

The MIND logo, featuring a large black 'D' with a white 'M' inside it, and the word 'MIND' in black capital letters below it. A green DNA double helix is positioned between the 'M' and 'N'. The background is a light blue and white DNA double helix.

MIND
Microbiology In Nuclear waste Disposal
(GRANT AGREEMENT: 661880)

DELIVERABLE D1.9
Final synthesis report for MIND WP1

Editor:	Joe Small (NNL)
Date of issue of this report:	29.05.2019
Report number of pages:	40 + 27 appendices
Start date of project:	01/06/2015
Duration:	48 Months

This project has received funding from the Euratom research and training programme 2014-2018 under Grant Agreement no. 661880

Dissemination Level

PU	Public	PU
PP	Restricted to other programme participants (including the Commission)	
RE	Restricted to a group specified by the partners of the MIND project	
CO	Confidential, only for partners of the MIND project	

Publishable Summary

The MIND project targets a number of high urgency and high importance topics identified in the Strategic Research Agenda, by IGD-TP 2011. The scientific technical work programme of MIND was divided into two operative Work Packages (WP 1 and 2) where WP1 addresses the behaviour of intermediate level wastes (ILW) and WP2 focusses on the engineered barrier system for high level waste (HLW). The objectives of MIND WP1 were to reduce the uncertainty of safety-relevant microbial processes controlling radionuclide, chemical and gas release from long-lived ILW containing organics. The outcome of MIND WP 1 research was presented in 9 deliverable reports. In addition 11 peer reviewed open access journal papers have been published to date. In this synthesis report the findings of MIND WP1 have been discussed in relation to the following topics:

1. Organic degradation
2. Gas generation / consumption
3. Radionuclide interactions
4. Limits of microbial life in an ILW repository

Organic degradation

The MIND project has examined the combined effects of irradiation and biodegradation of; cellulose, polyvinylchloride (PVC), bitumen and polystyrene based ion exchange resins under alkaline conditions. In this first study of this type the organic materials were mainly irradiated with maximal doses of gamma radiation (1 M Gy, ^{60}Co), in most cases in pH 12.5 $\text{Ca}(\text{OH})_2$ solution. The biodegradation of irradiated and hydrolysed materials was then examined. In the case of cellulose, dissolved organic carbon (DOC) and isosaccharinic acid (ISA) generation was enhanced by irradiation but this stimulated higher levels of microbial activity (fermentation), which led to a decrease in pH, to 11 which would be expected to reduce the rate of formation of ISA by alkaline hydrolysis. The MIND project has thus provided further evidence of the biodegradation of ISA under strongly alkaline conditions.

Pure PVC polymer was found to be not biodegradable under anaerobic alkaline (pH 10) conditions, but some additives (plasticisers and fire retardants) present in PVC sheet were biodegradable and capable of nitrate reduction at pH 10. Irradiation enables minor biodegradation of the polymer, but the biodegradation of additives is reduced slightly. Pure phthalate additives, which have potential to form complexes with radionuclides, were not found to be biodegradable under the pH 10 conditions. The rather limited overall biodegradation of such PVC materials suggests that these organic materials should not provide a significant source of microbial (CH_4) gas generation.

Study of nitrate containing bituminised wastes examined the ability of known organic bitumen degradation products to consume nitrate leaching from aged bitumen samples under Boom Clay and alkaline, cement buffered pH conditions. Acetate was found to be the most effective electron donor followed by formate and oxalate. Microbes were also observed to grow on the surface of the bitumen, possibly using it as a nutrient and energy source. In the case of a microbial inoculum adapted to alkaline conditions nitrate reduction was found to be most efficient at pH 10.5.

Studies of ion exchange resin degradation indicate that microorganisms are capable of utilising ion exchange resin radiolysis products (amines, benzene, ketones), especially by coupling their oxidation to reduction of sulfate, which itself will be readily sourced from cationic resins. Ion exchange resins thus have the potential to undergo biodegradation without addition of electron acceptors. Breakdown of the resin by radiolysis is an important step in the overall degradation process liberating soluble organic carbon. At high doses (1 M Gy) the resins develop strong acidity, which may

prevent subsequent biodegradation unless the waste resins are conditioned or radiolysis products are diluted or dispersed. At lower doses (100 kGy) amines released from anionic resins have been shown to be able to fuel sulfate reduction processes. Oxidation of the amine functional groups by such biodegradation may eliminate any radionuclide complexation effects of released amines. Hydrogen and CO₂ are the main gaseous radiolysis products detected, but CH₄ gas formation has not been observed in the current microcosm experiments undertaken due to the presence of sulfate.

Gas generation / consumption

Microbiological and modelling studies of a long term (18 y) Gas Generation Experiment (GGE) has provided further understanding of processes of CH₄ generation from cellulose and steel containing low level waste (LLW). The GGE illustrates how microbial processes can neutralise pH initially buffered by concrete by fermentation of organic waste. The GGE thus provides an insight into how LLW/ILW repositories with higher proportions of concrete may be neutralised over longer periods of time. A doubling of gas generation rate occurring after 8 years is attributed to neutralisation and homogenisation of pH and the growth of organic consuming methanogens in initial alkaline regions of the experiment. Initially, CH₄ generation mainly occurred in neutral pH waste drums by methanogens utilising H₂ generated by anaerobic corrosion. Sulfate reduction occurs in parallel to methanogenesis, but at a low level limited by its slow release from solid wastes. High concentrations of sulfide at the initial stages of the experiment may also have inhibited the initial rates of gas generation in the GGE. Higher rates of sulfate input, such as through groundwater would be expected to slow the rate of methanogenesis by competition for electron donors.

In the case of *in situ* H₂ injection in borehole experiments at the Mont Terri URL, reaction of H₂ with nitrate and sulfate electron acceptors has shown to be rapid. In the case of sulfate reduction it has been impossible to fully exhaust the supply of sulfate provided by diffusion from the Opalinus Clay. It is apparent, from microcosm experiments and also from observations from the GGE that sulfate concentration must be lowered to very low levels (<1 µM) before methanogenesis can be established. The configuration of a sand:bentonite column experiment allows a much greater proportion of H₂ to react with sulfate for the growth of methanogens to be observed. Reactive-transport modelling approaches developed through the MIND project have been used to interpret both the GGE and H₂ injection experiments.

Radionuclide interactions

Studies of radionuclide-microbe interactions were not a major component of the MIND project, nevertheless a number of scientific observations have been made that are relevant to the discussion of microbiology and nuclear waste disposal. For example, interpretation of geochemical data from the GGE is consistent with attainment of redox equilibrium of iron and sulfur species during degradation of steel and cellulose containing LLW after a period of 18 years. This observation supports the assumption made in performance assessment studies that redox processes, including multivalent radionuclide speciation (e.g. Se, Tc, U, Np, Pu), attain equilibrium over the timescales of nuclear waste disposal. In the case of the GGE the relatively rapid attainment of redox equilibrium has been mediated by microbial processes.

Microbes have an important role in the biodegradation of organic complexants, which can affect radionuclide solubility and sorption. In particular, this is highlighted by further study within MIND of the formation and biodegradation of the cellulose degradation product ISA. In addition, MIND research has relevance to the consideration of potential radionuclide complexants released from PVC, bitumen and ion exchange resins.

Other MIND studies have examined the speciation and reaction of radioelements and their chemical analogues. Spectroscopic techniques have been developed to examine the complexation of uranium

with cellulose degradation products. Studies of the fate of Ni and U complexed with the cellulose degradation product ISA during biodegradation reveal how such contaminants may be sequestered in secondary sulfide and other mineral phases that are formed by biodegradation of ISA and other organics released from ILW. Studies of direct interactions of radionuclides with bacteria isolated from bentonite illustrate mechanisms of bioprecipitation by the bacteria to control toxicity of Se and processes of biosorption where Eu is associated with phosphate groups present on the bacterial cell surface.

Limits of microbial life in an ILW repository

The principal uncertainty concerning microbial processes and the disposal of ILW is the ability of microbes to grow and metabolise under the high pH conditions that prevail in cementitious ILW waste packages and engineered barriers. Physical limitations, such as the requirement for space to grow and availability of water will also be relevant, but are likely less important than in the case of bentonite backfilled engineered barrier system for HLW considered by MIND WP2.

The ability of microbes to grow in high pH environments is linked to the amount of chemical energy available for metabolism. Under anaerobic conditions relevant to most disposal scenarios nitrate reduction provides the most energy. MIND project studies indicate that pH in the range 10-11 represents a realistic upper limit for microbial nitrate reduction process, including the consumption of ISA and gluconate and organics present in plasticised PVC and bitumen. Faster rates of nitrate reduction were observed for an inoculum collected from a former lime kiln site that has adapted to higher pH conditions than from a Boom Clay inoculum. The high pH adapted inoculum also showed some evidence of survival of pH 12.5 conditions.

Methanogenesis is the lowest energy yielding process of relevance to ILW disposal and is expected to have the lowest pH tolerance. Studies of the GGE imply that pH 9 represents a likely upper limit for active methanogenesis, although other toxicity effects and competition with sulfate reduction and fermentation processes may further reduce this threshold to pH 8. It is also evident from the GGE studies and other smaller scale high pH microcosm experiments that heterogeneity of pH and the occurrence of low pH micro-niches, where microbes may thrive, are important to defining such pH thresholds.

The MIND project studies also indicate that a number of other chemotoxic effects may result from the contaminants presented in ILW (e.g. Se, Cs) and that microbial processes may generate toxic species such as H₂S, which may exert a self-limiting effect on the rate of microbial activity.

Outlook

Understanding of CH₄ generation and H₂ consumption processes has benefitted from use of *in situ* experiments within the MIND project. Further study of the competition between sulfate reduction and methanogenesis is critical to developing predictive models of these processes. MIND has also revealed new insights into the degradation processes of organic wastes present in ILW. Further study of these processes under *in situ* conditions is warranted, including processes within wasteforms and using borehole experiments to study host rock interactions. Such studies should develop the microbiological techniques used within MIND, alongside geochemical, mineralogical characterisation; examination of radionuclide complexation, and modelling. pH thresholds for the activity of nitrate reduction and methanogenic processes have been defined through MIND WP1, but there is scope to define limits for sulfate reduction processes, which has wider relevance to MIND WP2. Study of the spatial scale and magnitude of pH heterogeneity in ILW wasteforms and engineered barriers and the occurrence of niches for microbial activity is however required to fully define such pH thresholds.

MIND		
Work Package: 1	MIND Document no.:	Document type:
Task: -	MIND D1.9	R = report
Issued by: NNL		Document Status:
Internal no.: NNL 15013		Final
Document Title: Final synthesis report for MIND Workpackage 1		

Contents

1	Introduction	7
1.1	Participating partners	7
1.2	Objectives and task list in work package 1	8
1.3	Deliverables and peer reviewed publications	10
1.4	Synthesis report structure	11
2	Organic waste degradation	13
2.1	Inventory and state of previous knowledge	13
2.2	Cellulose.....	14
2.3	Polyvinylchloride	15
2.4	Bitumen.....	17
2.5	Ion exchange resins	19
3	Microbial gas generation and consumption	21
3.1	Microbial gas generation from cellulose-containing wastes	21
3.1.1	GGE overview description	22
3.1.2	Geochemistry and modelling	22
3.1.3	Microbiological characterisation	23
3.1.4	Summary	24
3.2	Microbial consumption of hydrogen	25
3.2.1	Sulfate reduction and methanogenesis	25
3.2.2	Nitrate reduction	26
3.3	Methanogenesis and H ₂ consumption; summary and outlook.....	28
4	Radionuclide interactions.....	29
4.1	Observations of redox equilibrium.....	29
4.2	Speciation and interactions with cellulose degradation products	30
4.2.1	Spectroscopic studies.....	30
4.2.2	Biogeochemical interactions	31
4.3	Microbe interactions with Se and Eu.....	31
5	Limits of life in cementitious ILW.....	33
5.1	pH limits	33
5.2	Chemical toxicity effects	34
6	Implications and outlook	36
7	Acknowledgement	38

8	References	38
	Appendix A Published summaries of WP1 deliverables.....	41
	Appendix B Supplemental reporting of studies of ion exchange resin degradation	49

1 Introduction

The MIND project targets a number of high urgency and high importance topics identified in the Strategic Research Agenda, (SRA) (IGD-TP, 2011), focusing mainly on Key topic 2: Wasteforms and their behaviour and Key topic 3: Technical feasibility and long-term performance of repository components. The Scientific Technical Work Programme of MIND was divided into two operative Work Packages (WPs):

WP1 addresses SRA Key topic 2: Remaining key issues for the geological disposal of ILW concerning the long-term behaviour, fate and consequences of organic materials in the waste along with H₂ generated by corrosion and radiolysis. The objectives of WP1 consequently are to reduce the uncertainty of safety-relevant microbial processes controlling radionuclide, chemical and gas release from long-lived intermediate level wastes (ILW) containing organics.

WP2 addresses SRA Key topic 3: Remaining key issues for the geological disposal of HLW (High Level Waste) concern the factors controlling sulfide production in the geosphere, including to what extent microorganisms can accelerate canister corrosion in the near-field either by hydrogen scavenging or by sulfide and/or acetate production. Further, it is important to identify conditions under which relevant bentonites inhibit bacterial activity, and to understand whether microorganisms can accelerate degradation of bentonite-based buffers and influence the long-term behaviour of plug systems and seals.

1.1 Participating partners

There were 15 partners in MIND, of which 10 partners from 7 countries participated in WP1 (Table 1-1).

Table 1-1: MIND partners participating in WP1f

Acronym	Name	Country
NNL	National Nuclear Laboratory	United Kingdom
CV REZ	Centrum Vyzkumu REZ S.R.O..	Czech Republic
EPFL	Ecole Polytechnique Federale De Lausanne	Switzerland
HZDR	Helmholtz-Zentrum Dresden-Rossendorf e.V.	Germany
SCK•CEN	Belgian Nuclear Research Centre	Belgium
TUL	Technická Univerzita V Liberci	Czech Republic
TVO	Teollisuuden Voima Oyj	Finland
UGR	Universidad De Granada	Spain
UNIMAN	University of Manchester	United Kingdom
VTT	Teknologian Tutkimuskeskus	Finland

1.2 Objectives and task list in work package 1

Long-lived intermediate waste (ILW) requiring geological disposal can include a variety of anthropogenic polymers (including contaminated plastic materials and organic ion exchange materials) and natural (cellulose) organic wastes. In addition, bitumen has been used on an industrial scale in France and Belgium as an encapsulant for soluble reprocessing residues, and organic resins have been proposed to encapsulate reactive metal wastes. Remaining key issues for the geological disposal of ILW concern the long-term behaviour, fate and consequences of such organic materials along with H_2 generated by corrosion and radiolysis. These electron donors in combination with electron acceptors present in the repository or host rock (e.g. NO_3^- , Fe(III), SO_4^{2-} , CO_3^{2-}) provide potential energy sources or electron sinks to fuel microbial processes. Such processes have the potential to affect radionuclide release directly (3H , ^{14}C) by affecting gas generation and oxidation and indirectly by catalysing speciation-relevant chemical reactions e.g. involving Se, Tc, U, Np, Pu. In addition some microbial-influenced processes may affect the transport and mobility properties of ILW and engineered barriers and the re-saturation of the repository.

The behaviour of ^{14}C and 3H is directly intertwined with key reactions catalysed by microbial activity. Biodegradation of organic ILW with associated gas generation (CH_4 , CO_2 , H_2) and consumption depends on the presence of electron acceptors of which sulfate, whose reduction requires microbial catalysis, is in many cases the most relevant.

Organic degradation products have the potential to form aqueous complexes with radionuclides, which could enhance their release from the repository and facilitate radionuclide transport in water through the barrier systems. A better understanding of the role that microbial processes play in altering the chemical complexity of organic radiolysis and hydrolysis products, resulting in possible complete oxidation to CO_2 and bicarbonate, will help reduce uncertainties in safety case analysis. Similarly, further understanding of the role that microbial processes, fuelled by organic materials and H_2 , play in controlling ILW repository chemistry (pH, Eh) and related speciation of radionuclides will also help gain further confidence.

This work package addressed the behaviour of ILW containing a significant fraction of organic wastes emplaced in a deep geological disposal concept that includes cementitious materials in the engineered barriers (Figure 1-1). The experimental work examined processes of organic degradation under radiation and chemical conditions representative of such a repository, with explicit consideration of microbial viability under *in situ* conditions, considering representative alkaline pH conditions and utilising existing long-term waste degradation and underground rock laboratory (URL) experiments. Models coupling microbial growth with geochemistry have been developed to assist in the understanding of processes of microbial gas generation and consumption.

The main objective of MIND WP1 was:

- To reduce uncertainty of safety-relevant microbial processes controlling radionuclide, chemical and gas release from long-lived intermediate level wastes (ILW) containing organics (SRA Key topic 2 sub topic 2),

which, included a number of more detailed objectives:

- To quantify the combined rates of biodegradation, radiolysis and hydrolysis of anthropogenic organic polymers and cellulose present in ILW under disposal conditions.
- To identify key chemical species resulting from organic ILW biodegradation, radiolysis and hydrolysis and their effects on radionuclide speciation and mobility.
- To establish the *in situ* chemical and physical conditions that may limit microbial activity in EU repository concepts for ILW utilising cementitious materials within a neutral pH host rock.

- To examine the microbial generation and consumption of CH_4 and H_2 under ILW repository conditions.
- To understand the effect of ILW heterogeneity on bioprocess pathways, pH and redox conditions, barrier degradation and radionuclide release.

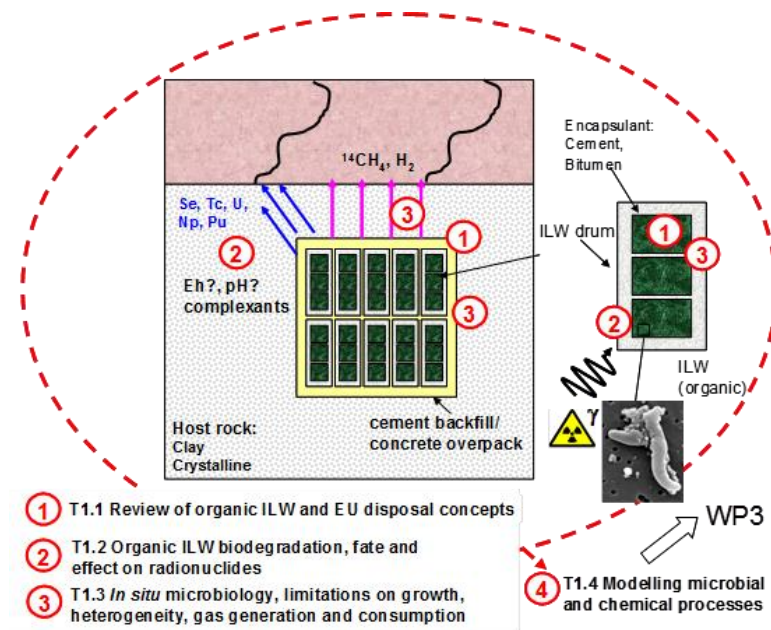


Figure 1-1. Schematic of key microbiological issues concerning geological disposal of ILW together with the four Tasks that comprised MIND WP1.

The work package was subdivided into four task areas (Figure 1-1):

- Task 1.1 Review of organic ILW and EU disposal concepts, which delivered (D1.1) a state of the art review of the nature and inventory of organic waste materials present in ILW/LLW, together with a review of current understanding of their degradation. The review completed during the first 6 months of the project also defined the conditions relevant to subsequent tasks.
- Task 1.2 Organic ILW biodegradation, fate and effect on radionuclides. This task examined the combined effects of radiation and biodegradation on a range of organic materials which are present in ILW, or which are used to encapsulate ILW (D1.2, D1.3, Appendix B). The studies examined processes between pH 9 to 12 conditions buffered by cementitious materials. The task included studies of radionuclide interactions and examined the fate of organic degradation products and their complexation effects (D1.4, D1.5).
- Task 1.3 *In situ* microbiology, gas generation and consumption. This task included studies of microbial gas generation from cellulose, utilising a 20 year large scale gas generation experiment (Small et al, 2008) delivering new data concerning the microbial communities present in the experiment (D1.6). The task also included studies of an existing H_2 injection experiment at the Mont Terri URL (Bagnoud et al, 2016a,b), which had the objective to study whether methanogenesis could be established under *in situ* Opalinus Clay conditions (D1.7).
- Task 1.4 Modelling microbial and chemical processes. This task undertook the development of coupled microbial kinetic, chemical speciation, and transport models that were applied to *in situ* experiments (Task 1.3). The deliverable from this task (D1.8) describes this modelling methodology and its potential for wider use by end users.

1.3 Deliverables and peer reviewed publications

The outcome of MIND WP 1 research was presented in 9 deliverable reports (Table 1-2). In addition 11 peer reviewed Open Access journal publications have been published (Table 1-3) and it is anticipated that further publications will arise in future.

Table 1-2: MIND WP1 Deliverables

Deliverable	Deliverable Description	Lead Beneficiary
D1.1	Review of organic wastes and their biodegradation under ILW repository conditions	NNL
D1.2	Effects of radiation and microbial degradation of ILW organic polymers	UNIMAN
D1.3	Radiation and microbial degradation of bitumen	SCK•CEN
D1.4	A Report of Uranium(VI) speciation associated with cellulose degradation products	HZDR
D1.5	Microscopy and element mapping characterization studies of radionuclide microbe organic interactions	UGR
D1.6	Relevant microbial metabolic processes and limits on chemical conditions leading to methane generation in LLW and ILW	VTT
D1.7	Methane generation in the Mt Terri URL and the underlying microbial processes	EPFL
D1.8	Modelling of microbial processes relevant to ILW disposal	NNL
D1.9	Final synthesis report for MIND WP1 (this report)	NNL

Table 1-3: Peer reviewed journal publications related to MIND WP 1

Ref	MIND WP 1 peer reviewed journal publications sorted by year and lead author
P1	Bassil, N.M. and Lloyd, J.R. (2017) Draft Genome Sequences of Four Alkaliphilic Bacteria Belonging to the <i>Anaerobacillus</i> Genus. <i>Genome Announcements</i> , Volume 5, Issue 3, American Society for Microbiology, 2017, e01493-16. https://doi.org/10.1128/genomeA.01493-16
P2	Sanchez-Castro, I., Bakkali, M. and Merroun, M.L. (2017) Draft Genome Sequence of <i>Stenotrophomonas bentonitica</i> BII-R7T, a Selenite-Reducing Bacterium Isolated from Spanish Bentonites. <i>Genome Announcements</i> , Volume 5, Issue 31, American Society for Microbiology, 2017, e00719-17. https://doi.org/10.1128/genomeA.00719-17
P3	Sanchez-Castro, I., Ruiz-Fresneda, M.A., Bakkali, M., Kämpfer, P., Glaeser, S.P., Busse, H.J., López-Fernández, M., Martínez-Rodríguez, P., and Merroun, M.L. (2017) <i>Stenotrophomonas bentonitica</i> sp. nov., isolated from bentonite formations. <i>International Journal of Systematic and Evolutionary Microbiology</i> 67:2779–2786. https://doi.org/10.1099/ijsem.0.002016

Ref MIND WP 1 peer reviewed journal publications sorted by year and lead author

- P4** Small, J.S., Nykyri, M., Vikman, M., Itävaara, M., Heikinheimo, L. (2017) The biogeochemistry of gas generation from low-level nuclear waste: Modelling after 18 years study under in situ conditions. *Applied Geochemistry*, 84, 360-372. <https://doi.org/10.1016/j.apgeochem.2017.07.012>
- P5** Bassil, N.M. and Lloyd, J.R. (2018) *Anaerobacillus isosaccharinicus* sp. nov., an alkaliphilic bacterium which degrades isosaccharinic acid International . *International Journal of Systematic and Evolutionary Microbiology*. <https://doi.org/10.1099/ijsem.0.002721>
- P6** Kuippers, G., Boothman, C. Bagshaw, H. Ward, M., Beard, R., Bryan, N. and Lloyd, J.R. (2018). The biogeochemical fate of nickel during microbial ISA degradation; implications for nuclear waste disposal. *Scientific Reports* Volume 8, Article number: 8753. <https://doi.org/10.1038/s41598-018-26963-8>
- P7** Nixon S.L., van Dongen B.E., Boothman C., Small J.S. and Lloyd J.R. (2018). Additives in Plasticised Polyvinyl Chloride Fuel Microbial Nitrate Reduction at High pH: Implications for Nuclear Waste Disposal. *Frontiers in Environmental Science*. 6:97. <https://doi.org/10.3389/fenvs.2018.00097>
- P8** Ruiz-Fresneda, M.A., Delgado Martín, J., Gómez Bolívar, J., Fernández Cantos, M.V., Bosch-Estévez, G., Martínez Moreno, M.F. and Merroun, M.L. (2018). Green synthesis and Biotransformation of amorphous Se nanospheres to trigonal 1D Se nanostructures: impact on Se mobility within the concept of radioactive wastes disposal. *Environmental Science: Nano* <https://doi.org/10.1039/c8en00221e>
- P9** Shrestha, R., Steinová, J., Ševců, A., Kovářová, H., Černoušek, T., Kokinda, J., Hadravová, R. (2018) The effect of caesium ions on a natural anaerobic microbial community. *Waste Forum*, Vol 2, CEMC, Prague, 2018 <http://odpadoveforum.cz/TVIP2018/prispevky/135.pdf>.
- P10** Boylan, A.A., Perez-Mon, C., Guillard, L., Burzan, N., Loreggian, L., Maisch, M., Kappler, A., Byrne, J.M. and Bernier-Latmani, R. (2019) H₂-fuelled microbial metabolism in Opalinus Clay. *Applied Clay Science* 174, p 69–76 <https://doi.org/10.1016/j.clay.2019.03.020>
- P11** Vikman, M., Marjamaa, K., Nykyri, M., Small, J.S., Miettinen, H., Heikinheimo, L., Haavisto, T. and Itävaara, M. (2019) The biogeochemistry of gas generation from low-level nuclear waste. *Applied Geochemistry*, 105, 55–67. <https://doi.org/10.1016/j.apgeochem.2019.04.002>
-

1.4 Synthesis report structure

This final synthesis report is structured to discuss the key research outputs from MIND WP1 in relation to the following processes and topics:

1. Organic degradation
2. Gas generation / consumption
3. Radionuclide interactions
4. Limits of microbial life in an ILW repository

A summary of this report is provided at the start of the document (Page1). Following this introduction (Section 1) each of the above topics comprises a major section of this report (Sections 2 to 5). Section 6 then discusses implications and highlights remaining uncertainties concerning microbiology and the disposal of low and intermediate level nuclear waste.

Appendix A provides copies of the summaries of each deliverable. Appendix B reports additional information concerning studies of ion exchange resin degradation

The four research areas align with topics discussed by the Implementers Review Board (IRB) that will be published in their evaluation report (MIND deliverable D4.6). Table 1-4 cross references the MIND WP 1 deliverables and current publications that are relevant to each of these topic areas.

Table 1-4: Cross reference of MIND WP 1 Deliverables and publications with research topics

Document	Process / topic			
	Organic Degradation	Gas Generation / Consumption	Radionuclide interactions	Limits of microbial life
D1.1	x	x	x	
D1.2	x			x
D1.3	x			x
D1.4	x		x	
D1.5			x	x
D1.6	x	x		x
D1.7	x	x		x
D1.8	x	x	x	x
D1.9	x	x	x	x
P1	x			x
P2			x	
P3			x	
P4	x	x		x
P5	x		x	x
P6	x		x	
P7	x			x
P8			x	
P9			x	x
P10		x		
P11	x	x		x

2 Organic waste degradation

2.1 Inventory and state of previous knowledge

Deliverable D1.1 (Abrahamsen et al, 2015; NNL lead) presents inventory information concerning the distribution of the different types of organic material present in ILW and some LLW collated from published national sources. The review confirmed that the materials selected for study in the MIND project; bitumen, organic ion exchange resins and halogenated polymers (PVC), are present in significant amounts in the national inventories. Cellulose is present in generally lower amounts in most inventories. The distribution of these main material types varies, halogenated polymers being present in the highest proportion in the French and UK inventories, with ion exchange resins dominating the inventories from Finland, Sweden and Switzerland. Bitumen is used for an encapsulant and is not strictly speaking a waste material. Bitumen is commonly used to immobilise ion exchange resins. In Belgium and France bitumen was used to encapsulate nitrate and sulfate salts that arise from fuel reprocessing.

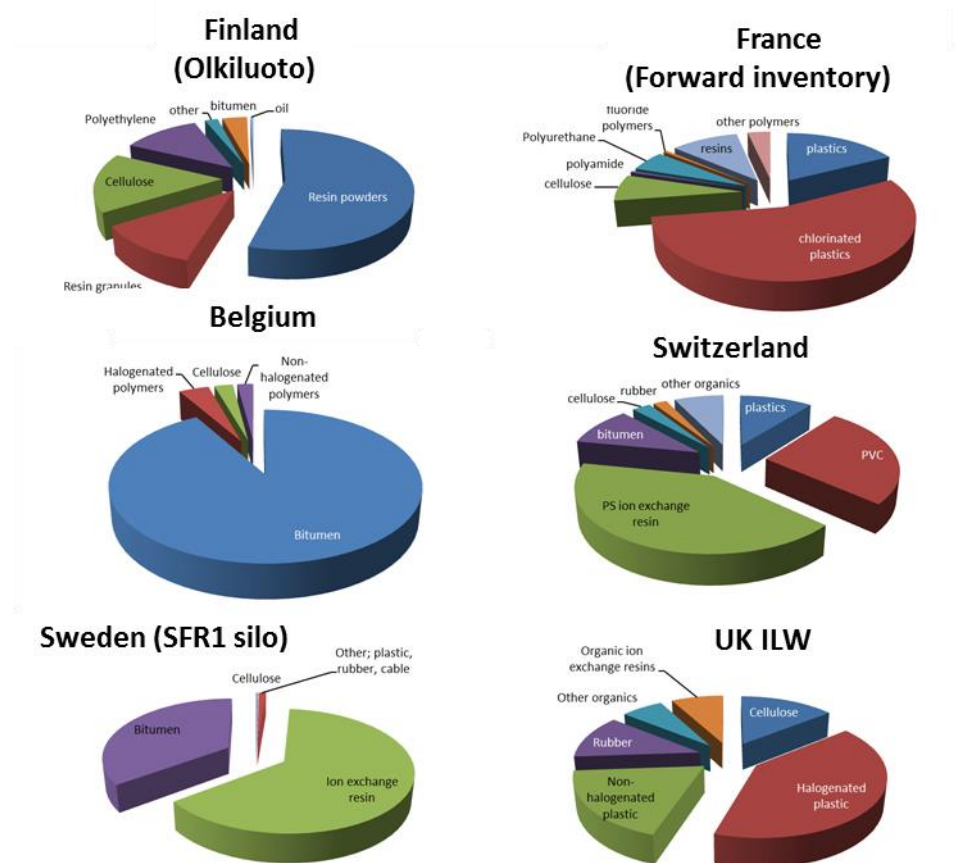


Figure 2-1 Examples of the distribution of organic materials (by mass) present in the ILW inventories of some European states (see D1.1 for source data and further information).

Cellulose is present in generally lower amounts in most inventories, but it is established that cellulose is of significance with regard to the strong complexation effect of its alkaline hydrolysis products, such as isosaccharinic acid (ISA). Cellulose is included in the Work Package 1 studies (Section 2.2) to consider the combined effects of irradiation and biodegradation on the hydrolysis process and the extent to which ISA and other hydrolysis products may be decomposed by microbial activity.

Deliverable D1.1 also reviews the knowledge base concerning the chemical and physical structure and degradation of ion exchange resins, halogenated plastics and bitumen. There is an extensive understanding of the radiolytic degradation of bitumen, which yields short chain carboxylic acids. Studies of bitumen therefore have focussed on the subsequent utilisation of these known degradation products as electron donors for the microbial reduction of nitrate present in the Belgian and French wastes (Section 2.4). For ion exchange resins and polyvinylchloride materials previous studies have identified a number of soluble organic species, but have not considered the potential for their biodegradation. The approach to consider these materials within MIND has involved irradiation under neutral and high pH conditions, followed by detailed characterisation, prior to biodegradation coupled to nitrate and sulfate reduction (Sections 2.3 and 2.5).

2.2 Cellulose

Deliverable D1.2 (Nixon et al, 2017; UNIMAN) reports combined irradiation, alkaline hydrolysis and biodegradation studies of cellulose tissue paper. In addition, publications P1 and P2 (Bassil and Lloyd; 2017; 2018) describe a novel bacteria that has been isolated that is capable of utilising the cellulose hydrolysis product ISA and other similar soluble organic compounds. These findings are also relevant to Topic 4, “Limits of microbial life in an ILW repository” and are further discussed in Section 5.

To study the combined effects of irradiation, hydrolysis and biodegradation, cellulose tissue paper (Kimwipes) was irradiated at a total dose of 1 MGy (^{60}Co , gamma) in a $\text{Ca}(\text{OH})_2$ buffered pH12.5 solution. Subsequently, two sets of microcosm experiments were set up with irradiated and non-irradiated cellulose at an initial pH of 12.5 and containing sediment from the Harpur Hill former lime kiln site (Rizoulis et al, 2012), which acted as a high pH adapted microbial inoculum.

Irradiation of Kimwipes induced physical degradation and a change in the colour of the tissue paper and the solution, and induced breakage of the cellulose fibres into short chains, as observed by light and scanning electron microscopy (presented in D1.2). Irradiation also enhanced the production of ISA by the alkali hydrolysis of cellulose (Figure 2-2), in addition higher levels of DOC were measured in the case of the irradiated cellulose (D1.2). The extent of microbial activity in the microcosms that included irradiated cellulose also appeared to be enhanced as apparent by the decline in pH and the higher concentrations of acetate and H_2 (Figure 2-2) that are consistent with enhanced fermentation of the increased levels of DOC, including ISA.

In these experiments the concentration of ISA and DOC would appear to be enhanced by irradiation by a decrease in the degree of polymerisation and an increase in mid chain scission. The higher levels of DOC and ISA have stimulated higher levels of microbial activity (fermentation), which is coupled with a decrease in pH, to 11 which would be expected to reduce the rate of formation of ISA by alkaline hydrolysis. The microcosm experiments with initial $\text{Ca}(\text{OH})_2$ buffered pH have shown that microbial fermentation is possible within a bulk solution pH of 12.5, presumably because of the existence of micro-niches within the solid sediment and cellulose tissue that have allowed the establishment of microbial processes. These findings provide further evidence for the importance of microbial processes in controlling the concentration of ISA by metabolising it via terminal electron accepting processes (Bassil et al, 2015; Rout et al, 2015). The enhanced fermentation that is apparent in the case of irradiated cellulose highlights the importance of lower pH micro-niches within cellulose materials encapsulated in a cementitious matrix that could initiate microbial activity in such wastefoms. This could lead to a lowering of the bulk pH and a reduction in the release of ISA from the wasteform. Further study of processes under *in situ* conditions of such cementitious wastefoms is required to assess the significance of microbial processes on cellulose degradation.

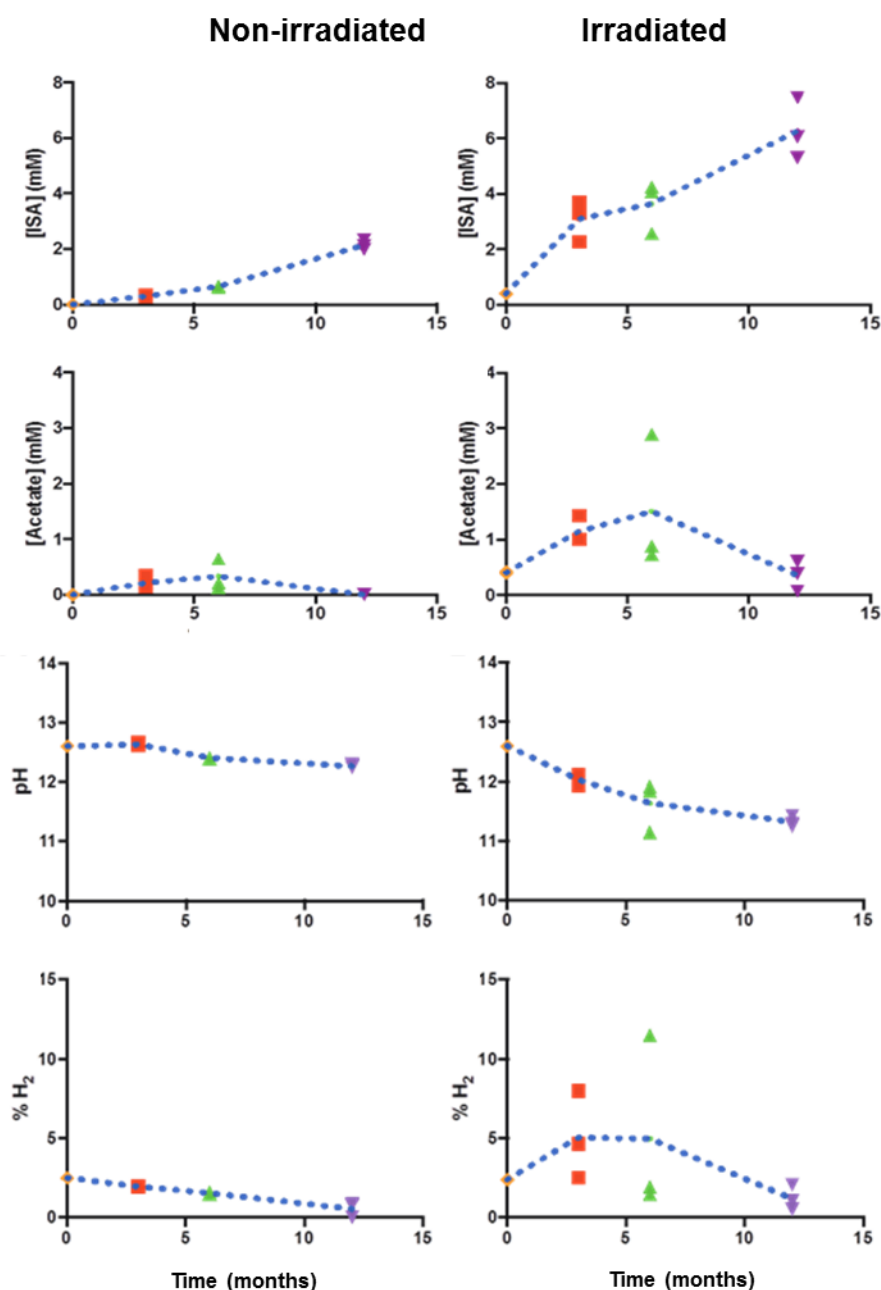


Figure 2-2 Selected data from D1.2 showing concentration of cellulose degradation products in microcosm experiments initially at pH 12.5 with irradiated cellulose (right) and non-irradiated cellulose (left).

2.3 Polyvinylchloride

Plastics represent a significant volumetric contribution of organic material in intermediate and low level waste inventories worldwide (D1.1; Abrahamsen et al., 2015). In the UK, halogenated plastics constitute the largest component by mass of the organic containing waste inventory (NDA, 2014). This waste arises from the use of plastics in maintenance and decommissioning operations at nuclear power plants, and from reprocessing plants and laboratories. PVC is widely used for glove box posting bags, protective suits, and tenting operations. The bulk of the PVC in the UK National Inventory is expected to be flexible films and sheets of PVC derived from these activities. Such PVC sheet contains plasticiser additives, together with fire retardants (D1.2; Nixon et al, 2018). Phthalate

ester additives present in PVC have been invoked as potential complexants of radionuclides, so their release and stability has been an important objective of studies within MIND.

D1.2 and P7 (Nixon et al, 2018) report studies where pure PVC powder and PVC sheet were irradiated (total dose 1M Gy ^{60}Co , gamma) in a saturated $\text{Ca}(\text{OH})_2$ pH 12.5 solution. Biodegradation of this irradiated material was then studied in pH 10 anaerobic microcosm experiments inoculated with Harpur Hill sediment (see Section 2.2) using nitrate as an electron acceptor.

Pyrolysis GC-MS was used to identify the organic moieties that could be released from a sample of plasticised PVC used at the UK Sellafield nuclear site, representing additives that are not covalently bonded to the PVC polymer. Analyses were made before and after leaching in $\text{Ca}(\text{OH})_2$ and irradiation indicated the presence of aryl phosphate ester (mostly triphenyl phosphate; TPP) and phthalate ester (mostly dinonyl phthalate) moieties in all sheet materials analysed. Consequently, additional microcosms were set up to examine whether TPP and phthalate ester could be utilised as electron donors for nitrate reduction.

The pH 10 microcosm experiments indicated that the PVC sheet material could support nitrate reduction to nitrite (Figure 2-3, B), with irradiated sheet (open symbols, dashed lines) showing less (30%; Nixon et al, 2018) nitrate reduction. Non irradiated, pure PVC powder showed no nitrate reduction, but irradiated pure PVC powder showed a small amount of reduction and nitrite formation (Figure 2-3, A).

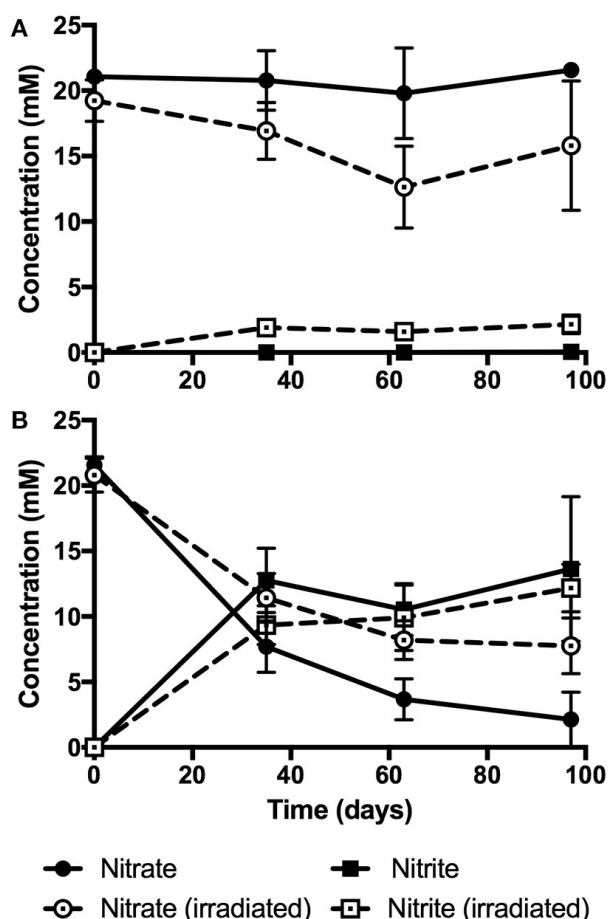


Figure 2-3 Results from pH 10 nitrate reduction enrichment experiments with non-irradiated and irradiated PVC powder (A) and sheet (B) (Nixon et al, 2018).

These results indicate that additives released from PVC sheet in $\text{Ca}(\text{OH})_2$ solution are biodegradable at pH 10. Additional microcosm experiments examining either TPP or phthalate ester as a carbon source indicate that these compounds are not responsible for the nitrate reduction (D1.2; Nixon et

al, 2018) so the exact nature of the additives that fuel the observed nitrate reduction is not known. In the case of irradiated sheet (at maximum dose levels likely for ILW) it is possible that the additives become bonded to the PVC polymer and are less easily leached. In contrast, irradiation of the pure PVC powder liberates some DOC that can be utilised as an electron donor and carbon source for nitrate reduction.

Overall, PVC polymer is not biodegradable under alkaline (pH 10) conditions, but some additives present in PVC sheet are biodegradable. The rather limited overall biodegradation of such PVC materials suggests that these organic materials should not provide a significant source of microbial (methane) gas generation. An important next step is to assess which additives, or combinations of additives, are ultimately fuelling nitrate reduction, what effect these additives have on radionuclide mobility, whether they can fuel gas generation processes, and therefore constrain their overall impact on the safety case for nuclear waste disposal.

2.4 Bitumen

The focus the MIND project research on bituminised waste (as present in the Belgian and French legacy waste inventories, Section 2.1) concerned the ability of known radiolysis and hydrolysis products (acetate, formate and oxalate) to fuel the reduction of nitrate as it leaches from salts embedded in the bitumen.

Deliverable D1.3 (SCK•CEN) reports a series of anoxic microcosm experiments that examined the efficiency of the Boom Clay borehole water microbial community to reduce nitrate leaching from regular and thermally aged non-radioactive Eurobitum in the presence or absence of known bitumen degradation products. It was shown that acetate is the most preferred electron donor for microbial nitrate reduction and the highest nitrate reduction rates were observed in the presence of acetate. Formate also seemed to be an easily accessible electron donor, but in terms of the kinetics of the reaction, not as efficient as acetate in reducing nitrate. Oxalate was the least preferred electron donor for nitrate reduction and was only completely degraded in one out of three replicates. However, calcium oxalate crystals were formed, indicating that if oxalate is present, it will probably be less (bio)available compared to other organic compounds. Next to the added bitumen degradation products, the microbial community was able to use organics that leached from the Eurobitum blocks as electron donors to carry out nitrate reduction. Moreover, a clear biofilm formation was observed on the solid bitumen block in experiments with Boom Clay borehole water, suggesting that microorganisms could enhance the degradation of Eurobitum.

A second set of microcosm experiments, examining a synthetic Boom Clay porewater (reported in D1.3) showed that the presence of natural organic matter in the borehole water did not affect the nitrate reduction experiments. However during these experiments it was found that addition of phosphate increased the rate of nitrate reduction, which is primarily attributed to the requirement for phosphate for microbial growth, although it is possible that phosphate addition may have an effect on the diffusive release of nitrate from bitumen.

The effect of pH on nitrate reduction was examined in a third set of experiments undertaken at pH 9 (Boom Clay) pH 10.5 (old cement water) and pH 12.5 (Ca(OH)_2 buffered) (D1.3). This set of experiments considered acetate as the electron donor and compared the Boom Clay inoculum with the high pH adapted Harpur Hill inoculum studied in D1.2 and by previous UK studies (e.g. Rizoulis et al, 2012). Results presented in Figure 2-4 show that at pH 9 acetate is quite rapidly consumed during the first 10 days and that the concentration of nitrate released is around half that of the sterile control experiments. The behaviour of the Boom Clay and Harpur Hill cases is quite similar except that the Harpur Hill case develops a higher nitrite concentration. In contrast, at pH 10.5 the Harpur Hill microbial consortium is much more efficient at reducing nitrate, and this experiment attains a lower nitrate concentration than at pH 9. At pH 12.5 a small amount of nitrite is detected at the start of the Harpur Hill experiment, but there is little effect on nitrate release from bitumen in either case.

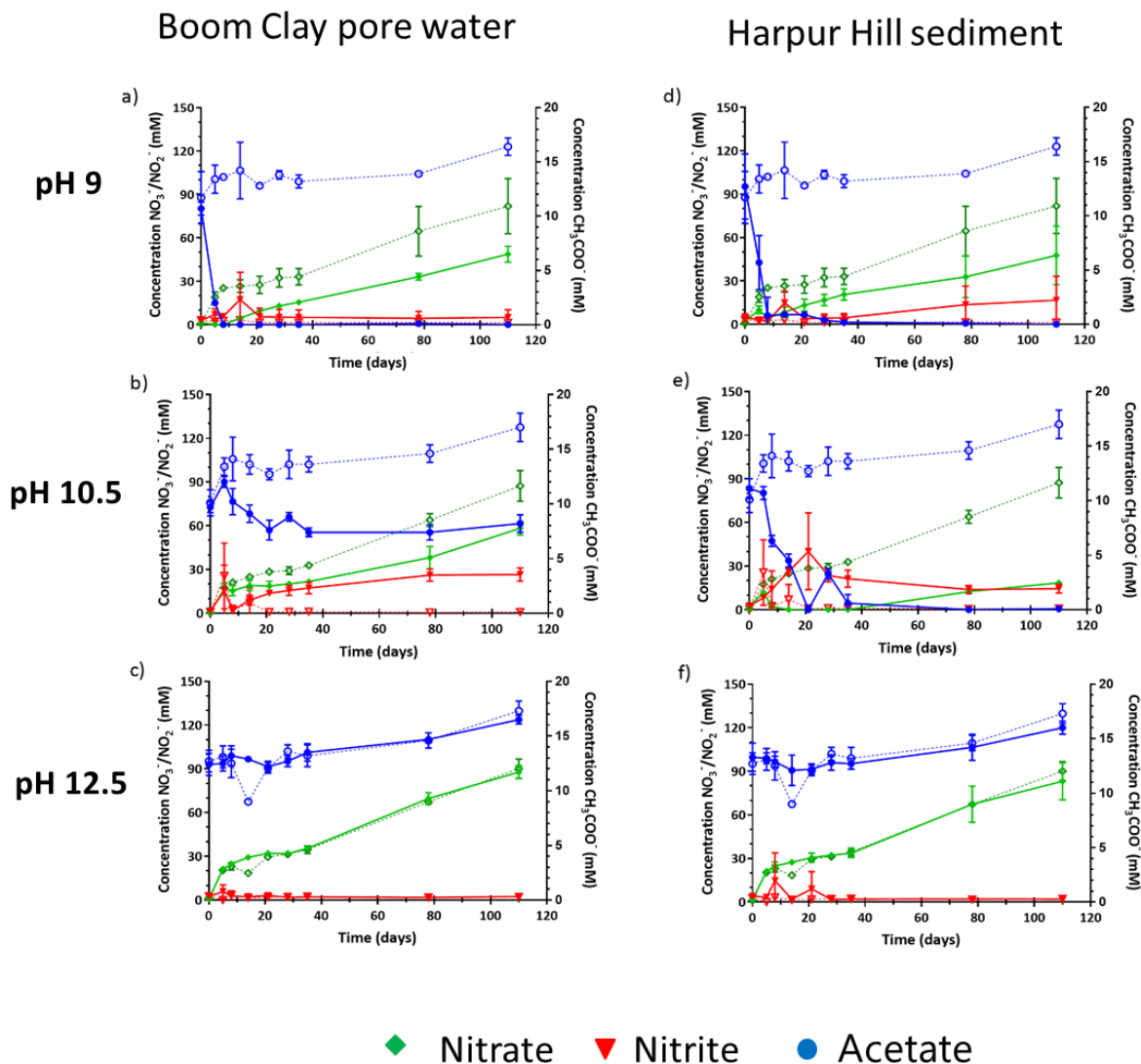


Figure 2-4 Results of anoxic microcosm experiments comparing the release of nitrate, consumption of acetate and formation of nitrite in bituminised waste leaching experiments as a function of pH and inoculated with either Boom Clay or high pH adapted Harpur Hill inocula. Solid symbols and lines represent inoculated experiments, dashed lines and open symbols represent sterile controls (D1.3)

The MIND project studies examining the microbial reduction of nitrate released from bituminised waste, builds upon previous work examining the effect of nitrate and nitrite on dissolved organic matter in Boom Clay (Bleyen et al, 2016) and studies at the Mont Terri URL that have examined microbial nitrate reduction under *in situ* borehole conditions, by acetate, H_2 and electron donors present in Opalinus Clay (Bleyen et al, 2017). A common observation in the experiments reported in D1.3 is the occurrence of a "biofilm" on the bitumen surface, suggesting that in addition to the added organic electron donors (acetate, formate, oxalate) the bitumen may be acting as an additional electron donor or source of nutrients for biomass growth. In addition the experiments examining the effect of pH indicate that microbially mediated denitrification readily occurs at pH 10-11 corresponding to the conditions likely at the interface (chemical disturbed zone) between a concrete barrier system and the host rock. Collectively these studies indicate that nitrate released from such bituminised wastes will be metabolised by the radiolysis and hydrolysis products of the bitumen. However, further *in situ* study is required to assess the significance of results obtained in high pH microcosm experiments, with high fluid content, to consider the *in situ* solid-liquid ratio, transport and chemical conditions of a disposal cell for such wastes.

2.5 Ion exchange resins

Ion exchange resins form a significant component of the organic inventory of LLW and ILW in EU member states. Resins represent the largest single component (>40%) of the organic material in the inventory of ILW and LLW in organic waste inventories from Czech Republic, Finland, Sweden and Switzerland. Within the MIND project, studies are being undertaken with material from Switzerland and the Czech Republic, reported in detail in Appendix B with key findings discussed here.

Ion exchange resins used in the nuclear industry to reduce radioactivity in reactor coolant water and in the treatment of effluents are based on the divinyl benzene (DVB) styrene copolymer (Figure 2-5). DVB styrene is composed of polystyrene chains cross linked by DVB. The styrene aromatic ring can contain either a sulfonyl group (Figure 2-5a) which is negatively charged (strongly acidic) and can be balanced by an exchangeable cation e.g. Na^+ . Alternatively, the styrene can contain a quaternary amine group (Figure 2-5b) and can act as a strong base balanced by an exchangeable anion e.g. Cl^- . The simple cation (or anion) present is easily exchanged by other cations (or anions) present in solution. In nuclear applications, sulfonyl cation resins are used to remove metallic radionuclides (e.g. $^{137}\text{Cs}^+$, $^{60}\text{Co}^{2+}$ and $^{90}\text{Sr}^{2+}$) while amine resins are used to remove anionic radionuclides (e.g. $\text{H}^{14}\text{CO}_3^-$, $^{36}\text{Cl}^-$).

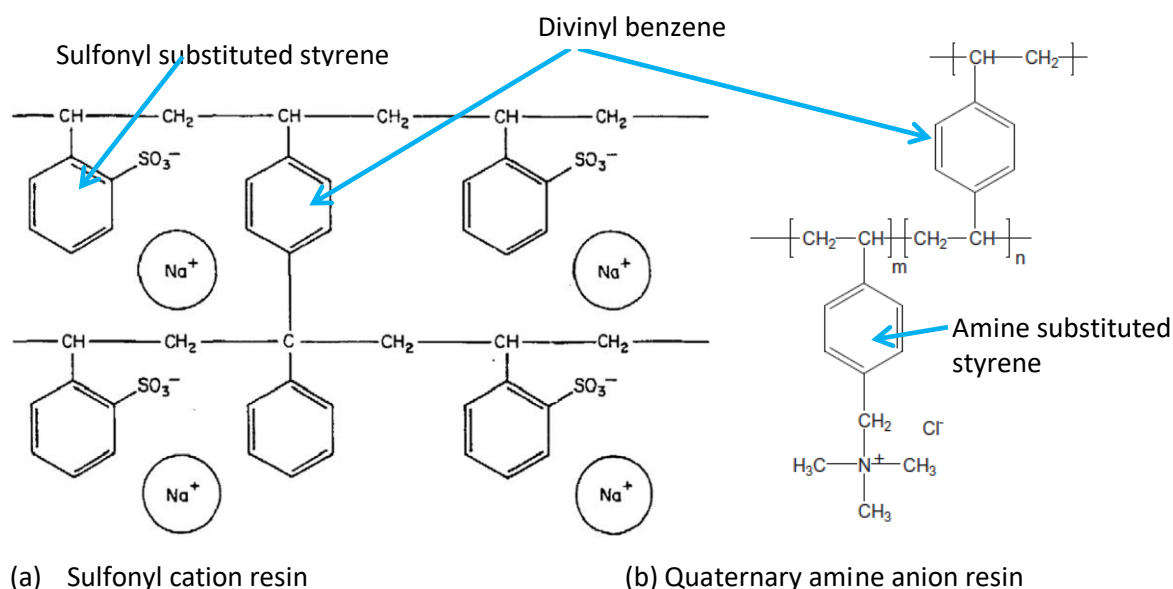


Figure 2-5 Structures of DVB-styrene resin with (a) sulfonyl and (b) quaternary amine functional groups (after Wang and Wan, 2015; Baidak and La Verne, 2010)(D1.1; Abrahamsen et al 2015)

MIND project studies in the Czech Republic (CV-REZ/TUL) and Switzerland (EPFL) (Appendix B) have studied representative mixtures of anionic and cationic resins, as such mixed resins are commonly used. The Czech studies have also examined anionic and cationic resins separately. The Czech study examined irradiation at a total dose of 1 MGy (^{60}Co gamma), while the Swiss study considered a lower dose (100 kGy, ^{60}Co gamma). Both studies examined anaerobic conditions, which are considered more representative of post closure conditions than a number of previous studies undertaken in air. Bead and powder resins were examined either in neutral groundwater or pH 12.5 cement water (Appendix B).

In considering the subsequent biodegradation of the irradiated resins' radiolysis products the Czech study performed microcosm experiments that included the resin materials and irradiated solutions at varying resin / water ratios. The Swiss study undertook microcosm experiments examining the ability of specific organic compounds (identified from the irradiation studies) to act as electron donors for sulfate reduction in Opalinus Clay porewater.

Both MIND studies identified radiolysis products consistent with previous work (see review within D1.1, Abrahamsen et al 2015). The Czech study indicated that anionic resins released around 5 times as much water soluble organic carbon than the cationic resins (Appendix B, Table B1-3), which is likely to represent loss of the amine functional groups (Figure 2-5b). Identification of trimethylamine, dimethylamine, acetonitrile and ammonia in the two studies is consistent with radiolysis of the amine functional group. Benzene was identified in both studies and indicates fragmentation of the styrene-DVB co-polymer. In the Czech study irradiated to 1 MGy benzene was detected by GC/MS analysis of the aqueous phase, whereas in the Swiss study at lower dose (100 kGy) benzene was only detected at low concentrations in the gas phase, which was mainly composed of CO₂ and H₂. The Czech 1 MGy irradiation study also identified ketones (2-propanone and 2-butanone), which may be attributed to fragmentation of the aliphatic chains present in the co-polymer (Figure 2-5). Liquid chromatography further proved the presence of several organic compounds of middle and high molecular weight probably originating from the polystyrene backbone structure of the ion exchangers, particularly the cationic variety. Significant fragmentation of the co-polymer would therefore only occur at high gamma radiation dose (1 MGy), which are likely to represent the extreme upper limits for ILW (see discussion in D1.1.).

The two MIND studies are consistent in demonstrating that ion exchange radiolysis products can be utilised by sulfate reducing microbes present in the two groundwater inocula studied. In the case of the inoculum from the Opalinus Clay (Appendix B2) sulfide formation has been observed in microcosm experiments examining benzene and trimethylamine as electron donors. Similar experiments in progress examining chloromethane and ammonia have not however shown evidence of sulfate reduction. The Czech study has shown that in microcosm experiments with varying resin/water ratio that microbial activity only developed in the case of low proportions of resin (0.2 and 2.0 g/l), which is attributed to the development of low pH (< 5) conditions at high resin content (20 g/l). qPCR (Appendix B1) was used to quantify the growth of microbes and showed an increased growth in irradiated resins at low resin/water ratio resulting from the utilisation of radiolysis products. Sequencing analysis revealed the presence of several microorganisms that might utilise the radiolysis products such as *Limnobacter*, *Hydrogenophaga*, *Sulfuritalea* and *Ceaenomonas* capable of using various reduced sulfur compounds as electron donors, methylotrophic *Methylothera* and *Methyloversatilis* metabolizing methane and methylamine or *Sphingobium* and *Novosphingobium* capable of metabolizing various aromatic organic compounds. The distribution of these microorganisms also varied between the initial population present in the inoculum and irradiated and non-irradiated samples.

These MIND project studies indicate that microorganisms are capable of utilising ion exchange resin radiolysis products, especially by coupling their oxidation to reduction of sulfate, which itself will be readily sourced from cationic resins. Ion exchange resins thus have the potential to undergo biodegradation without addition of electron acceptors. Breakdown of the resin by radiolysis is an important step in the overall degradation process liberating soluble organic carbon and at high dose (1 MGy) DOC increases from < 50 mg/l to up to 1000 mg/l in the case of anionic resins. At these high doses the resins develop strong acidity, which may prevent subsequent biodegradation unless the waste resins are conditioned or radiolysis products are diluted or dispersed. At lower doses (100 kGy) amine release from anionic resins has been shown to be able to fuel sulfate reduction processes. Oxidation of the amine functional groups by such biodegradation is important with regard to any possible radionuclide complexation effects of the amines. Hydrogen and CO₂ are the main gaseous radiolysis products detected and methane gas formation has not been observed in the current microcosm experiments undertaken, although in both cases the experiments have included high concentrations of sulfate, which is likely to outcompete methanogenesis. Evaluation of the extent of ion exchange resin degradation will require consideration of the radiation dose history of specific resins, both during their operational use in water treatment and during storage and final disposal.

3 Microbial gas generation and consumption

Processes of microbial gas generation have been examined within MIND WP1 utilising two *in situ* experimental systems:

1. The TVO gas generation experiment, which is focussed on microbial gas generation from LLW containing steel and cellulose waste materials.
2. Borehole experiments performed at the Mont Terri URL that have involved injection of hydrogen to study its reaction as an electron donor in studies of nitrate reduction and sulfate reduction.

Both these studies provide further insight into the controls on methanogenesis in LLW/ILW disposal systems, which have relevance for assessing, (i) gas generation rate and effects of pressurisation and (ii) in considering the pathway for gaseous ^{14}C transport to the biosphere.

3.1 Microbial gas generation from cellulose-containing wastes

The Gas Generation Experiment (GGE) located at the VJL Repository, Olkiluoto, Finland is operated by TVO to study and quantify the rate of gas generation from LLW arising from the nuclear power plants at the Olkiluoto site. The GGE has been in operation since 1997 and provides an extensive set of geochemical and microbiological data. The GGE was originally designed and configured as part of the European Community PROGRESS project (Rodwell, 2000). The primary aim of the experiment has been to quantify the rate of gas generation from representative LLW under the conditions of the VJL Repository. In this repository, drums of waste are emplaced in an underground cavern (Figure 3-1), which after closure will re-saturate and submerge the waste in groundwater. Experimental results from the first 9 years of GGE operation, together with a biogeochemical model of the experiment were published by Small et al (2008).

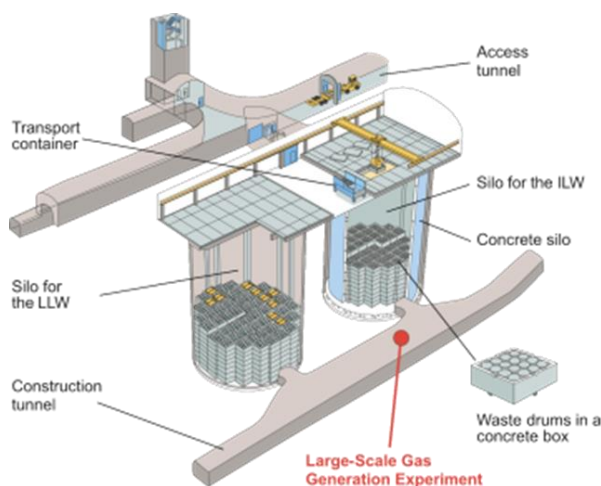


Figure 3-1 Schematic of the VJL Repository, Olkiluoto, Finland, showing the underground Silos for ILW and LLW and the location of the Gas Generation Experiment.

Further study of the GGE was included within the scope of WP1 of the MIND project and included further modelling of the 18 years dataset (as of 2017) (D1.8; P4; Small and Abrahamsen-Mills, 2018; Small et al, 2017) and also detailed microbiological characterisation of stored and new samples from the experiment (D1.6; P11; Vikman et al, 2018,2019). The studies undertaken within MIND WP1 had the objectives to;

- understand the reasons for an increase in gas generation that has been observed in the GGE occurring after around 9 years (2006),
- to examine coupled effects of pH buffering and microbial activity, and

- to further understand the controls on methanogenesis in LLW.

MIND publication P4 (Small et al, 2017) provides a detailed description of the 18 year results dataset from the GGE and results of updated GRM modelling.

3.1.1 GGE overview description

The GGE comprises 16 200 dm³ drums of LLW from the VUJ repository together with a concrete box (mass 4,000 kg) used to stack the waste drums in the repository that is enclosed in a gas-tight, acid-proof steel tank of around 20 m³. Figure 3-2 is a photograph taken through a window in the tank before it was filled with local river water that provided a microbial inoculum. Sample lines are present in the experiment that can be used to sample water from different locations, including within waste drums and in the free water outside the drums. An on-line sampling loop is also installed, which is used for continuous measurement of pH, Eh and conductivity.

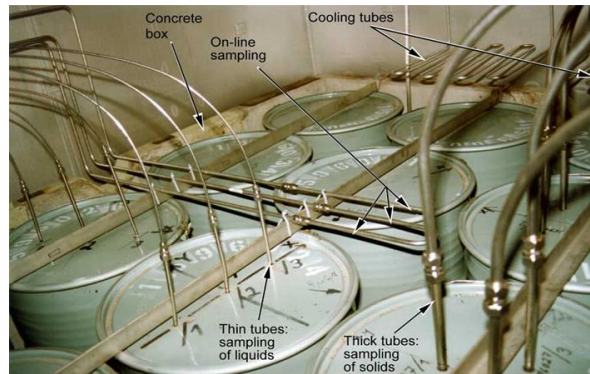


Figure 3-2 View inside the Gas Generation Experiment, before filling with water showing waste drums, concrete box and sampling lines (TVO).

3.1.2 Geochemistry and modelling

MIND publication P1 (Small et al, 2017) presents detailed gas generation and aqueous geochemical data obtained from the GGE over a period of 18 years operation. Results are also presented of an updated biogeochemical model of experiment that aids the interpretation of the processes of anaerobic cellulose waste degradation, which lead to methane generation. Figure 3-3 presents the gas generation data, where it is observed that the rate of CH₄ generation doubled after the first 8 years of the experiment.

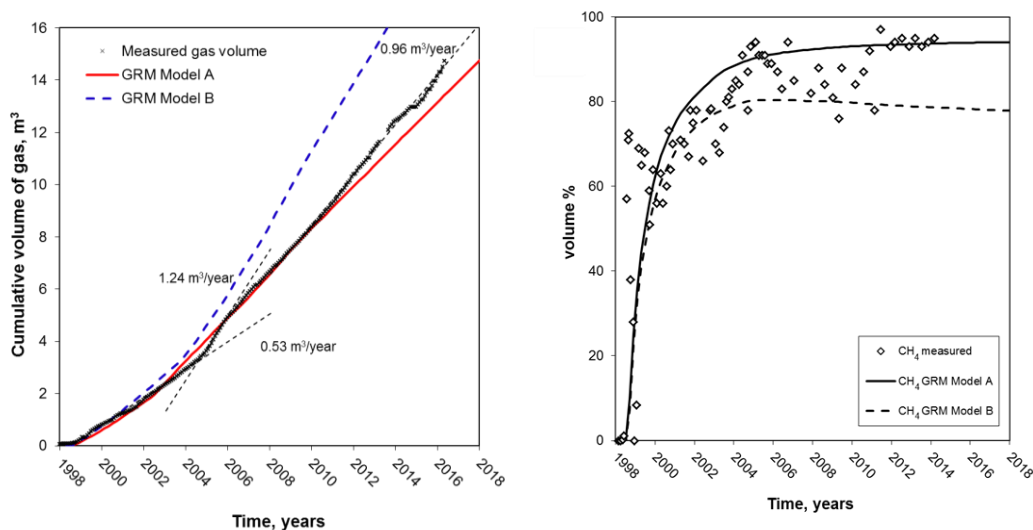


Figure 3-3 Cumulative volume of gas generated by the Gas Generation Experiment (Left) and CH₄ content (Right, measured data are plotted as symbols and model simulation plotted as lines (Small et al, 2017).

The increase in methane gas generation rate is attributed to an observed neutralisation of pH of the tank water present outside the waste drums, where pH declines from initial pH 11 to neutral conditions (Figure 3-4). The decline in pH of the tank water enables more wide spread microbial activity. The neutralisation of pH is further attributed to the initial development of microbial activity in the waste drums that have maintained a near neutral pH. The increase in gas generation rate at around 2008 also coincides with a significant decline in dissolved organic carbon in the tank water (Small et al, 2017).

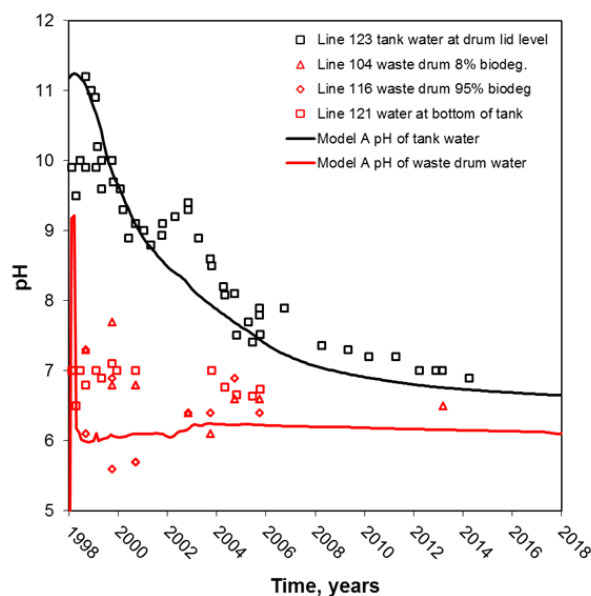


Figure 3-4 pH variation in the Gas Generation Experiment and its modelled evolution (Small et al, 2017)

A further factor that may explain the increase in gas generation rate concerns the effect of sulfide toxicity, since during the first two years of the experiment high concentrations of sulfide ($> 1 \times 10^{-4}$ M) are present, which are sufficiently high to inhibit sulfate reduction and methanogenesis. Sulfide concentration declines slowly over a period of 6 years as it attains equilibrium with mackinawite (FeS) at around 1×10^{-6} M (Small et al, 2017).

The biogeochemical model of the GGE represents well the increase in gas generation rate (Figure 3-3), the pH heterogeneity and neutralisation (Figure 3-4) and other chemical parameters such as variations in dissolved organics, sulfate and sulfide (see Small et al, 2017) and has enabled this interpretation of the evolution of geochemical and microbiological processes to be developed. Deliverable D1.8 (Small and Abrahamsen-Mills, 2018) provides a detailed description of the Monod kinetic and geochemical modelling methodology.

3.1.3 Microbiological characterisation

Results of genomic characterisation studies of stored samples prior to and collected during the MIND project are presented in MIND publication D1.6 and P11 (Vikman et al, 2018,2019). Detailed sequencing analysis of DNA and RNA extracted from water samples from the GGE reveal that the most significant microbial groups influencing the gas generation in GGE are cellulose and hemicellulose hydrolysing microbes, fermentative microbes and methanogens (Figure 3-5). Several microbial groups with potential to hydrolyse cellulose and hemicellulose, metabolise sugars to acetate and hydrogen or volatile fatty acids and produce methane were detected. The methane-producing step in the anaerobic degradation process is mainly performed by methanogenic archaea. Both acetoclastic and hydrogenotrophic methanogens were found in the GGE but the formation of CH_4 from H_2 and CO_2 appeared to be the more favourable metabolic route compared to the one

utilising acetate, especially at the beginning of the experiment. Methanosarcinales were first detected in 2015 and are acetoclastic methanogens; their appearance coincided with acetate consumption and increased gas generation as deduced from the biogeochemical modelling (Small et al, 2017).

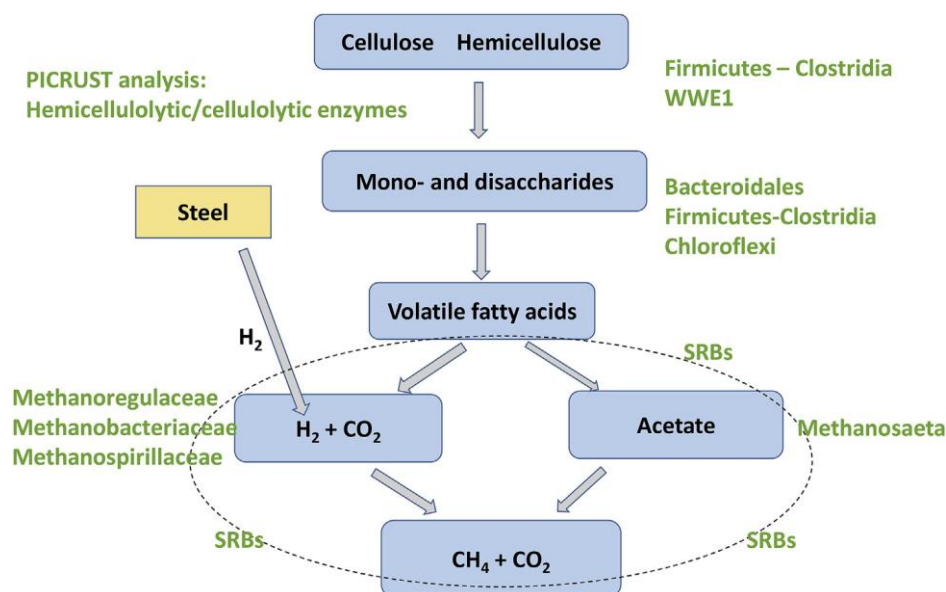


Figure 3-5 Summary of the microbiological processes developed in the GGE (Vikman et al, 2019)

Vikman et al (2017;2019) also characterised the sulfate reducing bacteria (SRB) present in the GGE. SRBs belonging to the orders Desulfobacterales, Desulfovibrionales, Desulfovibrionaceae and Desulfomonadales, which were detected by 16S amplicon sequencing. The relative ratio of SRBs compared to methanogens has decreased during the operation of GGE, but SRBs are still active even though the concentration of sulfate has been below detection since 2001. It is likely that sulfate may be slowly released from the LLW and be subject to reduction.

3.1.4 Summary

The chemical evolution of the GGE over a period of 18 years has provided information directly relevant to the understanding of gas generation within the VUJ repository and other similar repositories. The chemical data and modelling studies suggest that the initially strong heterogeneity in pH and other parameters has been reduced and homogenised and aqueous sulfide has decreased, resulting in a doubling of gas generation rate. As a consequence, further gas generation is expected to proceed at a steady state until the cellulose and steel materials are exhausted. Sulfate reduction appears to be occurring in parallel to methanogenesis, but at a low level limited by its slow release from solid wastes. Higher rates of sulfate input, such as through groundwater, would be expected to slow the rate of methanogenesis by competition for electron donors.

The neutralisation of the initial alkaline pH of the experiment, by microbially mediated organic waste degradation processes, also has relevance to other repository designs, where the cementitious materials provide a number of chemical and physical safety functions. Other repository concepts typically contain less water and a larger proportion of Portland cement material (e.g. the Swedish SFR repository is estimated to contain between 4 and 200 times as much concrete as the GGE; Small et al, 2017), which may maintain pH above pH 12.5 and hence inhibit microbial activity. However, the GGE has shown the potential for microbial activity to develop in lower pH regions where they generate acidic cellulose degradation products (acetate and other VFAs) that eventually neutralise the cement buffered regions of the experiment. The GGE thus provides an insight into how LLW/ILW repositories with higher proportions of concrete, and higher initial pH conditions, may be neutralised over longer

periods of time and where organic hydrolysis and fermentation products, including radionuclide complexants, may be degraded and contribute to gas generation.

3.2 Microbial consumption of hydrogen

Hydrogen is the most significant gas produced by abiotic processes in a deep geological repository for ILW, forming primarily by anaerobic corrosion of steel and other metals and by radiolysis of organic waste materials. The generation of hydrogen is highly significant to gas pressurisation and the potential for mechanical disturbance. In performance assessment the consumption of hydrogen by microbial processes is largely ignored, because of the uncertainty whether microbial processes could be established under the physical and chemical conditions of the engineered barrier and host rock systems. The potential for microbial processes to utilise H_2 is however recognised and in particular the Swiss programme (Leupin et al, 2016) have undertaken a detailed assessment of the potential sources and sinks of H_2 and other gases in their disposal concept for LLW/ILW disposal in Opalinus Clay.

In Opalinus Clay the principal microbial sink for H_2 is by sulfate reduction, as has been demonstrated by studies at the Mont Terri URL (Bagnoud et al, 2016a, b), which have shown that H_2 injection stimulates a complex consortium of microbial processes. Within MIND WP1 these studies have continued and developed to consider the potential for methane generation to be established (D1.7, Bernier-Latmani and Boylan, 2018).

A further experiment at the Mont Terri URL has examined the reaction of H_2 co-injected with nitrate within the Bitumen-Nitrate (BN) experiment (Bleyen et al, 2017). Here MIND WP1 modelling studies have assisted in the interpretation of the geochemical and microbiological processes associated with H_2 injection (D1.8, Small and Abrahamsen-Mills, 2018).

3.2.1 Sulfate reduction and methanogenesis

Building on previous work (Bagnoud et al, 2016a,b) D1.7 (Bernier-Latmani and Boylan, 2018) present the results of continuing use of the BRC-3 bioreactor borehole at the Mont Terri URL (Figure 3-6) to examine whether the concentration of sulfate present in the borehole could be lowered sufficiently so that acetogenic and methanogenic conditions could be established. Operation of the bioreactor over a one year period involved a series of pulsed injections of a pure H_2 gas phase (~3 L) at intervals of between 7 and 15 days forming a gas headspace in the borehole, at each injection around 800ml of water was removed for geochemical and microbiological study and was replaced by an equal volume of artificial Opalinus Clay groundwater devoid of sulfate.

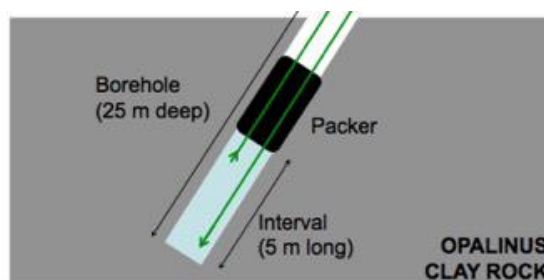


Figure 3-6 Schematic of the BRC-3 bioreactor at the Mont Terri URL (D1.7; Bernier-Latmani and Boylan, 2018)

Geochemical data presented in D1.7 indicated that the concentration of dissolved methane remained low at $< 20 \mu\text{M}$, with no sustained increase in concentration. Over the course of 350 days sulfate concentration declined from 14 mM to a minimum of 4 mM, which will partially be a consequence of dilution by the experimental procedure. Aqueous sulfide ranges between $< 50 \mu\text{M}$ and $400 \mu\text{M}$, which is similar to the range observed in the Gas Generation Experiment following the initial phase of sulfate reduction (Section 3.1; Small et al, 2017). pH fluctuates between 7.3 and 8.5,

which may be a consequence of mineral buffering reactions (D1.7) although in the BN H₂ injection experiment pH increase is attributed to an autotrophic denitrification process (see below Section 3.2.2).

The microbial community sampled from the BRC-3 bioreactor was dominated by sulfate reducing bacteria (SRB). Methanogens and acetogens were identified in some samples, but they comprised < 0.1% of the total reads during the sampling period (D1.7). Thus, it is clear that levels of sulfate and SRBs in the bioreactor remained too high for methanogenesis to be established.

In a further experiment, (MA-A) at Mont Terri studying H₂ interaction with a sand:bentonite column leached with Opalinus Clay groundwater sourced from the BMA-A1 borehole, methanogens, in particular *Methanosarcina*, and fermenters, such as *Pseudomonadaceae* have been shown to dominate the microbial community in specific regions of the column (D1.7; Burzan, 2019).

Geochemical analyses are currently ongoing and the extent of methane generation in this system is yet to be quantified. Compared to the BRC-3 bioreactor in Opalinus Clay this sand:bentonite experiment represents a more continuous input flow of H₂ gas, such that a significantly higher relative amount of reaction of H₂ with sulfate can be achieved.

Laboratory microcosm experiments have also been performed to attempt to establish methanogenesis in artificial Opalinus Clay groundwater (devoid of sulfate), in the presence or absence of Opalinus Clay and with a microbial inoculum obtained from Opalinus Clay groundwater. The batch microcosms included approximately equal volumes of solution and a gas headspace, which was flushed with a 20% H₂: 80% N₂ gas mixture prior to incubation (D1.7, P10; Boylan et al, 2019). While sulfate was not included in the artificial groundwater, sulfate is present in the Opalinus Clay and the inoculum, resulting in initial sulfate concentrations in the solution in the range of 200 to 900 µM depending on the amount of clay added. Similar to the BRC-3 bioreactor the microcosms were dominated by sulfate reducing organisms belonging to the *Desulfobulbaceae* family and there is no evidence of methanogenesis. This probably reflects the rather low proportion of H₂ gas that can be included in such a batch microcosm experiment compared to the sand:bentonite column experiment.

In the publication resulting from the batch microcosm experiments (P10; Boylan et al, 2019) the fate of reduced sulfate has been examined by X-ray absorption spectroscopy (XANES) and Mössbauer spectroscopy, to show that sulfide reacts with Fe²⁺ released from Fe containing minerals (siderite) present in the Opalinus Clay to reduce the aqueous concentration of sulfide to low levels, which is of relevance to studies of Microbial Induced Corrosion studied by MIND WP2 (D2.19; Pedersen, 2019).

D1.7 (Bernier-Latmani and Boylan, 2018) discuss a number of factors that may affect the development of methanogenesis in Opalinus Clay, summarised as follows:

1. Availability of electron donors; sufficient H₂ is expected to be available.
2. Bicarbonate concentration; thermodynamic indicate that higher levels may be required for methanogenesis.
3. The presence of more energetically favourable terminal electron accepting process; sulfate reduction will outcompete methanogenesis.
4. Availability of water and space; microbial activity only likely in fractures in Opalinus Clay.
5. Availability of trace elements and vitamins; optimum levels of nickel may be important for methanogenesis.

3.2.2 Nitrate reduction

The Bitumen-Nitrate (BN) experiment (Bleyen et al, 2017) concerns the fate of nitrate released from bituminised waste, disposed in a clay host rock repository alongside soluble organic and H₂ radiolysis and hydrolysis products of the bitumen (see Section 2.4). The BN experiment comprises a borehole

with three intervals in which different solutions can be circulated, with online pH, Eh and UV spectrophotometric analysis of nitrate and nitrite, together with sampling for further chemical and microbiological analyses (Figure 3-7).

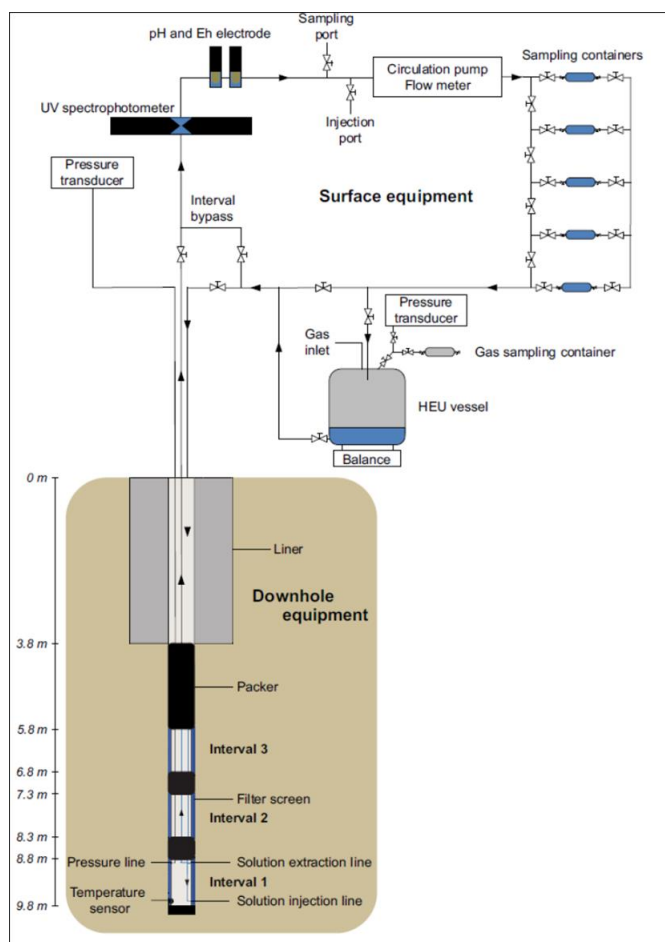


Figure 3-7 Schematic representation of the BN experiment, showing the three water injection intervals, sampling lines, hydraulic packing (black), of the borehole, together with circulation pump, sampling and analytical instruments and hydrogen equilibration unit (HEU) surface equipment (Bleyen et al, 2017).

In order to study the effect of H_2 as an electron donor a hydrogen equilibration unit (HEU) is also installed (Figure 3-7), which can be connected into the circulating fluid. Within the HEU, the circulating interval solution drips into a stainless steel vessel of 10 L, filled with ~ 1 L of solution and ~ 9 L of gas. The HEU serves both as a supply of H_2 and as a gas trap, collecting the gases produced by denitrification. The HEU has been used to provide a pulsed equilibration of H_2 within the circulation fluid by initially using an inert gas (argon) in the vessel and then changing the gas phase to 100% hydrogen before replacing the gas with argon (Bleyen et al, 2017). The experiment considered here injected nitrate into the borehole to attain a concentration of 17 mM after initial equilibration. Nitrate was then allowed to diffuse into the Opalinus Clay for a period of 54 days before the HEU was changed to H_2 , which stimulated a more rapid decline in nitrate concentration and formation of nitrite (Figure 3-8). The denitrification and H_2 oxidation process generated significant alkalinity in the borehole resulting in an increase in pH approaching pH 9.

Deliverable D1.8 (Small and Abrahamsen-Mills, 2018) describes the development of a reactive transport model implemented with the PHREEQC code (Parkhurst and Apello, 2013), that includes a two stage Monod kinetic model of the reduction of nitrate to nitrite and the subsequent further reduction of nitrite to N_2 gas. The transport model considers radial diffusion around the borehole parameterised against previous tests using a Br^- non-reactive anion tracer (Bleyen et al, 2017). The model is able to be fitted to the nitrate and nitrite data (Figure 3-8, upper) by adjusting the Monod

kinetic parameters. Representation of the significant pH increase required consideration of the processes as an autotrophic processes where additional alkalinity is generated by the synthesis of biomass from inorganic carbon. The best representation of the pH evolution (Figure 3-8, lower, Model 2) included a pH dependent Monod kinetic model, suggesting that the microbial process may be self limited by the effect of pH increase.

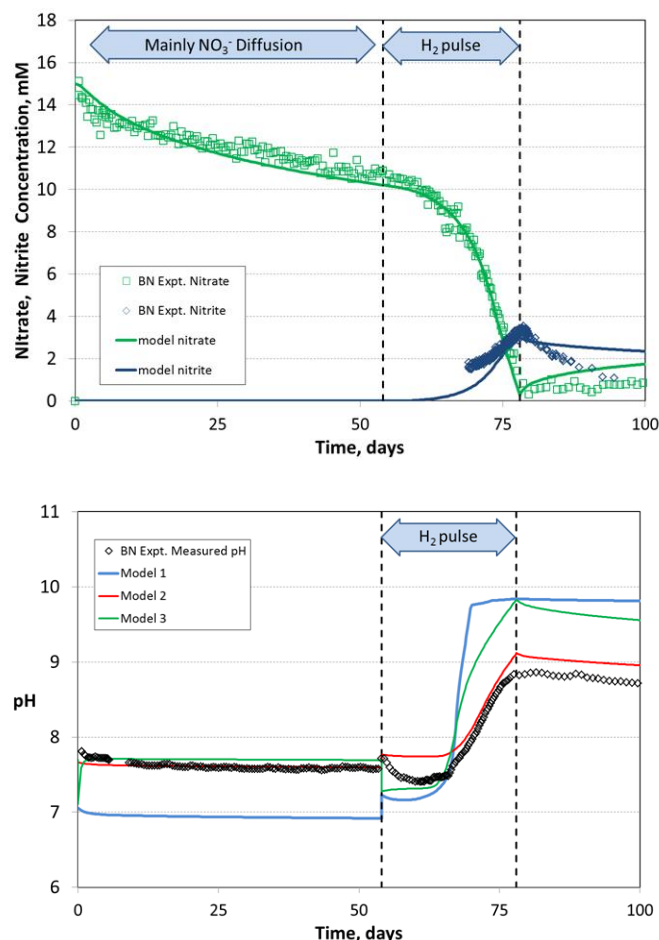


Figure 3-8 Upper figure, nitrate and nitrite concentration during a pulsed equilibration of hydrogen gas. Lower figure, pH variation. Symbols represent experimental data (Bleyen et al, 2017). Solid lines are the modelled concentrations of nitrate and nitrite and pH (Small and Abrahamsen-Mills, 2018).

3.3 Methanogenesis and H₂ consumption; summary and outlook

MIND WP1 studies have furthered the understanding of processes of microbial gas generation (methanogenesis) under (i) *in situ* waste conditions from cellulose and steel containing wastes (D1.6, D1.8, P4, P11) and (ii) under *in situ* host rock and engineered barrier conditions from H₂ and in competition with sulfate reduction processes (D1.7, P10). Methanogenesis liberates the lowest amount of energy for microbial metabolism and represents the end stage of anaerobic systems. Consequently, experimental simulation requires careful design and configuration to develop conducive chemical conditions to maintain an excess of electron donors. In addition, methanogens are sensitive to environmental conditions (pH, trace elements,) and toxicity.

The 20 year duration Finnish Gas Generation Experiment (GGE) illustrates that methanogenic processes require long periods of time to establish steady state conditions. The GGE includes a large quantity of metabolisable cellulose waste and mild steel which provide an excess and constant source of soluble organics and H₂ (through corrosion). The MIND project studies have shown that both these electron donors fuel methanogenesis, with H₂ more important during the early stages of

the experiment, perhaps driven by corrosion of waste drums. Subsequently, alkaline regions of the GGE have been neutralised and metabolism of soluble organics has more widely developed. Heterogeneity in the pH conditions and occurrence of low pH niches for microbial activity has been important to establish the homogeneous methanogenic system that was established after around 8 years.

In the case of *in situ* H₂ injection in borehole experiments undertaken at the Mont Terri URL, rapid reaction of H₂ with nitrate and sulfate electron acceptors occurs. In the case of sulfate reduction it has been impossible to fully exhaust the supply of sulfate provided by diffusion from the Opalinus Clay. It is probably impracticable that an URL borehole experiment could be configured that could safely deliver sufficient H₂ to overcome the supply of sulfate from such a clay host rock. It is apparent, from microcosm experiments and also from observations from the GGE that sulfate concentration must be lowered to below 1 µM before methanogenesis can be established. The configuration of the sand:bentonite column experiment discussed in D1.7 allows a much greater proportion of H₂ to react as an electron donor with sulfate present in Opalinus Clay groundwater for the growth of methanogens to occur. Further analysis of the geochemistry of this experiment and confirmation of methane generation should shed further light on the conditions and relative fluxes of H₂ and sulfate required to establish methanogenesis. Once these conditions are established then environmental, trace nutrient and toxicity effects on methanogenesis that are relevant to a specific host-rock or engineered barrier design can be evaluated.

Reactive-transport modelling approaches developed through the MIND project (D1.8) have been used to interpret both the GGE and H₂ injection experiments. In the case of the latter such models will be useful to design future *in situ* borehole, column or batch experiments targeted at further studies of methanogenesis and other microbial processes to ensure and demonstrate that the experiments will maintain conditions conducive for methane generation.

4 Radionuclide interactions

Direct interaction between microbes and radionuclides was not a major area of scope of the MIND project. Views canvassed from waste management organisations and other stakeholders during the definition of the project scope were that radionuclide speciation, especially over the long time scales considered by performance assessment, is assumed to be controlled by equilibrium with the major element chemistry (pH, Eh, [HCO₃⁻], etc). Complexation of radionuclides by organic degradation products (e.g. the cellulose degradation product ISA) was viewed as important and was included in WP1 Task 1.2 (Section 2) and included some spectroscopic studies of cellulose degradation product interactions with U and Eu (D1.4). Other studies examining the biogeochemical reactions of ISA with nickel under geosphere conditions were also included within research undertaken by UNIMAN (P6). Limited study of the association of radionuclides (Se, Eu) with microbial biomass was also included within the WP (D1.5, P2, P3, P8), but also has wider relevance to WP2. In a number of cases observations of radionuclide- microbes interactions made in these studies are serendipitous, and while they were not defined from the outset of the project they have value in demonstrating scientific understanding relevant to effects of microbiology on the safety case for nuclear waste disposal.

4.1 Observations of redox equilibrium

MIND publication P4 (Small et al, 2017) presenting data from the long term Gas Generation Experiment (GGE) has relevance to the general assumption regarding the attainment of redox equilibrium that is commonly made in considering the speciation of multivalent radionuclides such as Se, Tc, U, Np, Pu. As described in Section 3.1 the GGE has collected geochemical data associated with the microbial degradation of cellulose and steel containing LLW over a period of 20 years. Amongst this data set are redox potential (Eh) measurements made using a Pt electrode and individual

concentrations of sulfate (S(6)) and sulfide (S(-2)) made on 5 water sample from which Eh can also be calculated. Figure 4-1 plots Eh electrode measurements and Eh determined by calculation against pH on a Pourbaix diagram.

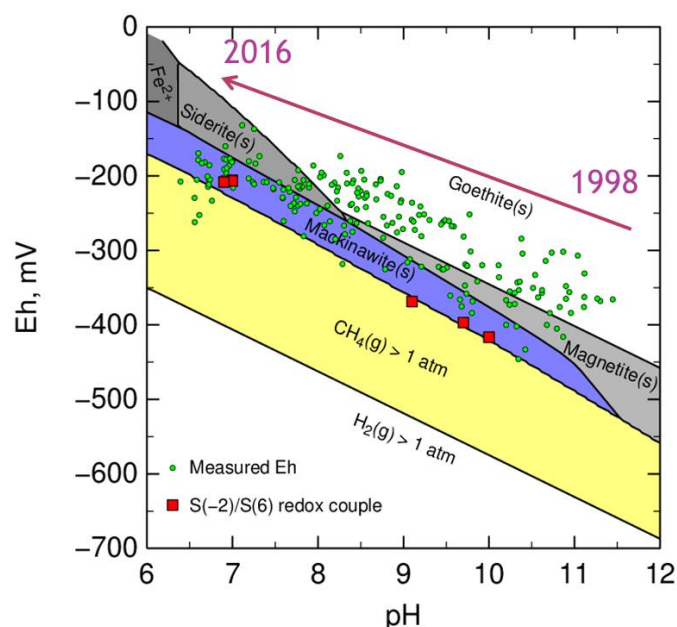


Figure 4-1 On line Eh and pH measurements from the GGE and Eh calculated by the sulfate/sulfide redox couple compared to an equilibrium predominance diagram for the Fe-C-S system calculated using Phreeplot (Small et al, 2017)

The trend in the Eh measured by the Pt electrode from right to left corresponds to the decline in pH measured over 18 years between pH 11 and pH 8 (see Figure 3-4) and is consistent with the formation of Fe(2) and Fe(3) containing corrosion products (goethite and magnetite). The redox potential calculated by the sulfate /sulfide couple (red symbols) at early times (pH 9-10) is significantly more reduced (~-100 mV) than the electrode measurements. However, for more recent samples collected in 2016 the two methods Eh determination are consistent and indicate equilibrium with FeS (mackinawite). These observations from this unique long term experiment are therefore consistent with the establishment of equilibrium of redox processes involving Fe and S over 18 years. It is also observed that the determined Eh and phase diagram are also in general agreement with the observed generation of CH₄ from the experiment. It is likely that in this experiment equilibrium is attained due to the mediation of the redox reactions by microbes.

4.2 Speciation and interactions with cellulose degradation products

4.2.1 Spectroscopic studies

D1.4 (Brinkmann et al, 2018) reports spectroscopic studies of uranyl (U(6)) interaction with cellulose degradation products; acetate and ISA. Acetate is formed from cellulose by fermentation processes at neutral pH conditions (see for example Small et al, 2017; Vikman et al 2019). MIND studies of cellulose degradation also indicate acetate formation is also possible under initial Ca(OH)₂ buffered conditions (see Section 2.2). The spectroscopic studies (TRLFS and UV-Vis) presented in D1.4 examined the existing uranyl acetate speciation model that includes 3 uranyl acetate complexes, stable under acidic conditions. Using these spectroscopic techniques it is apparent that uranyl speciation with acetate under acidic condition can be explained by the uranyl ion together with 2 uranyl acetate complexes. Similar spectroscopic studies of ISA, which is formed under hyperalkaline pH>12 conditions (Section 2.2), also revealed the detailed nature of uranyl-ISA complexes. Evidence

is presented in D1.4 for three dominant uranyl ISA complexes, formed under acidic conditions, which include binding motifs comprising 5 and 6 membered rings.

These specific results presented in D1.4 concerning acetate and ISA complexation under neutral and low pH conditions are not expected to have a significant impact on understanding the transport of uranium under the alkaline conditions prevailing in most ILW disposal scenarios. Nevertheless, the exploration of these spectroscopic methods applied and tested with this study may be useful in future studies to characterise a wider range of potential organic complexants, such as released by the range of anthropogenic organic polymers present in ILW (Section 2).

4.2.2 Biogeochemical interactions

MIND publication P6 (Kuippers et al, 2018) presents the result of a study examining the fate of the cellulose degradation product ISA, representing a scenario where ISA and complexed radionuclide (Ni) formed under the hyperalkaline near field conditions migrate to lower pH geosphere conditions. The study was devised to examine the potential for Fe(III) reducing processes to oxidise ISA under neutral pH conditions. While degradation of ISA was observed, both in the presence and absence of Ni, the complexed Ni was found to be sequestered during the biogeochemical processes stimulated by ISA biodegradation (Figure 4-2).

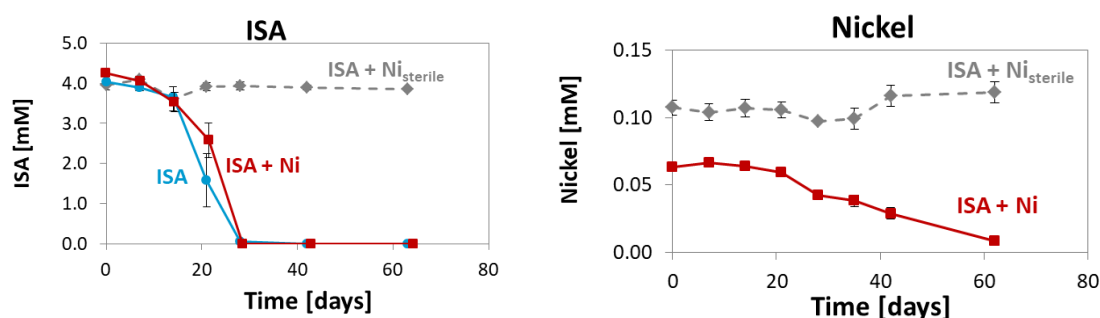


Figure 4-2 Aqueous concentrations of ISA and Ni during biodegradation of and ISA-Ni complex (Kuippers et al, 2018)

Detailed electron microscopy studies of the reduced iron phases formed in the microcosm experiments revealed that Ni was associated with an amorphous mixed Fe sulfide phase. The Fe sulfide phase had formed as a consequence of the reduction of low concentrations of sulfate present in the growth medium occurring alongside the main process of iron reduction.

A further similar study undertaken within MIND WP1 (Kuippers, 2019) examined the fate of uranium complexed with ISA and revealed the potential for complexed uranyl to be bioreduced to precipitate as U(4) or to precipitate as a U(6) phosphates once the ISA complex is oxidised. While, these findings regarding the fate of complexed radionuclides are somewhat serendipitous, they do illustrate the complex range of biogeochemical mechanisms that may be stimulated in the chemical disturbed zone (CDZ) associated with a hyperalkaline geological repository for ILW. Together with the process of ISA biodegradation the associated sequestration of radionuclides may be considered to represent a microbiological barrier for radionuclide migration that develops in the CDZ.

4.3 Microbe interactions with Se and Eu

D1.5 (Ruiz Fresneda et al, 2018) and associated publications (P2, P3, P8; Sanchez-Castro et al, 2017a,b; Ruiz Fresneda et al, 2018) have examined the role of the *Stenotrophomonas bentonitica*, isolated from Spanish bentonites on the mobility and migration of radionuclides (Se(4), Se(6) and Eu(3)) under relevant conditions. Selenium has rather complex redox speciation and is toxic to microbial processes and as ^{79}Se is a common fission product. Eu(3) was considered as an inactive analogue of trivalent actinides, Cm(3) and Am(3).

In the case of Se, the bacterial strain was able to reduce Se(6) and Se(4) to Se(0) forming Se nanostructures. Using a combination of number of microscopic techniques (STEM/HAADF, ESEM, FESEM, etc.), Ruiz Fresneda et al demonstrated that *S. bentonitica* reduced Se(4) to Se(0) aerobically at pH7 , forming initially amorphous Se(0) (a-Se) nanospheres which subsequently transform to one-dimensional (1D) trigonal selenium (t-Se) nanostructures with diverse crystallinities, morphologies and sizes. Time-dependent experiments showed that the cells of *S. bentonitica* and their proteins are involved into the transformation of amorphous Se(0) nanospheres (a-Se) to one-dimensional (1D) trigonal selenium (t-Se) nanostructures (hexagons, polygons and nanowires). (Figure 4-3).In addition, *S. bentonitica* is also able to reduce Se(4) under anaerobic and alkaline conditions (up to pH 10) producing a-Se nanospheres.

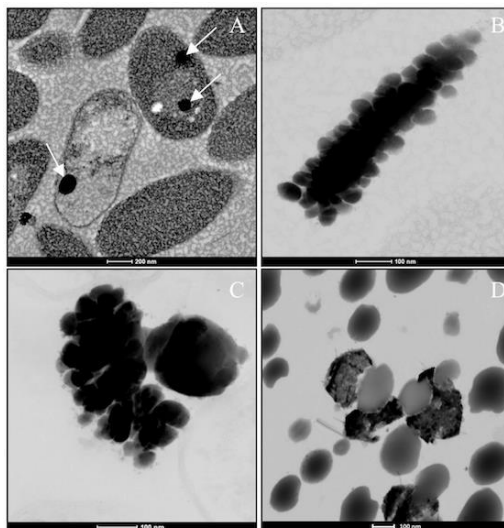


Figure 4-3 HAADF-STEM pictures showing individual a-Se nanospheres (white arrows) after 24 h (A) and forming aggregates after 48 h (B) and 72 h (C). Mixture of different t-Se nanostructures and a-Se nanospheres produced after 144 h (D). Scale bars: 200 nm (A), 100 nm (B-D)(D1.5; P8, Ruiz Fresneda et al, 2018)

The Se bioreduction mechanism appears to be related to the bacteria responding to the toxicity effects of Se. In contrast, association of Eu with *S. Bentonitica* appears to be a biosorption process. The results of potentiometric titration experiments indicated that the cell surface groups capable for metal binding sites involving carboxyl groups (pK around 3-5), sites involving phosphate groups (pK around 6-7), and sites involving hydroxyl and amine groups (pK > 8). Microscopy including X-ray mapping (Figure 4-4) illustrates the sorption of Eu with the cell surface and association with phosphorous.

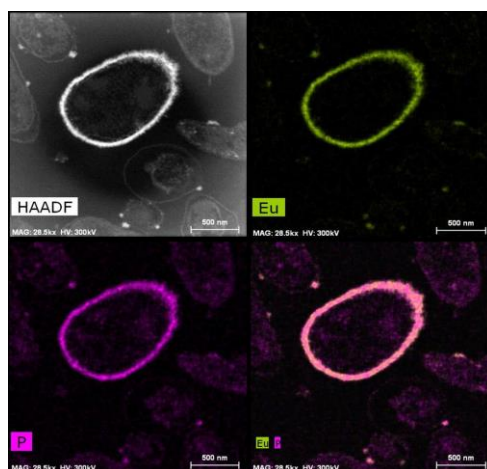


Figure 4-4 EDX element-distribution maps confirming that the electron-dense accumulations surrounding *S. bentonitica* cells are composed of Eu and P.

These observations of Se bioreduction and Eu biosorption are consistent with previous studies that illustrate the potential range of processes whereby radionuclides become associated with microbial biomass. The studies of *S. Bentonitica* isolated from bentonite deposits of course has more specific relevance to the use of bentonites as buffer and backfill materials in HLW and spent fuel disposal, which is the focus of MIND WP2 (Pedersen, 2019). While the association of Se and Eu with cell structures does imply the potential for radionuclide immobilisation and retardation, the potential for colloid facilitated migration of the nanoparticulate and biomass materials must also be considered in discussing the relevance of these findings to radiological safety.

5 Limits of life in cementitious ILW

An important uncertainty concerning the effect of microbial processes in the disposal of ILW is the ability of microbes to grow and metabolise under the high pH chemical conditions that are likely to prevail in ILW wastepackages and engineered barriers that comprise large quantities of cementitious material. Physical limitations, such as requirement for space to grow and availability of water will also be relevant, but are likely less important than in the case of the bentonite backfilled engineered barrier system for HLW and spent fuel that is considered by MIND WP2 (Pedersen, 2019). The level of physical containment of ILW wastepackages is less than that for HLW and spent fuel to allow for gas release, and ILW may contain a wide range of radionuclides and metal contaminants that may be toxic to or inhibit microbiological processes. The effect of radiation on microbial activity is also a consideration for ILW, which may limit or delay microbial activity occurring within some high activity wastepackages. However, the effect of radiation has largely been studied to examine the effects on organic polymer degradation (Section 2). At the outset a number of tasks within MIND WP1 were devised to study the pH limits of specific microbial processes, while observations and studies of other toxicity effects have also been made.

5.1 pH limits

The pH limit of specific microbial processes can be related to the chemical energy that is available, since microorganisms will require energy to maintain a near neutral internal cell pH. Aerobic soda lake systems thus provide the greatest diversity in alkaliphilic microorganisms (Grant et al, 1990), while microorganisms capable of growth at pH 13 have been characterised from a steel making industrial site coupling H₂ oxidation with oxygen in hyperalkaline slags (Roadcap et al 2006). Rizoulis et al (2012) in their study of anaerobic microbial processes under high pH conditions with inocula from the Harpur Hill site showing evidence of nitrate and Fe(III) reduction up to pH 11. Rizoulis et al further related their observations to the free energy available as a function of pH, indicating that the limits for sulfate reduction and methanogenesis would be expected to be at pH < 10.

Within the MIND project a pH for denitrification of at least pH 10 for the Harpur Hill inoculum has been confirmed through studies of PVC degradation (D1.2; P1, Nixon et al, 2018), indicating that the metabolism of anthropogenic additives is also supported as well as the lactate and acetate studied by Rizoulis et al (2012). Also bitumen degradation experiments with Harpur Hill inoculum showed the greatest amount of nitrate reduction at pH 10.5 (D1.3, Section 2.4). Furthermore, there is evidence for an increase in cell numbers at pH 12.5 (Figure 5-1). In comparison, microcosm experiments inoculated with Boom Clay pore water show a decline in cell numbers at pH 12.5 and lower cell concentration at pH 10.5 (Figure 5-1) indicating that adaptation of the microbial community is clearly important to the pH thresholds for survival and growth of nitrate reducers. The Harpur Hill inoculum has however adapted to the high pH conditions of the lime kiln waste over a relatively short period (100 years) compared to the timescales of geological disposal.

MIND publications P1 and P5 (Bassil and Lloyd, 2017,2018) have isolated the bacteria (*Anaerobacillus isosaccharinicus*) responsible for ISA degradation from Harpur Hill sediment. As part of this formal classification and description these publications define specific conditions for growth of the organism;

pH 9-11, optimum 9.8; 0-6% NaCl; 0-5% NaHCO₃. In addition *A. isosaccharinicus* it is capable of reducing nitrate and arsenate utilising several organic substrates including, ISA, gluconate (a known metal complexant and cement additive), lactate, pyruvate and succinate.

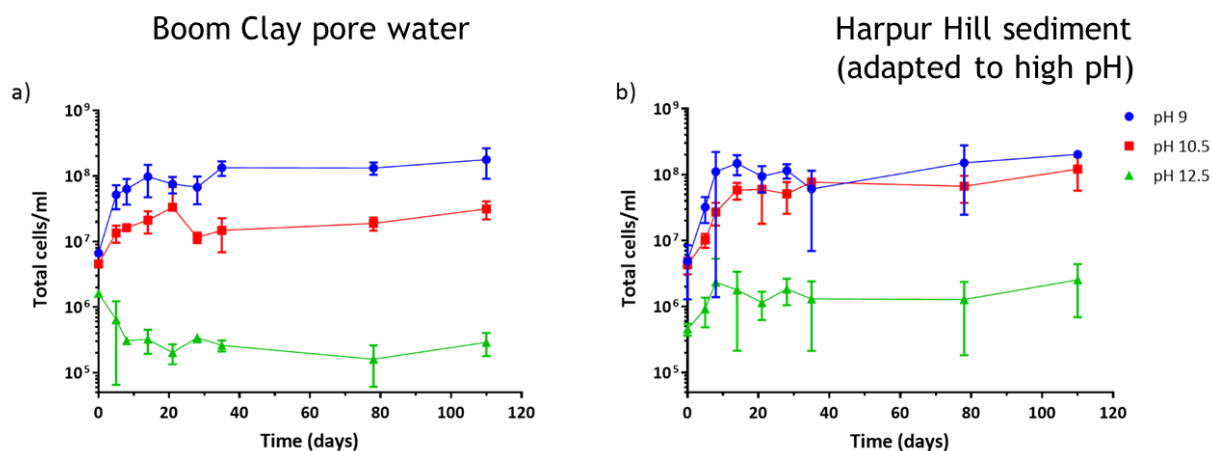


Figure 5-1 Comparison of the biomass concentration of microcosm experiments comparing nitrate reduction by Boom Clay and Harpur Hill inocula at pH 9, 10.5 and 12.5.

Studies of microbial gas generation involving the GGE, Finland (Section 3.1) indicate a threshold for methane generation from organic-containing LLW of around pH 8, which is the approximate pH where an increase in gas generation was observed that is attributed to neutralisation of the water region of the experiment. Given that other factors, such as competition with sulfate reduction and sulfide toxicity may have also affected the rate of CH₄ generation the threshold could be extended to pH 9 and this would still be consistent with thermodynamic considerations. An important observation from the GGE, and smaller scale high pH microcosm experiments performed within MIND, is the occurrence of lower pH niches, in which microbial activity may be first established, but which may then influence the wider experimental system. The pH limits defined above represent the bulk pH measured in experiments and may not reflect the pH developed in niches. Likewise, the occurrence of such niches may extend the apparent pH limits to more alkaline bulk conditions depending on the physical nature of the system. Further research concerning the scale of such spatial heterogeneity in pH is warranted.

5.2 Chemical toxicity effects

Chemical toxicity, such as by some specific contaminants present in ILW could inhibit or prevent microbial activity, including selenium as discussed in Section 4.3. Some ILW wastestreams may also contain specific contaminants that may need consideration on a case by case basis. In the context of the degradation of ion exchange resin in the Czech Republic a study has been made of the toxicity of Cs⁺ to nitrate and sulfate bacteria present in natural groundwater (VITA) collected from the Josef URL, Czech Republic (MIND publication P9, Shrestha et al, 2018). Here it was found from DNA extractions using specific gene markers for that nitrate reducing bacteria that growth was enhanced, relative to a control, in 0.5mM Cs, inhibited at 1mM Cs and toxic at 5mM Cs. The results were also confirmed by TEM examination (Figure 5-2). It is proposed, considering previous studies, that the toxic effect of Cs is most probably due to Cs induced K starvation and impairment of metabolic pathways comprising K dependent enzymes (Shrestha et al, 2018).

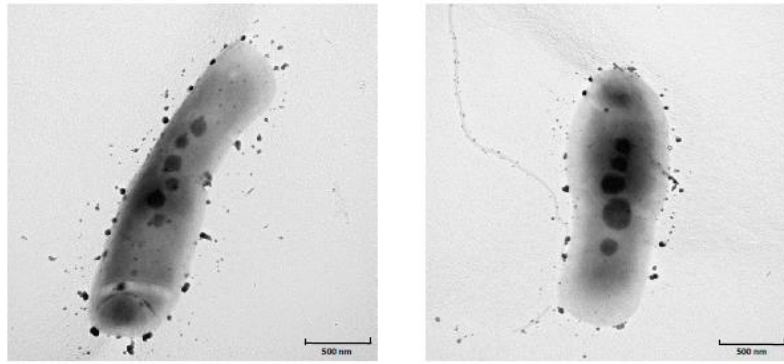


Figure 5-2 TEM image showing the presence of bacteria in 0.5 mM Cs+ after 8 days (Shrestha et al, 2018)

Another chemical toxicity effect that may require consideration is that of sulfide, which is known to inhibit both sulfate reduction and methanogenesis in anaerobic digestion systems (Lens et al 1998; Paulo et al, 2015). The effect is more significant at pH < 6, where the more toxic H₂S species is predominant. H₂S concentrations above 1mM severely limit sulfate reduction and methanogenesis (Paulo et al, 2015). Concentrations of total aqueous sulfide measured in the GGE (Section 3.1) approach this threshold early in the experiment, when sulfate reduction is most active (Figure 5-3). Sulfate concentration then declines significantly as FeS solubility approaches that of mackinawite. It is possible that sulfide toxicity slows the rate of CH₄ generation early in the GGE, although the effect cannot be delineated from the effect of pH (Small et al, 2017).

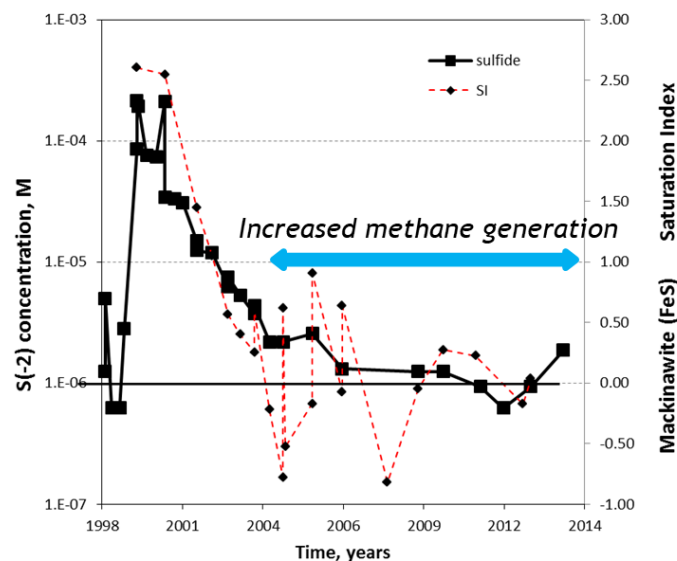


Figure 5-3 Concentration of aqueous sulfide developed during operation of the GGE and the calculated saturation index of FeS (after Small et al, 2017).

Similar observations of varying aqueous sulfide concentration and FeS precipitation are noted in other studies within MIND WP1 and in studies related to sulfate reduction in bentonite (WP2; Boylan et al, 2019; Pedersen, 2019). As apparent in Figure 5-3, potential effects of sulfide are most likely during the early stages of experimental systems. Sulfide toxicity is however unlikely to be of significance in a repository scenario, where equilibrium with mackinawite and pyrite is likely to be established.

6 Implications and outlook

In this synthesis report the findings of MIND WP1 have been discussed in relation to the following topics:

1. Organic degradation
2. Gas generation / consumption
3. Radionuclide interactions
4. Limits of microbial life in an ILW repository

In this section the implications of research in these areas is commented upon and any remaining uncertainties identified.

Organic degradation

The MIND project has examined the combined effects of irradiation and biodegradation of; cellulose, polyvinylchloride (PVC), bitumen and polystyrene based ion exchange resins under alkaline conditions. In this first study of this type the organic materials were mainly irradiated with maximal doses of gamma radiation (1 M Gy, ^{60}Co) and in most cases in pH 12.5 $\text{Ca}(\text{OH})_2$ solution.

The research concerning the degradation of these polymers indicates that the high levels of irradiation studied increase the extent of polymer degradation. Irradiation tends to enhance the susceptibility and rate of subsequent biodegradation. In the case of cellulose the enhanced microbial activity has an effect of lowering pH from $\text{Ca}(\text{OH})_2$ buffered conditions, which would ultimately reduce the effect of alkaline hydrolysis and ISA generation. Neutralisation of alkalinity by microbial activity is also demonstrated by the GGE on actual LLW initially buffered at pH 11 by concrete materials. Irradiation of PVC and ion exchange resin also develops acidity, which could contribute to the neutralisation of cementitious encapsulants enabling biodegradation of PVC additives and radiolysis products of resins as observed in the MIND studies.

The positive effects of microbes on cellulose degradation and ISA formation are not therefore limited to the utilisation of ISA as an energy and carbon source, but also fermentation processes, as observed by the MIND project, may affect the generation of ISA at source by lowering pH in micro-niche regions of cellulose wastes. Further study of processes under *in situ* conditions of cementitious wasteforms is required to assess the significance of microbial processes on the cellulose degradation process and ISA generation. While *in situ* study of the fate of ISA in URL borehole experiments may provide justification to include the effect of ISA degradation in performance assessments.

In the case of PVC, the PVC polymer can be neglected from the inventory of gas sources, however, some additives have been shown to be readily leached from plasticised materials and oxidised by nitrate. An important next step will be to assess which specific additives, or combinations of additives, are ultimately fuelling nitrate reduction, what effect these additives have on radionuclide mobility, whether they can fuel sulfate reduction and gas generation processes, and therefore constrain their overall impact on the safety case for nuclear waste disposal.

The primary objective of study of bitumen is in the context of disposal of nitrate containing wastes and the MIND project study together with previous and ongoing URL studies indicates the potential for nitrate to be readily reduced by organic and H_2 degradation products. However, further *in situ* study is required to assess the significance of results obtained in high pH microcosm experiments, with high fluid content, to consider the *in situ* solid-liquid ratio, transport and chemical conditions of a disposal cell for such wastes.

Irradiated ion exchange resins have been shown to yield water soluble and gaseous products that can fuel microbial sulfate reduction. The amine functional groups released at lower dose (100 k Gy) have also shown to be biodegradable. The presence sulfate in cationic resins and mixed resins is likely to

suppress the potential for CH₄ generation from irradiated resins materials, but this could be tested in further studies. Evaluation of the extent of ion exchange resin degradation will require consideration of the radiation dose history of specific resins, both during their operational use in water treatment and during storage and final disposal.

Gas generation / consumption

Microbiological and modelling studies of a long term (18 y) Gas Generation Experiment (GGE) has provided further understanding of processes of CH₄ generation from cellulose and steel containing LLW. The GGE illustrates how microbial processes can neutralise pH initially buffered by concrete by fermentation of organic waste. The GGE thus provides an insight into how LLW/ILW repositories with higher proportions of concrete may be neutralised over longer periods of time.

Sulfate reduction occurs in parallel to methanogenesis in the GGE but at a low level limited by its slow release from solid wastes. Higher rates of sulfate input, such as through groundwater would be expected to slow the rate of methanogenesis by competition for electron donors. This hypothesis could be examined by injection of sulfate into the GGE with monitoring of geochemistry and DNA/RNA as undertaken through the MIND project. Careful post mortem of the GGE is anticipated to yield further valuable scientific understanding of processes of cellulose degradation, anaerobic corrosion of steel and competition between sulfate reduction and methanogenesis.

In the case of *in situ* H₂ injection in borehole experiments undertaken at the Mont Terri URL, reaction of H₂ with nitrate and sulfate electron acceptors has shown to be rapid. In the case of sulfate reduction it has been impossible to fully exhaust the supply of sulfate provided by diffusion from the Opalinus Clay. It is apparent, from microcosm experiments and also from observations from the GGE that sulfate concentration must be lowered to very low levels (<1 µM) before methanogenesis can be established. The configuration of a sand: bentonite column experiment allows a much greater proportion of H₂ to react with sulfate for the growth of methanogens to be observed. Further analysis of the geochemistry of this experiment and confirmation of methane generation should shed further light on the conditions and relative fluxes of H₂ and sulfate required to establish methanogenesis. Once these conditions are established then environmental, trace nutrient and toxicity effects on methanogenesis that are relevant to a specific host-rock or engineered barrier design can be evaluated.

Reactive-transport modelling approaches developed through the MIND project have been used to interpret both the GGE and H₂ injection experiments. Such models are useful to design future *in situ* borehole, column or batch experiments targeted at further studies of gas generation /consumption and other microbial processes.

Radionuclide interactions

Studies of radionuclide-microbe interactions within MIND have yielded additional scientific understanding of radionuclide behaviour relevant to nuclear waste disposal, such as related to processes of radionuclide solubility and retardation. However, the detailed findings and observations will unlikely be directly incorporated into performance assessment calculations. Future studies of radionuclide interactions in this field of organic containing ILW should focus on assessing the significance of organic degradation products to act as complexants for radionuclides, under the range of pH and chemical conditions relevant for ILW disposal.

Limits of microbial life in an ILW repository

The MIND project has defined pH thresholds for microbial activity in ILW disposal facilities for different metabolic reactions, related to their energy yield, with pH 11 being the limit for nitrate reduction and pH 9 being the limit for methanogenesis. While the threshold for CH₄ generation supports the assumption made in safety assessments that pH 12.5 buffered by Ca(OH)₂ will suppress microbial activity and gas generation it should be recognised that the occurrence of lower pH niches

within the heterogeneous ILW may not eliminate microbial activity and methane generation in such cement encapsulated wastes. An important remaining uncertainty concerning microbial activity in an ILW repository concerns the extent and scale of waste and wastefrom heterogeneity that could provide such a microbial niche.

7 Acknowledgement

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880 The MIND-project. MIND WP1 partners; CV REZ, EPFL, HZDR, NNL, SCK•CEN, TUL, TVO, UGR, UNIMAN and VTT are thanked for their contribution to the work package. All MIND partners and members of the Implementers Review Board (IRB) are thanked for their discussion of the work package during project meetings. Liam Abrahamsen-Mills (NNL) and Genevieve Boshoff (NNL) are thanked for their review of this report.

8 References

- Abrahamsen, L., Arnold, T., Brinkmann, H., Leys, N., Merroun, M., Mijndendonckx, K., Moll, H., Polvika, P., Ševců, A., Small, J., Vikman, M., Wouters, K. (2015) Review of organic wastes and their biodegradation under ILW repository conditions. MIND project Deliverable D1.1
- Bagnoud, A., Chourey, K., Hettich, R.L., de Bruijn, I., Andersson, A.F., Leupin, O.X., Schwyn, B. and Bernier-Latmani, R. (2016a). Reconstructing a hydrogen-driven microbial metabolic network in Opalinus Clay rock. *Nat. Commun.* 7:12770
- Bagnoud, A., Leupin, O., Schwyn, B. and Bernier-Latmani, R. (2016b). Rates of microbial hydrogen oxidation and sulfate reduction in Opalinus Clay rock. *Applied Geochemistry* 72, 42-50.
- Baidak, A., La Verne, J.A., (2010) Radiation-induced decomposition of anion exchange resins. *J.Nucl.Mater.* 407, 211–219.
- Bassil, N.M. and Lloyd, J.R. (2017) Draft Genome Sequences of Four Alkaliphilic Bacteria Belonging to the *Anaerobacillus* Genus. *Genome Announcements*, Volume 5, Issue 3, American Society for Microbiology, 2017, e01493-16. <https://doi.org/10.1128/genomeA.01493-16>
- Bassil, N.M. and Lloyd, J.R. (2018) *Anaerobacillus isosaccharinicus* sp. nov., an alkaliphilic bacterium which degrades isosaccharinic acid *International Journal of Systematic and Evolutionary Microbiology*. <https://doi.org/10.1099/ijsem.0.002721>
- Bassil, N.M., Bryan, N., Lloyd, J.R. (2015). Microbial degradation of isosaccharinic acid at high pH. *ISME J* 9:310–320.
- Bernier-Latmani, R. and Boylan, A.A. (2018) Methane generation in the Mt Terri URL and the underlying microbial processes. MIND project Deliverable D1.7.
- Bleyen, N., S. Smets, J. Small, H. Moors, N. Leys, A. Albrecht, P. De Canniere, B. Schwyn, C. Wittebroodt, E. Valcke (2017). "Impact of the electron donor on in situ microbial nitrate reduction in Opalinus Clay: results from the Mont Terri rock laboratory (Switzerland)." *Swiss Journal of Geosciences* 110(1): 355-374.
- Bleyen, N., Vasil, M., Mariën, A., Bruggeman, C. and Valcke, E. (2016) Assessing the oxidising effect of NaNO₃ and NaNO₂ from disposed Eurobitum bituminised radioactive waste on the dissolved organic matter in Boom Clay. *Applied Geochemistry* 68, 29-38.
- Boylan, A.A., Perez-Mon, C., Guillard, L., Burzan, N., Loreggian, L., Maisch, M., Kappler, A., Byrne, J.M. and Bernier-Latmani, R. (2019) H₂-fuelled microbial metabolism in Opalinus Clay. *Applied Clay Science* 174, p 69–76 <https://doi.org/10.1016/j.clay.2019.03.020>

- Brinkmann H. Moll, H. Patzschke, M. Kaden, P. Arnold¹ T. (2018) Report of Uranium(VI) speciation associated with cellulose degradation products. MIND project Deliverable D1.4
- Grant, W.D., Mwatha, W.E. and Jones, B.E. (1990) Alkaliphiles: ecology, diversity and applications. FEMS Microbiology Reviews 75, 255-270.
- IGD-TP (2011) Implementing Geological Disposal of Radioactive Waste Technology Platform: Strategic Research Agenda 2011.
- Kuipers, G., Boothman, C. Bagshaw, H. Ward, M., Beard, R., Bryan, N. and Lloyd, J.R. (2018). The biogeochemical fate of nickel during microbial ISA degradation; implications for nuclear waste disposal. Scientific Reports Volume 8, Article number: 8753.
<https://doi.org/10.1038/s41598-018-26963-8>
- Lens, P.N.L., Visser, A., Janssen, A.J.H, Hulshoff Pol, L.W. and Lettinga, G. (1998) Biotechnological treatment of sulfate-rich wastewaters. Crit. Rev. Environ. Sci. Technol., 28 (1998), pp. 41-88
- Leupin, O.X. Zeyer, J. Cloet, V. Smith, P. Bernier-Latmani, R. Marschall, P. Papafotiou, A. Schwyn, B. Stroes-Gascoyne, S. (2016) An assessment of the possible fate of gas generated in a repository for low- and intermediate-level waste. NAGRA TR 16-05.
- Merroun, M.L. and Ruiz-Fresneda, M.A. (2018) Microscopy and element mapping characterization studies of radionuclide microbe organic interactions. MIND project Deliverable D1.5.
- Mijnendonckx, K., Van Gompel, A., Coninx, I., Bleyen, N. and Leys, N. (2018) Radiation and microbial degradation of bitumen. MIND project Deliverable D1.3
- Nixon S.L., van Dongen B.E., Boothman C., Small J.S. and Lloyd J.R. (2018). Additives in Plasticised Polyvinyl Chloride Fuel Microbial Nitrate Reduction at High pH: Implications for Nuclear Waste Disposal. Frontiers in Environmental Science. 6:97.
<https://doi.org/10.3389/fenvs.2018.00097>
- Nixon, S. Bassil, N.M. and Lloyd, J.R. (2017) Effects of radiation and microbial degradation of ILW organic polymers. MIND project Deliverable D1.2
- Parkhurst, D.L. and Appelo, C.A.J. (2013) Description of Input and Examples for PHREEQC Version 3—A Computer Program for Speciation, Batch-reaction, One-dimensional Transport, and Inverse Geochemical Calculations: U.S. Geological Survey Water-resources Investigations. Chapter 43 of Section A, Groundwater Book 6, Modeling Techniques: Techniques and Methods 6–A43, U.S Department of the Interior, U.S. Geological Survey (2013)
- Paulo, L.M., Stams, A.J.M. Sousa, D.Z. (2015) Methanogens, sulphate and heavy metals: a complex system. Rev. Environ. Sci. Biotechnol., 14, 537-553.
- Pedersen, K. (2019) Final synthesis report for WP2. MIND project Deliverable D2.19.
- Rizoulis, A., Steele, H.M., Morris, K., Lloyd, J.R. (2012). The potential impact of anaerobic microbial metabolism during the geological disposal of intermediate-level waste. Mineral Mag 76:3261–3270.
- Roadcap, G.S. Sanford, R.A. Jin, Q. Pardinas, J.R. and Bethke, C.M (2006) Extremely alkaline (pH >12) ground water hosts diverse microbial community. Ground Water, 44, 511-51.
- Rodwell, W.R. (Ed.), 2000. Research into gas generation and migration in radioactive waste repository systems (PROGRESS project). European Commission Report EUR 19133 EN.
- Rout, S.P., Charles, C.J. Garratt, E.J. Laws, A.P., Gunn, J. Humphreys, P.N. (2015) Evidence of the generation of isosaccharinic acids and their subsequent degradation by local microbial

consortia within hyper-alkaline contaminated soils, with relevance to intermediate level radioactive waste disposal PLoS One, 10 (3) , p. e0119164

- Ruiz-Fresneda, M.A., Delgado Martín, J., Gómez Bolívar, J., Fernández Cantos, M.V., Bosch-Estévez, G., Martínez Moreno, M.F. and Merroun, M.L. (2018). Green synthesis and Biotransformation of amorphous Se nanospheres to trigonal 1D Se nanostructures: impact on Se mobility within the concept of radioactive wastes disposal. Environmental Science: Nano <https://doi.org/10.1039/c8en00221e>
- Sanchez-Castro, I., Bakkali, M. and Merroun, M.L. (2017) Draft Genome Sequence of *Stenotrophomonas bentonitica* BII-R7T, a Selenite-Reducing Bacterium Isolated from Spanish Bentonites. Genome Announcements, Volume 5, Issue 31, American Society for Microbiology, 2017, e00719-17. <https://doi.org/10.1128/genomeA.00719-17>
- Sanchez-Castro, I., Ruiz-Fresneda, M.A., Bakkali, M., Kämpfer, P., Glaeser, S.P., Busse, H.J., López-Fernández, M., Martínez-Rodríguez, P., and Merroun, M.L. (2017) *Stenotrophomonas bentonitica* sp. nov., isolated from bentonite formations. International Journal of Systematic and Evolutionary Microbiology 67:2779–2786. <https://doi.org/10.1099/ijsem.0.002016>
- Shrestha, R., Steinová, J., Ševců, A., Kovářová, H., Černoušek, T., Kokinda, J., Hadravová, R. (2018) The effect of caesium ions on a natural anaerobic microbial community. Waste Forum, Vol 2, CEMC, Prague, 2018 <http://odpadoveforum.cz/TVIP2018/prispevky/135.pdf>.
- Small, J. Nykyri, M. Helin, M Hovi, U. Sarlin, T. Itävaara, M. (2008) Experimental and modelling investigations of the biogeochemistry of gas production from low and intermediate level radioactive waste. Applied Geochemistry, 23, pp. 1383-1418
- Small, J.S. and Abrahamsen-Mills, L. (2018) Modelling of microbial processes relevant to ILW disposal. MIND project Deliverable D1.8.
- Small, J.S., Nykyri, M., Vikman, M., Itävaara, M., Heikinheimo, L. (2017) The biogeochemistry of gas generation from low-level nuclear waste: Modelling after 18 years study under in situ conditions. Applied Geochemistry, 84, 360-372. <https://doi.org/10.1016/j.apgeochem.2017.07.012>
- Vikman, M. (2018) Relevant microbial metabolic processes and limits on chemical conditions leading to methane generation in LLW and ILW. MIND project Deliverable D1.6.
- Vikman, M., Marjamaa, K., Nykyri, M., Small, J.S., Miettinen, H., Heikinheimo, L., Haavisto, T. and Itävaara, M. (2019) The biogeochemistry of gas generation from low-level nuclear waste. Applied Geochemistry, 105, 55–67. <https://doi.org/10.1016/j.apgeochem.2019.04.002>
- Wang, J. and Wan, W., (2015) Treatment and disposal of spent radioactive ion-exchange resins produced in the nuclear industry. Progress in Nuclear Energy, 78, 47-55.

Appendix A Published summaries of WP1 deliverables

D1.1 A Review of Anthropogenic Organic Wastes and Their Degradation Behaviour (NNL lead)

Work Package 1 of the MIND project addresses remaining key issues for the geological disposal of ILW concerning the long-term behaviour, fate and consequences of organic materials in the waste along with H₂ generated by corrosion and radiolysis. This review begins this work by collating information concerning the inventory and nature of organic materials present in ILW and some LLW that requires geological disposal. The review has then considered the physical and chemical conditions that the organic materials will be subjected to during storage and geological disposal. The review then provides a summary description of the physical and chemical nature of the main organic materials present in ILW and their known chemical and radiolytic degradation behaviour. The effect of the organic degradation products on radionuclide speciation, which could affect how some radionuclides are transported in groundwater, is then considered to focus subsequent work in this field.

Inventory information concerning the distribution of the different types of organic material present in ILW and some LLW is collated from published national sources. This confirms that the materials selected for study in the MIND project; bitumen, organic ion exchange resins and halogenated polymers (PVC), are present in significant amounts in the national inventories. Cellulose is present in generally lower amounts in most inventories, but it is already established that it is of significance with regard to the strong complexation effect of its alkaline hydrolysis products. Cellulose is included in the Work Package 1 studies, but is not considered in detail by this review because comprehensive reviews exist in peer reviewed literature. The review of organic ILW also collates information relevant to the radiation dose, timescale and chemical conditions that the waste and encapsulant materials will be subjected to during storage and geological disposal. This information, together with information regarding the physical and chemical nature of the organic materials and current understanding of the effects of chemical and radiolytic degradation is used to refine the design of radiolytic and biodegradation experiments that are being undertaken in MIND Work Package 1.

In the next phase of the MIND project ion exchange resin, PVC, bitumen and cellulose materials will be irradiated using 60Co γ radiation at a range of dose rates. The first irradiation experiments will be undertaken under a range of relevant pH conditions with air and inert gas headspace. The first experiments will be to ensure that sufficient soluble organic material is produced in order to characterise the compounds present and to use the leachate in subsequent biodegradation experiments. Further experiments will then be undertaken at lower doses. The biodegradation experiments will be inoculated by various cultures, including consortia adapted to high pH conditions and indigenous microbes sampled from underground rock laboratories. Biodegradation studies will utilise a range of electron acceptors, including nitrate and sulfate identified by the review as being of most significance. The process of methane generation under cement buffered ILW conditions will also be explored. The soluble organic species will be characterised before and after biodegradation to identify compounds for complexation studies to draw conclusions regarding the degradation of known complexants such as the cellulose degradation product ISA. The solid residues and biomass materials will also be utilised for microscopy studies including interactions with radionuclides.

The information collated in this review is of prime importance to the design of experimental work undertaken in Task 1.2 of Work Package 1 of the MIND project. The review will also provide a basis of Task 1.3 that examines processes under in situ wasteform and repository conditions and modelling and integration in Task 1.4 and wider integration and dissemination through MIND Work Package 3.

D1.2 Effects of radiation and microbial degradation of ILW organic polymers (UNIMAN)

Work Package 1 (WP1) of the Microbiology in Nuclear waste Disposal (MIND) project addresses remaining key issues for the geological disposal of organic-containing intermediate level wastes. Specifically, its focus is on the long-term behaviour, fate and consequences of these organic materials. This report forms Deliverable 1.2 of the MIND project, which summarises WP1 research carried out by UNIMAN on the microbial degradation of two polymers and related compounds previously identified as priority targets for MIND research; cellulose and polyvinylchloride (PVC). In particular, the effects of gamma irradiation in combination with high pH on the microbial degradation of these materials were addressed. The results are presented here, along with discussion on their implications for the safe disposal of nuclear waste and recommendations for future study.

The impact of irradiation on the abiotic alkaline hydrolysis of cellulose was studied in batch experiments containing laboratory grade tissue paper immersed in a saturated Ca(OH)_2 solution (pH 12.7), and irradiated with 1 MGy of γ -radiation. Irradiation caused physical degradation of the tissue paper, and a change in the colour of the solid and solution. It also enhanced the rate of the abiotic alkaline hydrolysis of cellulose, through decreasing its degree of polymerisation and the number of crystalline domains. Batch sacrificial microcosms, containing a sediment sample from a high pH contaminated site, showed enhanced microbial activity in the samples that were supplemented with irradiated tissue paper compared to those containing non-irradiated tissue paper. The samples containing irradiated tissue paper showed a drop in pH, H_2 and acetate production compared to the samples containing non-irradiated tissue paper.

A series of microcosm experiments were conducted with the goal of assessing whether PVC could support microbial metabolism via nitrate reduction at high pH. In order to address the potential role of common PVC additives in these processes, a plasticised form of PVC sheet previously used in tenting operations within the nuclear industry was used, in addition to a pure PVC powder lacking additives. Samples of both forms were submerged in saturated calcium hydroxide at pH 12.4, and half of these were subject to a total cumulative dose of 1 MGy gamma irradiation. These PVC samples, all of which were subject to high pH conditions for several weeks, and some were additionally irradiated, were supplied to pH 10 microcosms as the sole source of organic carbon and electron donors for nitrate reduction. Microcosms

were inoculated with sediment from an anthropogenic high pH environment known to contain denitrifying bacteria. The results show that plasticised PVC is used to fuel nitrate reduction to nitrite, whether irradiated or not, though irradiated PVC sheet supported less nitrate reduction. In contrast, pure non-irradiated PVC powder did not support nitrate reduction, while irradiated powder supported minor amounts. Additional experiments with two of the PVC sheet additives identified with pyrolysis GC-MS (triphenyl phosphate and phthalate) did not support the extent of nitrate reduction observed with PVC sheet, indicating that other additives were fuelling this process. Measurements of dissolved organic carbon indicate that alkaline hydrolysis was occurring on all PVC materials, and no significant difference was observed between irradiated and non-irradiated, or sterile and live, microcosms.

Results from the cellulose degradation experiments provide further evidence of the role of microorganisms in cellulose degradation under cementitious ILW conditions that may overall reduce the complexation effect of the organic alkaline hydrolysis products generated. The study of PVC materials demonstrates the potential for alkaline degradation and biodegradation mainly of additives present. Further research may be required to examine the complexation properties and stability of specific PVC additives. Both studies provide data constraining the extent and rate of degradation that is of relevance to predicting gas generating processes from organic ILW.

D1.3 Radiation and microbial degradation of bitumen. (SCK•CEN)

Work Package 1 (WP1) of the Microbiology in Nuclear waste Disposal (MIND) project addresses remaining key issues for the geological disposal of organic-containing intermediate level radioactive wastes. Specifically, its focus is on the long-term behaviour, fate and consequences of these organic wastes. This report (deliverable 1.3 of the MIND project) summarizes all results obtained by SCK•CEN regarding microbial degradation of salts and organic compounds leaching from bituminized waste in relevant conditions during geological disposal. In first instance, the effect of different organic compounds expected to leach from bituminized waste on the efficiency of nitrate reduction was investigated. Afterwards, the effect of pH on the nitrate reduction rates of two microbial communities was investigated.

In Belgium, an important fraction of the current stored volume of long-lived intermediate level radioactive waste is immobilised in a bituminous matrix as Eurobitum, which contains besides bitumen and radionuclides, large amounts of soluble salts with sodium nitrate as the most dominant. Geological disposal of this waste in a water-saturated sedimentary formation such as Boom Clay will induce water uptake by the hygroscopic salts present in the bituminized waste. The dissolution of these salts will result in a further osmosis-induced water uptake through the semi-permeable bitumen membrane and thus in swelling of the Eurobitum. Additionally, sodium nitrate will slowly leach from the waste. The nitrate plume in the clay water could cause a geochemical perturbation of the surrounding clay, possibly affecting the redox conditions, causing ionic strength effects and cation exchange processes, which might result in an increase in the mobility of the radionuclides through the host rock. However, it is known that nitrate can also be removed inside the disposal gallery or in the near-field by various processes. Abiotically, the reduction of nitrate can occur with H₂ (produced during radiolysis of bitumen or water and during corrosion of steel) and/or steel acting as electron donors and/or with the steel or even pyrite in the clay possibly serving as catalytic surface. These abiotic reactions would lead to the production of ammonium, which can sorb onto clay minerals and would therefore compete with some radionuclides for sorption. Biotically, nitrate can be consumed as electron acceptor by microorganisms, if proper growth conditions are provided. Respiratory microbial consumption of nitrate leads to the intermediate production of nitrite, and finally to nitrogenous gases. Most of the other leachates from bituminized waste (e.g. acetate, H₂) are biodegradable and can be used as electron donor, to fuel this microbial nitrate reduction. Depending on the electron donor used in this denitrification process, the final overall result could be a gas pressure decrease or increase. During disposal conditions, the microbial population will be exposed to hyperalkaline conditions originating from the pore water from the concrete lining of the waste monolith and the backfill material, which could affect the viability and activity and thus denitrification potential of the microbial population.

The efficiency of the Boom Clay borehole water microbial community to reduce nitrate leaching from regular and thermally aged non-radioactive Eurobitum in the presence or absence of known bitumen degradation products was investigated in different series of anoxic laboratory batch experiments. It was shown that acetate is the most preferred electron donor for microbial nitrate reduction and the highest nitrate reduction rates were observed in the presence of acetate. Formate seemed also to be an easy accessible electron donor, but related to the kinetics of the reaction, not as efficient as acetate to remove nitrate. On the other hand, oxalate was the least preferred electron donor for nitrate reduction and was only completely degraded in one out of three replicates. However, calcium oxalate crystals were formed, indicating that if oxalate is present, it will probably be less (bio)available compared to other organic compounds. Next to the added bitumen degradation products, the microbial community was able to use organics that leached from the Eurobitum blocks as electron donor to carry out nitrate reduction. Moreover, a clear biofilm formation on the solid

bitumen block was observed in all conditions, suggesting that microorganisms could enhance the degradation of Eurobitum.

However, in the high pH conditions expected to prevail in a repository, no biofilm on Eurobitum or nitrate reduction was observed when the community was exposed to pH 12.5 for 110 days. In general, pH 12.5 seems the upper limit for microbial nitrate reduction for both the microbial community of the Boom Clay borehole water and the pH adapted Harpur Hill sediment. However, stress evoked by this high pH was not enough to eliminate the complete microbial community as intact cells remained present and could be resuscitated when lowering the pH to a less alkaline pH. In addition, the Harpur Hill sediment revealed an initial increase in cell numbers at this high pH 12.5 similar as observed in the other conditions. The microbial community present in the Harpur Hill sediment is clearly adapted to high pH conditions as twice as much nitrate was reduced at pH 10.5 compared to pH 9. The Boom Clay microbial population was also still able to carry out nitrate reduction in at pH 10.5, although rates were lower compared to pH 9. It must be noted, however, that phosphate can quickly become a limiting nutrient in such experimental batch conditions, hence it is important to distinguish between pH and nutrient limitation in these laboratory test. Nevertheless, phosphate is not expected to be a limiting nutrient in situ as phosphate in Boom Clay is bioavailable by the mineral fraction such as apatite. Beta diversity assessment of both communities based on flow cytometry profiles indicate that at pH can induce a shift in the microbial community which is different depending on the microbial community.

Altogether, this study indicates that microbes can make biofilms on Eurobitum, and can reduce the nitrate leaching of the bitumen to nitrite and nitrous gasses. To carry out this nitrate reduction, the microbial community is able to use organics leaching from the bituminized waste. High alkaline pH (up to 12.5) alone is not sufficient to eliminate microbial presence in a geological repository, but it can induce a significant shift in the microbial community and inhibit microbial activity. Where a less alkaline pH will occur, e.g. at the boundary of the Boom Clay and the concrete liner of the disposal gallery, microbes can be present and are able to perform nitrate reduction.

D1.4 A Report of Uranium(VI) Speciation Associated with Cellulose Degradation Products (HZDR)

Work Package 1 of the MIND project addresses remaining key issues for the geological disposal of ILW concerning the long-term behaviour, fate and consequences of organic materials in the waste along with H₂ generated by corrosion and radiolysis. Degradation products of organic components in nuclear waste can enter the environmental water pathways. They can act as complexing agents for released radionuclides. Thereby they can increase the concentration of radionuclides in environmental waters. Hence, a mobilization of toxic radionuclides can take place. This report deals with a detailed characterization of the uranium(VI) speciation in the presence of acetate and isosaccharinic acid (HISA) mainly by applying spectroscopic techniques. The motivation for this study was to provide spectroscopic parameters such as absorption-, luminescence- and infrared spectra as well as structural data, for each uranyl complex, which can then serve as reference data. It was further important to work with low, environmentally relevant uranium concentrations. Especially in the uranium(VI)-acetate system, it was difficult and partially not reasonable to interpret the spectroscopic data with the formation of three uranyl-acetate complexes. For this reason, especially the studies focusing on the determination of the speciation were critically reviewed. The combination of experimental results of the present and crucial revelations in previous studies results in a different point of view on the aqueous uranyl-acetate system. Moreover, the fate of uranium in a long-term tissue degradation experiment under hyperalkaline conditions was investigated.

The report will provide a basis for the new interpretation of soluble U(VI) species in the presence of acetate and isosaccharinate (ISA) as main products of cellulose degradation. This impacts also the modelling of U(VI) dissemination from nuclear waste repositories.

D1.5 Microscopy and element mapping characterization studies of radionuclide microbe organic interactions (UGR)

Deep geological repository (DGR) has been proposed as the safest option for the disposal of radioactive wastes. In this multi-barrier's system, the radioactive wastes will be encapsulated by metal containers surrounded by artificial and natural barriers. Bentonite clay formations have been selected as reference materials for safety barriers in a DGR. In Spain, bentonite formations located in Cabo de Gata Natural Park (Almeria) were selected for this purpose. Microbes can affect the safety and performance of future DGRs at 3 different levels by: i) transformation of clay minerals through Fe(III) reduction; ii) corrosion of metal canisters; and iii) alteration of radionuclide mobility through different interaction processes. For instance, microorganisms are able to interact with typical elements of radioactive waste like selenium (Se), uranium (U) or curium (Cm) through different processes like biomineralization, biosorption or reduction leading to their mobilization or immobilization.

The main objectives of this study is to determine the role of *Stenotrophomonas bentonitica*, bacterial strain isolated from Spanish bentonites on the mobility and migration of radionuclides (Se(IV), Se(VI) and Eu(III)) under DGR relevant conditions using a multidisciplinary approach combining microscopy, spectroscopy, cell biology, radiochemistry, microbiology, etc. Selenium is a common component of radioactive waste and Eu(III) was considered as inactive analogue of trivalent actinides, Cm(III) and Am(III).

In the case of Se, this strain was able to reduce Se(VI) and Se(IV) to Se(0) forming Se nanostructures. Using a combination of number of microscopic techniques (STEM/HAADF, ESEM, FESEM, etc.), we could demonstrate here that *Stenotrophomonas bentonitica* reduce Se(IV) to Se(0) aerobically at pH7, forming initially amorphous Se(0) (a-Se) nanospheres which subsequently transform to one-dimensional (1D) trigonal selenium (t-Se) nanostructures with diverse crystallinities, morphologies and sizes. Due to the low solubility of t-Se nanostructures compared to that of a-Se nanospheres and Se(IV), the mobility of selenium in the environment may be significantly reduced. Time-dependent experiments showed that the cells of *S. bentonitica* and their proteins are involved into the transformation of amorphous Se(0) nanospheres (a-Se) to one-dimensional (1D) trigonal selenium (t-Se) nanostructures (hexagons, polygons and nanowires). In addition, *S. bentonitica* is also able to reduce Se(IV) under anaerobic and alkaline conditions (up to pH 10) producing a-Se nanospheres. However, the cells synthesize only intracellular Se(0) nanowires when this strain grow aerobically in presence of Se(VI).

The production of different volatile methylated Se species produced by *S. bentonitica* confirmed the change in the chemical speciation from Se(VI) and Se(IV) to Se(-II).

On the other hand, *S. bentonitica* is able to interact with Eu(III) mainly by a biosorption process under both aerobic and anaerobic conditions.

The Se reduction and production of crystalline t-Se nanostructures would be of great significance within the DGR system since the mobility of Se through the surrounding environment may be reduced. The biosorption of Eu(III), as a non-radioactive analogous to An(III) characteristics of nuclear waste, would also be of importance within the DGR system due to its retention through the surrounding environment.

D1.6 Relevant metabolic processes and limits on chemical conditions leading to methane generation in LLW and ILW (VTT)

Results obtained from in-situ large-scale Gas Generation Experiment (GGE) in final disposal repository for operational LLW and ILW in Olkiluoto, Finland, are summarized in this report. The aim of the GGE was to study gas generation in LLW repository conditions using representative maintenance waste from nuclear power units. The results obtained from the GGE has been used as a basis of the conclusions presented in this report.

Gas generation can lead to overpressure in the repository and migration of water-borne or gaseous radionuclides to the biosphere. Gas generation in geological LLW and ILW repositories in anoxic conditions occur mainly by corrosion of steel and as a result of microbial degradation of organic polymers like cellulose.

Environmental conditions in the repository influence the microbial activity and the rate of gas generation. In addition to the water activity and temperature, one of the major factors influencing the gas generation is pH. Alkaline conditions limit microbial activity but microbes have been shown to adapt extreme environmental conditions. Suitable niches for microbial activity are formed in heterogenic chemical conditions. Microbes can also reduce pH by producing microbial metabolites as shown during operation of the GGE. Gas generation can also be reduced if methanogenesis is inhibited by the formation of microbial metabolites such as volatile fatty acids. Other typical chemical compound that is known to cause toxic effects and to inhibit methanogenesis is hydrogen sulphide. Methanogens compete with other microbial groups for electron acceptors and especially sulphate reducers (SRBs) can influence gas generation. The activity of SRBs is linked to sulphate which can be leached from the waste materials or enter the repository with groundwater.

D1.7 Methane generation in the Mt Terri URL and the underlying microbial processes (EPFL)

Methanogenesis is one of the potential microbial processes in deep geological repositories after closure. It could have several impacts on the repository environment due to the consumption and production of gases and induced changes in pH which can affect the surrounding geochemistry. It is potentially less harmful than sulfate reduction in a repository environment as it does not produce any corrosive substances and can consume hydrogen gas.

In-situ experiments at Mont Terri URL have shown that there is a diverse microbial community within borehole water which is capable of utilising hydrogen gas. Methanogens have been identified in this community at very low abundance (<0.1%), however the experiment showed no evidence for the onset of methanogenesis with no increase in methane concentrations over time. The composition of the microbial community varies with time, but there is no increase in the relative abundance of methanogens. Further *in-situ* experiments using a porous sand:bentonite medium showed dominance of methanogenic archaea and fermenting bacteria after nine months of incubation. Laboratory microcosm experiments where the initial sulfate concentrations were very low also show no evidence for methanogenesis even after sulfate depletion. There is no increase in methane concentration and at the end of the experiment the microbial community is dominated by one family of sulfate-reducing bacteria. Further analysis of the geochemical conditions in both experiments suggests that the bicarbonate concentration is too low in these systems which makes methanogenesis thermodynamically unfavourable. The effect of bicarbonate concentration on the onset of methanogenesis is being investigated using a series of laboratory microcosm experiments. Further evidence that methanogenesis is extant in the presence of sand/bentonite, experiments are also ongoing to assess the impact of trace metal availability, which are essential for enzyme co-factors in methanogenesis.

D1.8 Modelling of microbial processes relevant to ILW disposal (NNL)

Work Package 1 (WP1) of the Microbiology in Nuclear waste Disposal (MIND) project addresses remaining key issues for the geological disposal of organic-containing intermediate level radioactive wastes (ILW). Uncertainties examined by the WP include study of the role that anaerobic microbes play in the degradation of organic (e.g. cellulose) wastes and the metabolism of soluble organic degradation products by reaction with oxidised (electron acceptor) species (e.g. NO_3^- , Fe(III) , SO_4^{2-}) present in the repository system. Such reactions are important as they will establish reducing chemical conditions that stabilise the less mobile forms of some radionuclides (e.g. Se, Tc, U, Np, Pu). In addition, organic degradation products that may act as radionuclide complexants may be decomposed. Ultimately, organic biodegradation processes can lead to the establishment of methane gas generation, which is important in terms of its potential physical effects on the repository and for the transport of gaseous radionuclides such as C-14. The ability of microbes to mediate these reactions is, however, limited by a number of environmental constraints. In particular, for the case of ILW disposal, the alkaline pH conditions developed by the use of cementitious materials may limit microbial activity.

Methane may also be formed by hydrogenotrophic microbes that utilise hydrogen gas generated by anaerobic corrosion and radiolysis of organic wastes, which will lead to an overall reduction in the amount (moles) of gas produced. Microbes readily consume hydrogen as an energy source by reaction with electron acceptors such as sulfate present in groundwater. Sulfate reduction tends to out-compete the methanogenic processes and acts as a constraint on gas generation processes. Sulfide produced by sulfate reduction is of further importance with regard to the promotion of copper corrosion, widely proposed for containment of spent fuel, which is considered by MIND Work Package 2.

This report (MIND Deliverable D1.8) describes modelling approaches that can be used to elucidate the above complex set of microbial processes and which can be integrated with the wider chemistry of experimental systems and the ILW repository concept. The report first discusses the role that models of microbial processes can play in supporting the safety case for the disposal of low level waste and ILW, such as in the quantification of rates of gas generation and in underpinning the phenomenological understanding of the repository near-field processes. The computational approach to represent the main microbial kinetic processes of relevance to radioactive waste disposal is then described, including the coupling of microbial kinetics with chemical speciation and the representation of microbial processes in reactive-transport codes.

Three case studies are presented representing modelling studies undertaken with the MIND project, which serve as examples of the application of microbial modelling to large-scale and in-situ experiments. The first case study concerns modelling of a long-term and large-scale gas generation experiment located at the LLW/ILW repository, Olkiluoto, Finland and provides an example of how modelling can be used to predict rates of gas generation from cellulose-containing LLW and to further understand the controls on methanogenesis, such as the effect of alkaline pH. The model reproduces the variation in pH and other chemical variables observed and is consistent with microbiological characterisation studies of the experiment undertaken within the MIND project (D1.6).

Two further case studies model biogeochemical processes occurring in in-situ borehole experiments at the Mont Terri underground rock laboratory that have examined the fate of hydrogen injected into Opalinus Clay. The second case study models the diffusion and reaction of nitrate injected in the Bitumen Nitrate Clay (BN) experiment. Here the model is able to represent the reaction of a pulsed equilibration of hydrogen with nitrate previously injected into the borehole. The model represents the formation of nitrite as an intermediate in the overall denitrification process to form N_2 gas and the overall drop in gas pressure as H_2 gas is consumed. The model is able to represent the associated

increase in pH observed with the H₂ reaction and provide further confirmation of the autotrophic nature of the processes that appear to be stimulated. The third case study considers the Microbial Analysis (MA) experiment undertaken in the BRC-3 bioreactor. Detailed metagenomic and metaproteomic analyses indicate that hydrogen injection firstly stimulates an autotrophic process that fixes inorganic carbon, which is used as a carbon source for heterotrophic sulfate reduction. Such an acetogenic process has been represented in the model of the experiment that generates acetate that is used by the sulfate reduction process. Further H₂ injections with the BRC-3 bioreactor have been undertaken within the MIND project (D1.7) to examine whether methanogenesis could ultimately be developed by a hydrogenotrophic process in Opalinus Clay, however in these tests sulfate has remained in the bioreactor borehole. The model has the potential to quantify the rate of hydrogen generation required to exhaust the high concentrations of sulfate present within the bioreactor, which is replenished by diffusion from the Opalinus Clay. However, with an excess supply of hydrogen, other reactants may become limiting, such as inorganic carbonate.

The report concludes with a discussion of the potential wider use of microbiological models in studies to support the safety case for radioactive waste disposal.

Appendix B Supplemental reporting of studies of ion exchange resin degradation

B1 Survival of microorganisms in solutions of irradiated ion exchangers (CV Rez/TUL)

Kateřina Černá (TUL), Tomáš Černoušek (CV Rez), Alena Ševců (TUL), Petr Polívka (CV Rez)

B1.1 Introduction

Ion exchangers form a significant component of the organic inventory of LLW and ILW in EU member states (Abrahamsen et al., 2015). Ion exchangers are used in nuclear power plants to remove radionuclides from water. The ion exchanger inventory consists of both cationic and anionic exchangers that share a common polystyrene polymer chain cross-linked by divinyl benzene. The functional groups with ion-binding capacity are sulfonate groups (cation exchange) or amine groups (anion exchange) (Wang and Wan, 2015).

Because these ion exchangers bind radionuclides, it is expected that they will be exposed to significant gamma radiation, potentially resulting in their radiolytic degradation. During irradiation, the functional groups are released more easily, leaving the aromatic structure that will be more resistant to radiolysis and biodegradation (Figure B1- 1) (Rébuba et al., 2015; Traboulsi et al., 2012; Van Loon and Hummel, 1995).

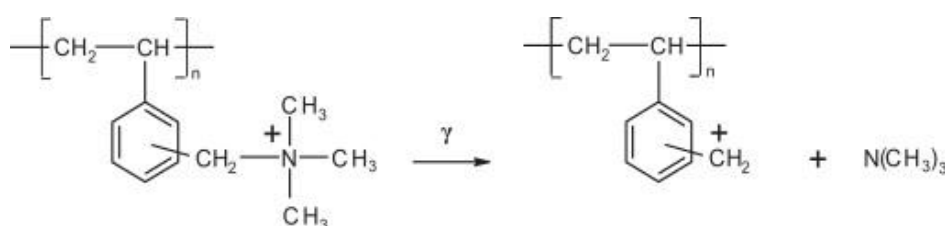


Figure B1- 1 Scheme of trimethylamine production by Gamma radiation from polystyrene ion exchangers (Traboulsi et al., 2012)

However, there are several limitations to the current state of knowledge regarding the radiolytic degradation of ion exchangers. Rébuba et al. (2015) showed, that trimethylamine (TMA) and H_2 production is generally higher under anaerobic conditions, than in aerobic environments, which is relevant for the ILW repository as it is expected to transition rapidly to anoxic conditions. Additionally, the range of analytical tools applied to characterize the products of ion exchanger irradiation has been limited in early work (Rébuba et al., 2015) and it is very likely that major groups of compounds have been overlooked.

The sulfate and amines released during the radiolysis can provide potential energy sources for microbial activity. Radiolysis of the polystyrene ion exchanger structure can further provide dissolved organic carbon for use as an electron donor. Although the microbial degradation of ion exchangers has not been studied in detail and remains uncertain (Kale et al., 2015; Van Loon and Hummel, 1995) we can expect that some of the indigenous microorganisms in the deep underground water surrounding repository or present within EBS might have the potential to further degrade (i.e., biodegrade) the radiolytic degradation products. These compounds could produce chelating agents for radionuclides increasing their release in geosphere, could fuel sulfate reduction or methanogenesis. More precise knowledge on the microbial role in the ion exchanger degradation

under repository relevant conditions is thus very important for the long-term safety of the ILW repositories.

B1.2 Materials and methods

B1.2.1 Irradiation experiment

B1.2.1.1 VITA water and ion exchangers characteristics

Natural groundwater (called VITA) was collected at the Josef Underground Research Centre (URC), Czech Republic a day before performing each experiment (Figure B1-2). VITA water is an anaerobic water with a high concentration of sulfate and rich in sulfate reducing bacteria (SRB) and fermenting bacteria that form important parts of the indigenous microbial community. The anaerobically collected VITA water was quickly poured into sterile bottles to minimize the contact time with air. The VITA water was then transported to the laboratory and kept under anaerobic conditions inside the glove box before the beginning of the experiments.



Figure B1- 2 Gallery in the Josef URC (on the left) and VITA source of anaerobic water with SRB (on the right)

For the experiments, ion exchangers were selected that are commonly used in pressurized water reactors (PWR): the strongly acidic cation exchanger based on a polystyrene skeleton with sulfonic groups (R-SO_3^-) and the strongly basic anion exchanger based on a polystyrene skeleton cross-linked with divinylbenzene with quaternary ammonium functional groups $\text{R-N}^+(\text{CH}_3)_3$. Ion exchangers had the shape of irregular spheres (diameter from 0.3 to 1.2 mm) (Figure B1-3) with a dry matter content of 49 wt%.

Prior to irradiation, ion exchangers were saturated to 90% of their capacity. Each type was saturated separately using sterile water solutions – sodium and potassium hydroxide for the cation exchanger and nitric and boric acid for the anion exchanger. After 24 hours of saturation the ion exchangers were filtered and further processed as described below.



Figure B1- 3 Appearance of cation exchanger (left) and anion exchanger (right)

B1.2.1.2 Ion exchanger irradiation

All the samples were prepared under anaerobic conditions in the glove box (oxygen < 1 ppm) and at room temperature. The saturated ion exchangers were transferred into the sterile 750 ml glass bottles with parallel walls under an inert atmosphere and ground glass joint was over sealed with paraffin shrink foil (Figure B1-4). Samples of cation exchanger, anion exchanger and a mixture of cation exchanger and anion exchanger in the ratio 2:1, to best simulate the material from the PWR reactor, were prepared this way.



Figure B1- 4 Samples of ion exchanger mixtures before irradiation in the glove box

Prepared samples were placed on the bottom of the irradiation chamber and were irradiated up to 1 MGy total dose by constant 2 kGy/h dose rate (Figure B1-5). Temperature during irradiation was constant 20 ± 1 °C. The content of oxygen in the irradiation chamber was under 0.1 vol.% O₂ after 3 days and did not exceed 0.3% during the irradiation. Bottles were placed in the glove box directly after irradiation and were used for further analyses and experiments.



Figure B1- 5 Bottles with saturated ion exchanger ion exchangers situated inside the irradiation chamber

B1.2.1.3 Characterization of radiolysis products

First, we analysed the composition of the irradiated ion exchangers for total organic carbon (TOC) in the sample and further gas (GC/MS) and liquid (LC/MS) chromatography coupled with mass spectrometry to detect the presence of dominating radiolysis products. The non-irradiated cation and anion exchangers were analysed in parallel and served as controls.

One gram of each exchanger was extracted in either 6 ml of water (TOC and GC/MS) or methanol (LC/MS) prior analysis. TOC was determined using a high temperature combustion TOC Multi N/C 2100S device (Analytik Jena, Germany) at 850°C. Only the water extracts were used for this analysis and the samples were diluted by a factor of 100 prior analysis resulting in the threshold detection limit 50 mg/l.

The water-extracted ion exchanger samples were measured on a gas chromatograph with an ion trap - GC/MS Varian, a Saturn 2200 model. The analysis was performed in headspace fullscan mode (all ions are monitored) with VF 624ms column. Sample agitation (homogenization and temperature stabilization for reproducible measurement) was at 80°C. The column temperature began at 45 and ended at 230°C. The chromatograms obtained were compared to the blank spectrum, which is caused by the natural presence of some volatile compounds in the laboratory and its equipment.

A Dionex Ultimate 3000 system with an AB Sciex 3200 QTRAP MS detector was used. For direct infusion an infusion speed of 10 µl/min was used. The declustering potential was set to 40 V, entrance potential to 10 V, Curtain gas to 30 psi, Gas 1 to 30 psi, Gas 2 to 0 psi and ion source temperature to ambient. Positive Fullscan was used in the mass range 100-1200 m/z. The ESI voltage was 5500 V and scan time 2 s. For Gel Permeation Chromatography (GPC) the mobile phase consisted of 70% methanol and 30% water with the addition of 1 mM ammonium acetate. The flow rate used was 0.6 ml/min. An Asahipak GF-310 HQ GPC column was used, the temperature of the column compartment was set to 40°C. The declustering potential was set to 40 V, entrance potential to 10 V, Curtain gas to 30 psi, Gas 1 to 50 psi, Gas 2 to 60 psi and ion source temperature to 500 °C. Positive Fullscan was used in the mass range 100-1200 m/z. The ESI voltage was 5500 V and scan time 2 s.

B1.2.2 Microbial degradation experimental set-up

Further, study of microbial degradation of a 2:1 (cation exchanger:anion exchanger) mixture was carried out. At the beginning, preliminary tests used several concentrations of ion exchanger

mixtures (not presented). Based on these preliminary results we performed batch experiments under anaerobic conditions for 18 months:

Amounts of 0.1 , 1 or 10 g of 2:1 mixture of either irradiated or non- irradiated ion exchangers were mixed with 500 ml inoculum water to obtain the concentration of 0.2, 2 or 20g/l. The inoculum water consisted of VITA water and degassed, sterile filtered tap water in ratio 1:4 (VITA:TW). Controls containing only inoculum water were also included to see the behaviour of inoculum over time without the effect of ion exchangers. The sample preparation and its long-time storage took place in the glove box under strictly inert atmosphere ($O_2 < 1$ ppm). The experiment was sampled immediately after the start (0 day), 3 days, 1 month, 6 months, 12 months and 18 months.

B1.2.3 Microbial community analyses

B1.2.3.1 Sample processing and chemical analysis

At the sampling times, the samples were homogenized and the solutions were filtered through sterile 0.22 μ m GV Durapore® filter membrane and the filters were immediately stored in a deep freezer (-80°C) and subsequently used for DNA extraction as described below. The filtrate was used for the analysis of volatile organic compounds - 10 ml vials with a septum were filled with the filtrate in an anaerobic glovebox for GC/MS chromatography as described in B1.2.1.3.

B1.2.3.2 DNA Extraction

The DNA from VITA water biomass concentrated at 0.22 μ m GV Durapore® filter membranes was extracted by means of PowerWater® DNA Isolation Kit from Mo Bio (QIAGEN) according to manufacturer's protocol.

B1.2.3.3 Quantitative real-time PCR (qPCR)

The method of qPCR was used to describe relative changes in bacterial abundance in the samples. Because obtaining standards fully representative of these samples is very difficult and absolute quantification without standardized calibration curves is not possible, a relative quantification (RQ) $\Delta\Delta C_q$ calculation method was used. It estimates the magnitude of difference in C_q values between the sample zero state at the beginning of experiment and the sample after treatment (or in time in no treatment control (NTC) samples) using the formula $RQ = \text{effectivity}^{(-\Delta C_q)}$. PCR effectivity for each marker was estimated beforehand by measuring the slope of curves constructed from a serial dilution of template DNA from five internal environmental standards. The measured C_q values were normalized by the sample mass used for DNA extraction prior to calculations.

We used a marker specific for total Eubacteria and this marker was amplified using primers described in Table B1-1 on a LightCycler® 480 Instrument (Roche Biochemicals, USA). Reaction mixtures were prepared in 10 μ l of reaction volume. The mixture contained 2 μ l of DNA template, 5 μ l KAPA SYBER FASTqPCR kit (Kapa Biosystems. Inc., MA, USA), 0.4 μ l of 2 μ M forward and reverse primer mixtures (Generi Biotech, Czech Republic, IDT, US) and 2.6 μ l ultra-pure water (Bioline, UK). For each DNA sample, qPCR reaction was performed in duplicate along with negative control where DNA template was replaced by nuclease free water. Reaction conditions consisted of an initial 5 min incubation at 95°C, followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 60°C for all primers except for Geobacteraceae, which was 55°C for 15 s and extension 72 °C for 20 s with final extension at 72°C for 3 min. Finally, a melting curve was set for 5 s at 95°C, 1 min at 65°C and final ranging from 60 to 98°C, with a temperature gradient of 40°C per 10 s. Purity of the amplified fragment was determined through observation of a single melting peak. Crossing point values were obtained using the 'second derivative maximum' method included in the LightCycler® 480 Software.

Table B1- 1 qPCR primers for total bacteria

Primer	Sequence 5' - 3'	Specificity	Description	Reference	Annealing temp.
16SqPCR-F	TCCTACGGGAGGCAGCAGT	All Eubacteria	Gen for 16S rRNA	(Clifford et al., 2012)	60°C
16SqPCR-R	GGACTACCAGGTATCTAATCCTGTT				

B1.2.3.4 Library preparation and next generation sequencing

The next-generation sequencing (NGS) of 16S rDNA amplicons was used to study microbial diversity in our samples. The size of the amplicons was kept below 400 bp to cover as much microbial diversity as possible by performing In silico analysis of primers (Němeček et al., 2017).

Two consecutive PCR reactions per sample were performed during library preparation. We used primers 530F and 802R (Table B1-2) in the first PCR reaction and the PCR conditions were as follows: 95°C for 3 min; 15 cycles at 98°C for 20 s, 50°C for 15 s and 72°C for 45 s; and a final extension at 72°C for 1 min. Subsequently we performed a second PCR reaction with tagged barcode fusion primers. We used 21 differently tagged bar code fusion primers in one library preparation which enabled us to sequence up to 20 samples (plus mock up) in one run. The second PCR was performed as follows: 95°C for 3 min; 35 cycles at 98°C for 20 s, 50 °C for 15 s and 72 °C for 45 s; with a final extension at 72°C for 1 min. The quality of the library product was checked by gel-electrophoresis technology. The PCR products were purified using the Agencourt Ampure XP system (Beckman Coulter, Brea, USA), and the concentration of the purified PCR products was measured with a Qubit 2.0 fluorometer (Life Technologies, USA). Following this step, the barcode-tagged amplicons from different samples were mixed in equimolar concentrations.

Sequencing of the amplicons was performed on an Ion Torrent PGM (Thermo Fisher Scientific, USA) using the Ion PGM Hi-Q Sequencing Kit with the Ion 314 Chip following the manufacturer's instructions (Thermo Fisher Scientific).

Table B1- 2 Primers for amplicon sequencing of 16S rRNA gene

Primer	Sequence 5' - 3'	Coverage			Reference
		Archaea	Bacteria	Eukaryotes	
530F	GTGCCAGCMGCNGCGG	54,9	96,9	94,0	(Dowd et al., 2008)
802R	TACNVGGGTATCTAATCC	91,8	92,5	0,9	(Claesson et al., 2009)

B1.2.3.4 NGS data processing

Raw reads were split to particular samples by Mothur software (Schloss et al., 2009). The split samples were subsequently processed by the DADA2 software package (Callahan et al., 2016). Low quality and short reads were removed as well as chimeric sequences. Taxonomy classification of detected operation taxonomic units (OTUs) by the DADA2 package used SILVA database (version 13, www.arb-silva.de). The accuracy of classification was verified and evaluated against a predefined artificial MOCK community sample with 4 different taxa: *Enterococcus*, *Bacillus*, *Klebsiella* and *Staphylococcus*. Dada2 output was transformed to a Phyloseq object in R and subsequent bioinformatics analyses were performed in the R software using the Phyloseq library (McMurdie and Holmes, 2013). PCoA (principal component) cluster analysis was performed using the Phyloseq library using Bray-Curtis dissimilarity.

B1.3 Results and discussion

B1.3.1 Ion exchanger radiolysis products

The irradiated samples of cation exchanger and anion exchanger were visually different from non-irradiated samples as they notably changed their colour during irradiation (Figure B1- 6) and irradiated anion exchanger samples also had very typical odour.



Figure B1- 6 Samples of irradiated (on the right) and non-irradiated (on the left) cation exchanger and anion exchanger

Irradiation decreased the water content in the samples and increased TOC in both cation exchanger and anion exchanger, compared to non-irradiated ion exchangers (Table B1- 3). The amount of TOC was lower than 50 mg/l (detection limit) in non-irradiated cation exchanger and anion exchangers, but was several times higher in irradiated samples. The detected TOC values were also several times higher in irradiated anion exchangers than in cation exchanger, indicating that the anion exchangers are more prone to radiolysis.

Table B1- 3 Amounts of leachable organic compounds in irradiated and non-irradiated samples of cation exchanger and annex

Sample	Dry matter content (%)	TOC (mg/l)
cation exchanger non-irrad	62	< 50
cation exchanger irrad	38	165
anion exchangers non-irrad	54	< 50
anion exchangers irrad	34	980

Consistent with the TOC analyses, GS/MS detected the presence of several organic radiolysis products such as trimethylamine (TMA), dimethylamine, acetatnitrile, acetone (2-propanon) a 2-butanon originating from anion exchangers and benzene originating from cation exchanger (Figure B1- 7, Figure B1- 8). The presence of TMA was also responsible for the characteristic odour of the irradiated annex samples and resulted from anion exchange radiolysis as described in the literature (Rébufa et al., 2015; Traboulsi et al., 2012).

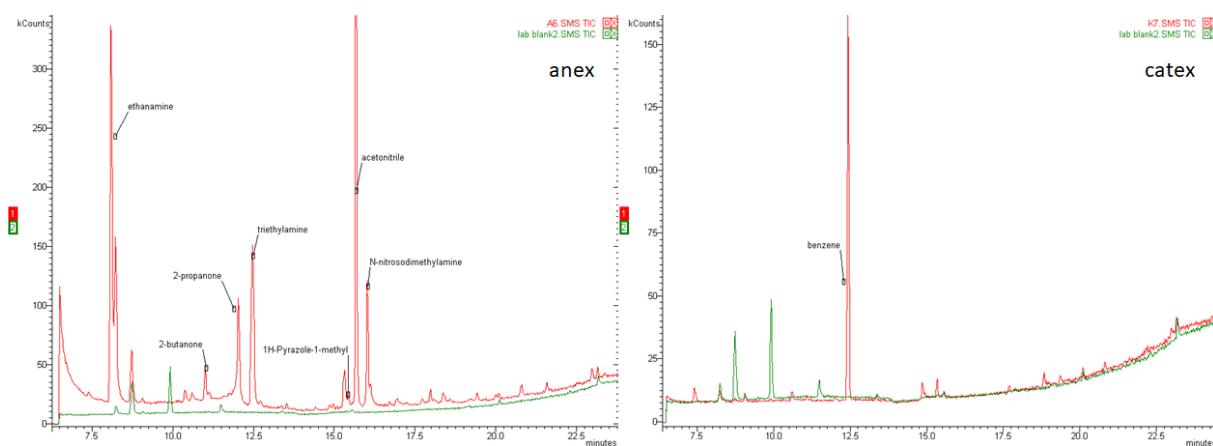


Figure B1- 7 GS/MS chromatograms of irradiated cation exchanger and annex with descriptions of major detected radiolysis products.

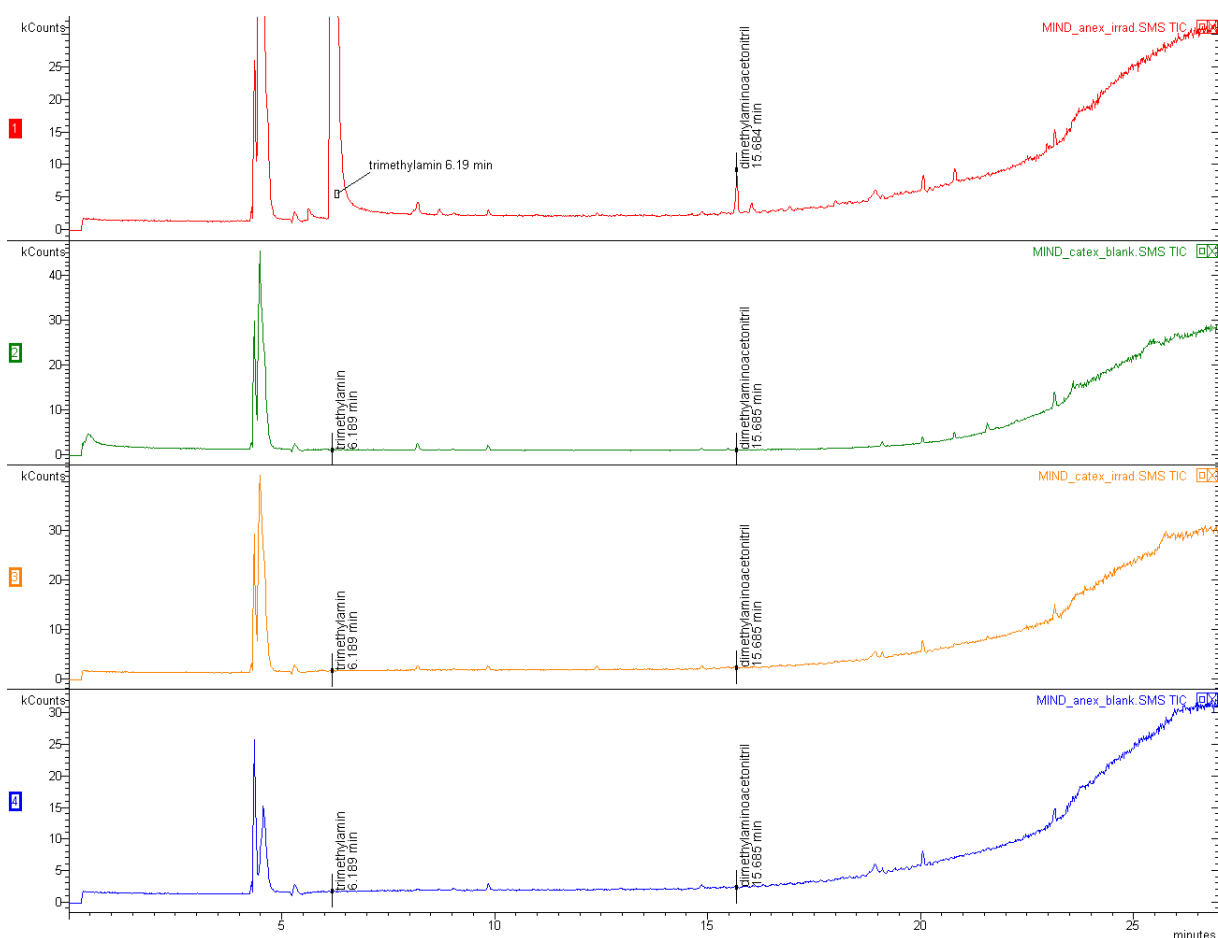


Figure B1- 8 GS/MS chromatograms of irradiated and non-irradiated cation exchanger and anion exchanger.

On the other hand, the results of LC/MS showed presence several larger organic degradation products especially in irradiated cation exchanger sample (Figure B1- 9), the composition of the anion exchange sample was rather similar to the non-irradiated sample. Differences between irradiated and non-irradiated cation exchanger were also detected in GPC mode (Figure B1- 10). The regular spacing between the detected peaks indicates that the organic molecules of various sizes originate from one polymeric source (polystyrene chain of the ion exchangers). However, the

detected organic radiolysis products were not structurally identified and the insoluble organic material was not analysed.

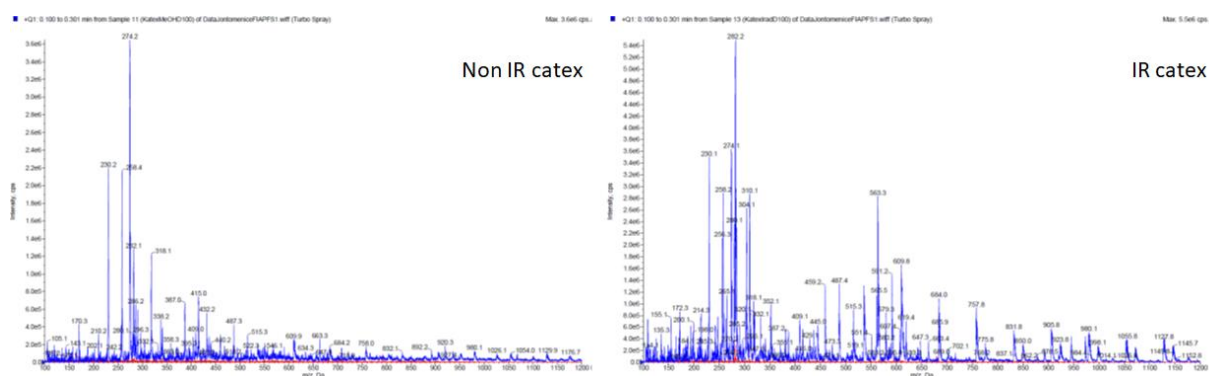


Figure B1- 9 LC/MS chromatograms of irradiated and non-irradiated cation exchanger.

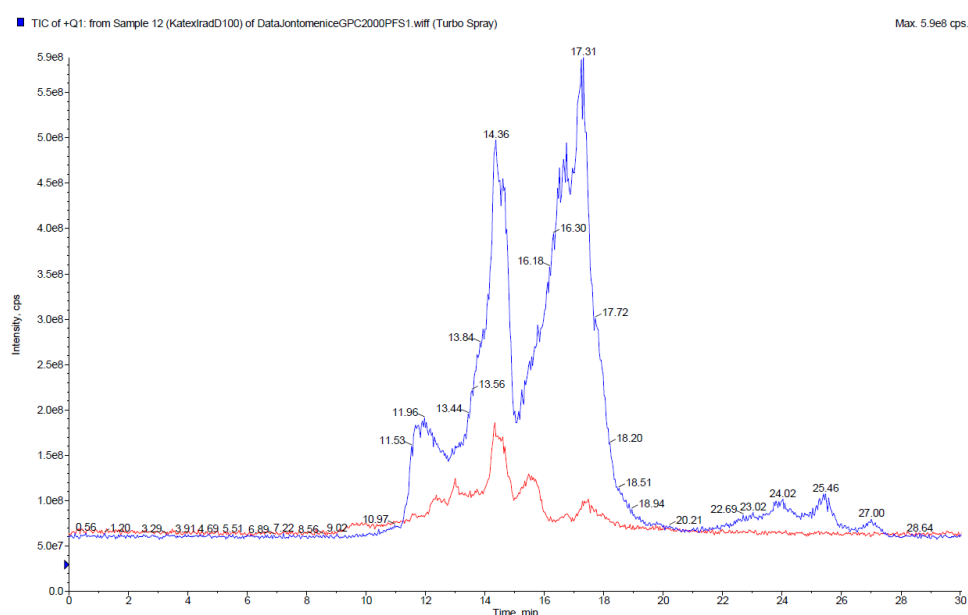


Figure B1- 10 LC/MS chromatograms in GPC mode – irradiated cation exchanger (blue) and non-irradiated cation exchanger (red).

The analyses revealed, that anion exchange resin is more sensitive to radiolysis caused by Gamma radiation than cationic exchanger. Resulting products of anion exchange resin radiolysis are mostly volatile, while radiolysis of cation exchanger results in the production of lower amounts of larger organic molecules, which agrees with the results of other studies (Rébufa et al., 2015; Traboulsi et al., 2012).

B1.3.2 Microbial biodegradation of irradiated ion exchangers

B1.3.2.1 GS/MS volatile organic compound analysis

Although we clearly detected the radiolysis products in both irradiated resins and also in their mixture (not shown), we did not detect the radiolysis products in filtrates from our experiment on microbiological degradation. We found probable presence of carbon disulphide or benzene by GS/MS in a few samples, but their concentration was at or below the detection limit and thus could not be reliably distinguished from the natural laboratory contaminants (Figure B1- 11).

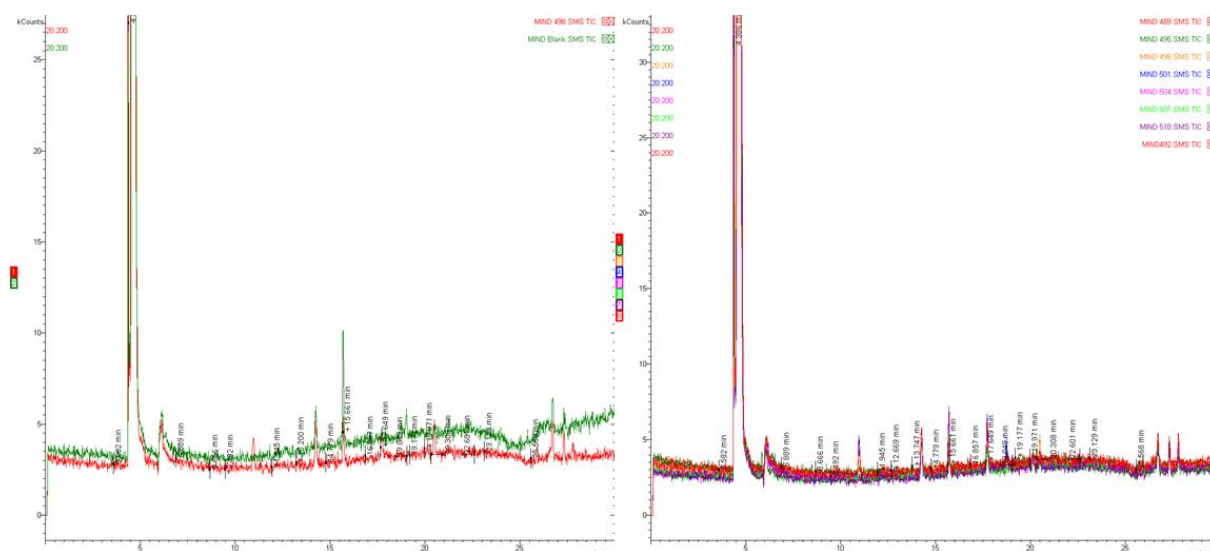


Figure B1- 11 Example of GS/MS of biodegradation experiment samples – on the left comparison of sample 489 (Irradiated 0.1 g/l ionex + inoculum) with blank showing no signal, similar results were obtained for most of the samples (example on the right). Results of other samples are very similar (not shown).

These results imply, that volatile compounds were either too diluted by the sample preparation or lost during the sample handling.

B1.3.3 Microbial community analyses

On the other hand, the results of qPCR confirm the possible presence of radiolysis products in samples with irradiated ion exchangers, because we detected highest increase in relative microbial abundance at the concentrations 0.2 and 2 g/l. In the non-irradiated ion exchanger samples of the same concentrations the increase in relative microbial abundance was lower (Figure B1-12). However, irradiated ion exchange ion exchangers at the concentration of 20 g/l proved to be lethal for the present microorganisms on the contrary to non-irradiated ion exchangers of the same concentration. We ascribe the lethal effect of higher concentrations of irradiated ion exchangers to a decrease in pH resulting from the irradiation and exchange processes (lowest measured pH in irradiated ion exchange samples was 4.76 compared to other samples with pH 7-9). The microbial abundance in control VITA water sample gradually decreased during the experiment (Figure B1- 12).

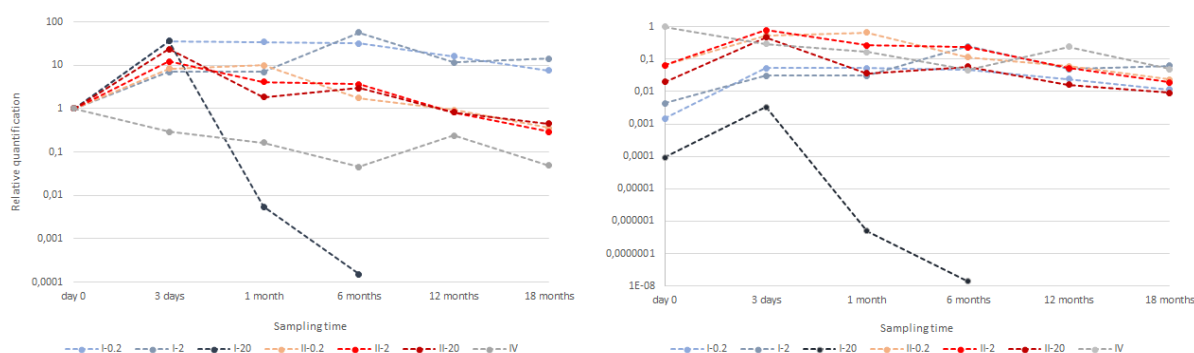


Figure B1- 12 Relative quantification of 16S rRNA gene copies (measure of total microbial biomass) in studied samples. On the left changes in relative quantification in each sample during the experiment, on the right relative quantification recalculated to the original Cq value of VITA water (sample IV), which better illustrates the original differences in relative abundances. . I – irradiated ion exchangers, II – non-irradiated ion exchangers, IV – VITA water. 0_2, 2, 20 – concentration of ionexchangers (g/l).

The microbial composition in samples analysed by NGS was partially determined by the experimental set-up. The overall microbial composition is shown in Figure B1- 13. The samples were dominated by the Gram-negative non-sporulating heterotrophic facultatively anaerobic microorganisms capable of nitrate respiration in the absence of oxygen. We detected the presence of several microorganisms that might utilise the radiolysis products such as *Limnobacter*, *Hydrogenophaga*, *Sulfuritalea* and *Ceaeomonas* capable of using various reduced sulphur compounds as electron donors, methylotrophic *Methylotenera* and *Methyloversatilis* metabolizing methane and methylamine or *Sphingobium* and *Novosphingobium* capable of metabolizing various aromatic organic compounds. However, we have not detected any specific pattern in their occurrence within the samples and thus no relation to the presumed presence of radiolysis products in sample with irradiated ion exchangers.

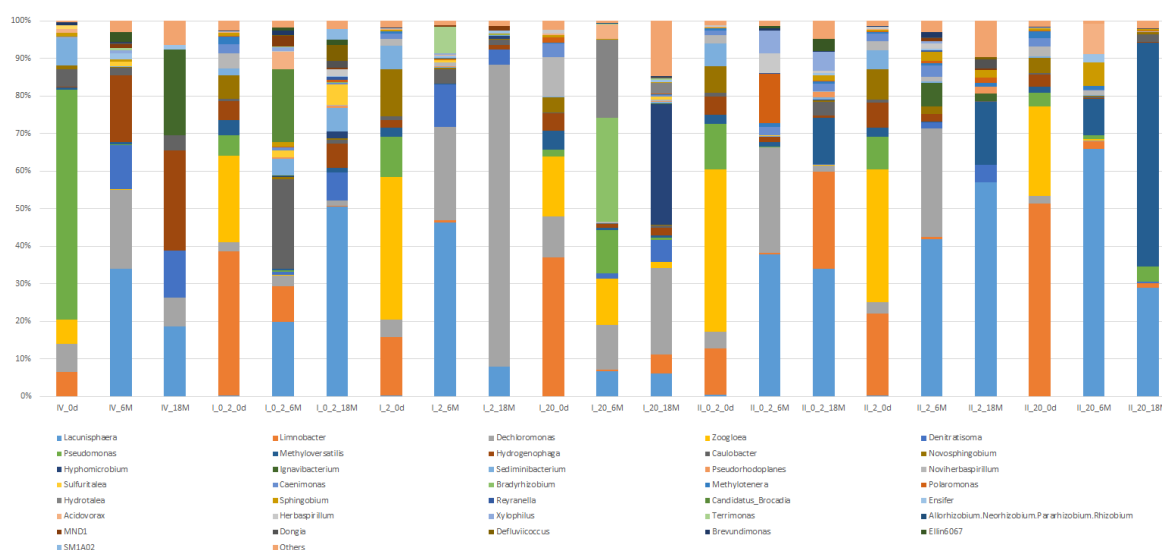


Figure B1- 13 Microbial composition in the samples - genera with mean of relative abundance above 0.2%. I – irradiated ion exchangers, II – non-irradiated ion exchangers, IV – VITA water. 0_2, 2, 20 – concentration of ion exchangers (g/l)

The PCoA analysis based on detected OTUs (Figure B1- 14) showed, that the time zero samples have clearly different microbial composition than other samples. Furthermore, most of the samples containing non-irradiated ion exchangers showed different microbial composition than the irradiated ion exchanger containing samples. The samples containing irradiated ion exchangers clustered with the VITA water samples together with two non-irradiated ion exchangers samples (II_2_18 and II_0.2_18). Interestingly, the microbial composition of samples with the highest concentration of irradiated ion exchangers (I_20_6M and I_20_18M) clearly does not differ from other samples with irradiated ion exchangers indicating the biomass was reduced evenly in most of the detected taxa.

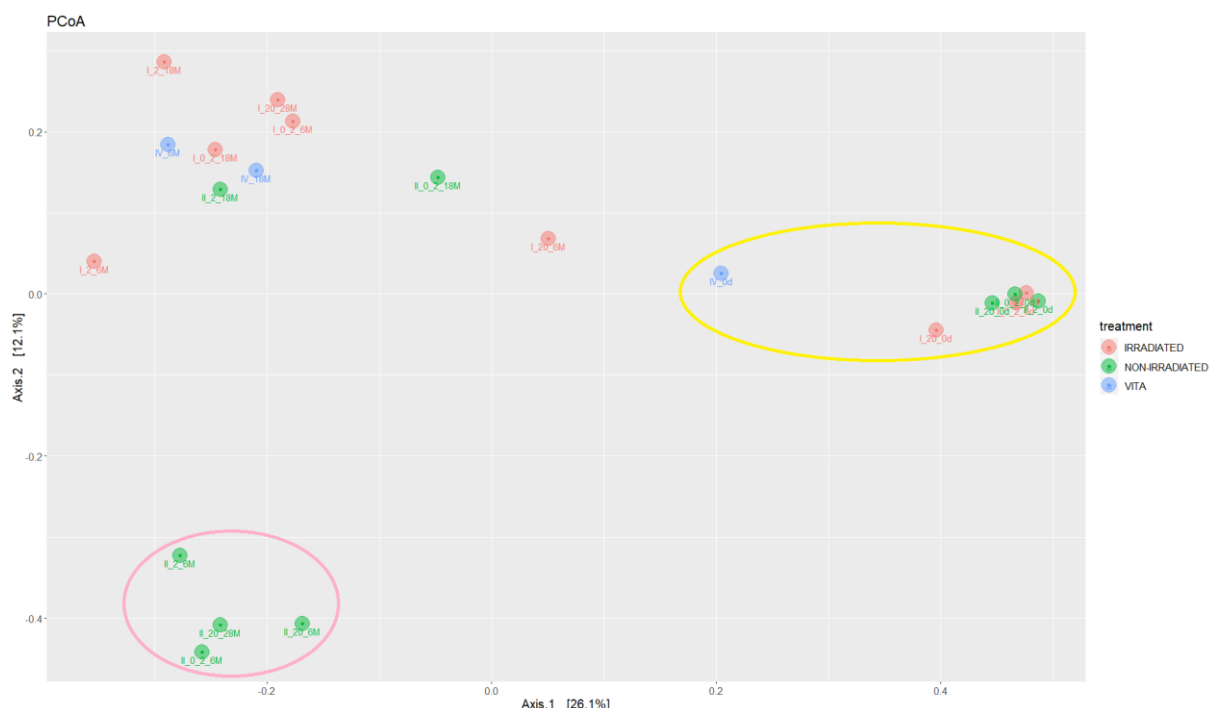


Figure B1- 14 PCoA analysis based on detected OTUs in the studied samples. I – irradiated ion exchangers, II – non-irradiated ion exchangers, IV – VITA water. 0_2, 2, 20 – concentration of ionexes (g/l). Yellow ellipse surrounds all the time zero samples, pink ellipse groups most of the samples containing non-irradiated ion exchangers.

B1.4 Conclusion

The quantity of total organic carbon was several times higher in irradiated cation exchanger and anion exchanger water extracts than the values in non-irradiated ion exchangers and blank samples that were below detection limit. These results clearly demonstrate, that irradiation increases the amount of available organic content in the ion exchanger samples. The gas chromatography confirmed the TOC analysis and we detected several volatile low molecular weight organic compounds such as trimethylamine, dimethylamin, acetatenitrile, acetone and 2-butanone in anion exchanger or benzene in cation exchanger. Liquid chromatography further proved presence of several organic compounds of middle and high molecular weight probably originating from the polystyrene backbone structure of ion exchangers especially in cation exchanger.

It is expected that some of the radiolysis products might serve as electron donors for microbial metabolism. In accordance with this we detected an increase in the overall microbial densities in samples containing irradiated ion exchangers compared to non-irradiated ion exchanger samples. However, high concentration of irradiated ion exchangers (20 g/l) had a strong negative effect on microorganisms probably due to low pH in these samples due to radiolysis. Although the detected increase in microbial activity implied the presence of radiolysis products in the samples inoculated with bacteria, we have not reliably detected the presence of volatile substances in the samples by gas chromatography. This could be caused either by the excessive dilution of the samples compared to preliminary analysis of non-diluted ion exchanger or the volatile compounds were lost during the sample handling.

Acknowledgement

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880 The MIND-project.

References

- Abrahamsen, L., Arnold, T., Brinkmann, H., Leys, N., Merroun, M., Mijndendonckx, K., Moll, H., Polivka, P., Ševců, A., Small, J., Vikman, M., Wouters, K., 2015. A Review of Anthropogenic Organic Wastes and Their Degradation Behaviour (DELIVERABLE No. D1.1). MIND.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. <https://doi.org/10.1038/nmeth.3869>
- Claesson, M.J., O’Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J.R., Smidt, H., de Vos, W.M., Ross, R.P., O’Toole, P.W., 2009. Comparative Analysis of Pyrosequencing and a Phylogenetic Microarray for Exploring Microbial Community Structures in the Human Distal Intestine. *PLoS ONE* 4, e6669. <https://doi.org/10.1371/journal.pone.0006669>
- Clifford, R.J., Milillo, M., Prestwood, J., Quintero, R., Zurawski, D.V., Kwak, Y.I., Waterman, P.E., Lesho, E.P., Mc Gann, P., 2012. Detection of Bacterial 16S rRNA and Identification of Four Clinically Important Bacteria by Real-Time PCR. *PLoS ONE* 7. <https://doi.org/10.1371/journal.pone.0048558>
- Dowd, S.E., Callaway, T.R., Wolcott, R.D., Sun, Y., McKeenhan, T., Hagevoort, R.G., Edrington, T.S., 2008. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *BMC Microbiol.* 8, 125. <https://doi.org/10.1186/1471-2180-8-125>
- Kale, S.K., Deshmukh, A.G., Dudhare, M.S., Patil, V.B., 2015. Microbial degradation of plastic: a review 10.
- McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE* 8, e61217. <https://doi.org/10.1371/journal.pone.0061217>
- Němeček, J., Dolinová, I., Macháčková, J., Špánek, R., Ševců, A., Lederer, T., Černík, M., 2017. Stratification of chlorinated ethenes natural attenuation in an alluvial aquifer assessed by hydrochemical and biomolecular tools. *Chemosphere* 184, 1157–1167. <https://doi.org/10.1016/j.chemosphere.2017.06.100>
- Rébufa, C., Traboulsi, A., Labed, V., Dupuy, N., Sergent, M., 2015. Experimental design approach for identification of the factors influencing the γ -radiolysis of ion exchange ion exchangers. *Radiat. Phys. Chem.* 106, 223–234. <https://doi.org/10.1016/j.radphyschem.2014.07.020>
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Horn, D.J.V., Weber, C.F., 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl. Environ. Microbiol.* 75, 7537–7541. <https://doi.org/10.1128/AEM.01541-09>
- Traboulsi, A., Dupuy, N., Rébufa, C., Sergent, M., Labed, V., 2012. Investigation of gamma radiation effect on the anion exchange ion exchanger Amberlite IRA-400 in hydroxide form by Fourier transformed infrared and ^{13}C nuclear magnetic resonance spectroscopies. *Anal. Chim. Acta* 717, 110–121. <https://doi.org/10.1016/j.aca.2011.12.046>
- Van Loon, L.R., Hummel, W., 1995. The radiolytic and chemical degradation of organic ion exchange ion exchangers under alkaline conditions: effect on radionuclide speciation (Nagra Technical Report 95-08 No. PSI Bericht Nr. 95-13).
- Wang, J., Wan, Z., 2015. Treatment and disposal of spent radioactive ion-exchange ion exchangers produced in the nuclear industry. *Prog. Nucl. Energy* 78, 47–55. <https://doi.org/10.1016/j.pnucene.2014.08.003>

B2 Ion exchange resin irradiation and biodegradation (EPFL)

Aislinn Boylan and Rizlan Bernier-Latmani, EPFL

B2.1 Introduction

Ion exchange resins form a significant component of the organic inventory of LLW and ILW in EU member states. Resins represent the largest single component (40%) of the organic material in the inventory of ILW and LLW in Switzerland. Within the MIND project, studies are being undertaken with material from Switzerland and the Czech Republic.

Ion exchange resins are used for the purification of various water streams during nuclear power production and during reprocessing. The resin inventory consists of cation exchange resins and anion exchange resins that share a common polystyrene polymer chain cross-linked by divinyl benzene. The functional groups with ion-binding capacity are sulfonate groups (cation exchange) or amine groups (anion exchange). Because these resins bind radionuclides, it is expected that they will be exposed to significant gamma radiation, potentially resulting in their radiolytic degradation. Chemical compounds released from the radiolytic degradation of resins could serve as electron donors, electron acceptors, or carbon sources for microorganisms in the repository, hence it is important to have a better understanding of their composition. Evaluating this process is the first phase of the proposed work.

Previous work has been carried out to investigate the radiolytic degradation of resins. However, there are several limitations to the current state of knowledge. First, much of the work has been carried out under oxic conditions (Van Loon and Hummel, 1995) and it is expected that the repository will transition to anoxic conditions rapidly. Additionally, the range of analytical tools applied to characterize the products of resin irradiation was limited (Rébufa et al., 2015) and may have overlooked major groups of compounds.

Additionally, the potential of microorganisms to further degrade (i.e., biodegrade) the radiolytic degradation products has not been evaluated. These compounds, if biodegradable by the microbial community that best represents repository conditions in Switzerland (i.e., that present in the Mt Terri underground rock laboratory), could produce chelating agents for radionuclides, could fuel sulfate reduction or methanogenesis. This potential is another aspect of the work that will be evaluated.

Early research on the degradation of resins under irradiation have focused on the physical changes which showed that the structures of resins were altered and in some cases destroyed by radiation (McConnell et al., 1993). Analysis of chemical products under oxic conditions suggests the formation of a variety of organic compounds (Van Loon and Hummel, 1999, 1995). The irradiation energy is known to be capable of breaking of bonds between the functional exchange group and the backbone structure of the resin of both cation and anion exchange resins results in increased concentrations of their functional groups (sulfate and ammonia respectively) in solution. The products of mixed resin degradation are less clear, with only small amounts of sulfate and ammonia present in solution after irradiation.

This study aims to identify the organic compounds produced during anoxic irradiation of ion exchange resins, the biodegradation of these compounds and then to assess the implications of these findings on the safe disposal of ion exchange resins in intermediate level waste.

B2.2 Methods

B2.2.1 Irradiation of resins

Resins were obtained that are identical to the ones used in the nuclear power plants in Switzerland. More specifically, 0.8g of a mixture of cation and anion bead resins (Lewatit M 800 KR and Lewatit S 200 KR, 30:70 ratio) as well as a 0.8g mixture of cation and anion powder resins (Powdex PAO and Powdex PCH, 50:50 ratio) were doped with 750 μL of a mixture of cations and anions (Li^+ , BO_3^{3-} , SiO_3^{2-} , SO_4^{2-} , Cl^- , Na^+ , Mg^{2+} , K^+ , Ca^{2+} , F^- , SO_4^{2-} , Br^-) to mimic the chemical state of the spent resins that will require disposal. The doped resins were placed in Opalinus Clay porewater at pH 8 and under alkaline conditions (pH 12.5). Irradiation of resins took place in March 2017 using a gamma (^{60}Co) irradiation source. The irradiation was carried out after degassing with argon, in order to ensure anoxic conditions. Sample holders were placed next to the radiation source and resins were subject to a dose of 100 kGy. Immediate analysis of the gas-phase was undertaken using a micro GC-MS at CEA, France. Unirradiated resin that was otherwise treated identically was also analysed. Aqueous samples were sent to EPFL for analysis.

B2.2.2 Microcosm experiments

Triplicate microcosms were prepared in 200 mL bottles containing $20\text{g}\cdot\text{L}^{-1}$ of Opalinus Clay from the excavation of the borehole BMA-A2 with 162 mL of N_2 sparged sterile artificial porewater (see Table B2- 1 for composition) with 18 mL of borehole water added to some experiments as inoculum and 100 μM of either benzene, chloromethane, trimethylamine and ammonia. Duplicate control microcosms were established in the absence of inoculum (uninoculated).

2 mL aqueous samples were taken under sterile conditions and needles were N_2 sparged to maintain anoxic conditions to monitor geochemical conditions over time. An additional 5 mL sample was taken from the benzene microcosms for analysis using the headspace sampler GC-MS.

Table B2- 1 Artificial Porewater composition

Compound	Amount g/L
NaCl	13.3185
$\text{MgCl}_2\cdot 6\text{H}_2\text{O}$	4.6800
KCl	0.1600
$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$	2.7800
Na_2SO_4	3.0300
NaHCO_3	0.0440

B2.2.3 Geochemical Measurements

pH was measured using Orion probe electrode calibrated at pH 4, pH 7 and pH 10 daily. Measurements of Fe^{2+} and S^{2-} were made using a UV-Vis spectrophotometer following methods by Stookey (1970) and Cline (1969) respectively. Sulfate, ammonia and trimethylamine were measured using ion chromatography. Chloromethane was analysed using GC with a flame ionisation detector. Benzene was measured using a headspace sampler GC-MS where 5 mL samples were heated to 80°C for 10 minutes prior to injection of the gas phase in to the GC.

B2.3 Results

B2.3.1 Irradiation of resins

The irradiation experiments with a cobalt gamma source at 100 kGy revealed that as well as H₂ and CO₂, chloromethane and benzene were measured in the gas phase. Additionally, based on the volatility of these compounds, we calculated that the detection limit afforded by the tool used was too low to detect these compounds in aqueous phase. Hence, gas phase analysis is the only reliable way to measure these volatile compounds. Radiolytic yields have been quantified for benzene and chloromethane in the gas phase by Sophie Le Caer at CEA which show only very low concentrations exist in the gas phase (approximately 10⁻⁸ M). Identification and quantification of organic compounds in the aqueous phase was undertaken using GC-MS and Ion Chromatography (IC).

Table B2- 2 Organic compounds detected after irradiation of resins.

Phase	Detection method	Compounds	Concentration (M)
Gaseous	GC-MS	Benzene	10 ⁻⁸
	GC-MS	Chloromethane	10 ⁻⁸
Aqueous	GC-MS	Trimethylamine	
	IC	Ammonia	10 ⁻⁴

B2.3.2 Microcosm experiments

Using the four organic compounds (benzene, chloromethane, ammonia and trimethylamine), experiments are now ongoing to evaluate the potential for biodegradation of these radiolytic resin degradation products under conditions relevant to the repository. Previous research suggests that all four organic compounds can be oxidised by microorganisms under varying redox conditions, including sulfate-reducing, nitrate-reducing and iron-reducing (Lovley et al., 2000; Doronina et al., 1996; Yang et al., 2012; Yang et al., 2009). Sulfate reduction is likely to be the main metabolic process in the repository in Opalinus clay due to the presence of sulfate as well as H₂ from anoxic steel corrosion and resin irradiation (Bagnoud *et al.*, 2016).

B2.3.2.1 Benzene

The pH for both the inoculated and uninoculated system remain at pH 7.7±0.3 for the duration of the experiment (50 days, Figure B2- 1). The concentration of Fe²⁺ increased after one day in both systems and continued to rise in the uninoculated system. The sulfate concentration of the inoculated system decreases after 50 days to 19 mM compared to an initial concentration of 22 mM. In the uninoculated system the concentration remains at 21±1 mM for the duration of the experiment. The inoculated experiment also shows an increase in sulfide concentration which is not seen in the uninoculated system.

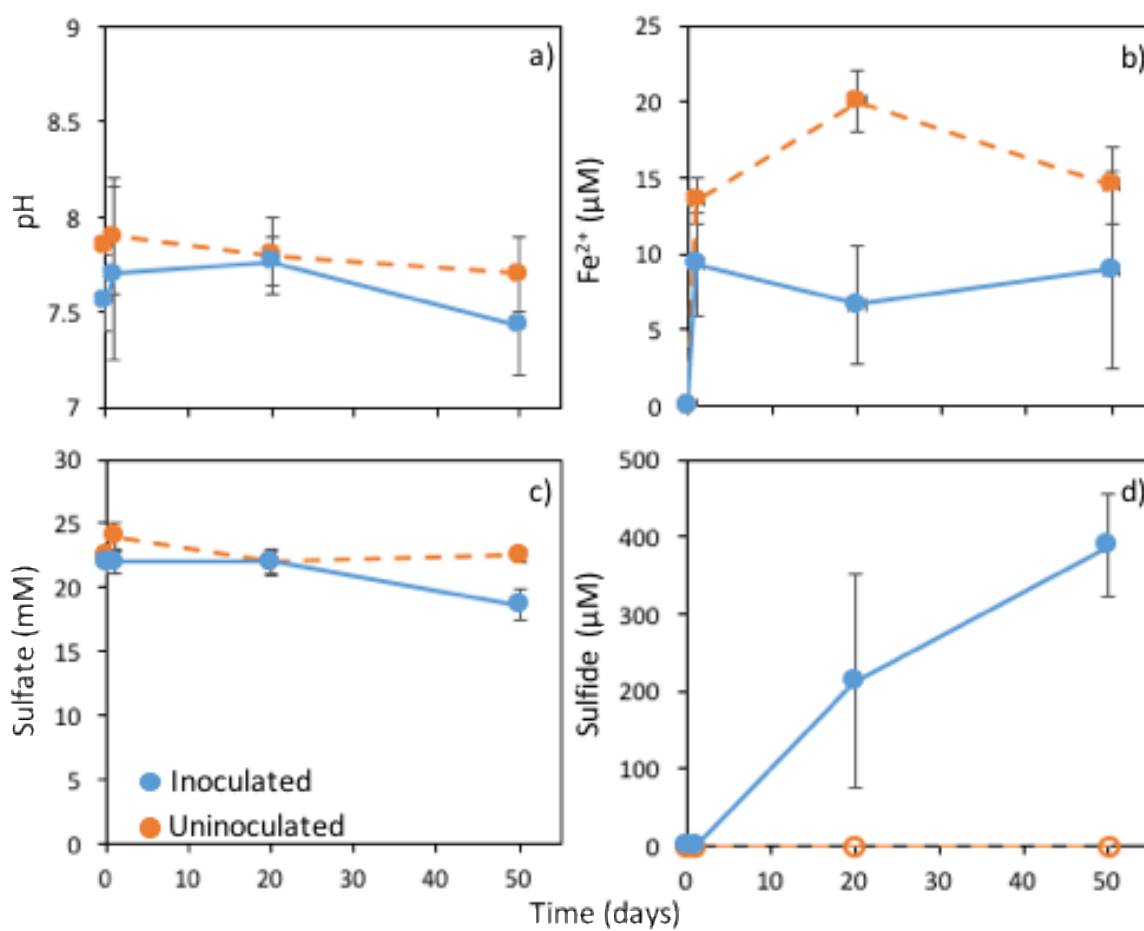


Figure B2- 1 Geochemical analyses of inoculated and uninoculated for microcosm experiments using benzene. a) pH; b) Fe²⁺; c) sulfate; d) sulfide. Inoculated samples shown in blue with continuous line, uninoculated shown in orange with dashed line. Unfilled data points represent values below the limit of detection for the method used. Error bars denote the standard deviation of replicate samples.

B2.3.2.2 Trimethylamine

The results of the pH analysis show a decrease in pH over time with the highest values recorded at the initial timepoint (Figure B2- 2). Fe²⁺ is measured in both systems, with the highest concentration associated with the uninoculated experiments. There is no significant change in sulfate concentration in either system, however sulfide production is recorded with approximately 350 μM measured in solution after 50 days. In the uninoculated system the sulfide concentration remained below the limit of detection.

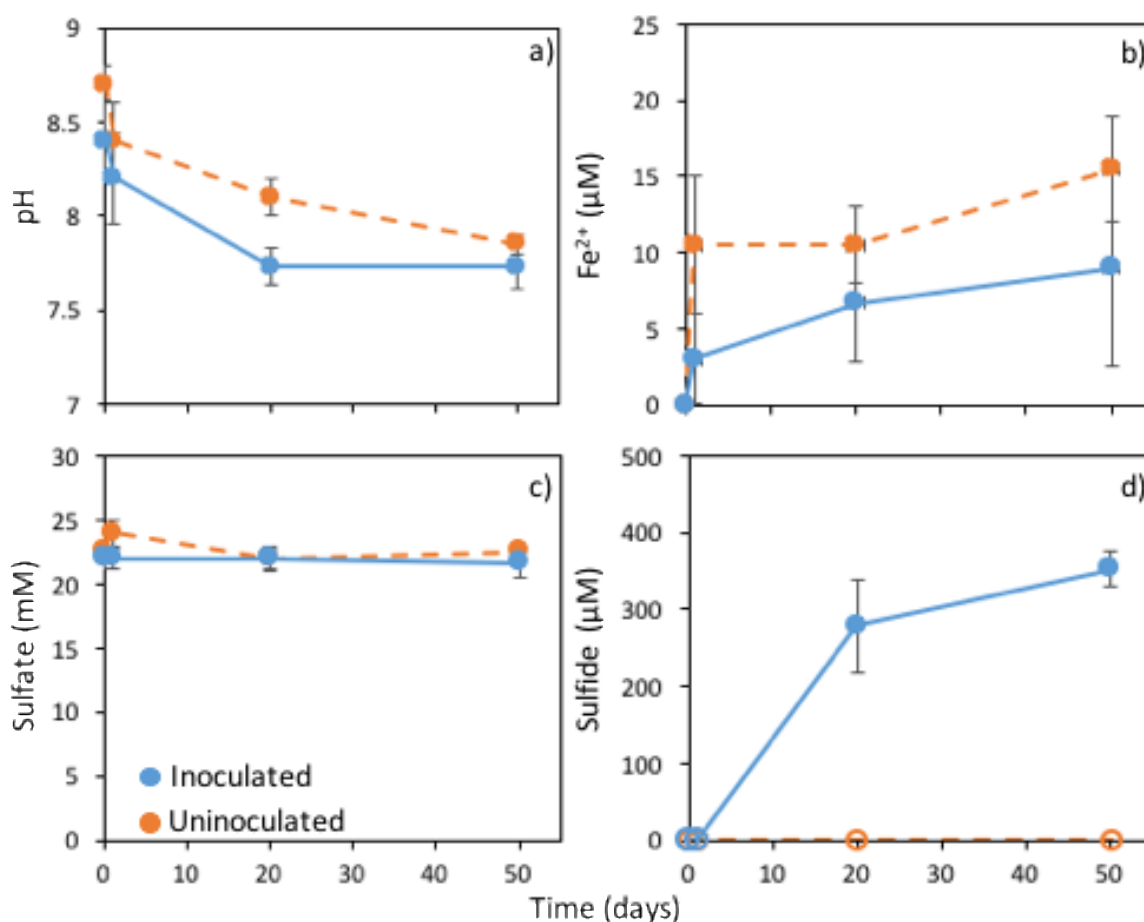


Figure B2- 2 Geochemical analyses of inoculated and uninoculated for microcosm experiments using trimethylamine. a) pH; b) Fe²⁺; c) sulfate; d) sulfide. Inoculated samples shown in blue with continuous line, uninoculated shown in orange with dashed line. Unfilled data points represent values below the limit of detection for the method used. Error bars denote the standard deviation of replicate samples.

Microcosm experiments are also ongoing for chloromethane and ammonia, currently no sulfide production has been detected and there has been no change to sulfate concentration in inoculated systems. This suggests that there is no active sulfate reduction occurring in these systems currently.

B2.4 Conclusions

Here, we have shown that the complexity of the composition of resin irradiation products is significantly greater than previously thought. This finding suggests that resins are likely to provide potential energy sources for microbial metabolic activity.

Preliminary data suggest that both benzene and trimethylamine are utilised by the microbial population found in the Mont Terri URL as hydrogen sulfide is measured in experiments where microorganisms are present, but it is not found in the absence of microorganisms. This suggests that sulfate-reducing bacteria are using the organic compounds as electron donors. These findings have not been replicated with ammonia and chloromethane. Work is ongoing to determine the products of this degradation, in particular to detect any intermediate products of the biodegradation which may have the potential to impact on the mobility of radionuclides stored in the waste.

Acknowledgements

This project has received funding from the Euratom, (EU) research and training programme 2014-2018 MIND project under grant agreement (No 661880).

References

- Abrahamsen L, Arnold T, Brinkmann H, Leys N, Merroun M, Mijnenonckx K, Moll H, Polvika P, Ševců A, Small J, Vikman M, Wouters K. 2015. A review of anthropogenic organic wastes and their degradation behaviour (D1.1) MIND project report. Available from <http://www.mind15.eu/wp-content/uploads/2015/11/MIND-Deliverable-1.1-Review-of-anthropogenic-organic-wastes.pdf>
- McConnell, J.W., Johnson, D. a., Sanders, R.D., 1993. Radiation degradation in organic ion exchange resins. *Waste Manag.* 13, 65–75. [https://doi.org/10.1016/0956-053X\(93\)90035-U](https://doi.org/10.1016/0956-053X(93)90035-U)
- Réboufa, C., Traboulsi, A., Labed, V., Dupuy, N., Sergent, M., 2015. Experimental design approach for identification of the factors influencing the γ -radiolysis of ion exchange resins. *Radiat. Phys. Chem.* 106, 223–234.
- Van Loon, L.R., Hummel, W., 1999. The Degradation of Strong Basic Anion Exchange Resins and Mixed-Bed Ion-Exchange Resins: Effect of Degradation Products on Radionuclide Speciation. *Nucl. Technol.* 128, 388–401. <https://doi.org/10.13182/NT99-A3039>
- Van Loon, L. and Hummel, W., 1995. The radiolytic and chemical degradation of organic ion exchange resins under alkaline conditions: effect on radionuclide speciation (No. PSI--95-13). Paul Scherrer Inst.(PSI).
- Stookey, L.L., 1970. Ferrozine---a new spectrophotometric reagent for iron. *Analytical chemistry*, 42(7), pp.779-781.
- Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters1. *Limnology and Oceanography*, 14(3), pp.454-458.
- Lovley, D.R., 2000. Anaerobic benzene degradation. *Biodegradation*, 11(2-3), pp.107-116.
- Doronina, N.V., Sokolov, A.P. and Trotsenko, Y.A., 1996. Isolation and initial characterization of aerobic chloromethane-utilizing bacteria. *FEMS microbiology letters*, 142(2-3), pp.179-183.
- Yang, W.H., Weber, K.A. and Silver, W.L., 2012. Nitrogen loss from soil through anaerobic ammonium oxidation coupled to iron reduction. *Nature Geoscience*, 5(8), p.538.
- Yang, Z., Zhou, S. and Sun, Y., 2009. Start-up of simultaneous removal of ammonium and sulfate from an anaerobic ammonium oxidation (anammox) process in an anaerobic up-flow bioreactor. *Journal of Hazardous Materials*, 169(1-3), pp.113-118.