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Microbial ecology and biogeochemistry of nuclear waste storage facilities

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ABSTRACT

The UK nuclear waste legacy consists of complex and heterogeneous wastes contaminated with both radioactive isotopes and toxic, stable co-contaminants. Microbial metabolism has the potential to drastically alter the chemistry of radioactively contaminated environments, complicate waste storage management, altering the structure of nuclear waste storage materials and controlling radionuclide speciation and mobility.

This study aims to explore the microbial ecology and biogeochemistry of extreme environments associated with radioactive waste forms, focusing mainly on two contrasting pond facilities used for nuclear fuel storage at Sellafield.

Water samples from the two ponds were collected and filtered through 0.2 µm membranes. DNA extraction and amplification of the highly conserved 16S rRNA phylogenetic maker gene preceded cloning and sequence analysis. Samples from the indoor pond revealed very low species diversity with an organism most closely related to an uncultured Fe(II)-oxidising *Gallionella* species detected as the dominant bacterial species. The outdoor pond water sample analysis conveyed a far greater species diversity with 72 different bacterial families detected and Bacteroidetes and Cyanobacteria the principle phylogenetic groups. This was consistent with several potential external inputs, including bulk and trace nutrients, into the pond from a range of sources, and also the stimulation of photosynthetic organisms by sunlight.

The identification of these dominant species of microorganisms in the ponds is an important first step in developing a greater understanding of the pond ecology will in turn help to develop a method to control the microbial productivity in the ponds. The results from this study will also lead to a greater understanding of the interactions of radioactive waste with the biosphere and the fundamental role of microorganisms in controlling the environmental behavior of radionuclides in key UK waste forms.

INTRODUCTION

Only in the last few years have we been able to obtain an indication of the level of microbial colonisation of radioactive environments (Lloyd and Renshaw 2005). Microorganisms have been shown to survive and populate extreme habitats (Rothschild and Mancinelli 2001; Cavicchioli 2002), including nuclear fuel waste disposal containers (Stroes-Gascoyne et al. 1997), the water and soils surrounding the damaged core reactor at the Three Mile Island Nucear Power Plant (Booth 1987; Romanovskaya, et al. 2002), and even radionuclide contaminated sediments at the DOE Hanford Site in Washington State, which were found to have a larger microbial biomass than uncontaminated sediment in the surrounding area (Fredrickson, et al. 2004).

This study focuses initially on two nuclear storage ponds at Sellafield, Cumbria, where microbial productivity is a concern.

The First Generation Oxide Fuel Storage Pond (FGSP) is an outdoor storage pond which experiences seasonal algal blooms, which greatly reduces the clarity of the pond water and therefore hinders pond operations. In addition, a combination of windblown particles and the recurring cycles of algal blooms has resulted in the build up of highly radioactive sludge on the bottom of the pond.

The Thorp Receipt and Storage (TR&S) Pond is contained within a building and has higher radioactivity levels than the FGSP pond. It too has biological growth on the pond walls, particularly in the areas with underwater lighting. As with FGSP there is also the presence of solid particulates on the pond floor, which may also contain biological matter, which in turn may be contributing to excessive fluctuations in pond chemistry.

METHODS AND MATERIALS

Sampling and water samples

A stringent sampling campaign was devised to target different locations within the two ponds including depth and proximity to lighting (artificial and natural) collecting water samples and biofilm scrapings from pond wall deposits and also pond floor sludge's.

At present one set of samples from the indoor TRSP has been received as well as weekly samples from the outdoor FGSP (May 2010-Present each sampling compromising of 2 x 500ml water samples).

DNA Extraction and PCR Amplification

Water samples from the ponds were collected in sterile 500ml bottles (from a few centimetres below the surface) and were delivered to the University of Manchester Centre for Radiochemistry Research. They were then filtered through 0.2µm pore size cellulose membrane filters and the DNA extracted; using PowerSoil® DNA Isolation Kit.

Full 16s rRNA gene was selectively amplified by the Polymerase chain reaction (PCR), using oligodeoxynucleotide primers, designed to anneal to conserved regions of the eubacterial16S rRNA (Eden et al. 1991).

Purified DNA (2 µl) and 1 µl of 25 µM
primer stocks were added to the reaction mix to a final volume of 50 µl. Primers used were the broad-specificity 8F forward primer; (5'-AGAGTTTGATCCTGGCTCAG-3'), and the reverse primer 1492R (5'-GTTACCTTGTTACGACTT-3) (Eden et al. 1991), and samples amplified using an iCycler (BioRad) thermal cycler.

The purity of the amplified product was determined by electrophoresis of 10 µl samples in a 1.5% agarose Tris-borate-EDTA (TBE) gel. DNA was stained with ethidium bromide and viewed under short-wave UV light.

Indoor pond samples were also subject to ARISA analysis, a DNA profiling technique that amplified the variable length intergenic spacer region between the genes encoding 16S and 23S rRNA in bacteria (Cardinale et al 2004).

RESULTS

Indoor pond (FGSP) Results

ARISA analysis conveyed that the microbial community in the indoor pond sample was of very limited diversity (each DNA band corresponding to a bacterial population) as shown in Figure 1.

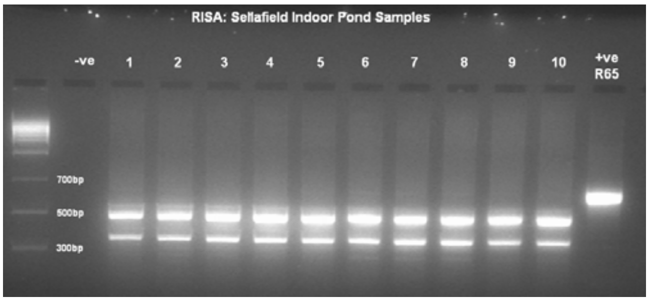

Figure 1 ARISA image for indoor pond

Following the ARISA analysis a full clone library was completed. Results from 39 clones yielded 3 different species as shown in table 1, with the most dominant species being most closely related to an Uncultured *Gallionella* sp (92.3%), in the Beataproteobacterial phylogenetic group being the most dominant phylogenetic class (Figure 2). Interestingly, this organism is implicated in Fe(II) oxidation using either oxygen or nitrate as an electron acceptor.

Table 1 Sellafield indoor pond sample full 16S Clone Library results

Clone Representative	No in Clone Library	Closest Matching Micro –organism	Identities (% Match)	% Present	Phylogenetic Class
S666-10	36	Uncultured Gallionella sp.	(97%)	92.3%	Betaproteobacteria
S666-26	2	Bradyrhizobium sp. II-47	(99%)	5.1%	Alphaproteobacteria
S666-46	1	Uncultured Comamonas sp.	(97%)	2.6%	Betaproteobacteria

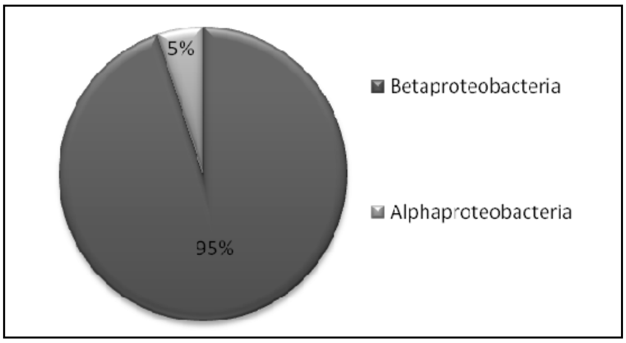

Figure 2 Phylogenetic class results for indoor pond sample.

Outdoor pond (TR&S) Results

Analysis of the first set of outdoor pond samples has shown that there is a much larger diversity of microorganism present, compared to the FGSP sample as shown in Figure 3. From 76 clones, there were 72 different bacterium families. The dominant organisms detected were most closely related to the Sphingobacteria class of Bacteroidetes (82%) and Cyanobacteria (100%) of the Cyanobacteria phylym.

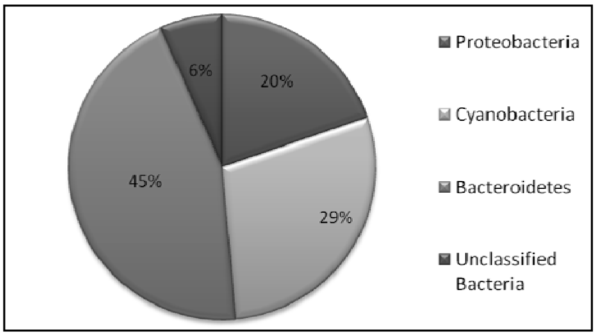

Figure 3 Phylogenetic class results for outdoor pond sample.

DISCUSSION

These preliminary studies have confirmed that the contrasting ponds at Sellafield support very different microbial communities. The indoor pond results suggested that an organisms most closely affiliated with *Gallionella sp.* dominates; a common aquatic iron-oxidizing, chemolithotroph which uses ferrous iron as energy source. Its presence in these ponds requires further study as it could play a role in the corrosion of Fe-based materials stored.

Outdoor pond (TR&S) results indicate a much richer diversity of common aquatic microorganisms, the most dominant plylogenetic class being Bacteroidetes, then Cyanobacteria and Proteobacteria. Ongoing work will help to identify the key organisms responsible for contributing to the increased turbidity during summer period, and how this in turn affects the pond chemistry.

CONCLUSION

Preliminary results have helped to develop the basis for the microbial ecological analysis, as working primer and enzyme sets for PCR work have been established, and a basic ecology of the ponds determined.

Further work will focus on further clone library work to acquire a better enhanced understanding of the microbial ecology of both ponds, including primer sets targeting additional functional groups including eukaryotes, and correlations of community dynamics with changes in pond water geochemistry. Further to clone library analysis, algal microscopy analysis will also be undertaken in conjunction with quantitative biomass analysis via ATP and fluorescence spectrophotometry for chlorophyll.

Biogeochemical characterisation of the samples will include anion and cation analysis using Dionex ion chromatography for electron acceptors (including nitrate and sulfate) and organic acids and ICP-AES/MS for trace metals, as well as Gamma spectroscopy for radiochemical analyses.

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