using nickel as an analogue to  $^{63}\text{Ni}$ . In the absence of complexant and biota, nickel sorbed to the surface of the sand, whilst as expected the introduction of ISAs resulted in the remobilisation of Ni from sand and its transport out of the system. The introduction of a microbial flocculate community saw the generation of a biofilm across the surface of the sand coupled to the metabolism of ISA which in turn resulted in a reduction in Ni transport and increased retention within the column. The biofilm was dominated by taxa of the Firmicutes, Bacteroidetes and Proteobacteria, with SEM observations indicating that the biofilm formed over and between the sand grains.

The flow system developed within the project will now be used to investigate the impact of biofilms on the ISA driven transport of Th and U. The findings to date are the first direct evidence of microbial activity reducing the impact of ISA on the transport of radioelements, a process that has been postulated but never before validated.

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## **BIOGENIC HYDROXYAPATITE CEMENT COATINGS – SYNTHESIS AND APPLICATIONS**

**RONALD J TURNER<sup>1\*</sup>**, JOANNA C RENSHAW<sup>1</sup>, SUSAN A CUMBERLAND<sup>1</sup>, PIETER BOTS<sup>1</sup>, ANDREA HAMILTON<sup>1</sup>

Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow, G1 1XJ

\* [Ronald.Turner@Strath.ac.uk](mailto:Ronald.Turner@Strath.ac.uk)

It has recently been established that a biogenic hydroxyapatite can be formed directly onto an Ordinary Portland Cement substrate by bacterial activity. In this experimental investigation, we describe how this biogenic hydroxyapatite layer is generated through the activity of *Pseudomonas fluorescens*. The deposition is dependant upon biofilm formation, which provides a scaffold for biomineral nucleation; and the metabolic activity of the bacteria leading to an increase in pH. This process additionally relies upon synergistic interactions with the cement substrate material, which provides a source of calcium.

We demonstrate that this bacterially generated hydroxyapatite produces a semi-crystalline hydroxyapatite with an 'open' crystal structure.. This is anticipated to facilitate uptake of environmental contaminants such as radionuclides and carbon dioxide. The formation of this biogenic hydroxyapatite coating has significance in a range of contexts. In medicine, hydroxyapatite coatings are linked to improved biocompatibility of ceramic implant materials. In the built environment, hydroxyapatite coatings have been proposed for the consolidation and protection of sculptural materials such as marble and limestone.

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***PSEUDORHIZOBIUM BANFIELDII* SP. STR. NT-26: A SUBTERRANEAN BACTERIUM ISOLATED FROM A GOLDMINE THAT LIVES ON ARSENIC**

Joanne Santini

University College London

[j.santini@ucl.ac.uk](mailto:j.santini@ucl.ac.uk)

Arsenic is ubiquitous in the environment; it exists in four oxidation states, two of which – arsenite (+3) and arsenate (+5) – are soluble and toxic. Arsenic is known as the silent killer as it is colourless and tasteless, and the effects of arsenic poisoning (arsenicosis) appear gradually, often over years. It kills and cripples both children and adults by causing cancers, heart and lung disease, and stillbirths. There is no medical cure: the solution to arsenicosis is preventing arsenic exposure, and testing arsenic levels in water is the first step of arsenic mitigation. More than 140 million people consume water with arsenic concentrations above the WHO limit of 10 ppb; 1/3 of Bangladesh's population is affected. Remarkably, microbes exist that can metabolise arsenic, coupling the oxidation of arsenite or reduction of arsenate to an electron transport chain involved in energy generation. This talk will focus on microbial oxidation of arsenite to arsenate by a facultative heterotrophic bacterium isolated from the Granites goldmine, Northern Territory. The mechanism of arsenite oxidation and its regulation by a three-component signal transduction system will be discussed. The final part of the talk will address the use of the metabolic enzyme, arsenite oxidase as a cheap and sensitive biosensor for arsenic.

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## CRYPTIC METHANE CYCLING IN MARINE SURFACE SEDIMENT

Ke-Qing Xiao

Cohen Geochemistry group, University of Leeds

[k.q.xiao@leeds.ac.uk](mailto:k.q.xiao@leeds.ac.uk)

Methane concentrations are generally very low in marine surface sediments and indicate net CH<sub>4</sub> oxidation and methane flux out of the sediment. Yet, methanogenic archaea are present in those sediments, suggesting a potential for methanogenesis. An isotope dilution method based on sediment bag incubation and spiking with <sup>13</sup>C-CH<sub>4</sub> was used to quantify CH<sub>4</sub> turnover rates in surface sediment from Aarhus Bay, Denmark. Highest CH<sub>4</sub> production and oxidation rates (>200 pmol cm<sup>-3</sup> d<sup>-1</sup>) were repetitively found in the top 0-2 cm, below which rates dropped below 100 pmol cm<sup>-3</sup> d<sup>-1</sup> (2-16 cm), leading to a cryptic cycling of CH<sub>4</sub>. Parallel <sup>14</sup>C-labelling experiments revealed that methanogenesis from the hydrogenotrophic pathway was below 20 pmol cm<sup>-3</sup> d<sup>-1</sup> throughout the surface sediment, and that there was no apparent contribution from the acetoclastic pathway. Our results show the existence of enhanced methanogenic activity and a dynamic recycling of CH<sub>4</sub> at low concentration in sulfate-rich marine surface sediment, where methanogenesis was dominated by the methylotrophic pathway.

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## BIOMINERALISATION-TARGETED GENOMICS OF THE GENUS *BACILLUS*: IMPLICATIONS FOR BIOGENIC $\text{CaCO}_3$ PRODUCTION AND BIOTECHNOLOGY

P. Loftus and A. Antunes\*

GEMM- Group for Extreme and Marine Microbiology, Biology Department, Edge Hill University, Ormskirk, L39 4PQ

\* [antunesa@edgehill.ac.uk](mailto:antunesa@edgehill.ac.uk)

Species of the genus *Bacillus* are key microbes in a variety of fields of biotechnology due to their high genetic and metabolic diversity. One particularly prolific field is the production of biogenic  $\text{CaCO}_3$ , which can be used in civil engineering, agriculture, bio-remediation, and even in  $\text{CO}_2$ -capture.

Microbially-induced carbonate production (MICP) has been reported in several microbial groups but seems particularly common in *Bacillus*. Accordingly, species of this genus are the most frequently explored regarding biotechnological applications of MICP. However, despite their wide-spread use, there is an over-reliance on a very limited number of species with very limited insights into the production mechanism itself or its genetic basis. MICP is most commonly linked with ureolysis so this feature is heavily used for selection of species for biotechnological application. *Bacillus* provides a large and well documented dataset, particularly from a genomic perspective, with whole genome sequence data from several species being readily available for analysis. This can provide new insights into a quicker selection method of novel species for biotechnology, based on genomics.

Here we explore the results of a genus-wide survey of MICP capacity within *Bacillus*, centred on genomic-based analysis and followed by laboratory-based confirmation. Our results identified phylogenetic subgroups within *Bacillus*, having characteristic and distinctive genomic profiles. Tested members within these subgroups share common features on their  $\text{CaCO}_3$ -producing capabilities, namely regarding crystal morphology and yield, confirming the applicability of our genomic-based approach. We identified a few clusters as being more proficient in MICP and highlighted a number of species with biomineral producing capabilities outcompeting those currently used in biotechnology. Notably, results uncovered the presence of a wide range of MICP-associated genes, including some lesser documented ones, questioning the prominence of urease activity for  $\text{CaCO}_3$  production.

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## MICROBIAL IRON CYCLING DURING PERMAFROST THAW IN A SUB-ARCTIC PEATLAND

Casey Bryce<sup>1</sup>, Monique-Sezanne Patzner<sup>1</sup>, Carsten Müller<sup>1</sup>, Verena Nikeleit<sup>1</sup>, Moritz Baur<sup>1</sup>, Thomas Borch<sup>1</sup>, Andreas Kappler<sup>1</sup>

<sup>1</sup>Geomicrobiology, Center for Applied Geoscience, University of Tübingen, Germany

<sup>2</sup>Lehrstuhl für Bodenkunde, Technical University of Munich, Germany

<sup>3</sup>Department of Soil & Crop Sciences, Colorado State University, US

\* [casey.bryce@uni-tuebingen.de](mailto:casey.bryce@uni-tuebingen.de)

High latitude peatlands store around 14% of Earth's carbon despite covering only 3% of the land surface. There is much concern that permafrost melt in these regions may release stored organic carbon and allow it to be emitted as CO<sub>2</sub> and CH<sub>4</sub>, potentially further exacerbating climate warming. The mobilization and release of carbon from permafrost peatlands depends on many factors. However, one key parameter, the stabilization of carbon by reactive soil minerals such as Fe(III) (oxyhydr)oxides, has received little attention in these systems.

Utilizing a permafrost thaw gradient in Abisko, Sweden, we have characterized the dynamic changes in iron and carbon cycling which occur as ice thaws and wetlands expand. Using both bulk (selective chemical extractions, EXAFS/XANES) and microscale analysis (correlative SEM and nanoSIMS), we have determined that up to 20% of the carbon in regions underlain by intact permafrost is associated with poorly crystalline Fe(III) minerals. However, redox conditions become more reducing as thaw progresses, with a corresponding increase in Fe(III)-reducing bacteria, loss of Fe(III) (oxyhydr)oxides and increased porewater Fe(II) concentrations (up to 1 mM). In the wettest soils, dissolved organic carbon is extremely high (120 mg/L) and up to 30% of the iron is present in aqueous Fe(II)-OM complexes.

Our results show that poorly crystalline Fe(III) minerals can limit the mobility of a sizable fraction of the total organic carbon in the early stages of permafrost thaw. However, these minerals are readily dissolved by Fe(III)-reducing bacteria, leading to mobilization of both iron and carbon, and potentially leading to C biodegradation and greenhouse gas release.

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## SEARCHING FOR EET-MICROBES FROM THE CRUSTAL DEEP BIOSPHERE OF NORTH POND, MID-ATLANTIC RIDGE, USING CATHODIC POISED POTENTIAL EXPERIMENTS

Rose M. Jones\*, Tim D'Angelo, Beth N. Orcutt

Bigelow Laboratory for Ocean Sciences, East Boothbay, Maine, US

\* [rosejones233@gmail.com](mailto:rosejones233@gmail.com)

The marine crustal sub-seafloor covers a large portion of the Earth's surface but is very poorly understood. This environment is very energy deficient and it is currently unclear what metabolisms are present that might support life in such extreme resource limitation. Yet, the deep marine crustal subsurface represents a significant portion of the earth's surface and therefore may be a large contributor to biogeochemical cycling by volume alone. There are microbes that can use solid rock for energy, and this study presents some of the first evidence that they are present in the cool, oxic marine crustal subseafloor on the western flank of the Mid-Atlantic Ridge. This evidence is from applying electrochemical techniques to pristine fluids from the crustal subsurface, poisoning electrodes at a particular voltage to provide electrons at an energy level that mimics the delivery of electrons from solid reduced minerals. In this way, microbes that use solid minerals for energy were selected for from the general community onto the electrode surface for identification by scanning electron microscopy and DNA sequencing. These results show that there are microbes capable of using solid minerals as an electron source, in the energy range equivalent to iron-oxidation. Microbial community identity shows that certain microbes are selected for with the metabolic potential to oxidize Ferrous iron coupled to reducing oxygen, though they are initially rare rather than common in environmental samples. Therefore, these microbes are a small part of the marine deep crustal subsurface. However, such bioelectrical techniques offer a new toolkit for expanding and exploring the metabolic function of uncultivated microbes from the largest potential habitat on Earth.

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## METHANOGENESIS IN SULPHATE-RICH ENVIRONMENTS AND ITS IMPORTANCE FOR MARS EXPLORATION

**M Toubes-Rodrigo**<sup>1</sup>, NK Ramkissoo<sup>1</sup>, T McGenity<sup>2</sup>, M Steinke<sup>2</sup>, M Patel<sup>1</sup>, A Poterfract<sup>3</sup>, K Olsson-Francis<sup>1</sup>

<sup>1</sup>Faculty of Science, Technology, Engineering and Mathematics, The Open University, Milton Keynes, (UK)

<sup>2</sup>School of Biological Sciences, The University of Essex, Colchester (UK)

<sup>3</sup>Earth, Atmospheric & Planetary Sciences, Massachusetts Institute of Technology, Cambridge (USA)

\* [mario.toubes-rodrigo@open.ac.uk](mailto:mario.toubes-rodrigo@open.ac.uk)

In its search for volatile signatures of biological or geological processes on Mars, NOMAD measures atmospheric trace gases (e.g. methane, ethane) at concentrations of parts per trillion. It has been postulated that a methane-to-ethane ratio over 1000 indicates biogenic gas production, whereas a ratio of less than 50 suggests a thermogenic source. Water is a requisite for life on Mars and growing evidence shows that brines could have existed or still exist. The compositions of martian brines have been inferred from the observations of evaporitic deposits, dominated by magnesium sulphate, both for past and present sub-surface aqueous environments on Mars. However, whereas our knowledge of life within chloride brines is extensive, comparatively there have been few studies in sulphate brines. To address these issues, we have used a combination of *in-situ* analogue site studies (sulphate-rich lakes in the interior of British Columbia) and laboratory simulations. Gas measurement showed a concentration of up to 2.1 ppm of methane from the lakes in February 2019. Enrichment cultures showed high concentrations of methane (whereas ethane levels were below limit-of-detection) when trimethylamine or a combination of lactate+acetate+butyrate were added, after 60 days. Martian analogue brines were simulated using different water:rock ratios. The concentration of Cl<sup>-</sup>, Na<sup>+</sup>, and SO<sub>4</sub><sup>-</sup> in the 1:1 ratio brine were comparable to that measured at the analogue site and was selected for further experiments (supplied with a CO<sub>2</sub>:H<sub>2</sub> (80:20) headspace). Our results demonstrate that life can thrive in highly concentrated brines, similar to the ones expected in the present-day martian sub-surface. The characterisation of VOCs from the laboratory simulation experiments and the martian analogue sites will be used to compare with the data obtained from the NOMAD mission to elucidate the potential origin of the gases and can inform future exploratory missions.

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## METAGENOMIC AND METATRANSCRIPTOMIC SEQUENCING PROVIDES NEW INSIGHTS INTO DIFFERENTIAL EXPRESSION OF S AND Fe METABOLISM GENES DURING BIOLEACHING

Jones, S. E.<sup>1,2\*</sup>, Osborne, T. H.<sup>2</sup>, Lane, K. R.<sup>3</sup>, Banfield, J. F.<sup>3</sup>, Santini, J. M.<sup>2</sup>

<sup>1</sup>Earth and Planetary Science, Birkbeck College, University of London

<sup>2</sup>Structural and Molecular Biology, University College London

<sup>3</sup>Earth and Planetary Science, University of California, Berkeley

\* [sarah.jones.15@ucl.ac.uk](mailto:sarah.jones.15@ucl.ac.uk)

The process of bioleaching is a cost-effective, low input method of metal extraction from sulfide minerals. Bioleaching exploits the sulfur (S) and iron (Fe) metabolisms of acidophilic prokaryotes to extract metals from ore. Despite the importance of S and Fe metabolisms in mineral breakdown, much remains unknown about both the exact mechanisms of S and Fe cycle processes, and the roles different community members play during the progression of bioleaching. Meta-omics studies allow us to accurately profile species abundance and gene expression within whole microbial communities, providing tools that could help us understand complex community interactions with regards to the bioleaching process.

SC3 is a bioleaching consortium enriched from the Phoukassa working copper mine in Skouriotissa, Cyprus. Genome resolved metagenomics was used to identify the types and abundance of microbes in the consortium when grown on the copper sulfide mineral chalcopyrite. Bacterial species present within the consortium include: *Leptospirillum ferrodiazotrophum*, several *Acidithiobacillus* species, and one member of the order *Rhodospirillales*. There are also archaeal species present: *Ferroplasma acidarmanus*, *Ferroplasma type II* and the *Thermoplasmatales* member dubbed “G-plasma”. Following this, the consortium was grown on a copper sulfide ore obtained from the Phoukassa deposit, in a minimal acid medium, at pH 1.5. Differential expression of genes associated with Fe and S metabolisms were established using RNA-seq analysis and mapped to the dissolution of the Phoukassa ore at 8 and 16 weeks’ growth. The results of this study could help inform the selection of microbes for industrial bioleaching.

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## **PERSISTENT MICROORGANISMS IN SHALE GAS WELLS: WHAT THE FRAC IS DOWN THERE?**

**Lisa Cliffe**<sup>1\*</sup>, Sophie Nixon<sup>1</sup>, Kelly Wrighton<sup>2</sup>, Mike Wilkins<sup>2</sup>, Bob Eden<sup>3</sup>, Kevin Taylor<sup>1</sup>, Jon Lloyd<sup>1</sup>

<sup>1</sup>School of Earth and Environmental Sciences, University of Manchester, Manchester, UK.

<sup>2</sup>Colorado State University, Fort Collins, USA

<sup>3</sup>Rawwater Engineering Company Limited, Culcheth, UK

\* [Lisa.Cliffe@manchester.ac.uk](mailto:Lisa.Cliffe@manchester.ac.uk)

Research suggests that the UK has substantial shale gas deposits that could help address the UK's energy demands. The exploitation of onshore shale gas is made possible by advances in hydraulic fracturing (HF) technology. HF involves high pressure injection of large volumes of water in order to fracture the shale and liberate methane from the rock. Conditions down well include moderately high temperatures, high pressures, brine-level salinities and low oxygen availability. Despite these conditions, there stands a growing body of evidence to support the existence of microbial life down well and produced waters collected during HF offer a window into the deep terrestrial biosphere.

This work aims to investigate the microbial ecology of HF systems, identifying dominant and persistent microbial taxa, with a focus on potential sulfide-generating microorganisms in produced water. Samples were collected from the Marcellus basin, West Virginia, at 29, 79, 142 and 303 days post-HF and stored at room temperature for up to 3 years prior to testing. Sulfide-producing microorganisms pose numerous threats to oil and gas recovery. Sulfide can "sour" oil and gas, a process that is costly to reverse. Sulfide is highly toxic and can lead to the corrosion of steel infrastructure. Within oil reservoirs sulfide is typically produced by sulfate-reducing bacteria (SRB). Previous studies have focussed on the presence of SRB in HF systems as a precursor for sulfide production, overlooking the importance of other microbial sulfide producers.

In this study, no SRB activity was detected using industry standard tests, nor were SRB detected using high-throughput 16S rRNA gene sequencing. Evidence of non-SRB sulfide-producing bacteria was found in the sequencing data, and the activity of persistent thiosulfate-reducing bacteria was established using bespoke media. This research demonstrates both the potential for sulfide production and the persistence of sulfide-producing microorganisms within HF systems.

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## DEFROSTING ARCTIC MICROBIOLOGY

Arwyn Edwards

Institute of Biological, Environmental & Rural Sciences (IBERS) Aberystwyth University

[aye@aber.ac.uk](mailto:aye@aber.ac.uk)

Rapid changes in the warming Arctic have consequences for the entire planet. Microbes act as "sentinels and amplifiers" of these changes in the Arctic by responding rapidly to the thawing or melting of their habitats. In many cases these microbes mediate processes which further accelerate climate change impacts. However, in many ways, the microbial ecology of Arctic environments is in its infancy when compared to contemporary fields of geomicrobiology, for example soil, marine, or subsurface microbiology. In this keynote I will explore some of the open challenges in Arctic microbiology which must be resolved if we are to understand the diverse microbial ecosystems of the Arctic before they are irrevocably changed by our warming of the climate.

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## INFLUENCE OF MICROBIAL BIOMASS ON MAGNETIC MINERAL FORMATION IN THE ENVIRONMENT

Xiaohua Han<sup>1,2,3,4</sup>, Elizabeth J. Tomaszewski<sup>2</sup>, Yongxin Pan<sup>1,3,4</sup> & Andreas Kappler<sup>2</sup>, **James M. Byrne<sup>2\*</sup>**

<sup>1</sup>Biogeomagnetism Group, Key Laboratory of Earth and Planetary Physics, Institute of Geology and Geophysics, Institutions of Earth Science, Chinese Academy of Sciences, Beijing, China

<sup>2</sup>Center for Applied Geoscience, University of Tuebingen, Germany

<sup>3</sup>Evolution and Development of Magnetotactic Multicellular Organisms, Chinese Academy of Sciences, Beijing, China

<sup>4</sup>College of Earth and Planetary Sciences, University of Chinese Academy of Sciences, Beijing, China

\* [james.byrne@uni-tuebingen.de](mailto:james.byrne@uni-tuebingen.de)

It is well established that the magnetic mineral magnetite ( $\text{Fe}_3\text{O}_4$ ) can form through both microbial and abiotic transformations of pure Fe(III) (oxyhydr)oxides (e.g. ferrihydrite, Fh) in terrestrial and marine sediments. However, it remains unclear whether biogenic Fe(III) minerals such as those produced by Fe(II)-oxidizing bacteria, and which contain a significant amount of microbial biomass, can also undergo such transformation to magnetite. We tested for magnetite mineralisation via either abiotic (by reaction with  $\text{Fe(II)}_{\text{aq}}$ ) or biotic (via reduction by Fe(III)-reducer *Shewanella oneidensis* MR-1) transformation of four types of Fh: 1) abiogenic Fh; 2) abiogenic Fh co-precipitated with humic acid (Fh-HA); 3) biogenic Fh produced by the phototrophic Fe(II)-oxidizer *Rhodobacter ferrooxidans* SW2, and 4) biogenic Fh treated with bleach to remove microbial biomass (Fh-bio-NaOCl). We found that the Fe(II)-catalyzed abiotic transformation of abiogenic Fh and Fh-bio-NaOCl led to complete transformation of Fh to magnetite; however, the transformations of Fh-HA and biogenic Fh to magnetite were incomplete. We hypothesize that the microbial biomass in biogenic Fh delayed and/or blocked the reaction between Fh and  $\text{Fe(II)}_{\text{aq}}$ , resulting in a stabilization of Fh. Of the Fh phases that did react with  $\text{Fe(II)}_{\text{aq}}$ , the transformation was faster compared to microbial transformation, implying that initial  $\text{Fe(II)}_{\text{aq}}$  concentration plays a dominant role in the rate of magnetite formation from Fh. Conversely, all Fh phases were transformed into magnetite to varying degrees during microbial reduction, suggesting that Fh remains bioavailable even when associated with microbial biomass. These results suggest that microbial biomass associated with biogenic Fh may influence secondary mineralization pathways, providing insights into biogeochemical cycling of Fe in both modern and ancient environments.

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## MICROBIAL DEGRADATION OF CELLULOSE UNDER HYPERALKALINE CONDITIONS; IMPLICATIONS FOR RADWASTE GEODISPOSAL

Naji M. Bassil\*, Jonathan R. Lloyd

Research Centre for Radwaste and Decommissioning & Williamson Research Centre for Molecular Environmental Science, School of Earth, Atmospheric and Environmental Sciences, The University of Manchester, Oxford Road, Manchester M13 9PL, UK

\* [naji.bassil@manchester.ac.uk](mailto:naji.bassil@manchester.ac.uk)

Intermediate-level radioactive waste from the nuclear fuel cycle, which is expected to contain cellulosic material encapsulated in cement, will be disposed of via a cementitious deep geological disposal facility (GDF). The GDF is expected to be dominated by a multiplicity of extreme conditions, including high pH, ionising radiation, low water content and high pressure. Despite this harsh environment specialised microorganisms may survive in such extreme environments potentially using organics in the radioactive waste as a carbon and energy source. Microcosms poised at pH 12.2, and inoculated using sediments from a legacy lime-workings, showed biodegradation of added cellulose and fermentation of the degradation products into acetate. Irradiation of cellulosic material enhanced this process which led to hydrogen production as a degradation product. Enrichment cultures prepared at pH 10 and inoculated with sediments from the same site showed that alkaliphilic bacteria degrade isosaccharinic acid (ISA), a product of the abiotic hydrolysis of cellulose by alkali, which can mobilise radionuclides. An ISA-oxidising obligate alkaliphile belonging to the genus *Anaerobacillus* was isolated from these cultures, and was found to precipitate soluble U(VI) from solution. Comparative genome and transcriptome analysis, coupled to transmission electron microscopy and X-ray absorption spectroscopy are helping identify the mechanisms of ISA degradation and radionuclide precipitation by this novel bacterium. Taken together, these results highlight the role that microorganisms may play in stabilising radioactive waste in the subsurface, despite the extreme conditions that are expected to dominate in the GDF after closure.

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## **UNDERSTANDING THE LIFECYCLE OF THE TIYENI DEEP-BED FARMING SYSTEM TO IMPROVE FOOD SECURITY IN MALAWI**

**Emma Ransom-Jones<sup>1</sup>, Simon Rout<sup>1</sup>, Adrian Wood<sup>2</sup>, Paul N. Humphreys<sup>1</sup>**

<sup>1</sup>Department of Biological and Geographical Sciences, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH.

<sup>2</sup>Huddersfield Business School, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH.

\* [E.Ransom-Jones@hud.ac.uk](mailto:E.Ransom-Jones@hud.ac.uk)

Approximately 800 million people deal with food shortages that affect their overall life expectancy and health on a day to day basis, particularly in low -income countries such as Malawi. In order to provide these people with secure access to food, agricultural processes need to be developed in a sustainable manner, such as the Tiyeeni deep-bed farming system. This system improves water infiltration and the root development for maize, the staple crop of the region, in comparison to conventional farming methods, making the deep bed system more resilient to the droughts that are predicted to be more frequent due to climate change. These beds are also lower maintenance as they do not require tilling for 4-5 years, and are therefore more economical for the farmers. However, there is currently no data on the cycling of nutrients or organic matter that occurs within these beds, and as a result the lifespan of the beds is unknown. Here, we sample beds of various ages (0-5 years) from four different areas across the Mzuzu region. Soil quality, moisture and pH were measured, and metagenomic sequencing used to determine the functional lifecycle of the beds, and the optimal timeframe for tilling and re-building these beds. These data will improve the management of the deep-bed farming system, and support farmers in developing a sustainable and resilient system for food production which can be transferred to other locations within Africa.

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## MICROBIAL DIVERSITY OF SUBTERRANEAN MINERAL-ASSOCIATED COMMUNITIES AT MYNYDD PARYS, ANGLESEY, WALES

Laura Catherine Kelly<sup>1,2,3\*</sup>, D. Barrie Johnson<sup>2</sup>, Si Creer<sup>3</sup>, Damian Rivett<sup>1</sup>

<sup>1</sup>Department of Natural Sciences, Manchester Metropolitan University, Manchester, M1 5GD

<sup>2</sup>Bangor Acidophile Research Team, School of Natural Sciences, Bangor University, Gwynedd

<sup>3</sup>Environment Centre Wales, Bangor University, Gwynedd

\* [Laura.C.Kelly@mmu.ac.uk](mailto:Laura.C.Kelly@mmu.ac.uk)

Mynydd Parys (Parys Mountain) in Anglesey, North Wales, once the largest supplier of copper in the world, is an abandoned mine site comprising two relic opencast voids and a subsurface network resulting from deep mining operations. The underground workings comprise two inter-connected mines, Parys and Mona, each comprising of kilometres shafts and adits containing exposed residual primary iron sulfide minerals such as pyrite and chalcopyrite. Both mines are consistently of low temperature (8-9°C) and acidic pH (2-3). Both mines also contain a variety of other primary (e.g. quartz and galena) and secondary minerals (e.g. pisanite, gypsum, sphalerite). Also present is anglesite, a secondary lead sulfate mineral first described at Mynydd Parys. While the composition of microbial communities colonising minerals in diverse terrestrial and aquatic environments has been demonstrated to be influenced by mineral chemistry, such an influence has not been investigated in such an extreme acid mine environment, which are typically characterised by low microbial diversity. By selecting diverse minerals from various levels throughout the underground workings, and through the use of cultivation techniques, microscopy and high-throughput sequencing, the PARMIN project focused on determining how mineralogy influences *Bacteria* and *Archaea* communities in the extreme Mynydd Parys environment. Analyses have revealed surprising microbial diversity, and the presence of microorganisms not previously detected in acid mine environments, some of which we have cultivated. The function of these organisms in the acid mine environment remains to be determined, however it is clear that these acid mine environments harbour more diverse and cultivable organisms than previously thought.

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## THE GEOMICROBIOLOGY OF METAL-RICH BIRNESSITE CAVE CRUSTS AND IMPLICATIONS FOR THEIR FORMATION

Laura Newsome<sup>1,2\*</sup>, Yunyao Luo<sup>2</sup>, Charles Bacon<sup>3</sup>, Jonathan R Lloyd<sup>2</sup>

<sup>1</sup>University of Exeter and Camborne School of Mines

<sup>2</sup>University of Manchester

<sup>3</sup>University of Bristol

\* [L.Newsome@exeter.ac.uk](mailto:L.Newsome@exeter.ac.uk)

Manganese(IV) oxide minerals such as birnessite are common in the environment and are subject to rapid biogeochemical cycling by microorganisms. In the presence of oxygen aqueous Mn(II) is oxidised to Mn(IV), predominantly by heterotrophic bacteria and fungi due to the high activation energy of the reaction. Although Mn(II)-oxidising microorganisms are phylogenetically diverse, we still do not fully understand why the process occurs; energy conservation from Mn(II) oxidation has yet to be observed. Instead Mn(II) oxidation occurs indirectly by microbially-generated superoxide, forming Mn(III) which then disproportionates to Mn(IV) and Mn(II). It has been proposed that this might act as a protection mechanism against oxidative stress. Mn(IV) oxide minerals are highly reactive and can scavenge toxic metals from the environment such as Pb, As, Ni, Cr, Co, Cd via sorption and incorporation.

Manganese oxides are often found in caves, typically as black or brown coatings and crusts. Massive crusts were found in limestone caves of the Mendip Hills, Somerset, and samples were collected and analysed. XRD patterns resembled nanoparticulate birnessite ( $\delta$ -MnO<sub>2</sub>) and EDX analysis showed that they were enriched in Pb (up to 56 wt. %) and Zn (up to 11 wt. %). Additional samples of cave crusts and waters were collected for geochemical and microbiological characterisation.

The microbial community was characterised in three Mn(IV) oxide cave crusts by 16S rRNA and ITS amplicon sequencing (Illumina MiSeq). The results showed that they contained diverse prokaryotic and fungal communities with sequences closely related to known Mn(II) oxidising prokaryotes present, as well as autotrophic and heterotrophic ammonia and nitrite oxidisers. To further investigate the potential for active Mn(II) oxidation, a series of isolation and enrichment experiments were performed. Six heterotrophic Mn(II)-oxidising bacteria (closely related to *Streptomyces* sp. *Phyllobacterium* sp. and *Terrimonas* sp.) were isolated, although these made up just < 2% of the sequenced prokaryotic community. One heterotrophic Mn(II)-oxidising fungus (closely related to *Cladosporium* sp.) was also isolated. All six bacterial isolates could grow in the presence of Pb and four were able to oxidise Mn(II) in the presence of Pb. *Streptomyces* spp. is known to have highly Pb-resistant strains.

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In summary the Mendips cave crusts comprised Mn(IV) oxides and contained a diverse microbial community. Although these Mn(IV) oxides sequester large quantities of metals from cave waters, it is unlikely that this would inhibit microbial Mn(II) oxidation, and suggests that that microbial activity likely contributed to their formation.

## **METAGENOMIC ANALYSIS OF OPEN-AIR AND INDOOR SPENT FUEL STORAGE PONDS AT SELLAFIELD, UK**

**S. Ruiz-Lopez<sup>1\*</sup>**, L. Foster<sup>2</sup>, N. Cole<sup>2</sup>, H. Song<sup>3</sup>, J. Adams<sup>4</sup>, J. Lloyd<sup>1</sup>

<sup>1</sup>Williamson Research Centre for Molecular Environmental and Research Centre for Radwaste Disposal. School of Earth and Environmental Sciences, University of Manchester, Manchester M139PL, UK

<sup>2</sup>Sellafield Ltd, Hinton House, Birchwood Park Ave, Warrington WA36GR, UK

<sup>3</sup>School of Biological Sciences, College of Natural Sciences, Seoul National University, Gwanak-ro, 08826 Seoul

<sup>4</sup>Nanjing University

\* [sharon.ruizlopez@manchester.ac.uk](mailto:sharon.ruizlopez@manchester.ac.uk)

Nuclear power is an important energy source that can compensate for carbon emissions from fossil fuel power plants. However, processing of radioactive waste from nuclear plants is a significant challenge. The current treatment prior to final geological disposal involves wet storage of spent fuel in designated ponds, and microbial colonisation of these ponds can complicate plant operation.

To help identify the key microbes that colonise hydraulically interlinked spent fuel storage ponds at Sellafield, UK, a series of samples were collected and analysed using next generation (Illumina) sequencing. Samples were taken from different areas at the facility's indoor spent fuel storage pond (INP), and also from the open-air First Generation Magnox Storage Pond (FGMSP). 16S rRNA gene sequencing revealed that the FHP is colonized mainly by Bacteria (99%), affiliated with species of *Curvibacter*, *Rhodoferrax*, *Sphingomonas* and *Roseococcus*, in addition to the hydrogen-oxidising bacterium *Hydrogenophaga*. In contrast the open-air FGSMP pond contained species of *Hydrogenophaga*, *Nevskia*, and *Roseococcus*, and also photosynthetic cyanobacteria (*Pseudanabaena*).

Biological function was also assessed by metagenomic sequencing and analyses. The most abundant genes were associated with carbohydrate and protein metabolism, cell wall and capsule synthesis, stress responses and respiration. Genes involved in respiration were also more abundant in the indoor pond microbiome, including genes underpinning hydrogen metabolism, whilst photosynthesis genes were more abundant in the open-air ponds. These datasets give valuable insight into the microbial communities inhabiting nuclear storage facilities, the metabolic processes that underpin their colonisation and can help inform appropriate control strategies.

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## THE BIOGEOCHEMICAL FATE OF POLYCARBOXYLIC ORGANIC DECONTAMINATION AGENTS IN HIGH PH, LOW LEVEL RADIOACTIVE WASTE DISPOSAL

Natalie Byrd<sup>1\*</sup>, Frank Taylor<sup>2</sup>, Joe Small<sup>3</sup> Jonathan Lloyd<sup>1</sup>, Katherine Morris<sup>1</sup>

<sup>1</sup>Research Centre for Radwaste Disposal and Williamson Research Centre for Molecular Environmental Science, School of Earth and Environmental Sciences, The University of Manchester, Manchester M13 9PL, UK

<sup>2</sup>Low Level Waste Repository Ltd, Pelham House, Seascale, Cumbria CA20 1DB, UK

<sup>3</sup>National Nuclear Laboratory, Chadwick House, Birchwood, Warrington WA3 6AE, UK

\* [natalie.byrd@manchester.ac.uk](mailto:natalie.byrd@manchester.ac.uk)

The Low Level Waste Repository (LLWR) in Cumbria is the UK's principal facility for disposal of low level radioactive waste (LLW). It is engineered to minimise waste contaminant mobility by generating an anoxic, high pH (~11), reducing environment once the facility is sealed (Small, *et al.*, 2011; Roch, 2015). Post closure the waste will remain in place indefinitely and is likely to be colonized by microbial communities. Indeed, previous work has already shown that microbes can proliferate under conditions relevant to a cementitious repository (Rizoulis *et al.*, 2012). Anaerobes present within the LLWR will derive energy either by fermenting the organics directly in the waste, or oxidising them concurrent with the reduction of inorganic species. These biogeochemical interactions may have significant implications for near-field geochemistry and contaminant fate. Furthering understanding of biogeochemistry is necessary to enhance the accuracy of model predictions regarding contaminant transport and manage associated risks to sensitive receptors.

In the context of LLW disposal, information regarding the high pH behavior and degradation of many organic electron donors is scant. Organic compounds are of interest due to their ability to act as complexing agents, potentially enhancing subsurface mobility of metal contaminants. The principal sources of organic complexing agents within the UK's LLW inventory are wastes from the chemical decontamination of material originating from nuclear sites (Randall, *et al.*, 2011). Of these, polycarboxylates (e.g. citrate and oxalate) are of keen interest as: (a) the LLWR has no capacity assigned for disposal of these compounds within the facility (b) the model that underpins the sites Environmental Safety Case (ESC) relies only on assumptions regarding their biodegradability at high pH. Therefore, this work aims to explore the biogeochemical fate of polycarboxylic acids under high pH conditions relevant to LLW disposal, using citric acid as a model compound. Furthermore, high pH microbial metabolism of citrate in the presence of cement is examined. Anaerobic microcosm experiments at pH 10-12 have shown that citrate is metabolism is possible under various anaerobic

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conditions, and in some instances citrate has enhanced the microbial metabolism of electron acceptors.

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**ABSTRACTS FOR POSTER PRESENTATIONS**

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## UNRAVELLING THE MOLECULAR BASIS OF HYDROXYAPATITE WEATHERING DRIVEN BY THE ECTOMYCORRHIZAL FUNGUS *PAXILLUS INVOLUTUS*

Christina Paparokidou<sup>1\*</sup>, Julie D. Scholes<sup>1</sup>, Jonathan R. Leake<sup>1</sup>, Stephen A. Rolfe<sup>1</sup> and David J. Beerling<sup>1</sup>.

<sup>1</sup>Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK.

\* [cpaparokidou1@sheffield.ac.uk](mailto:cpaparokidou1@sheffield.ac.uk)

Ectomycorrhizal (ECM) fungi form symbiotic partnerships with tree roots and can chemically weather inorganic phosphorus (Pi)-rich minerals, supplying Pi to their host in return for photosynthates. The fungal-driven nutrient cycling from mineral weathering plays a pivotal role in ecosystems and crops productivity, as well as geochemical cycles. The aim of this study is to unravel the molecular mechanisms by which the ECM fungus *Paxillus involutus* weathers the Pi-rich mineral hydroxyapatite (HAP), either non-symbiotically or in symbiosis with its host tree *Pinus sylvestris*. Hence, an artificial system was developed to study HAP solubilisation driven by *P. involutus*, which was confirmed by EDX spectroscopy data, showing depletion of Pi from the HAP crystals along with secondary minerals formation characteristic to fungal mineral weathering. Next, whole-transcriptomic RNA-seq analysis was used to identify fungal genes differentially expressed in response to HAP solubilisation. Besides, whole metabolomic profiling using UPLC-Q-TOF-MS was employed to detect the presence of metabolites secreted at the fungal-mineral interface. First, the RNA-seq analysis revealed that ECM symbiosis induces a different set of HAP weathering genes in *P. involutus* hyphae, compared to the fungus growing non-symbiotically, including the specific expression of organic acid metabolic and transporter genes. Second, the metabolomic analysis led to the identification of multiple metabolites enriched in the presence of HAP. Besides the identification of known fungal mineral weathering agents such as oxalic acid, this study has also led to the detection of putative novel ones, like glucuronic acid. Both transcriptomic and metabolomic data provide the first molecular evidence for the role of organic acids as drivers of ECM fungal mineral weathering. Last, these results were combined into a putative model illustrating the molecular mechanisms involved in HAP weathering driven by the ECM fungus *P. involutus*.

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## ARCTIC AND ANTARCTIC SNOWBALL WORLDS: A SURVEY OF CRYOCONITE ECOLOGY

Jaz Millar<sup>1,2\*</sup>, Anne Jungblut<sup>2</sup>, Liz Bagshaw<sup>1</sup>, Arwyn Edwards<sup>3</sup>

<sup>1</sup> School of Earth and Ocean Sciences, Cardiff University

<sup>2</sup> Department of Life Sciences, The Natural History Museum

<sup>3</sup> Institute of Biological, Environmental & Rural Sciences (IBERS) Aberystwyth University

\* [MillarJL@cardiff.ac.uk](mailto:MillarJL@cardiff.ac.uk)

Cryoconite holes, a glacial surface ecosystem, may provide an answer to how life survived the Cryogenian Snowball Earth 720–635 million years ago. Geological evidence suggests that during the Snowball Earth the planet was completely covered in ice from the poles to the equator. Not only did life persist, this time was a turning point — the beginning of multicellular life. As cryoconite holes are a cold-climate habitat that supports a range of organisms and biogeochemical processes, they are of particular interest to Snowball Earth researchers. In this study, we selected cryoconite samples from several locations across both the Arctic and Antarctic for 16S and 18S genetic amplicon sequencing. We present further evidence for the diversity of cryoconite across both poles and discuss the differences in community composition between Arctic and Antarctic cryoconite ecosystems. In addition, we report that cryoconite holes support key Cryogenian taxa.

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## ROLE OF FUNGAL AMINO ACID EXCRETION IN BIOMINERALIZATION OF COPPER CARBONATE NANOPARTICLES

Feixue Liu<sup>1\*</sup>, Laszlo Csetenyi<sup>2</sup>, Geoffrey Michael Gadd<sup>1</sup>

<sup>1</sup> Geomicrobiology Group, School of Life Sciences, University of Dundee, Dundee, DD1 5EH, Scotland, United Kingdom,

<sup>2</sup> Concrete Technology Group, Department of Civil Engineering, University of Dundee, Dundee, DD1 4HN, Scotland, United Kingdom

\* [f.y.liu@dundee.ac.uk](mailto:f.y.liu@dundee.ac.uk)

The ureolytic activity of *Neurospora crassa* results in an alkaline carbonate-rich culture medium which can precipitate soluble metals as insoluble carbonates. Such carbonates are smaller, often of nanoscale dimensions, than metal carbonates synthesized abiotically which infers that excreted fungal products can affect particle size. In this work, amino acid excretion was found to be a significant factor in affecting the particle size of copper carbonate. Eleven different amino acids were secreted by *Neurospora crassa*, and L-glutamic acid, L-aspartic acid and L-cysteine were selected for further study. Powder X-ray diffraction (XRPD), scanning electron microscopy (SEM), Fourier transmission infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA) were used to characterize copper carbonate samples. Copper carbonate nanoparticles with a diameter of 100 – 200 nm were produced with L-glutamic acid, which was found to stabilize the particles and prevent aggregation. Such processes may indicate a potential means to engineer the size and morphology of metal carbonate nanoparticles using biomass-free fungal systems. FTIR and TGA revealed that the amino acid moieties were intimately associated with the copper mineral particles. In addition, component analysis of the final products of TGA showed the ultimate formation of Cu<sub>2</sub>O, suggesting an abiotic synthesis method for producing another useful Cu-containing material.

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## SULFUR-ENHANCED REDUCTIVE BIO-PROCESSING OF CO-BEARING MATERIALS USING A CONSORTIUM OF ACIDOPHILIC IRON- AND SULFUR-OXIDISING BACTERIA

Ana Laura Santos\* and D. Barrie Johnson

School of Natural Sciences, Bangor University, Bangor (Gwynedd), UK

\* [ana.santos@bangor.ac.uk](mailto:ana.santos@bangor.ac.uk)

Biomining, as traditionally practised, uses aerobic, acidophilic bacteria and archaea to accelerate the oxidative dissolution of sulfide minerals present in ores and concentrates. Many acidophiles are also able to catalyse the dissimilatory reduction of ferric iron in anoxic or micro-aerophilic environments, accelerating the reductive dissolution of Fe<sup>3+</sup>-minerals, such as goethite. This approach can be used to extract metals, such as nickel, copper, cobalt and manganese from oxidised ores (e.g. limonitic laterites). With the demand for cobalt increasing worldwide (as a result of its use in rechargeable batteries, super-alloys, and chemical industries) a novel sulfur-enhanced reductive bio-processing technology has been developed focusing on the extraction and recovery of this metal. During the course of the EU-funded project Crocodile (GA no. 776743; “first of a kind commercial Compact system for the efficient Recovery Of CObalt Designed with novel Integrated LEading technologies”) reductive bioleaching experiments of two Co-containing processed materials (L1: dust and L2: slag) and three limonitic laterites (L3, L4 and L5) from nickel mines in Greece (operated by LARCO) were carried out in anaerobic bioreactors containing 5% solids (w/v), maintained at pH 1.5 and 35°C. Elemental sulfur (1% w/v) was added as electron donor for the acidophilic bacteria. Dissolution of the Co-bearing materials occurred anaerobically (using oxygen-free nitrogen) which was coupled to the oxidation of elemental sulfur. Sample L4 consumed far less sulfuric acid (60 mL) to maintain the bioreactor at pH 1.5 whereas all other samples consumed over 370 mL (biological dissolution of ferric iron minerals is proton consuming reaction). Analysis of the final leachates showed that alkaline, alkaline earth and transition metals other than cobalt were recovered in all bioleaching experiments. The percentage of cobalt recovered using this approach ranged between 39% and 50%.

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## **BIOGENIC SULFIDATION OF U(VI) CONTAINING IRON (OXYHYDR)OXIDES**

**L. T. Townsend\***, G. Kuippers, J. R. Lloyd, K. Morris

Research Centre for Radwaste Disposal & Williamson Research Centre for Molecular Environmental Science, School of Earth & Environmental Sciences, The University of Manchester, Manchester, M13 9PL, UK

\* [luke.townsend@manchester.ac.uk](mailto:luke.townsend@manchester.ac.uk)

Uranium is a key environmental contaminant that is present within the subsurface due to nuclear fuel cycle activities, from mining and accidental releases at nuclear sites. Furthermore, in the UK, government policy is that radioactive wastes, including a large inventory of uranium, will be disposed of in a deep geological disposal facility (GDF). In these environments, both iron (oxyhydr)oxide minerals and microbial activity will be ubiquitous, resulting in biogeochemical processes taking place. Of particular interest is the process of sulfidation, which occurs via microbial reduction of sulfate by sulfate reducing bacteria (SRB). The resultant sulfide can react with both iron (oxyhydr)oxides and contaminants like uranium, resulting in changes to the environmental mobility of uranium. Due to the complex nature of the sulfidation process, it is important to understand the mechanisms of reaction affecting U speciation and mobility. In this work, we have used a well characterised microbial consortium able to tolerate alkaline conditions from Harpur Hill sediment (Buxton, UK) to explore sulfidation processes in microcosm experiments. Here, sulfate reduction was mediated by the microbial consortium at pH 9.5, with U(VI) present under low (1 mM) and high (30 mM) carbonate conditions, in both the presence and absence of Fe(III). Analysis of the geochemistry and U speciation, using X-ray Absorption Spectroscopy, has allowed insights into U speciation and fate in these scenarios. In high carbonate conditions there was little or no removal of U(VI) from solution despite Fe(III)- and sulfate-reduction occurring. Under low carbonate conditions, U was removed from solution as Fe(III)- and sulfate-reduction occurred. The results from this study contribute to a more thorough understanding of how biogeochemical processes, such as sulfidation, affect the speciation and environmental fate of U in systems relevant to contaminated land remediation and disposal of radioactive waste.

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## GEOCHEMICAL INFLUENCE ON SELENITE AND TELLURITE REDUCTION BY YEASTS

Lara Codognotto\* and Geoffrey Michael Gadd

University of Dundee, Geomicrobiology Group

\* [l.codognotto@dundee.ac.uk](mailto:l.codognotto@dundee.ac.uk)

Selenium and tellurium are two metalloids essential for future green energy technologies due to their associated photovoltaic and photoconductive properties. In addition, selenium and tellurium oxyanions can be toxic in the environment and can potentially affect human health. This work aims to examine some geochemical influences on Se/Te reduction carried out by selected yeast strains to identify what limitations there are to the process, and their importance. Several yeast strains, capable of selenite or tellurite reduction, were isolated from environmental soil samples on solid media containing selenite or tellurite, reduction being detected by the color change of colonies to red (Se) or black (Te). Such reduction resulted in the formation of nanoparticles of elemental Se<sub>0</sub> or Te<sub>0</sub>. Growth was assessed in the presence of selenite or tellurite and minimum inhibitory concentrations determined. Rates of selenite and tellurite depletion were determined in different growth conditions and the production of elemental Se<sub>0</sub> or Te<sub>0</sub> was analyzed using energy dispersion X-ray analysis (EDXA), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). This work furthers understanding of selenium and tellurium transformation by yeasts also suggests potential routes for Se/Te biorecovery by the formation of Se/Te nanoparticles.

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## ACTIVE SULFUR AND CARBON CYCLING IN THE DEEP BIOSPHERE

Sophie Nixon<sup>1\*</sup>, Malin Bomberg<sup>2</sup>, Lillemor Claesson Liljedahl<sup>3</sup>, Tiina Lamminmäki<sup>4</sup>, Anne Kontula<sup>5</sup>

<sup>1</sup>School of Earth and Environmental Sciences, University of Manchester, UK

<sup>2</sup>VTT Technical Research Centre of Finland Ltd, Finland

<sup>3</sup>SKB, Sweden

<sup>4</sup>Posiva Oy, Finland

\* [sophie.nixon@manchester.ac.uk](mailto:sophie.nixon@manchester.ac.uk)

The deep terrestrial subsurface harbours most of the microbial diversity on Earth, and a significant proportion of the planet's biomass. Understanding how life operates in these extreme environments can help guide the search for life on other planetary bodies. However, most studies to date have catalogued diversity of deep biosphere microbial communities, rather than surveyed function and activity. As a result, we are left with a phylogenetic tree of deep biosphere life with little appreciation of how its foliage operates.

Here, we apply metagenomics and metatranscriptomics to probe microbial life in cold groundwater collected from two deep boreholes (191 m and 645 m deep) in the western region of Greenland. We present evidence for active carbon and sulfur cycling amongst diverse communities, dominated by Firmicutes and Proteobacteria. We also show evidence for active 'rare biosphere' microorganisms likely participating in carbon and sulfur cycling, and explore genomic evidence for cold adaptations and viral associations. These active microbial communities appear to be self-sustaining, with evidence of cryptic sulfur and carbon cycles, and are distinct from surface communities.

This work sheds light on deep cold subsurface communities that reside in environments analogous to those in the subsurface of other planetary bodies, especially ice moons of Jupiter and Saturn.

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## UNDERSTANDING THE EFFECTIVENESS AND ENVIRONMENTAL IMPACT OF ANTI-BIOFOULING STRATEGIES IN HYDRAULIC FRACTURING OPERATIONS

F. S. Nicol\*, C. W. Knapp, R. M. Kalin, V. R. Phoenix

Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow, G1 1XJ

\* [felix.nicol@strath.ac.uk](mailto:felix.nicol@strath.ac.uk)

Interest in developing black shales as a hydrocarbon reserve has grown significantly with the development of hydraulic fracturing combined with the use of horizontal drilling (Mouser *et al.* 2016). These methods allow access to previously inaccessible tight hydrocarbons trapped in low permeability shales. It is estimated that 38% of the US hydrocarbon energy will be provided from shale gas by 2040, with shale gas already accounting for 6% of natural gas globally (EIA 2013). Each “frac” consists of injecting 15-20 million litres of fracturing fluid at high pressure down the borehole, consisting of water, proppant, and chemical additives. These chemical additives are mainly organic compounds that alter the environment in the fracked subsurface, creating an opportunity for new microbial life to proliferate (Cluff *et al.* 2014). This proliferation of new species leads to various types of biofouling, such as reservoir souring through sulphate-reducing bacteria (SRB), reduced hydrocarbon volume and quality, bio-clogging by biofilm formation, and equipment corrosion caused by MIC (Microbially Induced Corrosion, Gaspar *et al.* 2014). To combat these effects, anti-microbial agents (biocides) are introduced in the subsurface along with the fracking fluids, however these are highly toxic and may be harmful to the environment if they migrate out of the shale formation. Additionally, there is evidence that biocides exhibit reduced effectiveness over time due to anti-microbial resistance when the water used for fracturing is recycled (Vikram *et al.* 2014).

Our work aims to reduce the negative effects of microorganisms on hydraulic fracturing operations by exploring innovative strategies to limit or remove the need for anti-microbial agents in fracking fluids. We started by observing the effect of fracking fluid on aerobic bacterium *Bacillus subtilis* growing planktonically in liquid media before testing biofilm formation in a bioreactor while exposed to fracking fluids. We will be using microfluidic techniques to create bioreactors that model fractures in shales in order to best create an analogue for the fracked subsurface. This work will allow us to characterise the microbial life spatially and temporally present within the fractures of a shale, which will in turn help better characterise and constrain the negative microbial effects. These analogues will be used to test the effectiveness of various anti-microbial and pro-biotic approaches to reduce the effects of biofouling. These strategies may either reduce total biocide concentration, trial new agents which have reduced environmental impact, or remove the need for biocides by formulating a pro-biotic approach promoting the occurrence of desirable species rather than problematic ones. This last approach is highly favourable due to its minimal environmental impact, and can be designed to enhance hydrocarbon extraction through production of biosurfactant (allowing improved liberation of hydrocarbon) and generation of biogenic (microbially produced) gas.

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## **MICROBIAL INDUCED CALCITE PRECIPITATION (MICP) AS A VIABLE GROUND ENGINEERING TECHNOLOGY**

**Anderson, M.<sup>1\*</sup>, Minto, J.<sup>1</sup> and Lunn, R.J.<sup>1</sup>**

<sup>1</sup> Department of Civil and Environmental Engineering, James Weir Building, University of Strathclyde, Glasgow, G1 1XJ.

\* [mary.m.anderson@strath.ac.uk](mailto:mary.m.anderson@strath.ac.uk)

In the past decade there has been extensive interest and laboratory research into microbial induced calcite precipitation (MICP), particularly for geotechnical purposes as the traditional techniques of ground improvement, such as grouting or compaction, can be invasive, energy demanding, and expensive. The process of MICP basically involves inducing a reaction between the common soil bacteria *Sporosarcina pasteurii* (*S. pasteurii*) and a solution mix of urea and calcium chloride, to produce calcite crystals (CaCO<sub>3</sub>). MICP is particularly effective when used with fine grained sands as the calcite crystals form a bridge between the individual grains, cementing them together and creating a weak sandstone.

A great deal of research has been conducted on the many factors that affect the efficiency of the process while the various opportunities for using MICP in the field have been discussed hypothetically in numerous research papers. However, the dilemma of how to transfer MICP to the field remains as very few projects have thus far been successfully scaled-up beyond the laboratory bench. The overall aim of this study is to support the up-scaling of MICP to eventual full-scale field implementation by developing an understanding of the influencing factors through a number of initial laboratory experiments. Results, conducted on short sand columns, have produced an indication of the optimum flow rate, bacteria concentration, and number of treatments likely required to produce effective calcite distribution and associated peak strengths. This paper reports on those findings and briefly touches on the initial progress made and how this will contribute to MICP up-scaling efforts.

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