

# State of Science Review: Modelling microbial effects to assess long-term performance of a DGR

**NWMO-TR-2019-15**

**October 2019**

**Liam Abrahamsen-Mills, Joe Small**

UK National Nuclear Laboratory

**nwmo**

NUCLEAR WASTE  
MANAGEMENT  
ORGANIZATION

SOCIÉTÉ DE GESTION  
DES DÉCHETS  
NUCLÉAIRES



**Nuclear Waste Management Organization**

22 St. Clair Avenue East, 6<sup>th</sup> Floor

Toronto, Ontario

M4T 2S3

Canada

Tel: 416-934-9814

Web: [www.nwmo.ca](http://www.nwmo.ca)

# **State of Science Review: Modelling microbial effects to assess long-term performance of a DGR**

**NWMO-TR-2019-15**

October 2019

**Liam Abrahamsen-Mills, Joe Small**  
UK National Nuclear Laboratory

This report has been prepared under contract to NWMO. The report has been reviewed by NWMO, but the views and conclusions are those of the authors and do not necessarily represent those of the NWMO.

All copyright and intellectual property rights belong to NWMO.

**Document History**

Title:	State of Science Review: Modelling microbial effects to assess long-term performance of a DGR		
Report Number:	NWMO-TR-2019-15		
Revision:	R000	Date:	October 2019
UK National Nuclear Laboratory			
Authored by:	Liam Abrahamsen-Mills, Joe Small		
Verified by:	Divyesh Trivedi		
Approved by:	Alan Wareing		
Nuclear Waste Management Organization			
Reviewed by:	Mehran Behazin		
Reviewed by:	Peter Keech		
Accepted by:	Paul Gierszewski		

**ABSTRACT**

**Title:** State of Science Review: Modelling microbial effects to assess long-term performance of a DGR  
**Report No.:** NWMO-TR-2019-15  
**Author(s):** Liam Abrahamsen-Mills, Joe Small  
**Company:** UK National Nuclear Laboratory  
**Date:** October 2019

**Abstract**

This report presents a state of science review of international literature and knowledge on the modelling of microbial processes in the context of nuclear waste disposal, with a focus on the Canadian disposal concept of copper-clad used fuel containers within a deep geological repository. Whilst general corrosion of the copper surface is expected to be minimal over the required timescales, there is a risk that microbiologically induced corrosion could occur. Sulfate reducing bacteria may be active in regions within or surrounding the repository, generating sulfide that may diffuse through the clay within the engineered barrier system towards the container surface, inducing corrosion.

Whilst microbial communities will be indigenous within the host rock formation and present in the engineered barrier system materials, studies have demonstrated that the disposal concept will limit microbial activity in the immediate vicinity of the container for prolonged periods. NWMO has developed computer models to simulate a number of these processes. The latest model, assumes a steady state sulfide flux at the far field, which diffuses to the container and induces general corrosion. The model results indicate that the extent of corrosion is expected to be manageable, but that the behaviour will be highly site-specific.

The scope of the review focusses on international approaches most relevant for evaluating microbiologically induced corrosion especially when applied to used fuel container. However, in order to provide comprehensive coverage in the wider context of microbiology considered in radioactive waste disposal, the review also covers the much more extensive literature relevant to low and intermediate level wastes.

The overall objective of this review is to recommend how the data collected through NWMO's experimental programs can be incorporated into NWMO's modelling program. The review recommends further experiments with compacted bentonite and a number of model developments, particularly ones related to representing the sulfide generation processes and a range of geochemical reactions.

**GLOSSARY OF TERMS**

NWMO	Nuclear Waste Management Organization (Canada)
APM	Adaptive Phased Management
DGR	Deep geological repository
EBS	Engineered barrier system
MIC	Microbiologically induced corrosion
UFC	Used fuel container
CANDU	Canada Deuterium Uranium, a Canadian pressurised heavy-water reactor design
SCC	Stress corrosion cracking
FEPs	Features, events and processes
PA	Performance assessment
ILW	Intermediate level waste
LLW	Low level waste
NAGRA	National Cooperative for the Disposal of Radioactive Waste (the Swiss research centre in the field of deep geological disposal of radioactive waste)
MIND	EU Horizon 2020 collaborative project 'Microbiology in Nuclear Waste Disposal', 2015-2019
MING	Microbial Impacts to the Near-Field Environment Geochemistry (Modelling code)
GRM	Generalised Repository Model, developed by NNL and predecessor organisations
TOUGH	Transport Of Unsaturated Groundwater and Heat, a suite of simulators for non-isothermal multiphase flow and transport in fractured porous media
SKB	Swedish Nuclear Fuel and Waste Management Company
IR	Iron reduction
SR	Sulfate reduction
Redox	The processes of chemical reduction and oxidation
REX	Redox Zone Experiment at the Äspö URL
DOC	Dissolved organic carbon
PA	Performance Assessment
POC	Particulate organic carbon
BIOCORE	Reactive transport code
KBS-3	Technology for disposal of high-level radioactive waste, developed in Sweden
GAMMON	A gas generation model for disposal environments developed by UK Nirex
T2GGM	Gas Generation Model coupled with TOUGH2 transport model
SRB	Sulfate reducing bacteria
PHREEQC	Chemical speciation model code, developed by US Geological Survey
PEST	Parameter ESTimation code, for fitting model parameters to experimental data
URL	Underground research laboratory
NOM	Natural organic matter
NMR	Nuclear magnetic resonance spectroscopy
MX-80®, National®, Canapril®	Types of bentonite under consideration for use as an EBS material for UFC
TEAP	Terminal electron accepting process
VFAs	Volatile fatty acids

## TABLE OF CONTENTS

	Page
<b>ABSTRACT .....</b>	<b>iii</b>
<b>1. INTRODUCTION .....</b>	<b>1</b>
<b>2. NWMO RESEARCH SUPPORTING ADAPTIVE PHASED MANAGEMENT .....</b>	<b>3</b>
<b>2.1 MICROBIAL ACTIVITY IN GEOLOGICAL AND DGR ENVIRONMENTS .....</b>	<b>3</b>
2.1.1 Nutrients required for microbial activity .....	4
<b>2.2 CONSTRAINING MICROBIAL ACTIVITY .....</b>	<b>4</b>
<b>2.3 MICROBIOLOGICALLY INFLUENCED CORROSION.....</b>	<b>5</b>
<b>2.4 QUANTIFYING MICROBIAL PROCESSES AND MIC IN A DGR .....</b>	<b>7</b>
2.4.1 Mass balance approach to the near field contribution .....	7
2.4.2 Numerical Models of microbial processes and MIC.....	7
2.4.2.1 Copper Corrosion Model, CCM.....	8
2.4.2.2 Nutrients and electron donors for sulfate reduction .....	10
2.4.2.3 Regions of microbial activity.....	10
2.4.2.4 CCM-MIC findings.....	10
2.4.2.5 Review of international modelling codes .....	11
2.4.2.6 Development of COMSOL model.....	11
<b>2.5 SUMMARY OF CURRENT NWMO POSITION.....</b>	<b>14</b>
<b>3. MODELLING AND QUANTIFICATION OF MICROBIOLOGICAL PROCESSES WITHIN INTERNATIONAL GEODISPOSAL PROGRAMMES .....</b>	<b>15</b>
<b>3.1 PERFORMANCE ASSESSMENT APPROACHES.....</b>	<b>16</b>
3.1.1 Mass balance / thermodynamic approaches to constrain microbial activity .....	16
3.1.2 Assessment of gas generation and consumption processes .....	18
3.1.3 Assessment of DGR redox stability .....	19
<b>3.2 MICROBIAL KINETIC MODELLING .....</b>	<b>20</b>
3.2.1 Gas generation models.....	20
3.2.2 Models of biogeochemical processes.....	21
3.2.2.1 Monod kinetic models of sulfate reduction microcosm experiments .....	21
3.2.2.2 Reactive-transport biogeochemical models of <i>in situ</i> URL borehole experiments .....	23
<b>4. MODELLING OF MICROBIAL PROCESSES AND COUPLING TO GEOCHEMISTRY .....</b>	<b>26</b>
<b>4.1 MICROBIAL GROWTH KINETIC MODELLING .....</b>	<b>27</b>
4.1.1 Biomass synthesis .....	28
4.1.1.1 Autotrophic processes .....	29
4.1.1.2 Biomass synthesis in the DGR concept .....	30
4.1.2 Organic hydrolysis processes .....	31
4.1.3 Microbial respiration and fermentation processes .....	32
<b>4.2 COUPLING TO GEOCHEMISTRY .....</b>	<b>36</b>
4.2.1 Use of the PHREEQC geochemical modelling code .....	36
4.2.2 Consideration of redox processes and determination of $E_h$ .....	39
<b>4.3 EXAMPLE MODELS OF SULFATE REDUCTION IN BENTONITE MICROCOSM EXPERIMENTS .....</b>	<b>40</b>

<b>5.</b>	<b>DISCUSSION OF MODELLING OF MICROBIAL PROCESSES IN THE DGR</b>	
<b>CONCEPT.....</b>		<b>46</b>
<b>5.1</b>	<b>FEATURES AND PROCESSES OF RELEVANCE TO SULFIDE GENERATION</b>	
<b>IN THE DGR .....</b>		<b>46</b>
<b>5.2</b>	<b>INTEGRATION OF MICROBIAL PROCESSES IN DGR TRANSPORT</b>	
<b>MODELS.....</b>		<b>49</b>
<b>6.</b>	<b>CONCLUSIONS, RECOMMENDATIONS AND REMAINING ISSUES .....</b>	<b>53</b>
<b>REFERENCES .....</b>		<b>55</b>



## LIST OF TABLES

	Page
Table 1: Model parameter values used to simulate microcosm experiments (values in bold indicate differences between the two bentonites considered) .....	42

## LIST OF FIGURES

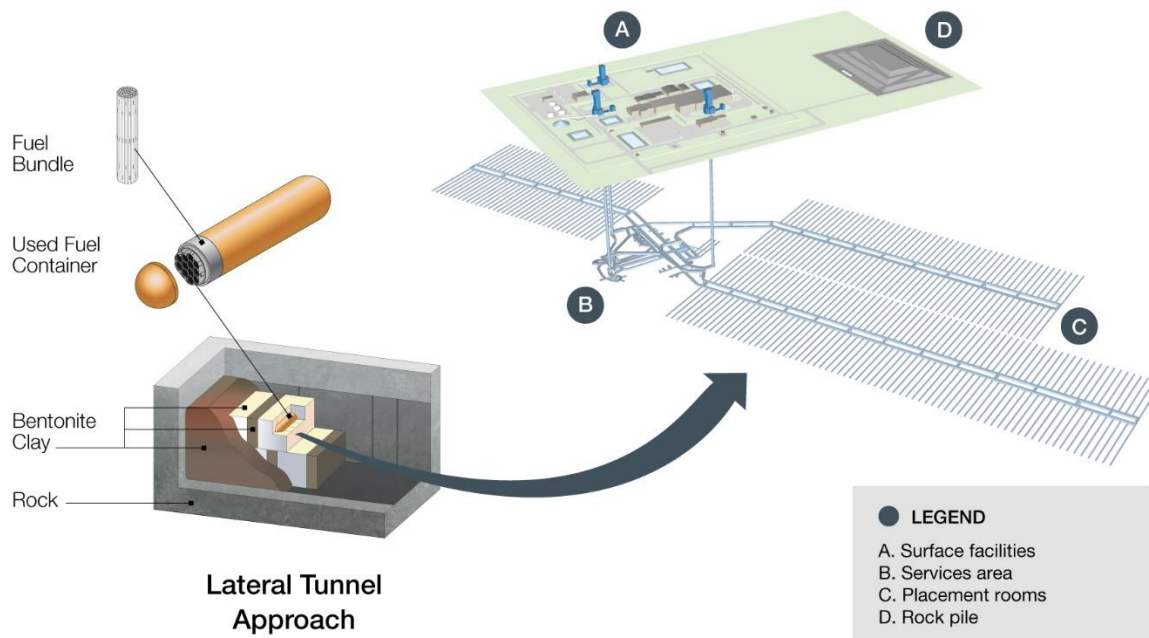
	Page
Figure 1: Proposed multiple barrier system in a Canadian deep geological repository, showing the redesigned Mark II used fuel container, with bentonite backfill .....	1
Figure 2: Culturable Heterotrophic Aerobes versus Measured Dry Density (Stroes-Gascoyne et al, 2014) .....	5
Figure 3: The abiotic reaction scheme utilised by CCM (King and Kolar, 1996).....	9
Figure 4: Microbial reaction scheme utilised by CCM (King and Kolar, 1996).....	9
Figure 5: COMSOL model results showing relative sulfide flux reaching the UFC surface, dimensions shown are metres (Briggs et al, 2017) .....	13
Figure 6: COMSOL model results showing relative sulfide flux at the UFC surface, effect of bentonite thickness (Briggs et al, 2017).....	13
Figure 7: Reference HLW design that formed the basis of the PA study of microbial activity in the Swiss DGR (McKinley et al, 1985), dimensions in metres .....	17
Figure 8: Schematic of possible gas generation and consumption reactions in a LLW/ILW generic repository in Opalinus Clay (Leupin et al, 2016).....	18
Figure 9: Experimental data for sulfide generation from microcosm experiments with Hydrogen, at different partial pressures, (top figure) and lactate at different concentrations (bottom figure) fitted modelled concentration (dashed lines) using a Monod kinetic model represented in PHREEQC (Maia et al, 2016) .....	22
Figure 10: Schematic of sector of the PHREEQC radial diffusion model of the BN experiment comprised of a series of concentric annular prisms (Small, 2019; Small and Abrahamsen-Mills, 2018).....	24
Figure 11: Nitrate and nitrite concentration (symbols) during a pulsed equilibration of hydrogen gas in the BN experiment (Bleyen et al, 2017). Solid lines are the modelled concentrations of nitrate and nitrite using PHREEQC with a two stage Monod kinetic model (Small, 2019; Small and Abrahamsen, 2018) .....	25
Figure 12: $F_{pH}$ function used in GRM to represent the effect of pH on microbial growth rate for $f=0.3$ and $f=1.0$ , for an optimum pH of 7 .....	28
Figure 13: Biological pathways to form nitrogen gas or ammonium from nitrate with initial reduction to nitrite (Bleyen et al, 2017) .....	33
Figure 14: PHREEQC RATES keyword definition of a dual Monod kinetic rate equation for sulfate reduction by acetate and biomass synthesis defined by the BASIC interpreter (# defines the start of comments) .....	38
Figure 15: PHREEQC KINETIC keyword definition for sulfate reduction by acetate considering heterotrophic biomass synthesis .....	39
Figure 16: Modelled sulfate reduction coupled with oxidation of acetate in microcosms at 37°C including Canapril bentonite, symbols indicate experimental data from Grigoryan et al, (2018).....	42
Figure 17: Modelled sulfate reduction coupled with oxidation of Lactate in microcosms at 37°C including Canapril bentonite, symbols indicate experimental data from Grigoryan et al, (2018).....	43

Figure 18: Modelled sulfate reduction coupled with oxidation of acetate in microcosms at 15°C including MX-80 bentonite, symbols indicate experimental data from Grigoryan et al, (2018).....45

Figure 19: Schematic (not to scale) of the diffusive transport processes (arrows) and microbiological and geochemical species and processes that require consideration in a model of sulfide generation in a bentonite backfilled DGR, where microbial activity may be restricted to an interface zone .....50

## 1. INTRODUCTION

The Nuclear Waste Management Organization (NWMO) is implementing Adaptive Phased Management (APM), the approach selected by the Government of Canada in 2007 for long-term management of used nuclear fuel. The goal of APM is long-term containment and isolation of used nuclear fuel in a Deep Geological Repository (DGR) constructed in a suitable rock formation in either sedimentary or crystalline rock at a depth of approximately 500 m. In support of APM, NWMO is pursuing an active Technical Program that is currently refining generic engineering designs and safety cases in support of APM. The Technical Program includes microbiology research to assess the significance of microbiological processes on the engineered barrier system (EBS) of a DGR, including the development of numerical models to evaluate the potential for microbiologically induced corrosion (MIC) of used fuel containers proposed for the DGR design (Figure 1).



**Figure 1: Proposed multiple barrier system in a Canadian deep geological repository, showing the redesigned Mark II used fuel container, with bentonite backfill**

The multiple barrier system proposed for a Canadian DGR includes a used fuel container (UFC) that must be strong enough to withstand geological pressures, including the hydrostatic load of glaciation events, and chemically resistant to long-term corrosion (Hall and Keech, 2017). The UFC is emplaced in tunnels backfilled with bentonite clay buffer, which following saturation are designed to have very low hydraulic conductivity. The average dry density specification of the bentonite ( $1.6 \text{ g/cm}^3$ ) is designed to limit microbial activity in the EBS, based on experimental studies (Stroes-Gascoyne and Hamon, 2008; Stroes-Gascoyne et al, 2010). The UFC has been optimised for the CANDU used fuel, with the current Mark II reference design comprising a steel container with 3 mm thick copper corrosion resistant coating (Hall and Keech, 2017), that exceeds the current conservative corrosion allowance of  $<1.3 \text{ mm}$  in the first million years (Kwong, 2011; Scully and Edwards, 2013). An ongoing corrosion research program is further

examining chemical thermodynamic controls of copper corrosion in pure water, concentrated chloride solutions, and in the presence of sulfide (Hall and Keech, 2017). In addition, the program considers a number of other physical phenomena related to the composite Mark II UFC design, including; comparison of the corrosion behaviour of copper coatings on steel with wrought copper; effects of  $\gamma$ -radiolysis (Ibrahim, 2018), galvanic corrosion of through-coating defects (Standish, 2017) and the localised corrosion of internal steel welds (Wu, 2017).

The principal effects of microbial activity on the Canadian DGR design concern the generation of sulfide species by microbial sulfate reduction that could affect UFC general corrosion by the formation of  $\text{Cu}_2\text{S}$ . In theory, stress corrosion cracking (SCC) could also be accentuated by the formation of high concentrations of bisulfide and other microbial product species such as acetate, ammonia and nitrite (King 1996). However, the potential for SCC is eliminated in the copper coated steel Mark II UFC design, as the shape of the UFC ensures that tensile stress on the copper is avoided (Hall and Keech, 2017); instead, only compressive stresses will develop in the DGR.

The effect of microbiologically induced corrosion (MIC) from sulfide however remains of primary importance to the DGR. While microbial sulfate reduction may be limited in the bentonite buffer surrounding a copper UFC, sulfide may be generated at the host rock interface or present at low concentrations within the host rock. An assessment of the extent of MIC assuming that sulfide does not chemically react in the bentonite and only diffuses through a 40 cm layer of bentonite, with a boundary condition of 3ppm  $\text{HS}^-$  in the host rock, indicates that 1 mm of corrosion would occur after 1 million years (King, 1996). Recent consideration of the transport of sulfide through the bentonite buffer has considered the 3-dimensional geometric effects of the Mark II UFC design, within the backfilled placement room (Figure 1). Further development of the models used to assess the extent of UFC corrosion requires more specific consideration of sulfide generation in the DGR and host rock, taking account of the kinetics of the microbially mediated processes together with geochemical constraints that may control the flux of sulfide through the EBS.

This report presents a state of science review of international literature and knowledge on the modelling of microbial processes in the context of nuclear waste disposal. The scope of the review focusses on approaches most relevant for specific consideration of MIC in the Canadian used fuel context, but also draws upon approaches developed to model processes relevant to low and intermediate level wastes. The overall objective of this review is to recommend how the data collected through NWMO's experimental programs can be incorporated into NWMO's MIC modelling program.

The report comprises the following sections:

1. Introduction to the Canadian DGR, context and structure of the review
2. NWMO research supporting Adaptive Phased Management
3. Consideration of modelling of microbiological processes within international geodisposal programs
4. Modelling of microbial processes and coupling to geochemistry
5. Discussions of modelling of microbial processes in the DGR concept
6. Conclusions, recommendations and remaining issues.

## 2. NWMO RESEARCH SUPPORTING ADAPTIVE PHASED MANAGEMENT

This section provides a review and summary of recent NWMO research into the effects of microbial activity within a DGR, with a focus on processes related to corrosion, and approaches to numerical modelling. NWMO has developed a comprehensive Proof Test Plan to evaluate the feasibility and safety of the DGR concept. This includes ongoing research to evaluate the possible extent of copper corrosion via a range of mechanisms: anoxic corrosion in pure water, concentrated chloride solutions (Senior, 2019), and in the presence of sulfide (Chen, 2011).

### 2.1 MICROBIAL ACTIVITY IN GEOLOGICAL AND DGR ENVIRONMENTS

Within the NWMO programme a number of state of science reviews have been performed to evaluate the most important microbiological considerations relevant to a DGR, including effects on copper corrosion, and identifying international best practice in representing these effects within numerical models.

In an assessment of far field processes, Sherwood Lollar (2011) summarises the types and classifications of microbial species expected within host rock geologies, their relevant metabolic processes and the potential effects on radionuclides in the far field. It is recognised that indigenous microbes exist in a broad range of geological environments. Factors affecting their presence, diversity and activity in geological settings include: the properties of the host rock (physical, chemical and mineralogical properties; transport properties; geologic and geochemical history of the site) and the properties of the groundwater (its role in supplying nutrients, energy sources and metabolites). When selecting a site, there will be a need to characterise the spatial and temporal variability of microbial activity. The review concludes that, in the far field,  $H_2$  oxidation will be an important microbiological process that may be coupled with Fe reduction, sulfate reduction, methanogenesis and acetogenesis. Other gases may be produced, e.g.  $H_2S_{(g)}$ . These microbial processes will drive the system to more anaerobic conditions and may affect local pH. They may also affect physical transport properties, which could have implications for radionuclides released from a DGR. Such property changes include: biofilm and colloid formation, clogging of pores and changes to permeability, hydraulic conductivity, etc. (Sherwood Lollar, 2011).

In NWMO assessments of the near field (Wolfaardt and Korber, 2012), it is similarly concluded that the repository environment will contain diverse communities of microbes. These will arise from sources including: the indigenous host rock communities, transportation into the vicinity by inflowing groundwater, those present in engineered barrier system (EBS) materials, and those introduced through repository construction and operations. The construction and operations phase will also introduce nutrients including  $O_2$ . However, upon repository closure, a substantial gradient of inhibition is expected to develop within the EBS. This is a biologically inactive zone extending from the container surface to tens of cm into the compacted clay buffer.

The regions expected to exhibit the greatest microbial activity are within interfacial regions between various EBS components: between the highly compacted bentonite buffer and the lower density bentonite backfill, and between the backfill and the host rock. Water transport throughout these interfaces will be controlled by saturation rates imposed by the host rock (Wolfaardt and Korber, 2012).

Initially, O<sub>2</sub> will be consumed by microbial reactions as an electron acceptor, and thereafter, increasingly anoxic conditions will develop and alternative electron acceptors, including nitrogen, iron and sulfate, utilised.

### 2.1.1 Nutrients required for microbial activity

In terms of the microbial population in the environment surrounding a DGR, both organotrophs that derive energy via the oxidation of organic compounds, and lithotrophs that derive energy from the oxidation of inorganic compounds may be active (Sherwood Lollar, 2011). Similarly, microbes can be classified by the source of C used to form biomass: heterotrophs utilise organic carbon, whilst autotrophs utilise inorganic CO<sub>2</sub>.

Marshall and Simpson (2014a) summarise the knowledge on the natural organic content of bentonite clays. They determine that this organic matter will be one of the largest inputs of organic C to the repository, which could act as a substrate for microbial activity, but that the concentration will be very low due to its limited bioavailability. Construction and operation activities will also introduce sources of organic C to the repository.

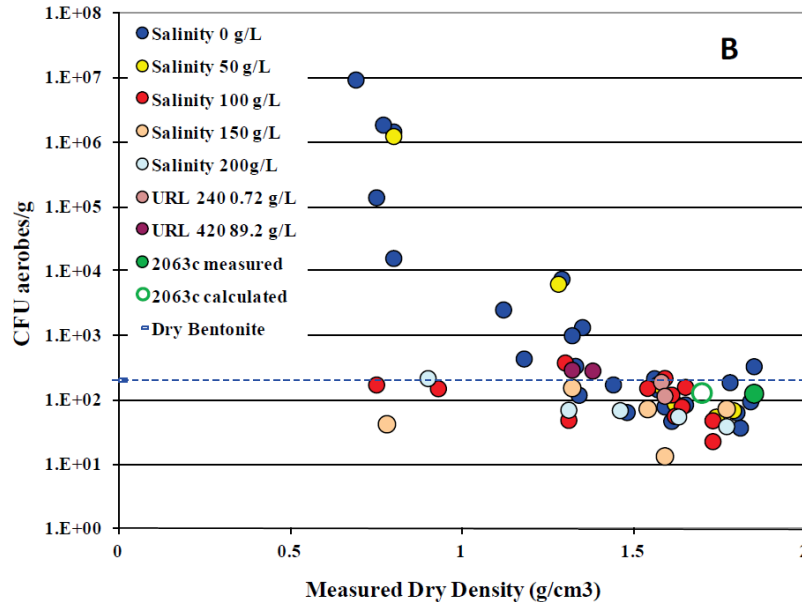
Sherwood Lollar (2011) noted that groundwaters typically contain dissolved gases including H<sub>2</sub>, CO<sub>2</sub> and CH<sub>4</sub>. The concentrations vary by site and rock type, but CO<sub>2</sub> concentration generally decreases with depth, whilst H<sub>2</sub> generally increases with depth in more saline fracture waters. Hydrogen driven microbial ecosystems are typical of the deep subsurface and have been observed over a wide range of geological environments. A wide range of microbes can utilise H<sub>2</sub> using several terminal electron acceptors, including nitrate, Fe(III), sulfate, and CO<sub>2</sub> (summarised in Sherwood Lollar, 2011).

Whilst the presence and bioavailability of organic C within or surrounding the DGR is important in controlling a range of microbial processes (Marshall and Simpson, 2014a), it should be recognised that autotrophic organisms may utilise CO<sub>2</sub> and H<sub>2</sub> as a carbon source and electron donor, respectively. In fact, the reduction of sulfate to sulfide is generally represented as occurring via the oxidation of hydrogen (Sherwood Lollar, 2011).

## 2.2 CONSTRAINING MICROBIAL ACTIVITY

There is a strong body of evidence that indicates microbial activity will not occur, or be severely limited, in highly compacted bentonite (Wolfaardt and Korber, 2012, Marshall and Simpson, 2014a). In fact, the suppression of microbial activity is considered to be an important role of the compacted bentonite (Stroes-Gascoyne et al, 2014). The bentonite will constrain microbial activity primarily by ensuring a low water activity in both unsaturated and saturated conditions. Experimental programmes have shown that a water activity of  $\leq 0.96$  in a bentonite buffer can effectively suppress microbial activity. In pure water, suppression of microbial growth can be achieved via a bentonite dry density of approximately 1.5 g/cm<sup>3</sup> (Figure 2), although this value is usually expressed as  $\geq 1.6$  g/cm<sup>3</sup> to account for non-homogeneity within the bentonite. A value of 1.6 g/cm<sup>3</sup> also ensures that the swelling pressure is  $\geq 2$  MPa and average pore size is  $< 0.02$   $\mu\text{m}$  (Stroes-Gascoyne et al, 2014). For systems containing salinity, microbial growth is suppressed at lower bentonite dry densities (Figure 2); although swelling pressure also reduces (Stroes-Gascoyne et al, 2014). Results indicate that any potentially culturable bacteria within highly compacted bentonite are present as inactive spores, and would not contribute to MIC or other detrimental effects (Stroes-Gascoyne et al, 2014). It has also been noted that the elevated

temperature and radiation field surrounding the UFC is expected to have a (temporary) sterilising effect (King and Kolar, 2006; Stroes-Gascoyne and Hamon, 2010), although these effects are less clear than for the density and water activity. As a result of these combined effects, it is thought that no microbial activity will occur close to the UFC at any stage during the evolution of the repository environment.

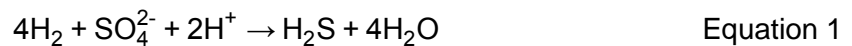


**Figure 2: Culturable Heterotrophic Aerobes versus Measured Dry Density (Stroes-Gascoyne et al, 2014)**

### 2.3 MICROBIOLOGICALLY INFLUENCED CORROSION

An extensive experimental and research programme has been performed in Canada into the potential corrosion of copper in a DGR environment. This has focussed on the mechanisms of uniform corrosion, SCC, and MIC, the latter having the greatest relevance to this review. Early studies (summarised in King, 1996) concluded that biofilm formation on the UFC surface is not credible and that microbial activity within the DGR, at a distance from the containers, presents the greatest risk of MIC. This would occur via the generation of potential corrosive agents at a distance from the UFC (in regions where microbial activity is less constrained), which then migrate to the UFC surface via diffusion through bentonite.

The reduction of sulfate to sulfide by sulfate reducing bacteria (SRB) is identified as the most likely cause of container corrosion (King, 1996). The overall reaction for sulfate reduction, with H<sub>2</sub> as an electron donor, is given in Equation 1 (for acidic conditions).



Depending on the local conditions, the sulfide formed may be in the gaseous form H<sub>2</sub>S<sub>(g)</sub> or the HS<sub>(aq)</sub><sup>-</sup> anion, bisulfide. The sulfide speciation will have a strong effect on its transport properties

within bentonite. These assessments have assumed that sulfide will be present and diffuse as the bisulfide.

In the presence of sulfide, Cu becomes thermodynamically unstable in water. A number of mechanisms have been proposed for sulfide-driven MIC of Cu (King, 1996), but most of these require the presence of a biofilm on the container surface. Since this has been discounted, the only credible MIC mechanism arising from sulfide generated at a distance from the container is the cathodic evolution of H<sub>2</sub> (given in Equation 2). In this mechanism, Cu dissolution is catalysed by the formation of highly insoluble Cu<sub>2</sub>S or CuS films, occurring at sufficiently negative potentials such that the anodic reaction can be supported by the reduction of H<sub>2</sub>O or HS<sup>-</sup>, defined in the equation as H<sub>2</sub>S (King, 1996).



A Cu<sub>2</sub>S layer may form on the Cu surface. At higher sulfide concentrations, this would be expected to form a passivating layer susceptible to localised breakdown and localised corrosion, but at lower concentrations, a more porous layer would lead to more uniform corrosion (Martino, 2017).

Therefore, the production of sulfide by SRB at a distance from the UFC during the prolonged anoxic period could result in container corrosion following the consumption of O<sub>2</sub>. It was determined that the most important factor controlling the extent of MIC is the extent of microbial activity within the vault, and hence the environmental parameters that will limit the extent and diversity of microbial activity (King, 1996). If the evolution of the physical and chemical vault environment can be predicted, then it should be possible to predict the likely extent of microbial activity at various times and locations in the vault.

Consideration was given to the spatial and temporal viability of microbial sulfate reduction within the DGR; this process is only expected to occur once all the O<sub>2</sub> has been consumed and anaerobic conditions have been established. Since SRBs are only expected to be active at a distance from the UFC, the transport of sulfide through the EBS is of crucial importance. These early studies concluded that uniform copper corrosion due to the presence of HS<sup>-</sup> produced by SRB is the only credible form of MIC (at least in the long term) and that rapid corrosion mechanisms are only credible during the relatively short oxic period (King, 1996).

The sulfide-driven uniform corrosion process will occur at a rate determined by the quantity of HS<sup>-</sup> produced by SRB and the rate of HS<sup>-</sup> diffusion to the container. King (1996) proposed a maximum [HS<sup>-</sup>] produced by SRB of 3 µg/g (3 ppm). This value is considered very conservative in more recent analyses (Garisto, 2017), as 5-90 ng/g (5-90 ppb) is the current estimate for sulfide by the NWMO. Ongoing and upcoming site characterization work will provide guidance to future estimates of the expected sulfide in groundwater.

Studies determined that diffusion of HS<sup>-</sup> to the UFC will be extremely slow and also identified the potential for HS<sup>-</sup> to react with Fe(II) in the buffer materials, further limiting its migration (King, 1996, Wolfaardt and Korber, 2012). Whilst it has been determined that these processes that may lead to corrosion will be slow, limited by sulfide diffusion and reaction with buffer minerals, it was recognised that they could continue for as long as SRBs are active (i.e. potentially indefinitely).



Overall, the studies on copper corrosion have concluded that the UFC will be primarily subject to uniform corrosion and that the degree of localised attack (pitting) and SCC will be negligible. It was concluded that, due to the expected low rates of MIC, adequate container lifetimes could be achieved through engineering practices (King, 1996, Kwong, 2011). To provide further confidence, efforts have been made to further quantify microbial activity and MIC processes, and to represent these within numerical models.

## **2.4 QUANTIFYING MICROBIAL PROCESSES AND MIC IN A DGR**

In order to quantify the impact of the microbially driven reactions described above, a number of models have been developed within the Canadian programme. These represent the important microbial processes that are expected to take place within a DGR or the surrounding host rock, with an emphasis on corrosion.

Preliminary assessments, based on steady state mass transport of  $\text{HS}^-$  from the far field, estimated a corrosion rate due to SRB of 1 nm/a, equating to 1 mm after 1 Ma (summarised in King, 1996).

### **2.4.1 Mass balance approach to the near field contribution**

One method that has been used to estimate the extent of near field MIC is a conservative mass balance approach (Krol and Sleep, 2013; 2014). The total quantity of sulfur within the repository (present as sulfate, sulfite, pyrite within the bentonite / emplacement materials) was calculated and assumed to be converted entirely to sulfide, and the corresponding total Cu corrosion depth calculated for both Mark I and Mark II UFC reference designs. For the Mark I design, the near field corrosion depth was 1.9 mm and 2.6 mm in a crystalline and sedimentary repository, respectively (Krol and Sleep, 2013a). For the Mark II design, depths of 1.21 and 1.14 mm were calculated (Krol and Sleep, 2014). The authors note that these are not intended to be a near field MIC allowance, but rather to bound worse-case scenarios that make highly conservative assumptions about microbial activity in the near field. These calculations do not credit the bentonite for suppressing microbial activity as described in section 2.2. Nitrogen and phosphorous were identified as limiting nutrients for biomass formation. These assessments recognise that, in addition to any near field MIC, the far field has the potential to supply sulfide, as noted above. Overall, the results indicate that MIC extents will be design- and site-specific.

### **2.4.2 Numerical Models of microbial processes and MIC**

It was recognised (King, 1996) that whilst models existed at the time to represent microbial kinetics and others to represent sulfide diffusion through porous media, no model existed that considered these processes together and linked them to copper corrosion. Starting with the assumption of a continuous far field  $\text{HS}^-$  source term (outer boundary condition), an approach to simulate the reactive transport of  $\text{HS}^-$  through the EBS to the UFC surface was proposed. Whilst it is not expected to be retarded by sorption, sulfide is expected to react and precipitate with metal ions such as Fe(II), Cu(I) and Cu(II), limiting its transport to the container. Additionally, during the oxic phase, it is thought that any sulfide produced in anoxic regions would be quickly oxidised when migrating to regions containing  $\text{O}_2$ .

#### 2.4.2.1 Copper Corrosion Model, CCM

Over a number of years the CCM (Copper Corrosion Model) group of models has been developed to estimate the extent of UFC copper corrosion over periods up to 1 Ma (King, 1996, King and Kolar, 2006, King et al, 2008). CCM utilises a 1-dimensional finite difference grid and simulates a number of abiotic and microbial processes that may contribute to copper corrosion. These processes include chemical, microbial and electrochemical reactions, precipitation/dissolution of solid phases, adsorption/desorption, and mass transport (diffusion).

The model comprises three modules: CCM-UC to represent uniform corrosion, CCM-SCC for stress corrosion cracking, and CCM-MIC for microbiologically influenced corrosion. A key assumption of CCM-MIC is that no microbial activity is expected in the highly compacted bentonite surrounding the UFC; the only effects of microbes on corrosion will be via diffusion of aggressive metabolic by-products ( $\text{HS}^-$ , acetate, nitrite, ammonia) in other regions of the DGR to the container surface (King and Kolar, 2006). There are 33 coupled second-order partial differential equations to represent the microbial reactions kinetically, using a Monod approach (see Section 4). The model also considers 18 inorganic species, three types of organic carbon (glucose, acetate, peptides) and 12 types of microbial species.

CCM utilises interfacial electrochemical reactions as boundary conditions, representing the surface of the UFC and allowing the time dependence of the corrosion potential ( $E_{\text{corr}}$ ) and the corrosion rate to be calculated (King and Kolar, 2006). The 1D spatial grid represents the natural and engineered barriers as a series of eight layers, through which reactive transport can occur. The abiotic processes considered are summarised in Figure 3. This reaction scheme includes interfacial electrochemical reactions, adsorption/desorption, precipitation/dissolution, redox reactions and diffusive mass transport. The oxidation of the Cu surface is shown for  $\text{O}_2$ , Cl,  $\text{NH}_4$ , as well as the reactive transport of those species to the surface. It includes the reaction of Fe(II) with  $\text{HS}^-$  and the precipitation of FeS.

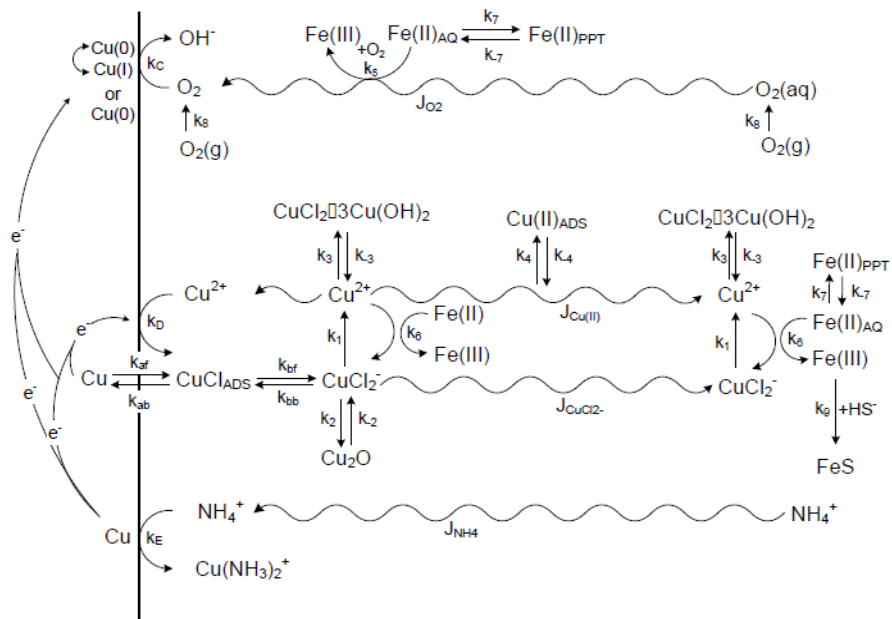


Figure 3: The abiotic reaction scheme utilised by CCM (King and Kolar, 1996)

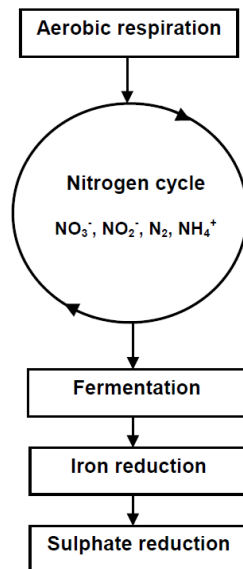


Figure 4: Microbial reaction scheme utilised by CCM (King and Kolar, 1996)

Figure 4 shows the microbial reaction scheme utilised by CCM, which follows a sequence in response to changing redox conditions, with aerobic respiration followed by the nitrogen cycle, followed by fermentation, Fe reduction and sulfate reduction. These processes are represented via 12 microbial species and 15 different reactions. Each of the reactions is represented by both an energy-generating equation and a cell-generating (biomass) equation. Biomass is formed from organic carbon and a nitrogen source (ammonia or nitrate) and has the assumed

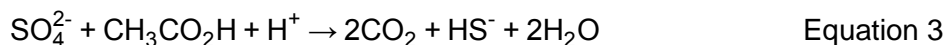
stoichiometry of  $C_5H_7O_2N$ . A conversion (or yield) factor determines the number of cells produced per mole of organic carbon (King and Kolar, 2006).

Monod kinetics define the maximum rates of microbial activity and a number of factors limit the rate: temperature, redox potential, and water activity. The effect of temperature is represented by both imposing an upper limit for microbial reactions and by having an activation energy for the reactions. The redox potential defines which microbial processes can occur. Low water activity imposes either dormant or dead microbial cells in the model.

Whilst the energy-generating microbial reactions are treated kinetically, the cell (biomass) forming reactions are treated as instantaneous, subject to the availability of organic C or N.

#### 2.4.2.2 Nutrients and electron donors for sulfate reduction

The source of organic carbon considered in the model is natural organic matter (NOM) present in the bentonite clay. This is assumed to provide a source of glucose and peptides, with acetate produced from their fermentation. Either organic carbon or hydrogen can be used as an electron donor to reduce sulfate to sulfide in the model. The equation for the former is given in Equation 3 and that for the latter process in Equation 1.



The model considers that  $H_2$  is generated via fermentation reactions, but it does not appear to account for any  $H_2$  dissolved in natural groundwaters.

Whilst CCM does account for lithotrophic sulfate reduction (without the requirement for organic C), the formation of biomass in the model does require organic C (i.e. heterotrophic growth is represented but autotrophic growth is not).

#### 2.4.2.3 Regions of microbial activity

In CCM, microbial activity primarily occurs in the two layers representing the excavated disturbed zone (EDZ) between the 'light backfill' and the 'near field rock'; the former is an obsolete term from a previous design that describes sealing material near the rock interface. A much greater initial microbial population is assumed for the EDZ than any other region (to represent the introduction of organic material during operations), and microbial activity within the EDZ will be less constrained than in the more highly compacted bentonite regions.

#### 2.4.2.4 CCM-MIC findings

King and Kolar (2006) present the results of a series of calculations using CCM-MIC version 0.1 for a base case and a number of sensitivity study cases. In the base case, organic matter is not considered to be limiting, and actually increases in concentration throughout the simulation. Despite this, the extent of microbial activity is predicted to be extremely low, due to the combined effects of the limiting factors discussed above. Sulfate reduction by SRBs is only predicted to occur significantly at times  $\gg 100,000$  a. The predicted effects on corrosion are predicted to be minimal over a 1 Ma timeframe (King and Kolar, 2006).

#### 2.4.2.5 Review of international modelling codes

Since MIC estimates are both design- and site-specific, it was recognised that a more flexible model, incorporating a 3D representation of the DGR, was required. A state of science review was performed to assess existing numerical models used by waste management organisations internationally (Krol and Sleep, 2013b).

17 models were reviewed and assessed with respect to:

- The ability to model temperature- and density-dependent groundwater flow and solute transport
- Biotic and abiotic reactions
- Gas generation and transport
- Easily changing model parameters
- Linking to the far field, and
- Transient capabilities.

The models reviewed were grouped into the categories of: mechanical stress models; transport models; reactive-transport models; corrosion models (including CCM); gas generation and transport models; and bulk calculation models. The review concluded that none of the models reviewed could simulate all of the processes relevant to a Canadian DGR and recommended that a new model be developed that has:

- 3D modelling capability with easily changeable parameters in order to provide scoping calculations
- Diffusive transport of corrosive agents through the buffer
- Ability to estimate MIC at the container.

Krol and Sleep (2013b) concluded that the COMSOL Multiphysics package was the most suitable for developing such a model, benefiting from a 3D simulator capable of parametric design that will allow easy design changes and various physics-based modules that can link to other packages. It was recognised that a 3D approach was required to correctly model the radial diffusion behaviour applicable to the geometry of the UFC Mark II design.

#### 2.4.2.6 Development of COMSOL model

A model was developed using the COMSOL Multiphysics package (Briggs et al, 2015a) and assumed a continuous far field  $\text{HS}^-$  concentration of 3 ppm. The mechanism for the production of sulfide was not included, as the model was tasked with identifying regions on the UFC that may be more susceptible to MIC. Instead, the boundary condition at the far field was considered to be continually producing sufficient sulfide to maintain a concentration of 3 ppm. At the UFC surface, the concentration was set to zero, based on the assumption that sulfide is consumed instantly via the copper corrosion reaction (Equation 2). At such a low concentration, SCC can be discounted; thus only general corrosion due to sulfide was modelled. Whilst it is implicitly assumed that sulfide arises in the far field due to microbial processes, this is not represented explicitly. Geochemical reactions, such as sulfide mineral precipitation, are not represented, as a conservative approach.

The model simulates sulfide diffusion (represented by Fick's first law) through the EBS towards the UFC surface using an effective  $\text{HS}^-$  diffusion coefficient of  $1 \times 10^{-11} \text{ m}^2/\text{s}$  within bentonite.

Initial modelling (Briggs et al, 2015a) considered steady state diffusion and did not account for the transient response of the DGR, such as re-saturation processes.

The depth of copper corrosion (at any given time) is calculated based on a molar conversion and stoichiometric conversion ratio of hydrogen sulfide to copper sulfide:

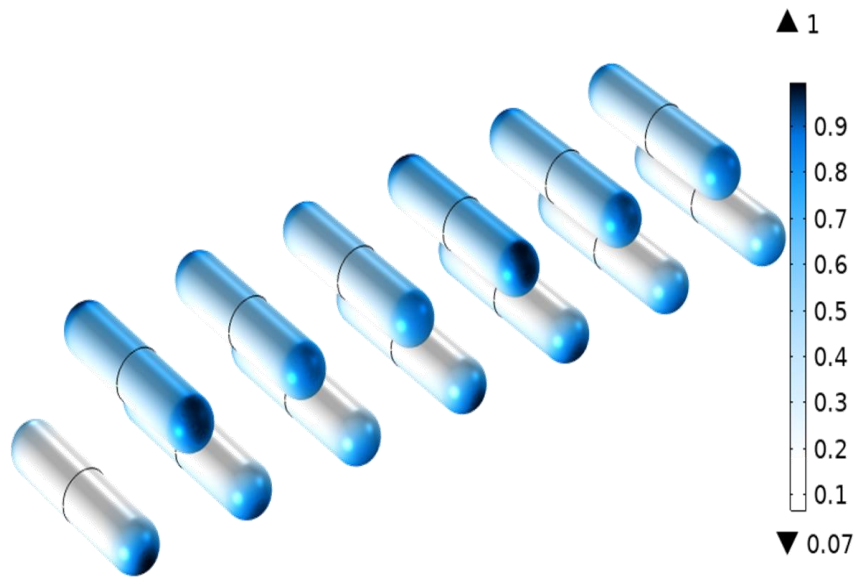
$$d_{\text{corr}} = \frac{N_{\text{HS}} f_{\text{HS}} M_{\text{Cu}}}{A_{\text{corr}} \rho_{\text{Cu}}} \quad \text{Equation 4}$$

where  $N_{\text{HS}}$  is the amount of sulfide,  $f_{\text{HS}}$  is the stoichiometric factor,  $M_{\text{Cu}}$  is the molar mass of copper,  $A_{\text{corr}}$  is the area exposed to corrosion and  $\rho_{\text{Cu}}$  is the density of copper.

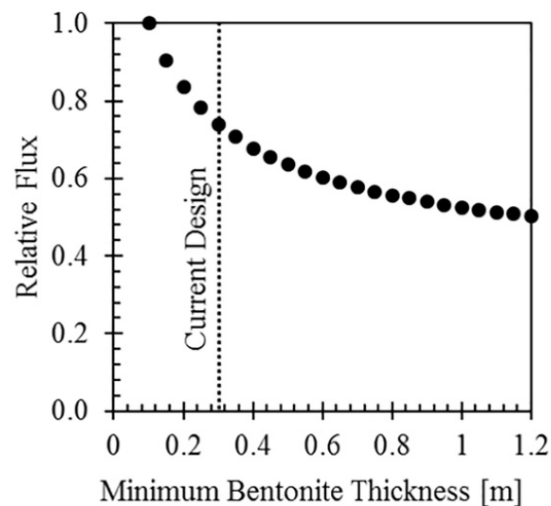
A 3D finite element mesh was developed and optimised to represent the geometry of the UFC containers and placement room within the DGR. A number of simulations considered a single UFC only, whereas others considered a 10 m section of the placement room containing 13 containers (Briggs et al, 2017). The diffusion model and finite element mesh were verified against a number of test cases with analytical solutions for 1D, 2D and 3D geometries.

Initial results indicated that, due to the geometry, the hemi-spherical end caps of the UFC would be subject to the greatest rates of corrosion, being greater by around a factor of two; the average corrosion rate was estimated at approximately 1 nm/a, and 2.44 nm/a for the end caps. This elevated corrosion rate, compared to the 1 nm/a proposed by King (1996) is attributed to geometry effects, highlighting the requirement for a 3D assessment. The predicted relative sulfide fluxes are shown in Figure 5. Additionally, the model considered a 30 cm bentonite thickness, whereas previous calculations assumed 40 cm. It is possible that this factor-of-two difference is less significant than the uncertainties in far field sulfide concentrations and other model parameters.

Sensitivity analyses developed the model further (Briggs et al, 2015b), introducing the time-dependent diffusion of sulfide from the far field into an initially sulfide-free EBS, and the sensitivity of corrosion results to the  $\text{HS}^-$  flux, the  $\text{HS}^-$  diffusion coefficient, and bentonite thickness. Calculations indicated that it would take around 2000 years for sulfide to reach a steady-state concentration within the EBS, which produced a minimal difference in corrosion compared to the base case simulations over the much longer timescales considered. The predicted extent of corrosion was found to be linearly dependent on the  $\text{HS}^-$  flux concentration and its diffusion coefficient. The effect of the bentonite thickness is more complex and requires a 3D solution, with increasing thickness having a diminishing improvement effect (Figure 6).



**Figure 5: COMSOL model results showing relative sulfide flux reaching the UFC surface, dimensions shown are metres (Briggs et al, 2017)**



**Figure 6: COMSOL model results showing relative sulfide flux at the UFC surface, effect of bentonite thickness (Briggs et al, 2017)**

Further modelling (Briggs and Krol, 2018) introduced the effects of temperature and water saturation, and examined the predicted evolution of the DGR environment via three cases:

1. Isothermal (25°C) and fully saturated
2. Non-isothermal and fully saturated, and
3. Isothermal and variably saturated

The temperature evolution of the repository was predicted, with long-term temperatures approaching 11°C. Variable water saturation was implemented using the Richards equation.

This does not simulate two-phase flow, but is a first approximation of variably saturated conditions. The variably saturated model geometry is currently limited to 2D due to the non-linear aspects of the Richards equation implementation (Briggs and Krol, 2018). This approximation results in a poorer representation of the variable saturation effects for the hemispherical endcaps of the UFC and qualitative comparisons must be made with the results from the 3D modules.

Since the previous simulations demonstrated the linear response to the far field sulfide concentration, the most recent reporting presents the results from a 1 ppm far field sulfide boundary condition, to allow for simple scaling to a site-specific condition. For the first case (isothermal and fully saturated) the calculated average corrosion rate was 0.76 nm/a/ppm HS<sup>-</sup>. For the second case (non-isothermal) the average corrosion rate was 0.53 nm/a/ppm HS<sup>-</sup>. In the third case, the model predicts that full water saturation of the DGR will occur after around 2000 years, with the first saturated pathways to the UFC appearing around 200 years (Briggs and Krol, 2018).

## 2.5 SUMMARY OF CURRENT NWMO POSITION

NWMO currently assigns an MIC allowance of 1 mm Cu corrosion over 1 Ma (Hall and Keech, 2017), calculated assuming 1D transport of a 3 ppm sulfide boundary concentration at the far field through the EBS to the container, resulting in uniform corrosion. The depth of corrosion was calculated using a simple conversion of HS<sup>-</sup> to Cu<sub>2</sub>S and the 3 ppm sulfide concentration is a conservative generic estimate until site-specific data is available.

To refine the MIC allowance, NWMO has supported the development of a 3D COMSOL Multiphysics model to predict the rates of sulfide diffusion from the host rock interface to the container and the resulting extents of corrosion. This model uses the same assumption of a constant 3 ppm sulfide flux at the host rock interface, which will need to be refined on a site- and design-specific basis. The model continues to be developed and there are plans to introduce (or better represent) the following processes/effects:

- Temperature
- Saturation of the bentonite buffer
- Mechanistic copper corrosion at the surface
- Microbial kinetic reactions, including
  - Consumption of O<sub>2</sub> and creation of an anoxic environment
  - Conversion of sulfate to sulfide
  - Gas production and consumption

It is intended that the model will also begin to incorporate data from experimental work with bentonite, diffusion experiments and site-specific geochemical and microbiological information. In addition, as new information regarding the concentration of sulfide becomes available, the model will be used to update the corrosion allowance due to sulfide.



### 3. MODELLING AND QUANTIFICATION OF MICROBIOLOGICAL PROCESSES WITHIN INTERNATIONAL GEODISPOSAL PROGRAMMES

A number of countries have active research programmes into how microbiological processes may affect the long-term safety of nuclear waste repositories. This section provides an overview of relevant programmes, including those related to LLW, ILW, HLW and spent fuel.

Across all disposal concepts, microbial processes may affect:

- the physical nature of the EBS, through effects on corrosion, backfill swelling, cement alteration etc;
- the generation and consumption of gases;
- the chemical speciation and hence mobility of radionuclides in the near field and far field; and
- the transport of radionuclides through sorption onto static and colloidal biomass.

In order to assess the significance of microbial processes on the safety of nuclear waste repositories it is necessary to consider microbial processes in a systematic analysis of the Feature, Events and Processes (FEPs, NEA, 2000) that are relevant to a specific disposal case. In some instances it is necessary to quantify the extent and rate of microbial activity either by simple calculations or models that represent the microbial growth processes.

Several international programmes have considered the effects of microbiological processes in geodisposal programmes. Sherwood Lollar (2011) presents a wider state of science review of microbiological processes relevant to the far field of the DGR, including summaries of the main international programmes. In Sweden and Finland, extensive microbiological characterisation of candidate host rocks for the development of repositories for spent fuel disposal has been undertaken (see Sherwood Lollar, 2011) as a means to assess the suitability of sites at Forsmark, Sweden and Olkiluoto, Finland. Similarly in Switzerland the geomicrobiology of the Opalinus Clay has been characterised (Leupin et al, 2016). Meleshyn (2011) and De Canniere and Meleshyn (2013) discuss microbial processes in a clay repository from a regulatory perspective.

Prior to the commissioning of site characterisation studies the Swiss (NAGRA) programme has developed a modelling methodology to quantify the potential for microbial activity in a DGR environment (McKinley et al, 1985; 1997). This approach has been more widely applied, such as in Canada (Stroes-Gascoyne, 1989) and in the U.S. Yucca Mountain project (Jolley et al, 2014). Pending a site selection process, the UK programme has considered FEPs approaches and literature review to define an approach to evaluate microbiological processes together with the development of kinetic models of gas generation processes applicable to ILW (Humphreys et al, 2010). The comprehensive review of Humphreys et al (2010) includes discussion of the integration of microbiology in PA and also delineates models that adopt a mass balance / thermodynamic approach (e.g. McKinley et al, 1985) from those that are kinetic-based models that simulate the microbial growth process, coupled with other processes. The first type of model can be used to assess the potential for microbiology processes over extended time scales whereas the latter, more mechanistic type of model may be used as a tool within a large PA study to evaluate or quantify specific processes. In the following subsections, approaches to consider microbial processes within PA (Section 3.1) are first described followed by examples of detailed microbial kinetic modelling of Geodisposal applications (Section 3.2).

### 3.1 PERFORMANCE ASSESSMENT APPROACHES

Humphreys et al (2010), defines PA and describes the systematic approach, following the international guidelines and FEP lists (NEA, 2000; IAEA, 2004), used commonly to identify key FEPs that require evaluation in a specific repository scenario. Humphreys et al delineates models and codes that are applicable to environments containing either high-organic content waste, or low-organic content, although their focus is on the former including gas generation and redox processes affecting LLW/ILW. Humphreys et al conclude that there is a wide range of available models that can be used to evaluate various aspects of microbial impacts on repository performance. However, they all have their limitations (e.g. significant data requirements) and, for deep environments, there is no single approach or code to quantify microbial influences on solute transport and effects on redox processes, consistent with the findings of Krol and Sleep (2013b).

Humphreys et al (2010) discuss the use of mass balance and thermodynamic approaches to assess the microbial activity in environments with low-organic content, including the approach that Stroes-Gascoyne (1989) developed to evaluate the potential for microbial activity in an earlier Canadian DGR design. Subsequently, this nutrient mass balance approach has been applied to the Mark I and Mark II UFC designs (Krol and Sleep, 2013; 2014).

Behrends et al (2012) discusses the implementation of microbial processes in the PA of spent nuclear fuel repositories, focussing on the behaviour of uranium through reduction and biosorption processes alongside microbial reduction of iron and sulfate. Significant uncertainties remain regarding the kinetics of microbial redox transformations and the effects of biofilm formation, especially in the application to *in situ* conditions. However, recent progress in implementing microbial kinetics in reactive transport models, such as in the context of near-surface uranium contamination (e.g. Lou et al, 2007) provides conceptual frameworks and opens possibilities to quantitatively assess direct and indirect effects of microorganisms on radionuclide mobility in the context of nuclear waste disposal (Behrends et al, 2012).

Assessment of the redox conditions of near surface and deep geological environments has been undertaken for several LLW/ILW disposal facilities (e.g. Neall, 1994; Humphreys et al, 1997; Small et al, 2008; Wersin et al, 2003; Duro et al, 2014). These quantitative approaches consider quantities of electron donor species (e.g. steel, organics) and implicitly consider the role of microbes in mediating redox reactions such as the oxidation of organic wastes (Duro, 2014). In the case of the UK LLW Repository, there is specific consideration of the kinetics of anaerobic microbial processes (Humphreys et al, 1997; Small et al, 2008).

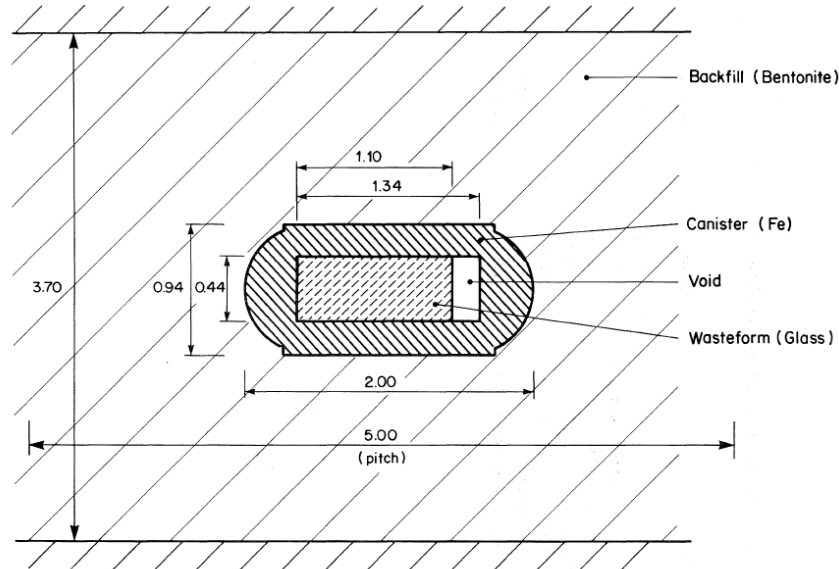
#### 3.1.1 Mass balance / thermodynamic approaches to constrain microbial activity

McKinley et al (1985) provides one of the first attempts to quantify the potential growth of microorganisms in a DGR concept, studying the potential for microbial growth in the Swiss HLW concept for a crystalline host rock (Figure 7). This approach considered the availability of nutrients and energy sources (C, N, P, S) in the near field, essentially comprising the bentonite backfill and groundwater input. Energy sources considered in this first model include that liberated by corrosion of the cast iron container forming magnetite ( $\text{Fe}_3\text{O}_4$ ) by anaerobic corrosion:



Equation 5

Further oxidation of the magnetite corrosion products led to hematite ( $\text{Fe}_2\text{O}_3$ ) formation. This arose from the initial trapped oxygen present and by  $\text{H}_2\text{O}_2$  formed by radiolysis, following mechanical failure of the canister. One of the key conclusions of this study was that energy from the corrosion process was probably limiting.



**Figure 7: Reference HLW design that formed the basis of the PA study of microbial activity in the Swiss DGR (McKinley et al, 1985), dimensions in metres**

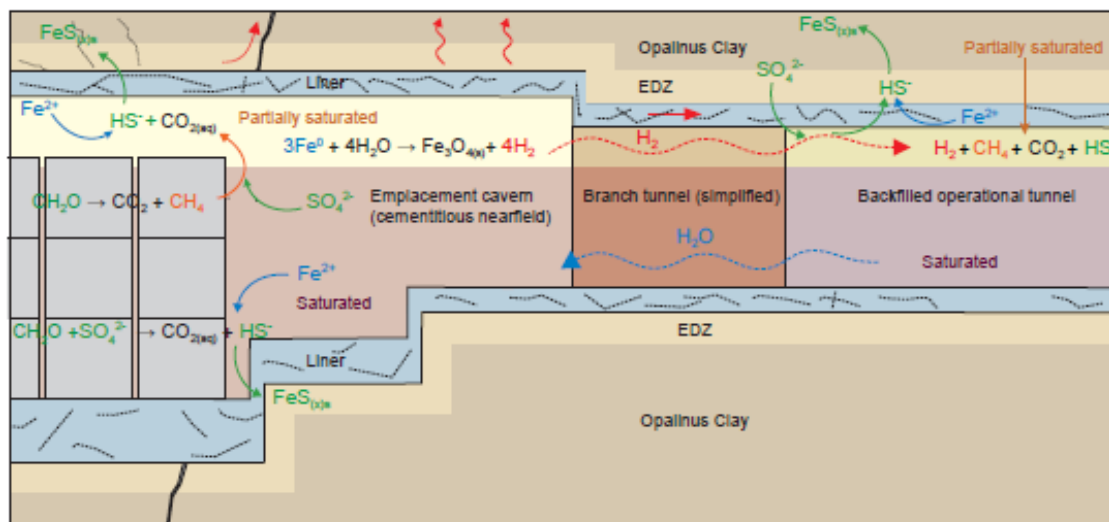
Subsequently, McKinley et al (1997) further developed this mass balance / thermodynamic approach to consider interfaces and redox reaction fronts that are particularly important for ILW disposal, where the energy yield is affected by greater pH variation. For HLW the authors conclude that microbial effects may be positive with respect to radionuclide release, where the main impact was considered as a consequence of organic complexation, since microbes may utilise soluble organics including radionuclide complexants. Importantly, McKinley et al (1997) recognise the potential for consumption of  $\text{H}_2$  formed by anaerobic corrosion by processes of sulfate reduction, but at that time MIC of the Fe canister was less relevant than for the concept of a Cu coated canister more recently being considered by the Swiss programme (Diomidis et al, 2017).

To evaluate the potential microbiological impacts affecting the Yucca Mountain repository concept for spent fuel disposal, Jolley et al (2003) developed the MING code (Microbial Impacts to the Near-Field Environment Geochemistry). MING further develops the approach of McKinley et al (1997) to limit microbial activity to below  $120^\circ\text{C}$  and above relative humidities of 90% in repository drifts. MING includes kinetic rates for the release of nutrients (C, N, P and S) from design materials, solubilised from the host rock and gaseous inputs in the unsaturated conditions that are used to calculate the number of microorganisms that can be formed as a function of time. A second calculation determines the energy available from optimally combined redox couples for the temperature and pH at that time. At each time step the nutrient and energy limits are compared to determine the maximum biomass growth possible.

It should be noted that this approach does not, however, consider the death of microbes, the recycling of biomass and also does not appear to consider the energy available to fermentative processes in such biomass recycling. Nevertheless, MING has been tested against laboratory experiments designed to determine limiting nutrients to microbial growth in the Yucca Mountain tuffaceous environment. The calculated biomass yields were generally accurate with one order of magnitude, although the model over-predicted the carbon-limiting case by two orders of magnitude (Jolley et al, 2003). Jolley et al (2003) discuss how biomass growth could be either a heterotrophic or autotrophic process, with the latter requiring a higher energy input. Organics present in waste materials and in cement additives were the main C source considered and along with P were growth limiting nutrients in the Yucca Mountain applications. MING has been applied to the Yucca Mountain concept to predict the quantities of CO<sub>2</sub> generated by microbial activity (and hence its effect on U and Np speciation) and to estimate the amount of uranium that is potentially sorbed to biomass, leading to migration by colloids (Jolley et al, 2003).

### 3.1.2 Assessment of gas generation and consumption processes

Leupin et al (2016) present a comprehensive review and assessment of potential gas generation and consumption reactions occurring in a generic repository for LLW and ILW located in Opalinus Clay (Figure 8). This review considered the chemical, microbiological and geoscientific understanding of processes of gas generation and consumption from LLW/ILW as a basis to assess the performance of engineered and natural barriers in order to ensure that gas generation does not compromise post-closure safety of the repository. The degradation of organic materials and the anaerobic corrosion of metals generate a range of gases (H<sub>2</sub>, CH<sub>4</sub>, CO<sub>2</sub>, H<sub>2</sub>S). Of these, H<sub>2</sub>S was considered to react with aqueous Fe and precipitate as FeS in a number of locations (Figure 8).



**Figure 8: Schematic of possible gas generation and consumption reactions in a LLW/ILW generic repository in Opalinus Clay (Leupin et al, 2016)**

Similarly, CO<sub>2</sub> was considered to be likely to react with concrete materials present in the nearfield. The gases H<sub>2</sub> and CH<sub>4</sub> are likely to be subject to slower oxidation reactions as they

are transported through gas-porous seals to backfilled operational tunnels where sulfate reducing microbial activity may be able to develop given the larger pores, higher water activity and circumneutral pH (Leupin et al, 2016). The assumed behaviour of this scenario is supported by information from natural and industrial analogues (Leupin et al, 2016) and *in situ* experiments at the Mont Terri URL examining sulfate reduction stimulated by H<sub>2</sub> injection (Bagnoud et al, 2016).

Assessment of the potential impact of sinks for repository-generated gas included mass balance calculations and multiphase gas transport modelling using TOUGH2. A series of calculations showed that both chemical and biological sinks for repository-generated gas may decrease the gas pressure build-up. However, the transport models also suggest that sulfate transport from the host rock may not be sufficiently fast to sustain an ongoing microbial oxidation of the hydrogen produced from anaerobic corrosion. A calculation case also examined the addition of sulfate to the backfill in the form of gypsum as a means to further mitigate gas overpressure.

### 3.1.3 Assessment of DGR redox stability

Excavation and construction of a DGR is likely to perturb the chemical conditions of the host rock, in particular the introduction of oxygen is likely to alter the redox conditions in the disturbed zone. In the case of a HLW / spent fuel disposal utilising Cu canisters, such residual oxygen will be important with regard to Cu corrosion. A further case of oxygen ingress concerns elevated levels of dissolved oxygen present in glacial melt water (Puigdomenech et al, 2001).

The Swedish (SKB) programme has examined the redox stability of crystalline rock in detail to understand the consumption of residual oxygen present in voids left by excavation, including the Redox Zone Experiment (REX) at the Äspö URL (Banwart, 1999; Puigdomenech et al, 2001; Samper et al, 2003). The REX experiment examined the depletion of dissolved oxygen following injection of oxygen in a fracture zone and included characterisation of the microbiology that developed (Samper et al, 2003). The main conclusions from the REX project were that microbial activity contributed substantially to the O<sub>2</sub> reduction and that O<sub>2</sub> uptake by the geologic medium was considered instantaneous over the time scale considered by PA (Puigdomenech et al, 2001). Banwart (1999) discusses that redox conditions can be buffered by Fe(III) reduction driven by natural organic matter infiltrating the fracture examined in the REX experiment. Puigdomenech et al (2001) further conclude that a substantial reducing capacity should be assigned to CH<sub>4</sub> and H<sub>2</sub>, which diffuse from deep geological sources and that they may be used by microbes as a redox buffer against the intrusion of O<sub>2</sub>-rich waters, independently of surface climatic conditions.

Samper et al (2003) present a numerical model of coupled reactive solute transport and microbial processes occurring in the REX experiment that was developed from previous hydrogeochemistry models that did not explicitly represent the microbial processes identified. Based on microbial characterisation studies, Samper et al (2003) included microbial processes of Fe(III) reduction by dissolved organic carbon (DOC) together with a fermentation process that generated DOC from particulate organic carbon (POC; humic and fulvic materials as described by Banwart, 1999). The fermentation process also included released sulfide (HS<sup>-</sup>) from sulfur present in the natural organic matter. Samper et al propose that by oxidation by reaction with Fe(III) and other electron acceptors (Mn(IV), NO<sub>3</sub><sup>-</sup>, O<sub>2</sub>) the sulfide is oxidised to form sulfate, which is observed to increase in concentration. In an initial model (Zhang, 2001 cited by Samper et al, 2003) the overall reaction of DOC and Fe(III) reduction and sulfide oxidation reaction was considered by a Monod kinetic formulation. This model reproduced increasing concentrations of HCO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup>. A further model presented in detail by Samper et al, 2003 represented the

fermentation of POC directly to sulfate and DOC together with a second process where DOC was oxidised by Fe(III). The Monod kinetic microbial reactions were represented in the BIOCORE reactive transport code (Zhang, 2001).

Yang et al (2007) have further developed the modelling approach using BIOCORE parameterised on the basis of the REX experiment to assess the depletion of oxygen in a bentonite backfilled reference case (KBS-3) Swedish design repository. The study considered six scenarios examining the reactivity of different minerals (e.g. chlorite, pyrite) present in a granite host rock and including microbial activity in the granite and in the bentonite, utilising DOC and CH<sub>4</sub> as electron donors. Model results showed that both geochemical and microbial processes are relevant for oxygen consumption. However, the time needed to consume the oxygen trapped in the bentonite buffer was reduced from several hundreds of years when only geochemical processes are considered to a few weeks when microbially mediated reactions were considered to occur within the bentonite (e.g. representing conditions prior to full saturation).

## **3.2 MICROBIAL KINETIC MODELLING**

In this subsection more detailed, mechanistic models of microbial processes are discussed that adopt a Monod kinetic approach to modelling the growth and metabolism of specific chemical energy sources, including sulfate reduction that is of direct relevance to the Canadian DGR application. Firstly, models of gas generation are summarised as these are perhaps currently the most established type of microbial model included in PA studies and safety cases.

### **3.2.1 Gas generation models**

Some of the first microbial kinetic growth models applied to nuclear waste disposal considered the process of microbial gas generation from organic-containing wastes. Such models were based models of anaerobic digestion and waste-water treatment processes (e.g. McCarty and Mosey, 1991). In particular codes were first developed in the UK programmes for deep geological disposal of ILW; GAMMON (Kidby and Rosevear, 1997 and near surface disposal of LLW; DRINK/GRM (Humphreys et al, 1997; Small et al, 2008). Both of these codes included a representation using the Monod kinetic approach of the growth and metabolism of aerobic and common sequence of anaerobic processes (e.g. denitrification, Fe(III) reduction, sulfate reduction), which lead ultimately in organic-rich systems to the development of methanogenic conditions. In addition, both GAMMON and DRINK/GRM included representation of corrosion processes that under anaerobic conditions generates H<sub>2</sub>, which is also represented as an electron donor and energy source for anaerobic microbial processes, including methanogenesis.

The DRINK/GRM code (Humphreys et al, 1997; Small et al, 2008) includes coupling to chemical processes (e.g. pH, Eh, speciation, mineral and gas solubility reactions) and has been successfully verified when tested against a long-term and large-scale experimental system at the Finnish VLJ Repository (Small et al, 2008;2017). The GRM code has been used in PA calculations for the UK Low Level Waste Repository to calculate a gas source term and to model the pH and Eh evolution of the repository (BNFL, 2002; LLWR, 2011).

The GAMMON code proved difficult to verify against a programme of laboratory based experimental research (Swift, 2016a) and has been replaced by a simpler empirical based model of gas generation (SMOGG) for deep geological PA applications (Swift, 2016b). SMOGG

has been tested against the Finnish gas experiment (Small and Dutton, 2009) and is used as a generic assessment tool in the UK deep disposal programme.

More recently, the GGM code has been developed by NWMO for assessment of gas generation in a DGR for LLW/ILW (Suckling, et al, 2015). GGM has similarities to DRINK/GRM in the representation of key microbial processes, although it does not include coupling of chemical processes. GGM has been verified against the Finnish gas experiment data set and in its PA application is coupled to the TOUGH2 multiphase flow and transport model T2GGM.

### 3.2.2 Models of biogeochemical processes

A variety of Monod kinetic models have been developed to represent key anaerobic biogeochemical processes, including sulfate reduction, to assist in the interpretation of experimental data and observations. Such models have also been used as part of wider PA studies directed at a specific issue, such as redoxiation processes and consideration of transport processes.

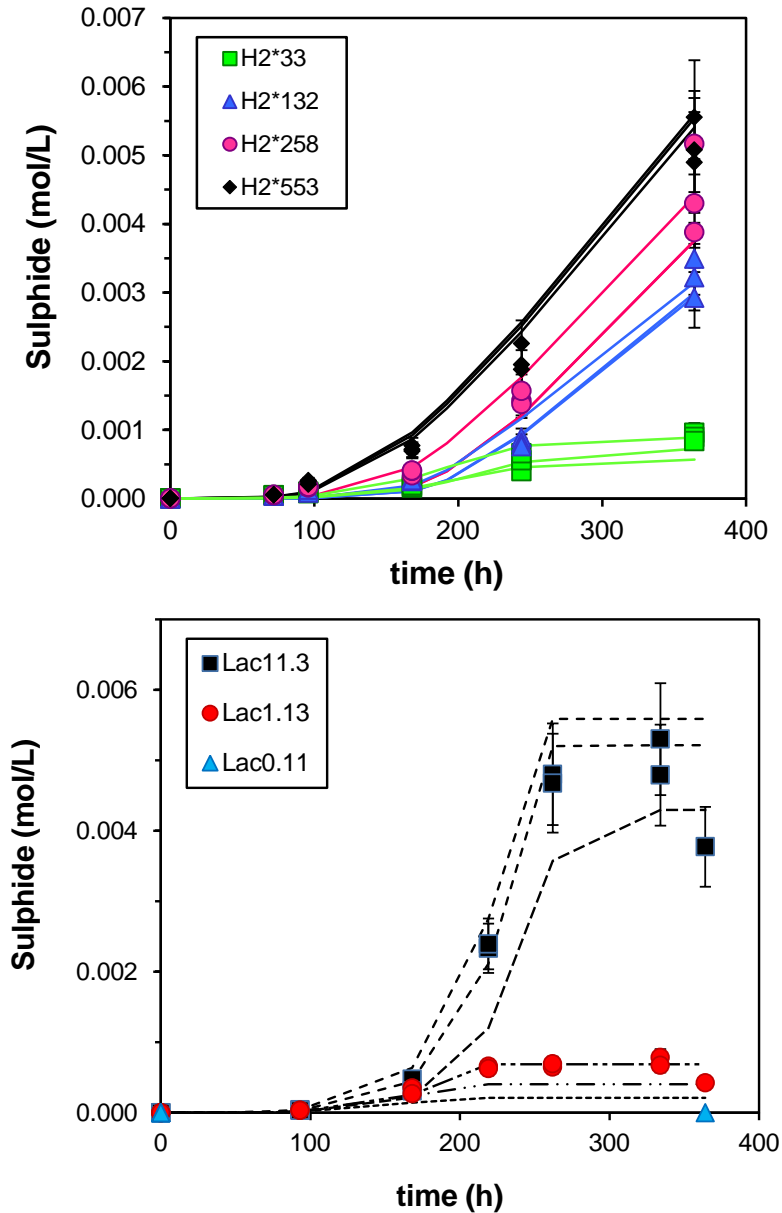
#### 3.2.2.1 Monod kinetic models of sulfate reduction microcosm experiments

Maia et al (2016) have developed models of sulfide generation in microcosm experiments (Hallbeck, 2014) that examined either lactate or acetate plus H<sub>2</sub> as substrates for the SRB *Desulfovibrio aespoensis* that was previously isolated from the Äspö URL. These experimental and modelling studies are highly relevant to the issue of sulfide generation in the Canadian DGR. They were undertaken on behalf of SKB to provide insight into copper corrosion processes in the KBS-3 design for spent fuel disposal adopted in Sweden and Finland.

Maia et al (2016) represented the sulfate reduction processes by a dual Monod kinetic rate law<sup>1</sup> that considered the concentration of both sulfate and the electron donor species (lactate or H<sub>2</sub>). For experiments with lactate, carbon for biomass synthesis was assumed to be sourced from lactate, which is known to promote the growth of SRB such as the genus *Desulfovibrio* (Maia et al, 2016). In experiments containing acetate and H<sub>2</sub> substrates Maia et al assumed that carbon for biomass growth was sourced from acetate and from CO<sub>2</sub> (representing a mixed autotrophic and heterotrophic process) based on observations of acetate consumption in the experimental data. Maia et al also included in their Monod kinetic model terms for sulfide inhibition and free energy (see Section 4.1), although the latter was found to be ineffective in these laboratory experiments that are far from chemical equilibrium. The Monod kinetic rate law was represented using the kinetic functionality of the PHREEQC geochemical speciation code (Parkhurst and Appelo, 2013; see later Section 4.2.1). The parameters for the Monod kinetic rate law (substrate utilisation rate, half saturation constants and yield coefficient; see Section 4.1) were fitted to the lactate and H<sub>2</sub> experiments by coupling PHREEQC with the Parameter ESTimation code PEST (Doherty 2010, 2015), using a least squares method (Maia et al, 2016). Good fits to the sulfide generation data (Figure 9) and other experimental data such as sulfate, lactate, acetate and biomass concentration were obtained.

---

<sup>1</sup> Further description and details of the Monod kinetic approach is provided later in this review (Section 4.1)



**Figure 9: Experimental data for sulfide generation from microcosm experiments with Hydrogen, at different partial pressures, (top figure) and lactate at different concentrations (bottom figure) fitted modelled concentration (dashed lines) using a Monod kinetic model represented in PHREEQC (Maia et al, 2016)**

Maia et al (2016) discuss that lactate will not be present in granitic groundwater, thus sulfate generation rates derived from lactate experimental studies (Hallbeck, 2014) has little direct relevance to the long-term safety of spent fuel repositories. Experimental data from the H<sub>2</sub> experiments studied may, however, have more relevance given the potential deep sources of H<sub>2</sub> (Sherwood Lollar et al, 2014). Maia et al also note that Monod kinetic parameters derived from literature display significant variation due to different data analysis techniques, differences in experimental conditions and different strains of SRB. Nevertheless, the ability to model the sulfate reduction processes using PHREEQC provides a promising tool to reproduce laboratory



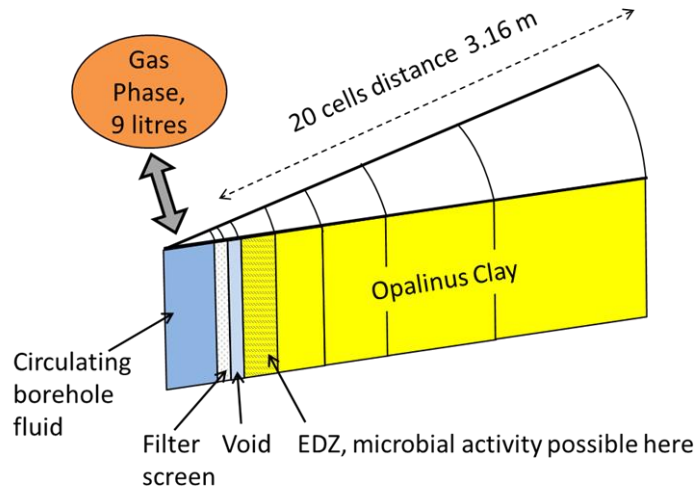
data and has potential for modelling sulfate reduction in the context of assessing the long-term safety of spent nuclear fuel repositories.

### 3.2.2.2 Reactive-transport biogeochemical models of *in situ* URL borehole experiments

The PHREEQC geochemical speciation code used by Maia et al (2016) to model kinetic processes in batch microcosm experiments has wider application to modelling of microbial processes in *in situ* URL experiments by considering transport processes. PHREEQC includes capabilities to represent 1-dimensional advective and diffusional transport processes typically used to represent a column experiment by a series of connected reaction cells (Parkhurst and Appelo, 2013). Solute transport can also be represented with PHREEQC by use of a MIX keyword function where the contents of two cells can be mixed; using this approach, processes of radial diffusion can be represented (Appelo and Wersin, 2007), which is particularly useful to represent transport processes associated with URL borehole experiments.

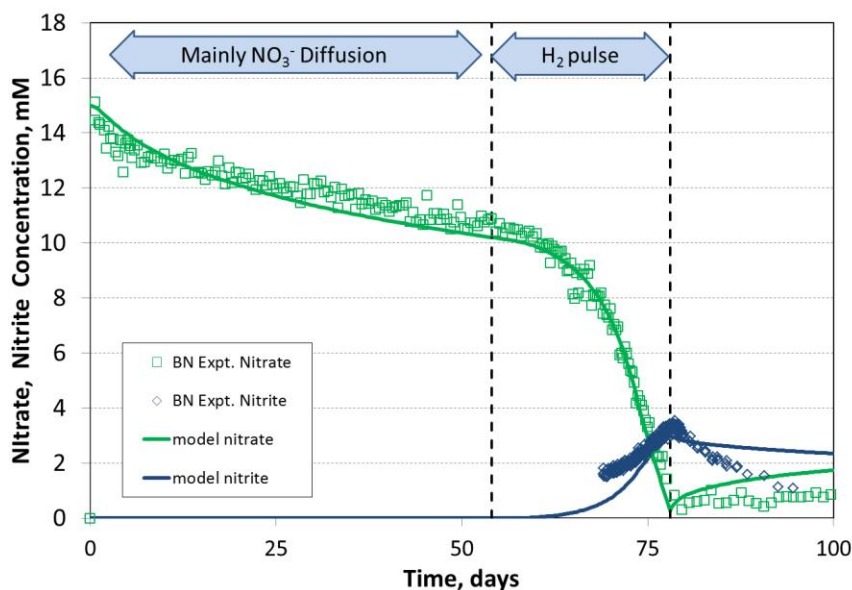
Tournassat et al (2011) used the approach of Appelo and Wersin (2007) to represent diffusion processes coupled to a range of biogeochemical processes observed in the porewater chemistry (PC) experiment at the Mont Terri URL. In the PC experiment microbial processes were stimulated inadvertently by glycerol that was released from a pH electrode. The released glycerol was utilised by sulfate reducing bacteria and also stimulated methane generation along with a number of geochemical reactions in the borehole and Opalinus Clay (see Wersin et al, 2011 and related papers cited therein). Following earlier modelling studies that adopted a Monod kinetic approach to represent the sulfate reduction process (reviewed by Tournassat et al, 2011), Tournassat et al represented the microbial processes as simpler zero order kinetic reactions fitted to the experimental data. The resulting biogeochemical model was able to represent the changes in chemical parameters measured; pH, redox potential, total organic C, dissolved inorganic C and SO<sub>4</sub> concentration. The model results were also consistent with data from the rock over-coring and demonstration that the Opalinus Clay has a high buffering capacity to the chemical effects of microbial activity by reactions of carbonates and the clay surfaces.

The Bitumen Nitrate clay interaction (BN) experiment (Bleyen et al, 2017) is a further borehole experiment at the Mont Terri URL that has examined how microbial activity is stimulated by injection of electron donor (either acetate or H<sub>2</sub>) or acceptor species (nitrate), representing reactive components of bituminised waste. The fate of nitrate is important in this context in terms of its effect on the reducing nature of the Opalinus Clay and the generation of gases (N<sub>2</sub>, N<sub>2</sub>O). A model of denitrification processes fuelled by acetate (co-injected with nitrate to represent soluble organic species from bitumen) or by electron donors present in Opalinus Clay has been implemented with the GRM model (Small 2015; Bleyen et al, 2017). The model considers a two-stage reduction of nitrate to N<sub>2</sub> gas via a nitrite intermediate (see also Section 4.1.3), which is observed experimentally as a transient species. The GRM model represents these processes using Monod kinetics, including the heterotrophic growth of biomass. The model simulates the volume of N<sub>2</sub>-rich gas evolved and enables quantification of the rate of denitrification by Opalinus Clay and the extent of nitrate and nitrite migration into the Opalinus Clay. The GRM model represented diffusive transport processes around the borehole in a simplified manner using a 1-dimensional model. Subsequently a radial diffusion model has been configured in PHREEQC following the approach of Appelo and Wersin (2007) and Tournassat et al (2011). The PHREEQC model of the BN borehole experiment was discretised to represent circulating fluid in the borehole and stagnant water present in a filter screen, together with a 3m thickness of Opalinus Clay (Figure 10).



**Figure 10: Schematic of sector of the PHREEQC radial diffusion model of the BN experiment comprised of a series of concentric annular prisms (Small, 2019; Small and Abrahamsen-Mills, 2018)**

The PHREEQC model of the BN borehole has been configured to represent an experiment where the circulating borehole was equilibrated with a gas phase that was used to examine the effect of hydrogen as an electron donor for denitrification processes (Bleyen et al, 2017). The model cell representing the circulating borehole fluid therefore has an associated gas phase of 9 litres to represent the gas equilibration apparatus installed. Figure 11 shows nitrate concentration data collected by an online spectrophotometer (Bleyen et al, 2017) following initial injection of 15 mM nitrate. Initially an inert gas (Ar) was present in the gas phase, which was changed to pure H<sub>2</sub> after 54 days and which was then replaced by Ar after a further 24 days. Prior to this H<sub>2</sub> pulse, nitrate concentration decreased by diffusion into the Opalinus Clay and is represented by the diffusion model parameterised by previous tests with a non-reactive Br<sup>-</sup> tracer. The H<sub>2</sub> pulse stimulated denitrification processes in the borehole that were apparent by the formation of nitrite. The Monod kinetic model representing a two-stage reaction forming nitrite as an intermediate in the complete reduction to nitrogen gas was fitted to the nitrate and nitrite experimental data (Figure 11). The Monod kinetic model considered that biomass growth was by an autotrophic process, where carbon was obtained from inorganic bicarbonate, which was observed to decrease during the H<sub>2</sub> pulse. This autotrophic model reproduced an observed increase in pH from pH 7.5 to pH 9 that is a consequence of the denitrification reaction and autotrophic biomass synthesis reactions (see later Section 4.1). The model also reproduced the decrease in gas pressure as the H<sub>2</sub> was consumed (Small, 2019; Small and Abrahamsen-Mills, 2018).



**Figure 11: Nitrate and nitrite concentration (symbols) during a pulsed equilibration of hydrogen gas in the BN experiment (Bleyen et al, 2017). Solid lines are the modelled concentrations of nitrate and nitrite using PHREEQC with a two stage Monod kinetic model (Small, 2019; Small and Abrahamsen, 2018)**

The Monod kinetic biogeochemical models of the BN experiment have aided the interpretation of this complex experiment and provide information concerning the fate of nitrate and nitrite that have the potential to oxidise the Opalinus Clay and affect the transport properties of redox sensitive radionuclides. In addition, the models have yielded rate constants for denitrification processes under *in situ* conditions for Opalinus Clay that may have wider application in PA models.

Based on the PHREEQC modelling of  $H_2$  reaction in the BN experiment, Small and Abrahamsen-Mills (2018) present a preliminary model of sulfate reduction processes that have been observed by  $H_2$  injection in the Mont Terri Microbial Analysis (MA) experiment undertaken in borehole BRC-3 (Bagnoud et al, 2016a, b). This borehole experiment has been used to quantify rates of sulfate reduction in Opalinus Clay by  $H_2$  (Bagnoud et al, 2016b). Detailed metagenomic and proteomic studies of the microbial populations developed by the  $H_2$  injection in the borehole experiment have also shed new light on the complex carbon cycle involved in sulfate reduction processes (Bagnoud et al, 2016a). Bagnoud et al, (2016a) conclude that the Opalinus Clay harbours the potential for a chemolithoautotrophic-based system, where hydrogen injection fixes carbon by stimulating autotrophic sulfate reduction by genomes belonging to the family *Desulfobulbaceae*. Resulting necromass is then processed by fermenters, followed by complete oxidation by heterotrophic SRBs to carbon dioxide by heterotrophic sulfate-reducing bacteria, which closes the carbon cycle. The work of Bagnoud et al (2016) is of wider significance to the generation of sulfide in the deep subsurface as it indicates that existing organic carbon is not a prerequisite of sulfate reduction driven by hydrogen.

Small and Abrahamsen-Mills (2018) have represented the complex carbon cycle associated with  $H_2$  driven sulfate reduction deduced by Bagnoud et al (2016a) by a two stage Monod

kinetic model considering an initial autotrophic process that fixes inorganic carbon as acetate followed by a heterotrophic sulfate reduction process. The model is coupled to the diffusive transport processes occurring in this borehole experiment, in particular which supply sulfate to the borehole and limit the overall extent to which sulfate concentration is lowered by around 10 % from the 12mM considered in the Opalinus Clay. The PHREEQC model also includes precipitation of sulfide as mackinawite (FeS), which limits the aqueous concentration of sulfide. This PHREEQC model of *in situ* sulfate reduction, whilst preliminary, indicates that it is possible to simulate relatively complex microbial reactions that may be important to a DGR facility. Such modelling can assist in the deconvolution of the combined effects of diffusive transport, kinetic controlled microbial processes and chemical/gas speciation.

#### 4. MODELLING OF MICROBIAL PROCESSES AND COUPLING TO GEOCHEMISTRY

Based on previous modelling approaches described above (Section 3) to model microbial growth kinetics and geochemistry, this section describes a modelling approach that has the potential to be applied to model sulfide generation in bentonite-containing experimental and repository systems, such as the Canadian DGR. The modelling approach is based on that originally developed to model anaerobic waste treatment systems (e.g. McCarty and Mosey, 1991) that have been further applied to model processes of microbial gas generation from cellulose-containing low and intermediate level waste (LLW/ILW) (Humphreys et al, 1997; Small et al; 2008; 2011; 2017). A full description of the microbial metabolic processes relevant to LLW/ILW disposal, which mainly utilise cellulose, is provided by Small and Abrahamsen-Mills (2018). This is based primarily on the Generalised Repository Model (GRM), which is used to study methane gas generation from cellulose containing LLW (Small et al, 2008; 2011; 2017). In the DGR system, much lower levels of organics will be present than for LLW/ILW disposal. However, the bentonite will contain organic matter and further natural organic matter (NOM) will be present in groundwater. Such organic matter may however be a limiting factor to the growth and metabolism of sulfate reducing microbes. In order to represent such heterotrophic processes it is therefore appropriate to include representation of various hydrolysis and fermentation processes involving NOM based on the approach developed for LLW/ILW (Small and Abrahamsen-Mills, 2018). Similarly, the main terminal electron accepting process of relevance to MIC is sulfate reduction, but it is important to also consider processes that yield greater energy. Initial aerobic processes, processes driven by nitrate contamination of bentonite, and Fe(III) reduction have the potential to reduce the extent of sulfate reduction by the consumption of electron donors (e.g. organic carbon and H<sub>2</sub>).

The following are discussed in this section:

- Microbial growth kinetics, including heterotrophic and autotrophic biomass synthesis, organic hydrolysis and anaerobic processes of relevance.
- Coupling to geochemistry, including determination of Eh and gas and mineral reactions.
- Representation of processes within the PHREEQC geochemical speciation code, including coupling to transport.
- A case study example of modelling sulfate reduction in bentonite slurry microcosm experiments.

#### 4.1 MICROBIAL GROWTH KINETIC MODELLING

A general equation for the growth of bacterial cultures was first proposed by Monod (1949), where the rate of microbial growth (R) is given by:

$$R = R_k \frac{C}{C+C_1} \quad \text{Equation 6}$$

Where  $R_k$  is the rate limit for increasing concentrations of nutrient (C) and  $C_1$  is the concentration at half the maximum rate.

In application of this equation to model, for example, to anaerobic digestion systems (McCarty and Mosey, 1991), the Monod equation is defined in terms of uptake of a single 'rate-limiting' substrate (S) that is required by the microbial process:

$$\frac{dS}{dt} = - \frac{V \cdot S \cdot X}{K_m + S} \quad \text{Equation 7}$$

Where V is the maximum substrate removal rate, S is the substrate concentration,  $K_m$  is the half saturation constant and X is the concentration of biomass. The rate of biomass formation is given by:

$$\frac{dX}{dt} = Y \frac{dS}{dt} - DX \quad \text{Equation 8}$$

Where Y is the yield coefficient and D is the death rate.

Depending on the system being represented, the Monod equation (Equation 6) may be extended to include more than one limiting substrate term (S1, S2) in a dual Monod equation:

$$\frac{dS}{dt} = - V \cdot X \left( \frac{S_1}{K_{m1} + S_1} \right) \left( \frac{S_2}{K_{m2} + S_2} \right) \quad \text{Equation 9}$$

Jin and Bethke (2002; 2003; 2005; 2007) have considered the thermodynamic aspects of microbial respiration and have introduced a thermodynamic potential factor ( $F_T$ ) into the Monod equation:

$$\frac{dS}{dt} = - V \cdot X \left( \frac{S_1}{K_{m1} + S_1} \right) \left( \frac{S_2}{K_{m2} + S_2} \right) F_T \quad \text{Equation 10}$$

The thermodynamic potential ( $F_T$ ) takes account of the Gibbs free energy of the electron transfer reaction ( $\Delta G_{\text{redox}}$ ) and also the energy required for the synthesis of ATP ( $\Delta G_p$ ):

$$F_T = 1 - e^{\left( \frac{\Delta G_{\text{redox}} + m \Delta G_p}{\chi R T} \right)} \quad \text{Equation 11}$$

Where  $\chi$  and m are coefficients specific to the microbial process, R is the gas constant and T is the absolute temperature. Inclusion of the  $F_T$  term allows the representation of reversibility in the electron transfer reaction when close to equilibrium. In systems that are far from equilibrium, such as in microcosm experiments (e.g. Maia et al, 2016),  $F_T$  is close to unity and the term can be ignored. In systems and chemical conditions that yield low amounts of energy, and natural

systems that are close to equilibrium, the thermodynamic potential effect may be more significant.

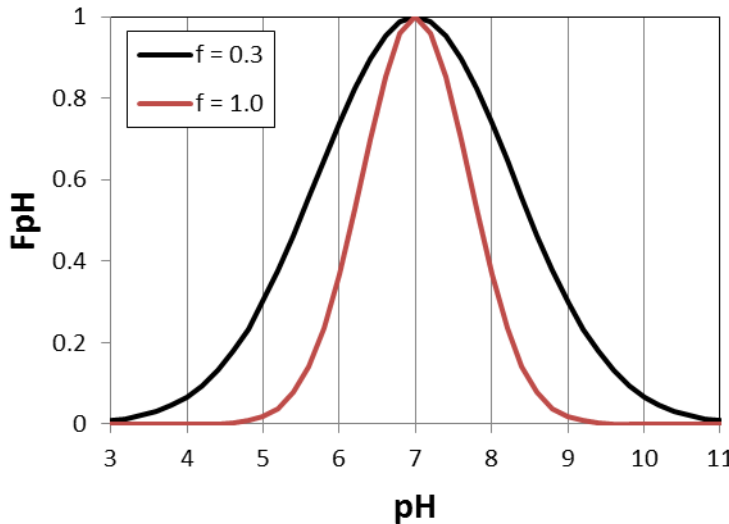
Additional terms may be included in the Monod equation to represent inhibition effects, such as related to the toxicity of a chemical species or the effect of pH. For example, Maia et al (2016) in modelling sulfate reduction in the context of LLW disposal include a term that represents the self-limiting effect of sulfide toxicity. To include such inhibition effects, additional factors (e.g.  $F_{H_2S}$ ,  $F_{pH}$ ) are included, where the factors vary between zero and 1:

$$\frac{dS}{dt} = -V \cdot X \left( \frac{S_1}{K_{m1} + S_1} \right) \left( \frac{S_2}{K_{m2} + S_2} \right) F_{H_2S} \cdot F_{pH} \quad \text{Equation 12}$$

In the GRM model developed to simulate anaerobic microbial processes in LLW (Humphreys et al, 1997; Small et al, 2008; 2011; 2017), where pH varies between different types of LLW wastefoms, the Monod equation includes a factor to represent the effect of pH. The factor ( $F_{pH}$ ) is defined by:

$$F_{pH} = e^{-((pH_{opt} - pH)^2 f)} \quad \text{Equation 13}$$

Where  $f$  is a parameter that controls how  $F_{pH}$  decreases away from the optimum pH for microbial growth ( $pH_{opt}$ ) as illustrated in Figure 12.



**Figure 12:  $F_{pH}$  function used in GRM to represent the effect of pH on microbial growth rate for  $f=0.3$  and  $f=1.0$ , for an optimum pH of 7**

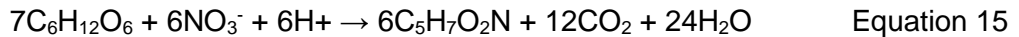
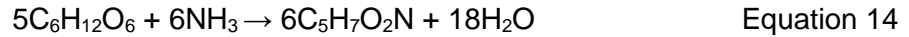
#### 4.1.1 Biomass synthesis

Microbial biomass is typically assumed to have the simplified chemical formula  $C_5H_7O_2N$  (McCarty, 1975). Biomass growth is simulated through Equation 8, where the yield coefficient defines the moles of biomass formed per mole of substrate consumed. The biomass formula ( $C_5H_7O_2N$ ) considers carbon and nitrogen as essential elements alongside hydrogen and

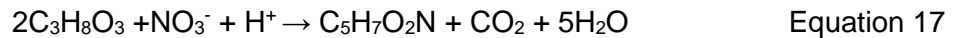
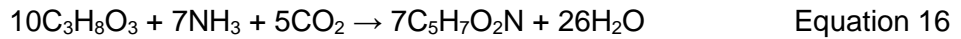
oxygen present in water. Around 30 further elements including sulfur, phosphorous and potassium are also essential for growth; this level of detail is not normally considered in modelling, where the assumption is made that such elements will be present in sufficient quantity. Carbon for biomass synthesis may be obtained either from inorganic carbon (autotrophy) or from organic carbon (heterotrophy). Nitrogen can be obtained from ammonia or nitrate chemical species in such model systems, although in near surface environments nitrogen fixation may also utilise N<sub>2</sub> gas.

In the case of anaerobic digestion systems and indeed degradation of cellulose containing LLW organic carbon is abundant and readily utilised for biomass synthesis. For the GRM model developed for such studies (Humphreys et al 1997; Graham et al, 2003; Small et al, 2008; 2011) several heterotrophic biomass synthesis reactions are considered, each utilising ammonia or nitrate.

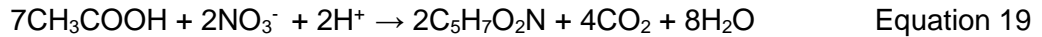
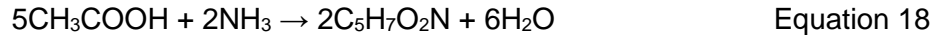
Glucose, considered as an enzymatic hydrolysis product of cellulose:



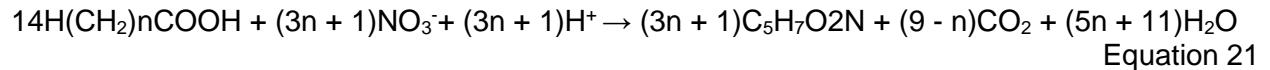
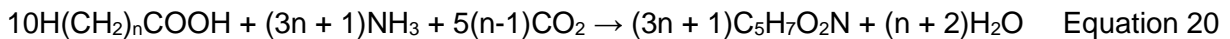
Glycerol, considered as a hydrolysis product of fats, including those formed during recycling of biomass:



Acetic acid, a fermentation product:



Volatile fatty acids (VFAs) fermentation products:



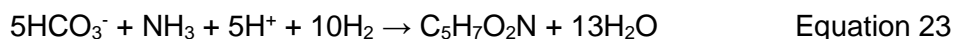
In GRM, dead biomass is recycled, forming peptides by hydrolysis that are subsequently reused in biomass synthesis, without the addition of nitrogen.



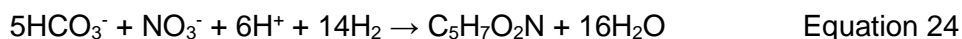
#### 4.1.1.1 Autotrophic processes

Autotrophic biomass synthesis is considered in the GRM model for methanogenesis from hydrogen and re-oxidation processes which potentially do not require organic carbon. An

autotrophic biomass synthesis reaction assuming ammonia as a nitrogen source can be written as:



Similarly assuming nitrate:



Both these reactions require  $\text{H}_2$ , which acts as an electron donor reducing inorganic carbon. In the case of LLW/ILW  $\text{H}_2$  can be formed by anaerobic steel corrosion and by radiolysis in the case of ILW. These autotrophic synthesis reactions consume significantly more protons per mole of biomass (5 and 6 for Equation 23 and 24 respectively) than the heterotrophic reactions (Equations 14-22) where the reactions that utilise nitrate consume 1 proton per mole of biomass formed and the reactions that utilise ammonia do not involve protons. Bagnoud et al (2016a) have studied sulfate reduction processes fuelled by hydrogen injection in the Opalinus Clay in the MA experiment at the Mont Terri rock laboratory. Using metagenomic and metaproteomic analysis of the microbes present during hydrogen injection, Bagnoud et al. conclude that the hydrogen injection firstly stimulates an autotrophic process that fixes inorganic carbon that is subsequently used as a carbon source for heterotrophic sulfate reduction. Similarly, Bleyen et al (2017) have studied denitrification by hydrogen as an electron donor in the Mont Terri BN experiment. Here a significant increase in pH is observed consistent with an autotrophic process stimulated by the presence of hydrogen. Models of these autotrophic reactions are presented in Small and Abrahamsen-Mills, 2018.

#### 4.1.1.2 Biomass synthesis in the DGR concept

The organic carbon content of bentonite and other engineered and natural barrier materials that comprise the DGR is limited, compared to the ILW cellulose systems for which Monod kinetic modelling approaches have been developed. In addition, biomass synthesis could be limited by the other major elements required. Assessments of the major elements (e.g. C, N, P, S, Fe) that might limit the sulfide production process in the DGR concept for Mark I and Mark II UFC designs has been undertaken (Krol and Sleep, 2013, 2014), which proposes that the availability of nitrogen and phosphorous (relative to carbon) could limit sulfide generation. However, this approach does not appear to fully constrain the limits on microbial biomass synthesis since it does not consider the yield coefficient (Equation 8) inherent in the Monod kinetic approach which controls how much biomass is formed for each mole of sulfate substrate that is reduced to sulfide. Since the yield coefficient for sulfate reduction typically has a value less than 1.0, the mass balance approach of Krol and Sleep, that effectively considers a yield coefficient of 1.0, may overestimate the amount of biomass required to be formed. This may also mean that the level of microbial activity required may not be limited by N or P in the DGR concept. Nevertheless, the approach detailed here, that considers nitrogen limitation, could be extended to consider phosphorous.

The nature of organic matter present in the DGR is also of key importance to the development of models and biomass synthesis reactions (Equations 14-22). NWMO research supporting the development of the DGR has included a state of science review of natural organic matter (NOM) present in bentonite materials and in groundwater (Marshall and Simpson, 2014a) and detailed NMR characterisation of solid and solvent extractable NOM present in three bentonite materials (MX-80, National and CCP; Marshall and Simpson, 2014b). NOM present in these



bentonite comprises both aromatic and aliphatic carbon, the latter being related to plant derived waxes. MX-80 showed lower amounts of total organic and solvent extractable carbon and evidence of oxidation of the NOM. The aromatic component of NOM showed signs of advanced degradation such as through diagenesis. The NOM present in bentonite is hypothesized to be more recalcitrant as compared to other labile NOM sources (sugars, proteins, and small organic acids) which were not detected (Marshall and Simpson, 2014b). It is however noted that n-alkanols and n-alkanoic acids with C<20 are predominant.

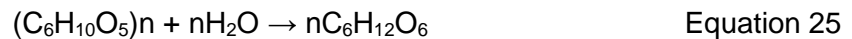
In application of the above biomass formation reactions derived from cellulose systems (Equations 14-21), Equation 21 for synthesis from VFAs would be most appropriate. However, further research concerning the bioavailability of NOM may be required to understand which components of NOM could be utilised. Appropriate biomass synthesis reactions could then be represented in models. Additionally, hydrolysis reactions may be stimulated by microbial activity which may yield shorter chain species, as described below for cellulose hydrolysis. The NWMO DGR microbiology programme has largely focussed on heterotrophic processes and the requirement of organic carbon to synthesise biomass for active sulfate reduction processes. The potential for autotrophic processes should not however be overlooked given evidence from URL studies (e.g. Bagnoud et al, 2016a) and in the wider literature concerning biogeochemical processes driven by deep H<sub>2</sub> sources (Sherwood Lollar et al, 2014; Truche et al, 2018). Equations 23 and 24 provide a basis to represent such autotrophic reactions in a Monod kinetic reaction scheme.

#### 4.1.2 Organic hydrolysis processes

NOM present in the DGR may be partially available for biomass synthesis and metabolism, acting as an electron donor for sulfate reduction. The bioavailability of such material is limited by its hydrolysis to smaller water soluble molecules that can be assimilated by microbes. Such hydrolysis processes are recognised to be important rate limiting processes in gas generation from cellulose containing LLW/ILW (Small et al, 2008).

Whilst cellulose will not be present in the DGR, the established approach to represent cellulose hydrolysis forms a basis by which the hydrolysis of NOM may be represented.

Under neutral pH conditions<sup>2</sup> cellulose hydrolysis is mediated by extracellular enzymes produced by cellulolytic bacteria such as the genus *Clostridium* (Leschine, 1995; Van Dyke and McCarthy, 2002). Anaerobic fungi have also been invoked as being actively involved in cellulose degradation at landfill sites and in the UK LLWR (Lockhart et al., 2006). Enzymatic hydrolysis of cellulose can be considered to be represented by the formation of glucose, where the soluble sugar is assimilated:



In the GRM the rate of enzymatic cellulose hydrolysis is represented by a first-order kinetic process:

$$\frac{dS}{dt} = -v F(T,pH) \theta S \quad \text{Equation 26}$$

<sup>2</sup> Under high pH conditions (>12) cellulose proceeds by an alkaline hydrolysis mechanism, but this is not relevant to the DGR concept discussed here.

Where S is the concentration of the polymer substrate (cellulose), v is the hydrolysis rate ( $s^{-1}$ ) and  $\Theta$  is the moisture content (porosity). The rate of hydrolysis is dependent on temperature and pH as defined by the function:

$$F(T, \text{pH}) = \frac{T \cdot e^{-((\text{pH}_{\text{opt}} - \text{pH})^2 f)}}{1 + e^{\left(\frac{T}{4} - 18\right)}} \quad \text{Equation 27}$$

Where T is temperature (Celsius) and  $\text{pH}_{\text{opt}}$  is the optimum pH for hydrolysis and f is a pH control factor as discussed above for Equation 8 and illustrated in Figure 12.

In the GRM, the approach detailed above for enzymatic hydrolysis of cellulose (Equation 26) is also applied to fats, which hydrolyse to form glycerol ( $\text{C}_3\text{H}_8\text{O}_3$ ) and to proteins, forming peptides ( $\text{C}_{46}\text{H}_{77}\text{O}_{17}\text{N}_{12}$ ). Fats and proteins may be used to represent putrescible materials present in LLW, and are also used to represent the recycling of dead microbial biomass. Such a recycling process also has application to modelling microbial activity in the DGR, where nutrients for biomass synthesis are likely to be limiting.

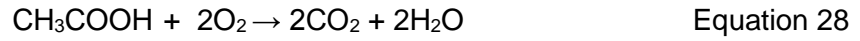
#### 4.1.3 Microbial respiration and fermentation processes

In this section microbial metabolic energy sources are discussed, providing example reactions between electron donor and electron acceptor species. In application to the DGR, sulfate reduction is of primary importance as a terminal electron accepting process (TEAP) since it provides a source of sulfide that is of key importance to corrosion of the copper clad UFC. The amount of energy that can be liberated by sulfate reduction is however relatively low and thus more energetically favoured TEAPs are likely to outcompete sulfate reduction and thus it is important to consider these processes in modelling the DGR even if concentration of dominant electron acceptors ( $\text{O}_2$ ,  $\text{NO}_3^-$ , Fe(III)) are likely to be limited).

Fermentation processes may have relevance to the DGR in the context of generating organic acids (acetate) that may act as agents promoting stress corrosion cracking (SCC) although this effect is mitigated by the Mark II UFC design (see Section 1). Fermentation processes are perhaps of more direct relevance to the bioavailability of natural organic carbon present in the DGR. URL studies of hydrogen interaction with Opalinus Clay have highlighted the role that autotrophic processes may have in fixing organic carbon that is subsequently used as an energy and carbon source for sulfate reduction (Bagnoud et al, 2016a). Thus in developing models of the sulfate reduction processes for application to the DGR it is important to consider a full range of respiration and fermentation processes.

Below are example reactions of the main microbial processes that may require consideration, generally ordered by energy yield and based on the approach of the GRM to model anaerobic processes leading to methane generation (Humphreys et al, 1997; Graham et al, 2003; Small et al, 2008; 2011). Methanogenesis is not included in this sequence as it yields the least energy and is normally outcompeted by sulfate reduction processes.

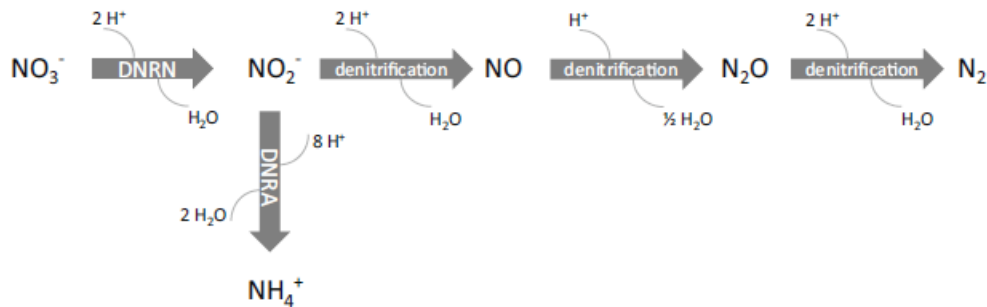
**Aerobic respiration** encompasses a very wide range of processes and those that are the most energetically favourable. However, in most disposal scenarios aerobic respiration is limited by the availability of oxygen. Aerobic respiration is of relevance to the operational phase of a DGR. Aerobic respiration of organic matter simply yields  $\text{CO}_2$  as shown in the example reaction below for acetic acid.



A wide range of other more complex natural organic substrates such as those present in bentonite may also be subject to aerobic respiration. Hydrogen, potentially formed by radiolysis, and by steel corrosion in anaerobic regions of a DGR will also be consumed where it migrates to aerobic regions. Such reaction of hydrogen is however likely to be spontaneous, but should be accounted for in considering the significance of aerobic respiration during the operational phase of a DGR.

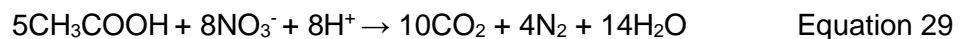
**Denitrification** is an important process occurring in shallow aquifer systems, which limits nitrate contamination from agriculture and waste water treatment. In the DGR environment nitrate will not be present naturally, but is likely to be introduced through its presence in bentonite, where between 2 and 50 mg/kg nitrate have been measured in bentonites examined by Marshall and Simpson (2014b).

Denitrification processes yield high amounts of energy and are the first anaerobic process to occur under anoxic conditions. The overall denitrification process occurs via a series of intermediate species (Figure 13) producing either nitrogen gas or ammonium with the initial reduction of nitrate to nitrite being common to both end products (Bleyen et al, 2017).



**Figure 13: Biological pathways to form nitrogen gas or ammonium from nitrate with initial reduction to nitrite (Bleyen et al, 2017)**

This series of reactions to form  $\text{N}_2$  gas can be represented utilising acetic acid:

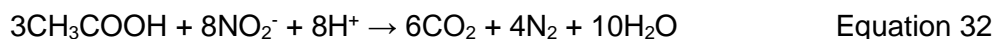
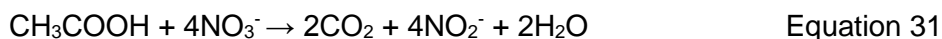


Or, utilising hydrogen as an electron donor:

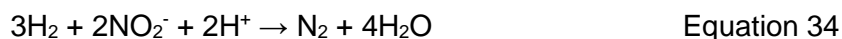


The reaction to form ammonium could also be represented, although Bleyen et al (2017) observed that the ammonium formation was rather limited compared to nitrogen gas, although higher concentrations were observed for the case where hydrogen was the electron donor. In studying denitrification under *in-situ* conditions in the BN experiment at the Mont Terri underground rock laboratory Bleyen et al (2016, 2017) have measured the occurrence of the nitrite intermediate. The formation of nitrite is important in the context of the BN experiment as nitrite may have the potential to react and oxidise the host rock abiotically (Bleyen et al, 2016).

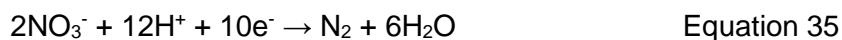
In the context of the DGR nitrite is of importance to SCC. The formation of nitrite is common in microcosm experiments (e.g. Nixon et al, 2018) and can inhibit further reaction due to its toxicity. To represent the formation of the nitrite intermediate, the following two reactions can be considered for the case of acetic acid acting as an electron donor:



Similarly, where hydrogen is considered as the electron donor:



Denitrification may also be driven by the oxidation of reduced mineral phases such Fe(II) containing clay minerals and sulfides such a pyrite ( $\text{FeS}_2$ ) where both Fe and S may act as electron donors. Here it is convenient to represent the process by half reactions for the nitrate and nitrite reduction reactions where electrons are included:



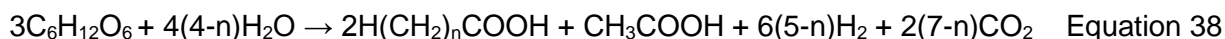
These half reactions can then be coupled to a mineral oxidation reaction, such as for pyrite ( $\text{FeS}_2$ ) where both iron and sulfur are oxidised:



Since organic carbon is not required for these reactions the processes can be assumed to be autotrophic e.g. as Equations 23 and 24.

**Fermentation** is an important process involved in the degradation of cellulose, typically producing a range of low molecular weight carboxylic acids, volatile fatty acids (VFAs), including acetic acid. Fermentation of cellulose is not directly relevant to the DGR, but analogous processes could be important to consider in the utilisation of natural organic matter (NOM) alongside hydrolysis processes (Section 4.1.2). In addition, fermentation is a process that may require in consideration in the recycling of biomass.

Fermenters gain energy through mediating redox reactions of carbon present in sugars (in the case of cellulose) and other soluble organic hydrolysis products. A general equation for the generation of VFAs by fermentation processes as represented in GRM is:

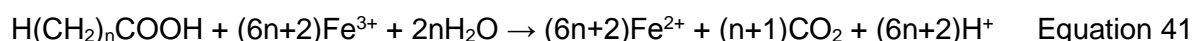
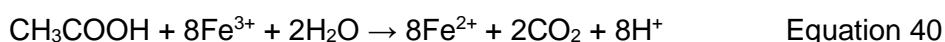


Here, n is the average number of aliphatic carbons present in the VFAs produced and typically has a value of between 2 and 3 representing the mixture of propionic, butyric and valeric acids formed. Fermentation also generates hydrogen, which along with the VFAs acts as an electron donor for other TEAPs. Acetogenesis, where VFAs are converted to acetic acid liberating further hydrogen is a further fermentation processes included in the GRM (Small and Abrahamsen-Mills, 2018).

For application to the DGR, Equation 38 can be used to consider the fermentation of sugars from hydrolysis of biomass. In order to consider utilisation of NOM the equation will require modification to include a representative NOM composition instead of glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>).

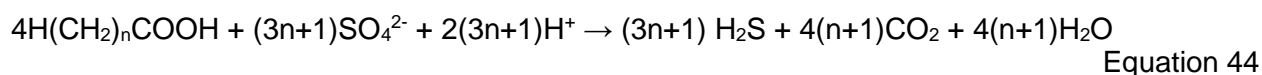
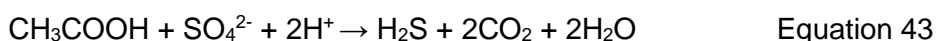
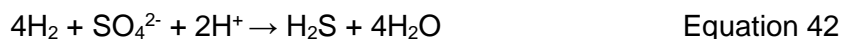
**Iron(III) reduction** is a well-known TEAP studied in environmental geochemistry, which is important to controlling the mobility of other metals and contaminants (e.g. U, Tc, Cr, As) either by reduction to less soluble oxidation states or through sorption and co-precipitation processes (Newsome et al, 2014). In the context of a DGR, reduction of Fe(III) present in bentonite is of current interest because of the possibility that reduction of Fe(III) present in octahedral sites within the smectite clay mineral structure could result in a decrease in the bentonite swelling capacity (Kim et al, 2004). Microbial reduction of such structural Fe(III) however remains tenuous (Perdrial et al, 2009; Haynes et al, 2018; Matschiavelli et al, 2018) and it is likely that Fe(III) present in smectite is less bioavailable than that in discrete Fe oxide/hydroxide phases that may be present at trace levels in bentonites.

Iron reduction may be driven by hydrogen, acetic acid or VFA electron donors as follows:



These reactions consider the aqueous Fe<sup>3+</sup>, Fe<sup>2+</sup> species rather than solid phases because so they can be coupled to geochemical speciation (see later Section 4.2) which considers the effect on mineral solubility. Iron mineral solubility effectively controls the bioavailability of Fe(III) for these processes, with poorly-soluble phases such as hematite being less easily reduced than more soluble phases such as ferrihydrite. The approach could, in principle, be extended to consider the solubility of Fe(III) present in smectite, as thermodynamic data exists (e.g. Blanc et al, 2012) for smectite phases including the end-member nontronite Fe(III) phase.

**Sulfate reduction** generally occurs after Fe(III) reduction. Sulfate reduction to sulfide involves the transfer of 8 electrons and is a multiple step process, with intermediate oxidation states such as S(0) being formed. However, for modelling it is convenient to represent the reaction as a single overall process for hydrogen, acetic acid or VFA electron donors:



The sulfide species formed can be toxic to the microbial reduction process and so may limit the extent of reaction (see Section 4.1). However, sulfide has low solubility in the presence of iron due to the formation of iron sulfides. Reactions of the sulfide with iron should therefore be included in the chemical speciation calculations as detailed in Section 4.2.

Sulfate reduction utilising both soluble organic and hydrogen electron donors are the main TEAPs that are relevant to DGR. An example model of sulfate reduction in bentonite containing microcosm experiments is illustrated below (Section 4.3).

## 4.2 COUPLING TO GEOCHEMISTRY

Microbial processes can have significant effects on geochemistry, especially in catalysing slow redox reactions between common multivalent elements e.g. N, Fe, S. The redox reactions of these elements often involve protons ( $H^+$ , e.g. Equations 29-44 Section 4.1.3) and thus have the potential to affect pH or induce changes in chemical speciation and mineral reactions.

In the case of sulfate reduction, which is of primary importance to the DGR, the generation of sulfide is likely to have a significant effect on geochemistry through the precipitation of iron sulfides, which generally have low solubility. Importantly, iron sulfide precipitation will also limit the aqueous concentration of sulfide, which is of prime importance to UFC corrosion.

As discussed in the preceding section the solubility of Fe(III) containing minerals will also influence the bioavailability of Fe(III), whose reduction may compete with sulfate reduction. Fe solubilities will be influenced by pH and Eh; pH is normally calculated by charge balance, while approaches to model Eh are discussed below. The total solubility of Fe is also important to determining the solubility of aqueous sulfide through a solubility product relation for iron sulfide. The solubility product of Fe(III) oxide / hydroxide minerals and iron sulfides is however variable, depending on their crystallinity, which can increase through ageing and Ostwald ripening processes (Bonneville et al, 2009). To fully consider the geochemical effects on aqueous sulfide generation in such bentonite systems it will be important to characterise the mineralogy of iron phases present initially and of iron sulfide phases formed in experimental systems.

While geochemical effects such as FeS precipitation are important to consider in the estimation of the level of sulfide formed in the DGR geochemistry may also influence the rate of microbial processes such as through effects of pH and of toxicity effect by sulfide.

### 4.2.1 Use of the PHREEQC geochemical modelling code

PHREEQC (Parkhurst and Appelo, 2013) is a versatile speciation, batch reaction and one-dimensional transport code that is widely used in geochemistry and in applications to nuclear waste disposal. It is a standard tool used to calculate the aqueous speciation and solubility of radionuclides and to represent mechanistic ion-exchange and surface complexation sorption processes. The code utilises thermodynamic data from a range of optional databases. The database used in the current work was a modified version of the Thermochimie Davies version 9b0 (Giffaut et al. 2014).

The PHREEQC approach to modelling kinetic processes is flexible in that it allows different rate equations and constraints (e.g. inhibition terms) to be examined. The stoichiometry of the electron transfer and biomass synthesis reactions can also be changed to consider different electron donors and carbon and nitrogen sources for biomass synthesis.

The code can be used to represent batch reactor systems and can consider reaction with a gas phase of variable volume or pressure. Such a configuration has direct application to represent microbial microcosm experiments. It can also simulate 1-dimensional transport, including

advection and diffusion and has been used to simulate a number of in-situ borehole experiments at the Swiss Mont Terri Laboratory (Small & Abrahamsen-Mills, 2018).

Microbial processes can be represented in PHREEQC using kinetic controlled reactions, where rate equations are defined by the user using the BASIC interpreter incorporated within the code. Figure 14 shows an example of the PHREEQC coding developed to model sulfate reduction in bentonite microcosm experiments (Abrahamsen-Mills, 2019; Grigoryan et al, 2018), where the rate equation for sulfate reduction by acetate (see Equation 43) is first defined. Then, the biomass synthesis rate (see Equation 14) is defined using the rate calculated and stored by the first equation. Note that 'Biomassb' represents the acetate-consuming biomass.

As shown in Figure 14, parameter values are entered in BASIC code lines 315, 320, 325, 330, 331, 332, 352, 405 and 410, corresponding to the Monod kinetic half saturation constants and maximum substrate removal rate, yield coefficient and death rate (See Equations 8 and 9). The stoichiometry of each microbial kinetic controlled reaction is defined by the PHREEQC KINETICS keyword. Figure 15 shows the keywords and statements entered in PHREEQC that define the sulfate reduction and biomass synthesis rate equations that were used in the coding shown in Figure 14.

```

RATES
#----- SULFATE REDUCTION, ACE e-DONOR -----
# REACTION CH3COO- + SO4-2 = 2HCO3- + HS-

SO4_red
-start
300 moles = 0
305 rate = 0
310 IF(TOT("S(6)")<=1E-10 OR TOT("Ace")<=1E-9 OR TOT("Biomassb")<=1E-15) THEN GOTO 395

315 Ka = 7e-3          # half saturation constant. for Ace (mol of Ace/L)      (KAce)
320 Ks = 1e-3          # half saturation constant for SO4 (mol of SO4/L)      (KSO42-)
325 Kmax2 = 5e-4       # max. rate of SO4 reduction by SRB (1/second)      (VAce)
330 Ki = 4.6700000E-04 # half sat. inhibition for H2S (mol of H2S/L).
#
#----- pH CONTROL -----
331 pHopt =7.5
332 f_pH = 0.35
333 FpH = EXP(-(pHopt+LA("H+"))^2)*f_pH
#
335 f1 = 1
340 IF(TOT("Ace") > 0 AND Ka > 0) THEN f1 = TOT("Ace")/(Ka + TOT("Ace"))
345 f2 = 1
350 IF(TOT("S(6)") > 0 AND Ks > 0) THEN f2 = TOT("S(6)")/(Ks + TOT("S(6)"))
#
#----- SULFIDE INHIBITION CONTROL -----
#
351 f3 = 1 # term for inhibition by HS-
#352 IF(TOT("S_") > 0 AND Ki > 0) THEN f3 = Ki/(Ki + TOT("S_"))
#
355 IF(TOT("Biomassb") > 0 AND Kmax2 > 0) THEN rate = -Kmax2 * f1 * f2 * f3*
(TOT("Biomassb"))*FpH
360 moles = rate * TIME
365 IF(moles<0 AND (TOT("S(6)")+moles)>1e-10 AND (TOT("Ace")+moles)>1e-10) THEN GOTO 395
370 moles = 0
375 rate = 0
395 PUT(rate, 2)
399 SAVE moles
-end
#----- Biomassb (HETEROTROPHIC) -----
# REACTION 2.5CH3COO- + NH4+ + 1.5H+ = Biomassb + 3H2O          (Biomassb = XAce)

Biomassb
-start
400 moles = 0
401 IF(TOT("Ace")<=1E-9 OR TOT("N(-3)")<=1E-9 OR TOT("S(6)")<=1E-10 OR
TOT("Biomassb")<=1E-15) THEN GOTO 499

405 Y2 = 0.03 # specific yield factor for Biomassb      (YAce)
410 D2 = 1E-15 # Decay coefficient (second-1) for Biomassb      (DAce)

415 IF(Y2 < 0) THEN Y2 = 0
420 R2 = GET(2) # uses rate calculated in rate SO4_red
425 IF(R2 >0) THEN R2 = 0
430 rate = -Y2 * R2 - D2 * TOT("Biomassb")
435 moles = - rate * TIME
499 SAVE moles
-end

```

**Figure 14: PHREEQC RATES keyword definition of a dual Monod kinetic rate equation for sulfate reduction by acetate and biomass synthesis defined by the BASIC interpreter (# defines the start of comments)**



```

KINETICS 1

SO4_red
# Ace + SO4-2 = 2HCO3- + HS-

-formula Ace 1    SO4 1    HCO3 -2    HS_ -1
-m0 0
-tol 1e-012

Biomassb
# 2.5CH3COO- + NH4+ + 1.5H+ = Biomassb + 3H2O

-formula Ace 2.5    NH4 1    H 1.5    Biomassb -1    H2O -3
-m0 0
-tol 1e-012

```

**Figure 15: PHREEQC KINETIC keyword definition for sulfate reduction by acetate considering heterotrophic biomass synthesis**

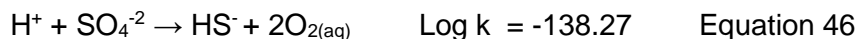
#### 4.2.2 Consideration of redox processes and determination of $E_h$

One of the principal calculations performed by a geochemical speciation model (e.g. PHREEQC, Parkhurst and Appelo, 1999) is to determine the distribution and concentration of individual aqueous species that comprise the total concentration of the chemical element. Each individual species is made by reactions of “master species”. In order to consider the speciation of multivalent elements where the reactions between different oxidation states is mediated by a microbial reaction it is necessary to define separate master species for each oxidation state, otherwise the speciation model will override the microbial process.

For example, in the case of sulfur the master species may be defined as the sulfate anion ( $\text{SO}_4^{-2}$ ). Depending on pH and the concentration of other ions the total sulfate concentration may be distributed amongst other ions and metal complexes such as  $\text{HSO}_4^-$ ,  $\text{NaSO}_4^-$ ,  $\text{CaSO}_4^0$ . Such secondary species are defined by their mass action equation and equilibrium constant (Log k), e.g. for  $\text{NaSO}_4^-$ :



Under strongly reducing conditions ( $E_h < -200\text{mV}$  at pH 7) and assuming equilibrium, sulfide will be thermodynamically stable and will dominate the speciation as defined by its mass action equation:



Thus, to correctly represent kinetically controlled microbial processes, sulfide must be defined as a separate master species to that of sulfate. Similarly, the other major multivalent elements involved in the microbial process model must be decoupled and separate master species represented for each species considered by the microbial model:

- Oxygen species:  $\text{O}_2$ ;  $\text{H}_2\text{O}$
- Nitrogen species:  $\text{NO}_3^-$ ;  $\text{NO}_2^-$ ;  $\text{N}_2$ ;  $\text{NH}_3$
- Iron species: Fe(II); Fe(III)
- Sulfur species:  $\text{SO}_4^{-2}$ ;  $\text{HS}^-$  (note,  $\text{SO}_3^{-2}$ ,  $\text{S}_2\text{O}_4^{-2}$ ,  $\text{S}_2\text{O}_3^{-2}$ , S(0) considered in equilibrium with  $\text{SO}_4^{-2}$ )

- Carbon species  $\text{CO}_3^{2-}$ ;  $\text{CH}_4$ ;  $\text{CH}_3\text{COOH}$ ; VFAs; Glucose

Having defined separate master species for each major multivalent element the concentrations of each oxidation state can be used to calculate a redox potential ( $E_h$  or  $pe$ ). For example, by rearranging Equation 42 in terms of electrons ( $e^-$ ) the sulfate/sulfide redox couple is defined by the reaction:



and the mass action expression is:

$$K_{\text{SO}_4/\text{HS}} = \frac{[\text{HS}^-][\text{H}_2\text{O}]^{4.0}}{[\text{SO}_4^{2-}][\text{H}^+]^9[e^-]^8} \quad \text{Equation 48}$$

Assuming that the concentration (activity) of  $\text{H}_2\text{O}$  is 1.0 then the redox potential ( $pe$ ,  $E_h$ ) can be calculated from the activities ( $a$ ) of sulfate and sulfide and the equilibrium constant for the couple:

$$pe = \log K_{\text{SO}_4/\text{HS}} - 9p\text{H} + \log a \{ \text{SO}_4^{2-} \} - \log a \{ [\text{HS}^-] \} \quad \text{Equation 49}$$

The  $pe$  is the negative logarithm of the electron activity and can be converted to redox potential ( $E_h$ ) by:

$$E_h = pe \frac{2.303 RT}{F} \quad \text{Equation 50}$$

Where  $F$  is Faraday's constant,  $R$  is the gas constant and  $T$  is absolute temperature. At  $25^\circ\text{C}$

$$E_h(\text{volts}) = pe \times 0.05916 \quad \text{Equation 51}$$

This method is used in the GRM code to simulate the evolving  $E_h$  conditions during degradation of cellulose waste and gas generation (Small et al, 2008;2017; Small and Abrahamsen-Mills, 2018).

In modelling microbial sulfate reduction processes in the DGR, including processes of chemical speciation and sulfide precipitation sulfide and sulfate will require representation as separate master species. In experimental systems, where sulfate and sulfide aqueous concentrations are measured an estimate of the redox potential can be made. Such an approach is used in the case study described below to assess the significance of  $\text{FeS}$  precipitation in sulfate reduction bentonite microcosm experiments.

### 4.3 EXAMPLE MODELS OF SULFATE REDUCTION IN BENTONITE MICROCOSM EXPERIMENTS

In this subsection, example numerical models developed in PHREEQC are presented to illustrate how microbial processes describing sulfate reduction can be coupled with geochemical reactions that may limit the concentration of generated sulfide. The models simulate microcosm experiments of sulfate reduction in the presence of un-compacted bentonites undertaken by Ryerson University and the University of Saskatchewan for NWMO (Grigoryan et al, 2018). The models utilise a Monod kinetic approach, implemented in PHREEQC, which can simulate a

number of linked microbial processes and the growth and decay of microbial biomass. This approach allows the reactions and their rates to be defined in terms of initial biomass populations, growth yield factors, and a number of kinetic parameters. The models were configured to represent experiments where either acetate (Figure 16 and Figure 18) or lactate (Figure 17) were used to stimulate sulfate reduction. Models were constructed for two of the bentonite materials studied by Grigoryan et al (2018); MX-80® and Canapril®.

The key variables employed in these models are the initial biomass present, the Monod kinetic parameters (for both acetate and lactate consuming populations): maximum substrate removal rate ( $V_{Ace}$ ,  $V_{Lac}$ ), half saturation constants for sulfide ( $K_{SO_4^{2-}}$ ), acetate ( $K_{Ace}$ ) and lactate ( $K_{Lac}$ ), the pH control factor ( $F_{pH}$ ) and the biomass yield coefficients ( $Y_{Ace}$ ,  $Y_{Lac}$ ) and death rates ( $D_{Ace}$ ,  $D_{Lac}$ ).

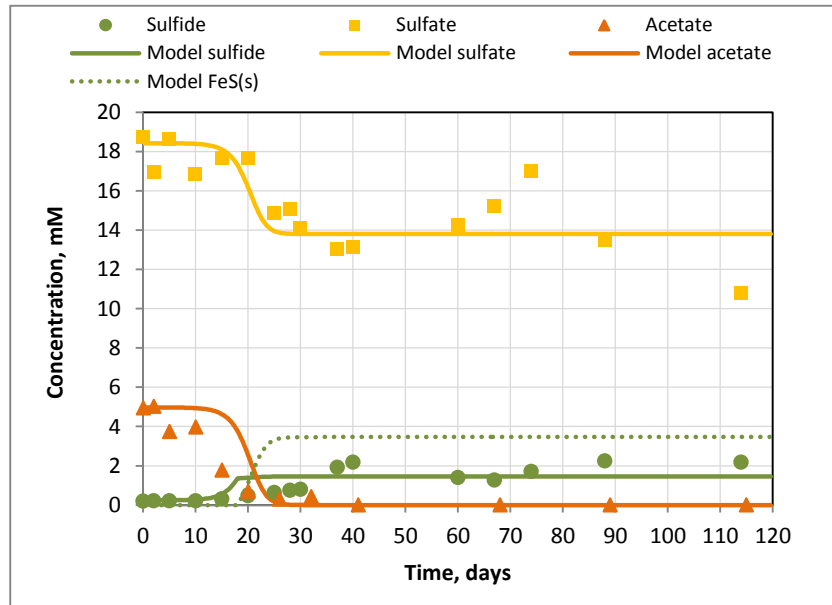
Where possible, these model parameters should be based on known or estimated values for the system (such as literature data for Monod kinetic parameters). Following this, the parameters can be adjusted in order to achieve the best match to experimental data. The approach utilised in this modelling exercise was to start with a set of parameters used for modelling similar systems (Small & Abrahamsen-Mills, 2018) and then to adjust key parameters to fit the model to one particular data set (the Canapril acetate experiment at 37 °C, shown in Figure 16). This set of parameter values (with zero/minimal adjustment) was then applied to a selection of the other experiments in order to test the model. The parameter values utilised are presented in Table 1. See Section 4.1 for an explanation of the terms.

Grigoryan et al. (2018) provide a quantification of microbial cells in terms of colony-forming units per gram dry weight ( $CFU\ gdw^{-1}$ ) and most probable number (MPN) estimations, which could be used in modelling as the biomass concentrations at the start of the microcosm experiments. However, this estimation appears to have been performed for the MX-80 bentonite only ( $42.4\ cells\ gdw^{-1}$ ). For the modelling of the MX-80 experiments, this was converted to a molarity assuming a biomass molecular weight of 113.115 g/mole and an average single cell mass of  $9.5E-13\ g$  (Neidherdt et al., 1990) giving a solution biomass molarity of  $3.97E-11\ M$  in the microcosm experiments. For modelling the Canapril experiments, the initial biomass concentration was estimated by fitting this parameter to the experimental data.

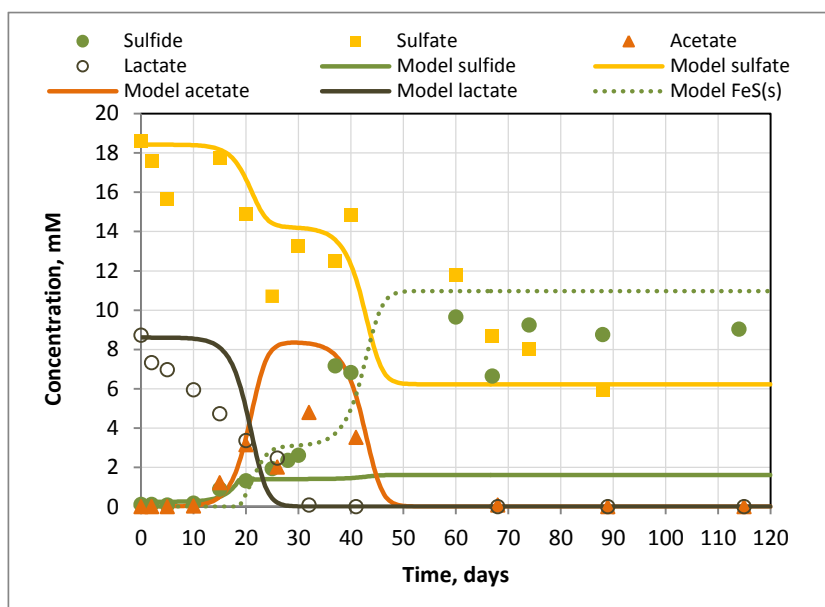
The only parameters that were altered between the models for different bentonites were the initial biomass concentrations and quantities of iron present in the bentonite.

**Table 1: Model parameter values used to simulate microcosm experiments (values in bold indicate differences between the two bentonites considered)**

Model Parameter	Canapriill Value	MX-80 Value
$X_{Ace}$ , initial biomass concentration (M)	1E-5	3.97E-11
$X_{Lac}$ , initial biomass concentration (M)	1E-5	3.97E-11
Hematite content of bentonite (wt %)	4.43	3.25
Converted hematite content in experiments (M)	3.08E-02	2.26E-02
$V_{Ace}$ ( $s^{-1}$ )	5E-4	5E-4
$V_{Lac}$ ( $s^{-1}$ )	5E-4	5E-4
$K_{SO42-(Ace)}$ (M)	1E-3	1E-3
$K_{SO42-(Lac)}$ (M)	4E-3	4E-3
$K_{Ace}$ (M)	7E-3	7E-3
$K_{Lac}$ (M)	1E-2	1E-2
$Y_{Ace}$ (unitless)	0.03	0.03
$Y_{Lac}$ (unitless)	0.03	0.03
$D_{Ace}$ ( $s^{-1}$ )	1E-15	1E-15
$D_{Lac}$ ( $s^{-1}$ )	1E-15	1E-15
pH inhibition parameters	pHopt = 7.5, f = 0.35	pHopt = 7.5, f = 0.35



**Figure 16: Modelled sulfate reduction coupled with oxidation of acetate in microcosms at 37°C including Canapriill bentonite, symbols indicate experimental data from Grigoryan et al, (2018)**



**Figure 17: Modelled sulfate reduction coupled with oxidation of Lactate in microcosms at 37°C including Canapriill bentonite, symbols indicate experimental data from Grigoryan et al, (2018)**

The approach adopted to model the bentonite microcosms was to fit the Monod kinetic parameters to the experimental data for Canapriill bentonite (e.g. Figure 16, Figure 17) which showed the clearest trends in experimental data, and then apply the model to the other bentonites examining differences in iron content and the initial biomass concentration (e.g. Figure 18). Models were configured for acetate assuming its complete oxidation (see Equation 43). In the case of lactate ( $\text{CH}_3\text{CH}(\text{OH})\text{COOH}$ ) a two-stage model was implemented considering first the oxidation to acetate:



followed by the oxidation of acetate. In addition, some lactate or acetate was utilised for biomass synthesis.

To account for geochemical controls on sulfide levels, the models included the potential effect for mackinawite ( $\text{FeS}$ ) precipitation, assuming that all the iron measured in the bentonites is available for reaction. Based on analysis data presented in Grigoryan et al (2018), iron was assumed to be present in the bentonites in the form of hematite ( $\alpha\text{-Fe}_2\text{O}_3$ ), a crystalline and low solubility  $\text{Fe}(\text{III})$  oxide. The models for Canapriill and MX-80 bentonite considered  $\text{Fe}_2\text{O}_3$  concentrations of 4.43 wt% and 3.25 wt% respectively, which are equivalent to hematite ( $\alpha\text{-Fe}_2\text{O}_3$ ) mineral concentrations per litre of porewater of  $3.08\text{E-}02 \text{ M}$  and  $2.26\text{E-}02 \text{ M}$  respectively.

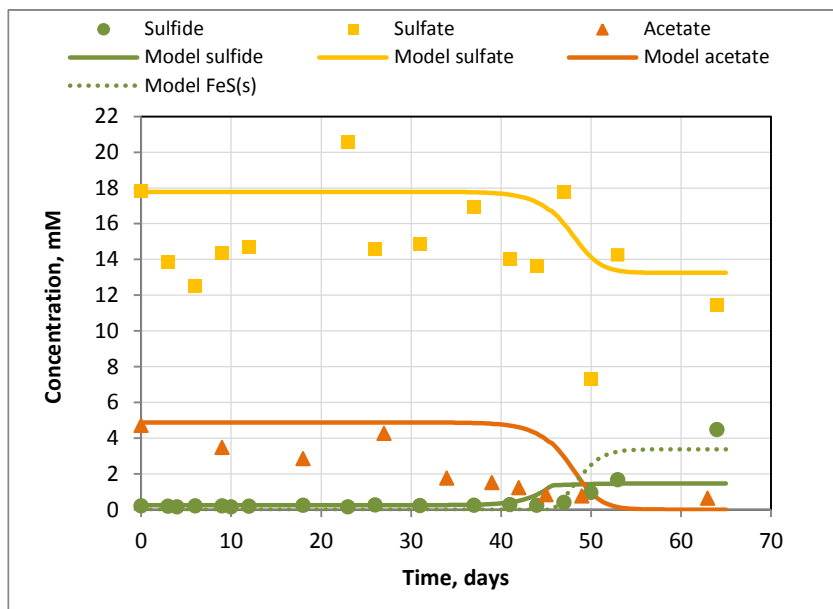
In order to calculate the solubility of hematite during sulfate reduction (where  $\text{Fe}(\text{II})$  will be stabilised) the Eh of the microcosms was estimated from the sulfate / sulfide ( $\text{S}^{\text{VI}}/\text{S}^{\text{II}}$ ) redox couple (Section 4.2.2). The average sulfate and sulfide concentrations (13.9 mM and 1.85 mM

respectively) from day 39 onwards in Figure 3D of Grigoryan et al, (2018), representing the resulting  $S^{VI}/S^{II}$  redox couple after acetate had been consumed in this experiment. This concentration ratio equated to an Eh of -0.213 V, which was imposed on the model systems to control iron speciation. This approach is consistent with observations that sulfide can reduce Fe(III) within bentonite (Pedersen et al., 2017), although the models do not account for the postulated reoxidation of sulfide to elemental sulfur.

Consideration of FeS solubility leads to a maximum predicted solution sulfide concentration of around 1.5 mM, with the rest precipitating as a reduced FeS phase. For one experiment considering Canapril bentonite (Figure 16) this matches the measured data well, but for others, greater sulfide levels are observed, suggesting that not all of the iron present in the bentonites is available for reaction, which may reflect differences in the reactivity of the iron-bearing minerals present.

Some of the models that have been constructed are able to represent the microbial metabolic processes reasonably well. For example, Figure 16 considering acetate as an electron donor correctly predicts the time and extent of sulfate reduction and consumption of acetate; the good fit is shown by the model lines being well within the scatter of the experimental data. In the case of lactate (Figure 17) the model shows a two-stage reaction described above where lactate is first oxidised to acetate and the peak in acetate concentration measured in the microcosms is represented. The extent of sulfate reduction in Figure 17 with lactate is greater than that for acetate Figure 16 and the sulfide formed appears to be greater than can be buffered by the available iron present in the bentonite.

The models predict a lag phase for all of the systems; this is the period in which the quantity of biomass increases from a very low initial amount at an exponential rate. Once sufficient biomass is present, the sulfate reduction reaction rates become significant. It is not clear from some of the Canapril data whether such lag periods were present in these microcosm systems, due to the level of scatter. The MX-80 data, however, do indicate a lag period, with sulfide levels becoming significant after around 50 days (Figure 18). Grigoryan et al (2018) provides a quantification of microbial cells of the MX 80 bentonite as 42.4 colony-forming units per gram dry weight of the MX 80 bentonite. This value has been used as an initial biomass concentration ( $3.97E-11$  moles/kg water) in the model and results in a good representation of the lag phase observed in the case of MX-80 (Figure 18). While the initial population of Canapril bentonite has not been quantified the model supports the view of Grigoryan et al (2018) that the different reactivity of the bentonites studied is primarily due to differences in initial biomass populations and different communities of microorganisms present in each bentonite. The modelling study supports this view, and also indicates the importance of the availability of iron in the bentonites in controlling the resulting solution sulfide concentration. The maximum predicted solution sulfide concentration of around 1.5 mM in these experiments equates to approximately 51 ppm  $HS^-$ . However, this value cannot be applied to the DGR environment directly, due to the much greater solution:bentonite ratio employed in these experiments compared to saturated highly compacted bentonite.



**Figure 18: Modelled sulfate reduction coupled with oxidation of acetate in microcosms at 15°C including MX-80 bentonite, symbols indicate experimental data from Grigoryan et al, (2018)**

## 5. DISCUSSION OF MODELLING OF MICROBIAL PROCESSES IN THE DGR CONCEPT

In this section, modelling microbial processes in the Canadian DGR concept is discussed, considering previous NWMO work undertaken in supporting APM (Section 2), other international approaches (Section 2) and consideration of the Monod kinetic approach to microbial processes (Section 4). This section firstly considers the features and processes that are of potential relevance, before considering the most appropriate representation of microbial and geochemical constraints on sulfide generation in NWMO's current PA model (Briggs et al, 2017).

### 5.1 FEATURES AND PROCESSES OF RELEVANCE TO SULFIDE GENERATION IN THE DGR

In the DGR environment, aqueous sulfide will be contributed by groundwater present in the host rock, where it will likely be present at low concentrations. For the Forsmark site in Sweden, sulfide appears to be influenced by microbial and geochemical processes and in most locations has a concentration of around 0.4 ppm, saturated with respect to  $\text{FeS}_{(\text{am})}$  (Tullborg et al 2010). However, a small number of samples have higher sulfide concentration (4 ppm; Tullborg et al 2010). Modelling and assessment studies of the Canadian DGR have indicated that assumed and pessimistic host rock boundary conditions (3 ppm, King, 1996) result in Cu corrosion of less than 1 mm over 1 million years (Kwong, 2011; Scully and Edwards, 2013; Briggs et al, 2017).

The generation of high concentrations of sulfide (> 3 ppm and above  $\text{FeS}_{(\text{am})}$  solubility) could be invoked by the development of an active microbial sulfate reduction process stimulated by the construction of a DGR, including bentonite and other engineered materials that will introduce organic carbon (recognised by Wolfaardt and Korber, 2012; Marshall and Simpson, 2014a), providing an electron donor and carbon source for the growth of SRB.

Any increase in sulfide concentration is however likely to be transitory, since there are likely to be sources of iron present in the DGR bentonite to precipitate the sulfide generated by SRB. The mineralogy of such secondary iron sulfide is also likely to evolve, from initially formed amorphous  $\text{FeS}$  to more crystalline mackinawite ( $\text{FeS}$ ) with an associated decrease in solubility by several orders of magnitude as observed in long-term (20 year) experiments of sulfate reduction systems (Small et al, 2017). A further transformation of mackinawite ( $\text{FeS}$ ) to greigite ( $\text{Fe}_3\text{S}_4$ ) and pyrite ( $\text{FeS}_2$ ), involving Fe oxidation and sulfidation (Lennie et al, 1997) is also possible and would be associated with a further reduction in the sulfide solubility. Consideration of the kinetics of sulfide precipitation and other geochemical (sorption) processes that may limit the aqueous concentration of sulfide are therefore viewed as of equal importance to the microbially mediated sulfate reduction process itself.

Organic carbon, mainly present in bentonite, is viewed as the most significant electron donor present in the DGR that could drive sulfate reduction (Marshall and Simpson, 2014a,b). Detailed characterisation of the natural organic matter (NOM) present in candidate bentonites (Marshall and Simpson, 2014b) indicates that it may be largely recalcitrant, with no labile species (sugars, proteins, and small organic acids) detected.

Pedersen (2017) analysed (qualitatively) a wider range of bentonites and detected a number of compounds (alcohols, esters, ketones, aldehydes, fatty acids, alkanes) that could serve as an electron donor for microbial activity. Furthermore, Pedersen (2017) observed that in pressure cell experiments, using lactate as an added electron donor for SR, that the levels of acetate



generated were higher than predicted for partial lactate oxidation. This suggests that organics present in the bentonite were being fermented. Depending on the nature of NOM present in bentonite, hydrolysis and fermentation may be required before it could be fully utilised by SRB, such as in the CCM-MIC model of King and Kolar (2006). Natural organic matter from the host rock will also have the potential to act as an electron donor for SR in the DGR. Such NOM is however likely to be recalcitrant to SR since it would be expected that the labile component would have been consumed by extant SRBs. Perturbation of the host rock biogeochemistry by DGR construction and operation could induce a greater reaction of host rock NOM by the introduction of oxygen and other high energy yielding TEAPs that might be able to utilise and reprocess the NOM. Subsequently, the biomass formed could be recycled and utilised by SRB. However, the extent of such activation is likely to be limited.

A recent *in situ* URL experiment in Opalinus Clay (Bagnoud et al, 2016a) has highlighted that SR can be stimulated by the injection of H<sub>2</sub>, stimulating an initial autotrophic SR process, apparently without the requirement of organic carbon. Biomass created by the initial autotrophic SR process is further processed by fermentation, yielding organic carbon for heterotrophic SRBs, to provide a closed carbon cycle.

The role of H<sub>2</sub> in fueling deep subsurface microbiology is well established (e.g. Lovley and Chapelle, 1995; Pedersen, 1997), including the occurrence of autotrophic processes. Significant sources of H<sub>2</sub> may be developed in the deep Precambrian basement from serpentinisation and radiolysis (Sherwood Lollar et al, 2014). In addition, deep sources of biogenic and abiogenic methane (CH<sub>4</sub>) (Sherwood Lollar, 2006) have been shown to act as an electron donor for SR in the deep subsurface (Pedersen et al, 2008). While natural SR processes driven by such deep H<sub>2</sub> and CH<sub>4</sub> may be occurring in prospective DGR sites at a low rate, they are likely to have achieved a steady state with respect to sulfide precipitation processes resulting in the observed low sulfide concentrations. Nevertheless, it is conceivable that the perturbation of deep geological host rocks during DGR construction could disrupt such a steady state of H<sub>2</sub> / CH<sub>4</sub> driven SR and sulfide precipitation, which might affect the levels of aqueous sulfide. DGR construction and operation are likely to introduce higher energy yielding electron acceptors (e.g. O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, Fe(III)) into the system, which will tend to consume deep H<sub>2</sub> / CH<sub>4</sub> sources and outcompete SR. However, once these added electron acceptors are consumed, the SR process should be reestablished, perhaps at an increased rate stimulated by the microbial activity and organic carbon fixed as a consequence of the DGR construction.

The extent of any deep H<sub>2</sub> / CH<sub>4</sub> source to drive SR processes in a DGR will of course be highly site dependent and detailed characterisation of the indigenous microbial processes at such sites is being undertaken within the NWMO programme. Such site characterisation should establish the potential for deep H<sub>2</sub> / CH<sub>4</sub> to drive SR processes. It is also possible that H<sub>2</sub> may be sourced from the DGR itself, such as by radiolytic processes and by anaerobic steel corrosion. The extent of H<sub>2</sub> generation is however expected to be significantly lower than for an ILW repository (e.g. Leupin et al, 2016, Section 3.1.2) as radiolysis of water will be limited by the shielding effect of the UFC and minimal amounts of steel are likely to present in the DGR construction.

The process of sulfate reduction to sulfide yields a relative low amount of chemical energy for microbial metabolism compared to aerobic respiration, denitrification and Fe(III) reduction (Stumm and Morgan, 1995; Lovley and Chapelle, 1995). Fermentation of organic species is also likely to occur prior to or contemporaneous with heterotrophic SR due to the energy yield and also due to the interdependencies of these microbes (Bagnoud et al, 2016a). The model of King and Kolar (2006) simulates that oxygen would be consumed in around 200 years by chemical processes. Subsequently, nitrate present in bentonite (Marshall and Simpson, 2014b) is likely to

be consumed as an electron acceptor. Fe(III) that may be present in bentonite as a component of the octahedral sheet of smectite clay minerals that form the bulk of the bentonite, or as impurities in the form of discrete oxide/oxyhydroxide phases such as goethite or hematite. Significant amounts of Fe may be present in bentonite. For example, Pedersen (2017) reports between 3 and 13 wt% Fe<sub>2</sub>O<sub>3</sub>. However, some iron may be in reduced form (e.g. pyrite, FeS<sub>2</sub>) while Fe(III) present in crystalline phases such as hematite will have limited bioavailability (Lovley and Chapelle, 1995; Bonneville et al, 2009).

Reduction of Fe(III) present in the smectite crystal structure has been invoked to affect the swelling properties of bentonite (Kim et al, 2004; Perdrial et al, 2009), although recent studies within the EU MIND project (Matschiavelli, et al, 2018) do not indicate any change in crystal structure based on X-ray diffraction and electron microscopy studies. Bentonite bioreduction experiments tend to indicate a dominance of SR despite the presence of Fe(III) in bentonite and Pedersen et al (2017) has proposed a reaction where sulfide generated reacts with Fe(III) in bentonite, immobilising the sulfide as FeS. Haynes et al (2018) also observed that the growth of iron reducing bacteria (IRB) relative to SRB is correlated with the amount of Fe extractable in 0.5 M HCl from different bentonites. Several independent studies of anaerobic microbial growth with bentonite within the EU MIND project indicate the coexistence of SRB and IRB processes stimulated by organic electron donor (lactate) and H<sub>2</sub> (Pedersen, 2017; Machiavelli et al, 2018; Vikman et al, 2018; Haynes et al, 2018). Competition between IRB and SRB in bentonites therefore provides a further potential limiting process on sulfide generation. Characterisation of the mineralogical nature of iron in bentonites will be important to assessing this limitation and also to the subsequent generation and precipitation of sulfide.

Bentonite backfill provides an important safety function in the DGR to prevent microbial activity and in particular MIC of the UFC. Consequently, factors such as swelling pressure (related to dry density), temperature and salinity have been examined in detail e.g. Stroes-Gascoyne et al (2010, 2014); Pedersen, (2017). Factors such as swelling pressure and temperature are most appropriately represented in models as a threshold maximum condition under which microbial activity is viable (e.g. King and Kolar, 2006). In the case of temperature, which will decline due to radioactive decay, microbial activity may initially be prevented close to the UFC, but then develop on cooling. Temperature and swelling pressure will evolve during the re-saturation phase. During re-saturation of bentonite, water will be absorbed into the interlayer region of the smectite clay and may not be available for microbial activity and hence may limit microbial activity during this phase. Generally, it is expected that the scenario will be considered in PA where the bentonite develops sufficient swelling pressure to prevent microbial activity in the buffer, but that microbial activity may develop at the interface with the host rock.

Diffusive transport processes control the migration of aqueous sulfide through the bentonite and also will affect and possibly limit the supply of nutrients for microbial growth and metabolism. Physical properties of the bentonite, such as porosity and tortuosity in addition to external factors such as temperature, will affect the diffusion (Briggs et al, 2017). In addition, chemical related effects such as anion exclusion will affect the migration of ions of differing charge and radius (e.g. Appelo and Wersin, 2007). The smectite clay will also provide a sorption substrate. Bentonite is a potential source of organic carbon for microbial growth and a potential electron donor for SR (Marshall and Simpson, 2014a,b), but since microbial activity is not thought to be viable in fully saturated bentonite, then the diffusion of soluble organic carbon to the host rock interface could be an important rate limiting process on SRB growth. The potential for enzymatic hydrolysis and fermentation of organic material within bentonite will be limited where microbial activity is prevented by the physical properties of the DGR.

In summary, a combination of microbiological, geochemical and transport processes will affect the generation of aqueous sulfide in and around the DGR and the extent of MIC of the Cu coated UFC. The relative rates of these main processes will determine the flux of sulfide reaching the UFC. Sulfide generation in the DGR could be driven by organic matter present in the DGR and by deep sources of  $H_2$  and  $CH_4$ , the extent of the latter being dependent on the host rock geology. Wherever a site is developed, DGR construction is likely to perturb the steady state deep geomicrobiology and geochemistry by introduction of oxygen and other high energy yielding electron acceptors that could stimulate microbial activity and synthesise carbon for SR processes. Construction will also introduce some organic carbon directly. Iron reduction processes (e.g. of Fe present in bentonite) may also be stimulated that will compete with SR. Several physical constraints, such as bentonite swelling pressure, have the potential to limit microbial activity in bentonite and such physical FEPs may be coupled to the microbiological and geochemical processes occurring in the DGR.

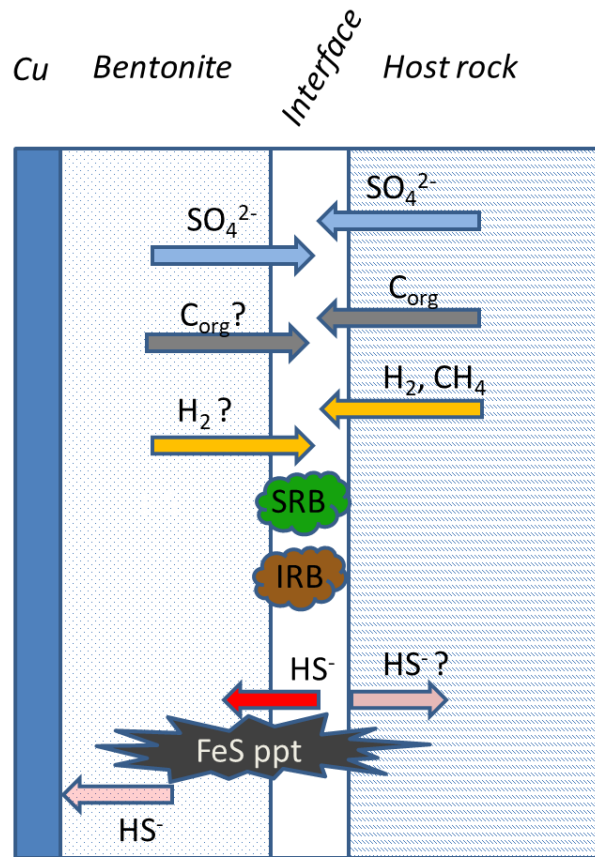
## 5.2 INTEGRATION OF MICROBIAL PROCESSES IN DGR TRANSPORT MODELS

Previous assessments of the potential for sulfide generation in the Canadian DGR concept have been undertaken (Stroes-Gascoyne, 1989; Krol and Sleep, 2013a; 2014) based on the mass balance approach first proposed by McKinley et al, (1985). The assessments of Krol and Sleep (2013a; 2014) consider worst case scenarios where all sulfate present in bentonite is able to be reduced to sulfide and is able to mediate corrosion of the Cu surface of the UFC. This worst case calculation results in corrosion of around 2 mm after 1 Ma, for crystalline and sedimentary host rocks and is around double that of the current reference case MIC allowance of 1 mm. Krol and Sleep concluded from their calculations that nitrogen and phosphorous were limiting nutrients and that the inventory of elements in the DGR should be further quantified. Krol and Sleep (2013a) further recognised the need to consider the kinetics of the sulfide generation and transport processes and have considered modelling approaches and tools (Krol and Sleep, 2013b).

Subsequently, a model has been developed with COMSOL to represent the diffusion processes (Briggs et al, 2017). The main objective of this current review has been to consider how this COMSOL model can be developed to represent sulfide generation processes. The review has indicated that the sulfide generation process is not simply a consequence of microbial SR, but that wider consideration of geochemical processes is required, highlighted by the process of sulfide reaction with iron minerals and precipitation as FeS. In addition, SR may be driven by a variety of electron donors including organic matter present in bentonite together with  $H_2$ ,  $CH_4$  and NOM sourced from the host rock geological formation. A substantial body of work has been developed by the Canadian and other international programmes to investigate the function of the bentonite backfill to suppress microbial activity in the vicinity of the DGR (e.g. Stroes-Gascoyne et al, 2014; see Section 2).

Thus, if microbial activity is able to develop in the DGR it is more likely to develop at the bentonite backfill / host rock interface as illustrated in Figure 19. Such an interface may comprise the excavation damaged zone (EDZ), which may have higher porosity than the undamaged host rock and backfill regions of the engineered barrier system (Wolfaardt and Korber, 2012). The interface zone might also include a wider range of materials that provide nutrient sources for microbial activity including oxygen introduced during excavation and cementitious shotcrete and steel reinforcements that may be required to stabilise some excavated host rocks. Initially, prior to full water saturation, microbial activity may be possible within the bentonite, which could consume residual oxygen by oxidation of bioavailable organic

matter. Thus if the interface zone were considered a zone of microbial activity it could vary with time and could initially include the bentonite buffer. During saturation, anaerobic fermentation, IR and SR processes may develop in the bentonite, similar to those observed in microcosm and low pressure experiments (Pedersen, 2017; Grigoryan et al, 2018; Haynes et al, 2018; Machievelli et al; 2018) Following saturation and development of target swelling pressure, microbial activity in the bentonite will be suppressed and so the liberation of soluble organics by enzymatic hydrolysis and fermentation for SR processes occurring in the interface is anticipated to be severely limited. NOM present in groundwater is therefore likely to dominate as an organic electron donor after saturation.



**Figure 19: Schematic (not to scale) of the diffusive transport processes (arrows) and microbiological and geochemical species and processes that require consideration in a model of sulfide generation in a bentonite backfilled DGR, where microbial activity may be restricted to an interface zone**

Sulfate is likely to be readily sourced from groundwater and also by soluble sulfate present in the bentonite. The concentration gradients of sulfate across the disposal cell will determine the direction of diffusive transport. The case illustrated in Figure 19 corresponds to a case of relatively low sulfate concentration and high rate of SR in the interface zone. In cases of high sulfate concentration in groundwater there may be relatively little SR and diffusion of sulfate after the re-saturation phase. Limited amounts of  $H_2$  are expected to be generated by anaerobic corrosion of steel and by radiolysis of water. Depending on the geological setting of the DGR,  $H_2$

and CH<sub>4</sub> could be sourced from the host rock formation and may be a limiting control on the SR process.

The interface zone illustrated in Figure 19 represents a niche in the DGR where reactive chemical species from the host rock and engineered barriers mix and where the physical conditions (space, pressure, water availability and temperature) are supportive of microbial growth. The development of SR processes in the interface zone will be dependent on the supply of an electron donor such as NOM, H<sub>2</sub>, or CH<sub>4</sub>. The supply of sulfate and nutrients for biomass growth (C, N, P, S etc.) are viewed as being of lesser importance given the conclusions of previous assessments (Stroes Gascoyne, 1989; Krol and Sleep 2013a; 2014). In addition, it is likely that microbial biomass is recycled and organic carbon may be synthesised by autotrophic processes that are stimulated by deep H<sub>2</sub> sources (Bagnoud et al, 2016a). Given that rates of sulfate reduction in laboratory microcosm experiments (e.g. Hallbeck, 2014; Grigoryan et al 2018), in bentonite pressurised experiments, below the saturation pressure threshold for microbial activity, (Bengtsson and Pedersen (2017) and in in situ URL experiments (Bagnoud et al, 2016a,b) occur over short timescales (e.g. days, weeks) then it would seem unlikely that the rates of microbial SR process would limit sulfide generation in the DGR. Rather, the supply of electron donors (NOM, H<sub>2</sub>, or CH<sub>4</sub>; Figure 19) are more likely to be rate limiting.

The rate of supply of H<sub>2</sub> and CH<sub>4</sub> from deep geological sources will be dependent on the geological setting and should be constrained by interpretation of detailed microbiological and geochemical characterisation of prospective DGR sites to quantify such deep biosphere processes (e.g. Sherwood Lollar et al, 2014). Consideration of the supply of metabolisable NOM is perhaps more problematic, since complex NOM material will require processing by hydrolysis and fermentation before it can be utilised by SRB. Significant research has examined the nature of NOM in bentonite and groundwater in the context of a Canadian DGR (Marshall and Simpson, 2014a,b). Development of this research to constrain the mechanism and conditions by which NOM can fuel SR processes under *in situ* saturated DGR conditions is apparent.

While a description of the Monod kinetic approach to represent the microbial biomass growth and metabolic processes has been provided in this review (Section 4.1) such a detailed representation is not considered appropriate for development of a PA model to assess processes of sulfide generation and MIC based on the current COMSOL model (Briggs et al, 2017). Such detailed Monod kinetic models have significant input data requirements (see Humphreys et al, 2010; King and Kolar, 2006) and are more appropriate to model shorter duration experimental data.

To date, Monod type models have been used to simulate more reactive systems of organic waste degradation and microbial gas generation (e.g. Humphreys et al, 1997; Small et al, 2008; 2017; Suckling et al, 2015). Such gas generation models describe the sequence of anaerobic processes of organic-containing LLW/ILW and have little or no coupling with transport and are not dependent on the supply of energy or nutrients. The Monod kinetic approach is however viewed as being useful to assist in the interpretation of laboratory and URL type experiments where the model is able to distinguish an initial lag phase, corresponding to microbial biomass synthesis, from the main steady state rate of metabolism, which is more appropriate to use within a PA model. As described in Sections 4.2 and 4.3, representation of Monod kinetic models in geochemical codes such as PHREEQC enables chemical speciation and mineral precipitation processes to be examined together with microbial kinetic processes.

The subsequent reaction of sulfide generated by SR processes with iron bearing minerals present either in bentonite or the host rock is important to represent in the PA model (Figure

19). Precipitation of FeS could be represented in COMSOL by a simple solubility model, parameterised on the basis of more detailed geochemical speciation and mineral reaction modelling with a code such as PHREEQC. This code could determine sulfide solubility by considering reaction with a range of iron oxides / hydroxides based on appropriate Eh conditions (see Section 4.3 and Abrahamsen-Mills, 2019). A PHREEQC 1D diffusive transport model of the conceptual model represented in Figure 19 could also be developed to examine in more detail the geochemical interactions with microbial kinetics and diffusion-controlled supply of electron donors. It is also possible to directly couple PHREEQC with COMSOL with iPHREEQC library (Charlton and Parkhurst, 2011; Nardi et al, 2014) to represent the detailed biogeochemistry of the DGR system, including the 3D geometry effects discussed by Briggs et al (2017). However, such a coupled COMSOL-PHREEQC model including representation of microbial kinetic processes is likely to have a high computational requirement. A more practicable approach may be to use 1D PHREEQC models in scoping calculations to investigate the significance of: limiting electron donor supply, microbial kinetics, sulfide speciation, solubility and precipitation kinetics. Such a PHREEQC model may then be used to parameterise a range of sulfide solubility and SR rate in a COMSOL model that can examine the effect of electron donor supply in a range of geological scenarios.

Development of a complex kinetic reactive transport model with PHREEQC and COMSOL should ideally be accompanied by an experimental system that can be used to parameterise and validate the model approach. In the case of gas generation, large-scale and long-term experiments with detailed geochemical and microbiological characterisation have proved invaluable in validating models used in PA studies (Small et al, 2008, 2017; Vikman et al, 2019; Suckling et al, 2015; Small and Dutton, 2009). To validate a model of sulfide generation in a DGR, pressure cell experiments, such as those described by Bengtsson and Pedersen (2016) would provide a useful means to demonstrate understanding of the biogeochemical processes in the DGR. Ideally, these would include a microbially active interface zone and potentially specific host rock materials. Such experiments would provide microbiological, geochemical and mineralogical data to validate a biogeochemical model of sulfide generation and MIC.

## 6. CONCLUSIONS, RECOMMENDATIONS AND REMAINING ISSUES

Through collaborative programmes of research with academia and the wider research community, NWMO has developed a strong body of evidence to support its Adaptive Phased Management approach to used nuclear fuel. This includes developing an improved understanding of the risk of MIC of the copper-coated UFC. Whilst stress corrosion cracking and localised pitting corrosion are not expected due to the container Mark II design, general (uniform) corrosion is expected to occur, and MIC may contribute to this via the reduction of sulfate to sulfide, which may migrate to the container surface.

Whilst microbial communities will be indigenous within the host rock formation and present in the engineered barrier system materials, studies have demonstrated that the disposal concept will limit microbial activity in the immediate vicinity of the container for prolonged periods via a number of mechanisms. Primarily, the use of highly compacted bentonite is expected to limit water activity, preventing growth. Also, the radiation and heat emitted by the container will likely provide a (temporary) sterilising effect.

Microbial activity is expected to take place within interface zones, between regions of highly compacted bentonite and more porous regions of backfill and the excavation damaged zone of the host rock. Here, the most important constraints on growth and activity are expected to be the availability of nutrients: electron donors, acceptors and sources of carbon. Sulfide generated in these regions may then migrate through the engineered barrier system towards the container via diffusion processes. NWMO has developed computer models to simulate a number of these processes. The latest model, developed in COMSOL Multiphysics, assumes a steady state sulfide flux at the far field, which diffuses (as  $\text{HS}^-$ ) to the container and induces general corrosion. The model continues to be developed to include processes of repository evolution, including re-saturation and temperature effects.

Following this review of NWMO's research and modelling programme, and considering international approaches to model and quantify the effect of microbial processes in nuclear waste disposal, the following recommendations are made regarding the further development of the model to include microbial sulfide generation processes:

- The overall PA model should include representation of geochemical processes of sulfide precipitation alongside the microbial sulfide generation process.
- The microbial sulfide generation process represented in the model should include consideration of all potential electron donors present in the DGR and host rock, including NOM,  $\text{H}_2$  and  $\text{CH}_4$ .
- The model should consider the rate of supply/release of these electron donors from the DGR and host rock, since these factors may control the rate of sulfide generation, rather than the kinetics of microbial growth and metabolism.
- A suite of modelling approaches should be used to assess the significance of sulfide generation in a DGR.
  - The existing 3D COMSOL model (Briggs et al, 2017) should be developed to assess the effect of; electron donor supply and transport; a simple representation of the rate of SR, and the effect of sulfide precipitation.
  - 1D reactive transport models may be developed in PHREEQC to investigate in more detail; the diffusive controlled supply of electron donors; microbial kinetics of the SR process, competing iron reduction (IR) processes, and sulfide reaction and precipitation kinetics. The PHREEQC biogeochemical model should provide parameter input to the COMSOL model.

- NWMO's experimental research programme should include simulation of the conceptual model developed in this review (Figure 19) to represent *in situ* conditions, such as using a pressure cell. The experiment should enable net rates of MIC and sulfide generation to be measured, together with microbiological and geochemical data to validate the models.

The review has identified a number of uncertainties that remain to be resolved before the model of the system can be fully parameterised and applied to a specific DGR site as follows:

- The nature of iron-bearing phases in bentonite and in candidate host rocks and their reactivity with sulfide. This has bearing on (i) the solubility of iron sulfide formed (ii) competition of IR processes with SR and (iii) an effect of IR on bentonite swelling properties.
- The bioavailability of NOM present in bentonite, including under fully saturated conditions, where microbial activity may occur external to the bentonite, but limited by hydrolysis and diffusion of organics from the bentonite.
- The significance of H<sub>2</sub> and CH<sub>4</sub> electron donors for SR sourced from the host rock, which should be evaluated during the microbiological and geochemical characterisation of candidate sites.



**REFERENCES**

- Appelo, C.A.J. and P. Wersin. 2007. Multicomponent Diffusion Modeling in Clay Systems with Application to the Diffusion of Tritium, Iodide, and Sodium in Opalinus Clay. *Environmental Science and Technology*, 41, pp 5002-5007. (Available at [doi.org/10.1021/es0629256](https://doi.org/10.1021/es0629256))
- Bagnoud, A., K. Chourey, R.L Hettich, I. de Bruijn, A.F. Andersson, O.X. Leupin, B. Schwyn and R. Bernier-Latmani. 2016a. Reconstructing a hydrogen-driven microbial metabolic network in Opalinus Clay rock. *Nature Communications*, 7:12770. (Available at [doi.org/10.1038/ncomms12770](https://doi.org/10.1038/ncomms12770))
- Bagnoud, A., O. Leupin, B. Schwyn and R. Bernier-Latmani. 2016b. Rates of microbial hydrogen oxidation and sulfate reduction in Opalinus Clay rock. *Applied Geochemistry*, 72, p 42-50. (Available at [doi.org/10.1016/j.apgeochem.2016.06.011](https://doi.org/10.1016/j.apgeochem.2016.06.011))
- Banwart, S. 1999. Reduction of iron(III) minerals by natural organic matter in groundwater. *Geochimica et Cosmochimica Acta*. 63, pp 2919–2928. (Available at [doi.org/10.1016/S0016-7037\(99\)00267-7](https://doi.org/10.1016/S0016-7037(99)00267-7))
- Behrends, T., E. Krawczyk-Bärsch and T. Arnold. 2012. Implementation of microbial processes in the performance assessment of spent nuclear fuel repositories. *Applied Geochemistry* 27, pp 453–462. (Available at [doi.org/10.1016/j.apgeochem.2011.09.014](https://doi.org/10.1016/j.apgeochem.2011.09.014))
- Bengtsson, A. and K. Pedersen. 2016. Microbial sulfate-reducing activity over load pressure and density in water saturated Boom Clay. *Applied Clay Science*, 132–133, pp 542–551. (Available at [doi.org/10.1016/j.clay.2016.08.002](https://doi.org/10.1016/j.clay.2016.08.002))
- Bengtsson, A. and K. Pedersen. 2017. Microbial sulfide-producing activity in water saturated Wyoming MX-80, Asha and Calcigel bentonites at wet densities from 1500 to 2000 kg m<sup>-3</sup>. *Applied Clay Science*, 137, pp 203–212. (Available at [doi.org/10.1016/j.clay.2016.12.024](https://doi.org/10.1016/j.clay.2016.12.024))
- Blanc, P., A. Lassin, P. Piantone, M. Azaroual, N. Jacquemet, A. Fabbri and E.C. Gaucher. 2012. Thermoddem: A geochemical database focused on low temperature water/rock interactions and waste materials: *Applied Geochemistry*, 27, pp 2107-2116. (Available at [doi.org/10.1016/j.apgeochem.2012.06.002](https://doi.org/10.1016/j.apgeochem.2012.06.002))
- Bleyen, N., S. Smets, J. Small, H. Moors, N. Leys, A. Albrecht, P. De Canniere, B. Schwyn, C. Wittebroodt and 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) pp 355-374. (Available at [doi.org/10.1007/s00015-016-0256-x](https://doi.org/10.1007/s00015-016-0256-x))
- Bonneville, S., T. Behrends and P. Van Cappellen. 2009. Solubility and dissimilatory reduction kinetics of iron(III) oxyhydroxides: A linear free energy relationship. *Geochimica et Cosmochimica Acta*, 73, pp 5273–5282. (Available at [doi.org/10.1016/j.gca.2009.06.006](https://doi.org/10.1016/j.gca.2009.06.006))
- Briggs, S., M. Krol and B. Sleep. 2015a. Preliminary modelling of diffusive transport of sulfide in a deep geological repository for used nuclear fuel. University of Toronto and York

- University, Report No. VR1-DS 253\_R00. NWMO APM-REP-04520-0001. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Briggs, S., B. Sleep and M. Krol. 2015b. Sensitivity analysis of diffusive transport of sulfide in a deep geological repository for used nuclear fuel. York University and University of Toronto, Report No. VR1-DS 453\_R00. NWMO APM-REP-04520-0002. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Briggs, S., J. McKelvie, B. Sleep and M. Krol. 2017. Multi-dimensional transport modelling of corrosive agents through a bentonite buffer in a Canadian deep geological repository. *Science of the Total Environment*, 599–600, pp 348–354. (Available at [dx.doi.org/10.1016/j.scitotenv.2017.04.242](https://doi.org/10.1016/j.scitotenv.2017.04.242))
- Briggs, S. and M. Krol. 2018. Diffusive transport modelling of corrosion agents through the engineered barrier system in a deep geological repository for used nuclear fuel. NWMO-TR-2018-06. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Charlton, S.R., and D.L. Parkhurst. 2011. Modules based on the geochemical model PHREEQC for use in scripting and programming languages: Computers and Geosciences, 37(10), pp 1653-1663. (Available at [doi.org/10.1016/j.cageo.2011.02.005](https://doi.org/10.1016/j.cageo.2011.02.005))
- Chen, J., Z. Qin, D.W. Shoesmith. 2011. Long-term corrosion of copper in a dilute anaerobic sulfide solution. *Electrochimica Acta*, 56, 7854–7861. (Available at [doi:10.1016/j.electacta.2011.04.086](https://doi.org/10.1016/j.electacta.2011.04.086))
- De Caniere, P. and A. Meleshyn. 2013. Microbial processes in a clay repository. EUROS SAFE Conference, Cologne, Germany 4 – 5 November 2013. (Available at [eurosafeforum.org/sites/default/files/Eurosafeforum2013/Seminar%202/2.06\\_Microbial\\_Processes\\_Clay\\_Repository\\_FANC\\_GRS\\_Paper.pdf](http://eurosafeforum.org/sites/default/files/Eurosafeforum2013/Seminar%202/2.06_Microbial_Processes_Clay_Repository_FANC_GRS_Paper.pdf))
- Diomidis, N., L.H. Johnson, P. Bastid and C. Allen. 2017. Design development of a copper-coated canister for the disposal of spent fuel in a deep geological repository in Opalinus Clay, *Corrosion Engineering, Science and Technology*, 52:sup1, pp 31-39. (Available at [doi.org/10.1080/1478422X.2017.1292200](https://doi.org/10.1080/1478422X.2017.1292200))
- Doherty, J. 2010. PEST, Model-independent parameter estimation. User manual. 5th ed. Brisbane: Watermark Numerical Computing. (Available at [nrc.gov/docs/ML0923/ML092360221.pdf](http://nrc.gov/docs/ML0923/ML092360221.pdf))
- Doherty, J. 2015. Calibration and uncertainty analysis for complex environmental models. Brisbane: Watermark Numerical Computing. (Available at [doi.org/10.1111/gwat.12360](https://doi.org/10.1111/gwat.12360))
- Duro, L., C. Domènech, M. Grivé, G. Roman-Ross, J. Bruno and K. Källström. 2014. Assessment of the evolution of the redox conditions in a low and intermediate level nuclear waste repository (SFR1, Sweden) *Applied Geochemistry*, 49, pp 192–205. (Available at [doi.org/10.1016/j.apgeochem.2014.04.015](https://doi.org/10.1016/j.apgeochem.2014.04.015))
- Garisto, F. 2017. Sixth Case Study: Features, Events and Processes. TR-2017-08. Nuclear Waste Management Organization. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Giffaut, E., M. Grivé, P. Blanc, P. Vieillard, E. Colàs, H. Gailhanou, S. Gaboreau, N. Marty, B. Madé and L. Duro. 2014. Andra thermodynamic database for performance

- assessment: ThermoChimie. Applied Geochemistry, 49, pp 225-236. (Available at [doi.org/10.1016/j.apgeochem.2014.05.007](https://doi.org/10.1016/j.apgeochem.2014.05.007))
- Graham, J., R. Plant, J. Small and D. Smalley. 2003. Program User's Guide for the code GRM, Version 4.1. BNFL Report00/EN0127/7/1. Drigg 2002 Post Closure Safety Case Report DTP/150. (Available at [webarchive.nationalarchives.gov.uk/20170412103307/http://llwrsite.com/wp-content/uploads/2016/03/2.-RP-103169-PROJ-00140-B-DTP-150-Program-users-guide-code-GRM-version-4.1.pdf](http://webarchive.nationalarchives.gov.uk/20170412103307/http://llwrsite.com/wp-content/uploads/2016/03/2.-RP-103169-PROJ-00140-B-DTP-150-Program-users-guide-code-GRM-version-4.1.pdf))
- Grigoryan, A.A., D.R. Jalique, P. Medihala, S. Stroes-Gascoyne, G.M. Wolfaardt, J. McKelvie, and D. Korber. 2018. Bacterial diversity and production of sulfide in microcosms containing uncompacted bentonites. Heliyon, 4(e00722). (Available at [doi.org/10.1016/j.heliyon.2018.e00722](https://doi.org/10.1016/j.heliyon.2018.e00722))
- Hall, D.S and P.G. Keech. 2017. An overview of the Canadian corrosion program for the long-term management of nuclear waste, Corrosion Engineering, Science and Technology, 52:sup1, 2-5. (Available at [dx.doi.org/10.1080/1478422X.2016.1275419](https://dx.doi.org/10.1080/1478422X.2016.1275419))
- Hallbeck, L. 2014. Determination of sulfide production rates in laboratory cultures of the sulfate reducing bacterium *Desulfovibrio aespoensis* with lactate and H<sub>2</sub> as energy sources. SKB Technical Report TR-14-14. (Available at [skb.com/publication/2693771](http://skb.com/publication/2693771))
- Haynes, H., C.I. Pearce, C Boothman and J.R. Lloyd. 2018. Response of bentonite microbial communities to stresses relevant to geodisposal of radioactive waste. Chemical Geology, 501, pp 58–67. (Available at [doi.org/10.1016/j.chemgeo.2018.10.004](https://doi.org/10.1016/j.chemgeo.2018.10.004))
- Humphreys, P., R. McGarry, A. Hoffmann and P. Binks. 1997. DRINK: a biogeochemical source term model for low level radioactive waste disposal sites. FEMS Microbiology Reviews, 20, pp 557–571. (Available at [https://doi.org/10.1016/S0168-6445\(97\)00034-X](https://doi.org/10.1016/S0168-6445(97)00034-X))
- Humphreys, P., J.M. West and R. Metcalfe. 2010. Microbial Effects on Repository Performance. Technical Report. UK Nuclear Decommissioning Authority. Quintessa Report QRS-1378Q-1, Issue 3. (Available at [eprints.hud.ac.uk/id/eprint/7613/](http://eprints.hud.ac.uk/id/eprint/7613/))
- IAEA. 2004. Safety assessment methodologies for near surface disposal facilities, results of a co-ordinated research project. Vol 1. Review and enhancement of safety assessment approaches and tools. IAEA, Vienna. (Available at [iaea.org/publications/6971/safety-assessment-methodologies-for-near-surface-disposal-facilities](http://iaea.org/publications/6971/safety-assessment-methodologies-for-near-surface-disposal-facilities))
- Ibrahim, B., D. Zagidulin, M. Behazin, S. Ramamurthy, J.C. Wren, D.W. Shoosmith. 2018. The Corrosion of copper in irradiated and unirradiated humid air. Corrosion Science, 141, pp 53-62. (Available at <https://doi.org/10.1016/j.corsci.2018.05.024>).
- Jin, Q. and C.M. Bethke. 2002. Kinetics of electron transfer through the respiratory chain: Biophysical Journal, v. 83, pp 1797-1808. (Available at [doi.org/10.1016/S0006-3495\(02\)73945-3](https://doi.org/10.1016/S0006-3495(02)73945-3))
- Jin, Q. and C.M. Bethke. 2003. A new rate law describing microbial respiration: Applied and Environmental Microbiology, v. 69, pp 2340-2348. (Available at [dx.doi.org/10.1128/AEM.69.4.2340-2348.2003](https://dx.doi.org/10.1128/AEM.69.4.2340-2348.2003))

- Jin, Q. and C.M. Bethke. 2005. Predicting the rate of microbial respiration in geochemical environments: *Geochimica et Cosmochimica Acta*, v. 69, pp 1133-1143. (Available at [doi.org/10.1016/j.gca.2004.08.010](https://doi.org/10.1016/j.gca.2004.08.010))
- Jin, Q. and C.M. Bethke. 2007. The thermodynamics and kinetics of microbial metabolism. *American Journal of Science*, Vol. 307, pp 643–677. (Available at [doi.org/10.2475/04.2007.01](https://doi.org/10.2475/04.2007.01))
- Jolley, D.M., T.F. Ehrhorn and J. Horn. 2003. Microbial impacts to the near-field environment geochemistry: a model for estimating microbial communities in repository drifts at Yucca Mountain. *Journal of Contaminant Hydrology*, 62, pp 553-575. (Available at [doi.org/10.1016/S0169-7722\(02\)00187-0](https://doi.org/10.1016/S0169-7722(02)00187-0))
- Kidby, D.W. and A. Rosevear. 1997. The microbiological basis of the gas generation program GAMMON. NSS/R369, UK Nirex, Ltd.
- Kim, J., H. Dong, J. Seabaugh, S.W. Newell and D.D. Eberl. 2004. Role of Microbes in the Smectite-to-Illite Reaction. *Science*, 303, pp 830-832. (Available at [doi.org/10.1126/science.1093245](https://doi.org/10.1126/science.1093245))
- King, F. 1996. Microbially Influenced Corrosion of Copper Nuclear Fuel Waste Containers in a Canadian Disposal Vault. (AECL-11471). Atomic Energy of Canada Ltd., Pinawa.
- King, F. and M. Kolar. 2006. Consequences of Microbial Activity for Corrosion of Copper Used Fuel Containers – Analyses Using the CCM-MIC. (0.1 Code. 06819-REP-1300-10120-R00). ON Power Generation, Toronto.
- King, F., M. Kolar and P. Maak. 2008. Reactive-transport model for the prediction of the uniform corrosion behaviour of copper used fuel containers. *Journal of Nuclear Materials*, 379, pp 133-141. (Available at [doi.org/10.1016/j.jnucmat.2008.06.017](https://doi.org/10.1016/j.jnucmat.2008.06.017))
- Krol, M. and B. Sleep. 2013a. Limiting nutrients and bounding microbiologically influenced corrosion calculations for Mark I crystalline and sedimentary reference designs. NWMO APM-TM-04520-P. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Krol, M. and B. Sleep. 2013b. Modelling of biogeochemical and hydrogeological processes in a repository for used nuclear fuel – State of science review. NWMO APM-TM-04520-P. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Krol, M. and B. Sleep. 2014. Limiting nutrients and bounding microbiologically influenced corrosion calculations for Mark II crystalline and sedimentary reference designs. NWMO APM-TM-04520-P. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Kwong, G.M. 2011. Status of corrosion studies for copper used fuel containers under low salinity conditions. TR-2011-14. Nuclear Waste Management Organization. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Lennie, A.R., S.A.T. Redfern, P.E. Champness, C.P. Stoddart, P.F. Schoefield and D.J. Vaughan. 1997. Transformation of mackinawite to greigite: An in situ X-ray powder

- diffraction and transmission electron microscope study. *American Mineralogist*, 82, pp 302–309. (Available at [doi.org/10.2138/am-1997-3-408](https://doi.org/10.2138/am-1997-3-408))
- Leschine, S.B. 1995. Cellulose degradation in anaerobic environments. *Annual Reviews in Microbiology*, 49, pp 399-426. (Available at [doi.org/10.1146/annurev.mi.49.100195.002151](https://doi.org/10.1146/annurev.mi.49.100195.002151))
- Leupin, O.X., J. Zeyer, V. Cloet, P. Smith, R. Bernier-Latmani, P. Marschall, A. Papafotiou, B. Schwyn and S. Stroes-Gascoyne. 2016. An assessment of the possible fate of gas generated in a repository for low- and intermediate-level waste. NAGRA TR 16-05. (Available at [nagra.ch/display.cfm/id/102541/display\\_type/display/filename/e\\_ntb16-05.pdf](https://nagra.ch/display.cfm/id/102541/display_type/display/filename/e_ntb16-05.pdf))
- Leupin, O.X., R. Bernier-Latmani, A. Bagnoud, H. Moors, N. Leys, K. Wouters, S. Stroes-Gascoyne and J.M. Soler. 2017. Fifteen years of microbiological investigation in Opalinus Clay at the Mont Terri rock laboratory (Switzerland). *Swiss Journal of Geosciences*, 110, p 343–354. (Available at [doi.org/10.1007/s00015-016-0255-y](https://doi.org/10.1007/s00015-016-0255-y))
- LLWR. 2011. The 2011 Environmental Safety Case: Near-field, LLWR/ESC/R(11)10021. (Available at <https://webarchive.nationalarchives.gov.uk/20170412103307/http://llwrsite.com/wp-content/uploads/2016/03/L2-LLWR-ESC-R-11-10021-Near-Field-MASTER-April-2011.pdf>)
- Lockhart, R.J., M.I. Van Dyke, I.R. Beadle, P. Humphreys and A.J. McCarthy. 2006. Molecular biological detection of anaerobic gut fungi (Neocallimastigales) from landfill sites. *Applied Environmental Microbiology*, 72, pp 5659-5661. (Available at [doi.org/10.1128/AEM.01057-06](https://doi.org/10.1128/AEM.01057-06))
- Lovley, D.R. and F.H. Chapelle. 1995. Deep subsurface microbial processes. *Reviews of Geophysics*, 33, pp 365-381. (Available at [doi.org/10.1029/95RG01305](https://doi.org/10.1029/95RG01305))
- Luo, J., F.-A. Weber, O.A. Cirpka, W.-M. Wu, J.L. Nyman, J. Carley, P.M. Jardine, C.S. Criddle and P.K. Kitanidis. 2007. Modeling in-situ uranium(VI) bioreduction by sulfate-reducing bacteria. *Journal of Contaminant Hydrology*, 92, pp 129–148. (Available at [doi.org/10.1016/j.jconhyd.2007.01.004](https://doi.org/10.1016/j.jconhyd.2007.01.004))
- Maia, F., I. Puigdomenech and J. Molinero. 2016. Modelling rates of bacterial sulfide production using lactate and hydrogen as energy sources. SKB Technical Report TR-16-05. (Available at [skb.com/publication/2484957/](https://skb.com/publication/2484957/))
- Marshall, M.H.M. and M.J. Simpson. 2014a. State of science review: Natural organic matter in clays and groundwater. NWMO TR-2014-05. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Marshall, M.H.M. and M.J. Simpson. 2014b. Characterization of natural organic matter in bentonite clays. NWMO TR-2014-10. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Martino, T., J. Chen, Z. Qin, D.W. Shoesmith. 2017. The kinetics of film growth and their influence on the susceptibility to pitting of copper in aqueous sulfide solutions. *Corrosion Engineering, Science, and Technology*. 52, pp 61–64. (Available at <https://doi.org/10.1080/1478422X.2017.1297575>)

- Matschiavelli, N., A. Cherkouk, H. Miettinen and M. Vikman. 2018. Long-term stability of bentonite in the presence of microorganisms. MIND Project Deliverable D2.8, EC Grant Agreement 661880. (Available at [mind15.eu/deliverables/](http://mind15.eu/deliverables/))
- McCarty, P.L. and F.E. Mosey. 1991. Modelling of anaerobic digestion processes (A discussion of concepts). *Water Science Technology*, 24, pp 17-33. (Available at [doi.org/10.2166/wst.1991.0216](https://doi.org/10.2166/wst.1991.0216))
- McCarty, P.L. 1975. Stoichiometry of Biological Reactions, *Progress in Water Technology* 7, 157-172.
- McKinley, I.G., I. Hagenlocher, W.R. Alexander and B. Schwyn. 1997. Microbiology in nuclear waste disposal: interfaces and reaction fronts. *FEMS Microbiology Reviews*, 20, pp 545-556. (Available at [doi.org/10.1016/S0168-6445\(97\)00033-8](https://doi.org/10.1016/S0168-6445(97)00033-8))
- McKinley, I.G., J. West and H.A. Grogan. 1985. An analytical overview of the consequences of microbial activity in a Swiss HLW repository. NAGRA TR 85-43. Baden, Switzerland. (Available at [nagra.ch/data/documents/database/dokumente/\\$default/Default%20Folder/Publikatione n/NTBs%201985-1986/e\\_ntb85-43.pdf](http://nagra.ch/data/documents/database/dokumente/$default/Default%20Folder/Publikatione n/NTBs%201985-1986/e_ntb85-43.pdf))
- Meleshyn, A. 2011. Microbial processes relevant for long-term performance of radioactive waste repositories in clays. Gesellschaft für Anlagen-und Reaktorsicherheit (GRS) mbH, GRS – 291, ISBN 978-3-939355-67-0. (Available at [inis.iaea.org/search/search.aspx?orig\\_q=RN:43063101](http://inis.iaea.org/search/search.aspx?orig_q=RN:43063101))
- Monod, J. 1949. The growth of bacterial cultures. *Annual Reviews in Microbiology*, 3, pp 371-394. (Available at [doi.org/10.1146/annurev.mi.03.100149.002103](https://doi.org/10.1146/annurev.mi.03.100149.002103))
- Nardi, A., A. Idiart, P. Trincherro, L.M. de Vries and J. Molinero. 2014. Interface COMSOL-PHREEQC (iCP), an efficient numerical framework for the solution of coupled multiphysics and geochemistry. *Computers and Geosciences*, 69, pp 10-21. (Available at [doi.org/10.1016/j.cageo.2014.04.011](https://doi.org/10.1016/j.cageo.2014.04.011))
- NEA. 2000. Features, Events and Processes (FEPs) for Geological Disposal of Radioactive Waste – An International Database. OECD, Paris, France. (Available at [oecd-ilibrary.org/nuclear-energy/features-events-and-processes-feps-for-geologic-disposal-of-radioactive-waste\\_9789264187511-en](http://oecd-ilibrary.org/nuclear-energy/features-events-and-processes-feps-for-geologic-disposal-of-radioactive-waste_9789264187511-en))
- Neall, F. 1994. Modelling of the Near-Field Chemistry of the SMA Repository at the Wellenberg Site: Application of the Extended Cement Degradation Model. NAGRA NTB-94-03. (Available at [inis.iaea.org/collection/NCLCollectionStore/Public/26/007/26007756.pdf](http://inis.iaea.org/collection/NCLCollectionStore/Public/26/007/26007756.pdf))
- Neidherdt, F.C., J.L. Ingraham and M. Scharchter. 1990. *Physiology of the Bacterial Cell: A Molecular Approach*. Sinauer Associates, Sunderland, MA. ISBN 0 87893 608 4
- Newsome, L., K. Morris and J.R. Lloyd. 2014. The biogeochemistry and bioremediation of uranium and other priority radionuclides. *Chemical Geology*, 363, pp 164–184. (Available at [doi.org/10.1016/j.chemgeo.2013.10.034](https://doi.org/10.1016/j.chemgeo.2013.10.034))

- Nixon, S., N.M. Bassil and J.R. Lloyd. 2017. Effects of radiation and microbial degradation of ILW organic polymers. MIND Project Deliverable D1.2, EC Grant Agreement 661880. (Available at [mind15.eu/deliverables/](http://mind15.eu/deliverables/))
- Parkhurst, D.L., and C.A.J. Appelo. 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 Techniques and Methods, book 6, chap. A43, 497 p. (Available at <https://pubs.usgs.gov/tm/06/a43/>)
- Pedersen, K. 1997. Microbial life in deep granitic rock. FEMS Microbiology Reviews, 20, pp 399-414. (Available at [doi.org/10.1111/j.1574-6976.1997.tb00325.x](https://doi.org/10.1111/j.1574-6976.1997.tb00325.x))
- Pedersen, K. 2017. Bacterial activity in compacted bentonites. MIND Project Deliverable D2.4, EC Grant Agreement 661880. (Available at [mind15.eu/deliverables/](http://mind15.eu/deliverables/))
- Pedersen, K., J. Arlinger, S. Eriksson, A. Hallbeck, L. Hallbeck and J. Johansson. 2008. Numbers, biomass and cultivable diversity of microbial populations relate to depth and borehole-specific conditions in groundwater from depths of 4–450m in Olkiluoto, Finland. The ISME Journal, 2, pp 760–775. (Available at [doi.org/10.1038/ismej.2008.43](https://doi.org/10.1038/ismej.2008.43))
- Pedersen, K., A. Bengtsson, A. Blom, L. Johansson and T. Taborowski. 2017. Mobility and reactivity of sulfide in bentonite clays – Implications for engineered bentonite barriers in geological repositories for radioactive wastes, Applied Clay Science, 146, pp 495-502. (Available at [doi.org/10.1016/j.clay.2017.07.003](https://doi.org/10.1016/j.clay.2017.07.003))
- Perdrial, J.N., L.N. Warr, N. Perdrial, M.-C. Lett and F. Elaiss. 2009. Interaction between smectite and bacteria: Implications for bentonite as backfill material in the disposal of nuclear waste. Chemical Geology, 264, pp 281–294. (Available at [doi.org/10.1016/j.chemgeo.2009.03.012](https://doi.org/10.1016/j.chemgeo.2009.03.012))
- Puigdomenech, I., J.P. Ambrosi, L. Eisenlohr, J.E. Lartigue, S.A. Banwart, K. Bateman, A.E. Milodowski and J.M. West. 2001. O<sub>2</sub> depletion in granitic media: The REX project. SKB Technical Report, TR-01–05, Stockholm, Sweden. (Available at [skb.com/publication/18352/](http://skb.com/publication/18352/))
- Samper, J., J. Molinero, C. Yang and G. Zhang. 2003. Redox Zone II. coupled modeling of groundwater flow, solute transport, chemical reactions and microbial processes in the Äspö Island. SKB Technical Report, TR-03–16, Stockholm, Sweden. (Available at [skb.se/upload/publications/pdf/TR-03-16.pdf](http://skb.se/upload/publications/pdf/TR-03-16.pdf))
- Scully, J.R. and M. Edwards. 2013. Review of the NWMO copper corrosion allowance. NWMO TR-2013-04. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Senior, N., R.C. Newman, D. Artymowics, W.J. Binns, P.G. Keech, D.S. Hall. (2019). Communication—A method to measure extremely low corrosion rates of copper metal in anoxic aqueous media. 166, pp 3015-3017. (Available at doi: 10.1149/2.0031911jes)
- Sherwood Lollar, B. 2011. Far-field microbiology considerations relevant to a deep geological repository – state of science review. NWMO TR-2011-09. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))

- Sherwood Lollar, B., G. Lacrampe-Couloume, G.F. Slater, J. Ward, D.P. Moser, T.M. Gihring, L.-H. Lin and T.C. Onstott. 2006. Unravelling abiogenic and biogenic sources of methane in the Earth's deep subsurface. *Chemical Geology*, 226, pp 328– 339. (Available at [doi.org/10.1016/j.chemgeo.2005.09.027](https://doi.org/10.1016/j.chemgeo.2005.09.027))
- Sherwood Lollar, B., T.C. Onstott, G. Lacrampe-Couloume and C.J. Ballentine, C.J. 2014. The contribution of the Precambrian continental lithosphere to global H<sub>2</sub> production. *Nature* 516, pp 379–382. (Available at [doi.org/10.1038/nature14017](https://doi.org/10.1038/nature14017))
- Small, J.S. M. Dutton. 2009. Review of research on cellulose degradation and input data for the Simplified Model of Gas Generation (SMOGG). National Nuclear Laboratory Report to the NDA, NNL (09) 8870, Issue 3. (Available at [rwm.nda.gov.uk/publication/review-of-research-on-cellulose-degradation-and-input-data-for-the-simplified-model-of-gas-generation-smogg/](http://www.nda.gov.uk/publication/review-of-research-on-cellulose-degradation-and-input-data-for-the-simplified-model-of-gas-generation-smogg/))
- Small, J., C. Lennon and L. Abrahamsen. 2011. GRM Near-field Modelling for the LLWR 2011 ESC NNL (10) 11233 Issue 2.0. (Available at [webarchive.nationalarchives.gov.uk/20170412103307/http://llwrsite.com/wp-content/uploads/2016/03/3.-11233-GRM-Near-Field-for-the-LLWR-ESC-Issue-2-MASTER-21-04-11.pdf](http://webarchive.nationalarchives.gov.uk/20170412103307/http://llwrsite.com/wp-content/uploads/2016/03/3.-11233-GRM-Near-Field-for-the-LLWR-ESC-Issue-2-MASTER-21-04-11.pdf))
- Small, J. 2015. BN Experiment: GRM biogeochemical modelling during Phase 18 and Phase 19 of the Bitumen-Nitrate-Clay interaction experiment. Mont Terri Technical Note, TN 2013-44 Federal Office of Topography (swisstopo), Wabern, Switzerland.
- Small, J. 2019. BN Experiment: Biogeochemical modelling of H<sub>2</sub> equilibration tests during Phase 23 of the Bitumen-Nitrate-Clay interaction experiment Mont Terri Technical Note, 2018-42, Federal Office of Topography (swisstopo), Wabern, Switzerland.
- Small, J. and L. Abrahamsen-Mills. 2018. Modelling of microbial processes relevant to ILW disposal. MIND Project Deliverable D1.8. EC Grant Agreement 661880. (Available at [mind15.eu/deliverables/](http://mind15.eu/deliverables/))
- Small, J., M. Nykyri, M. Helin, U. Hovi, T. Sarlin and M. Itävaara. 2008. Experimental and modelling investigations of the biogeochemistry of gas production from low and intermediate level radioactive waste. *Applied Geochemistry*, 23, p 1383-1418. (Available at [doi.org/10.1016/j.apgeochem.2007.11.020](https://doi.org/10.1016/j.apgeochem.2007.11.020))
- Small, J.S., M. Nykyri, M. Vikman, M. Itävaara and L. Heikinheimo. 2017. The biogeochemistry of gas generation from low-level nuclear waste: Modelling after 18 years study under in situ conditions. *Applied Geochemistry*. 84, pp 360-372. (Available at [doi.org/10.1016/j.apgeochem.2017.07.012](https://doi.org/10.1016/j.apgeochem.2017.07.012))
- Standish, T.E., D. Zagidulin, S. Ramamurthy, P.G. Keech, J.J. Noel, D.W. Shoemsmith. 2017. Galvanic corrosion of copper-coated carbon steel for used nuclear fuel containers. *Corrosion Engineering, Science and Technology*. 52, pp 65-69. (Available at <https://doi.org/10.1080/1478422X.2017.1306972>)
- Stroes-Gascoyne, S. 1989. The Potential for Microbial Life in a Canadian High-Level Nuclear Fuel Waste Disposal Vault: a Nutrient and Energy Source Analysis. *Whiteshell Nuclear*



- Research Establishment. AECL Report 9574. (Available at [iaea.org/inis/collection/NCLCollectionStore/Public/22/061/22061210.pdf?r=1](http://iaea.org/inis/collection/NCLCollectionStore/Public/22/061/22061210.pdf?r=1))
- Stroes-Gascoyne, S. and C.J. Hamon. 2008. The effect of intermediate dry densities (1.1–1.5 g/cm<sup>3</sup>) and intermediate porewater salinities (60–90 g NaCl/L) on the culturability of heterotrophic aerobic bacteria in compacted 100 % bentonite. NWMO TR-2008-11. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Stroes-Gascoyne, S. and C.J. Hamon. 2010. The effects of elevated temperatures on the viability and culturability of bacteria indigenous to Wyoming MX-80 bentonite. NWMO TR-2010-08. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Stroes-Gascoyne, S., C.J. Hamon, D.A. Dixon and D.G. Priyanto. 2010. The effect of CaCl<sub>2</sub> Porewater Salinity (50–100 g/L) on the culturability of heterotrophic aerobic bacteria in compacted 100% bentonite with dry densities of 0.8 and 1.3 g/cm<sup>3</sup>. TR-2010-06 11. Nuclear Waste Management Organization. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Stroes-Gascoyne, S., C.J. Hamon, D. Priyanto, D. Jalique, C. Kohle., W. Evenden, A. Grigoryan and D.K. Korber. 2014. Microbial analysis of a highly compacted Wyoming MX-80 bentonite plug infused under pressure with distilled deionised water over a period of almost eight years. NWMO TR-2014-20. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Stumm, W. and J.J. Morgan. 1995. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters, 3rd Edition. Wiley.
- Suckling, P., J. Avis, N. Calder, O. Nasir, P. Humphreys, F. King and R. Walsh. 2015. T2GGM Version 3.2: Gas Generation and Transport Code. NWMO-TR-2015-13. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Swift, B.T. 2016a. Specification for SMOGG Version 7.0: A Simplified Model of Gas Generation from Radioactive Wastes. RWM007409, AMEC/204651/001, Issue 2. (Available at [rwm.nda.gov.uk/publication/specification-for-smogg-version-7-0-a-simplified-model-of-gas-generation-from-radioactive-wastes/](http://rwm.nda.gov.uk/publication/specification-for-smogg-version-7-0-a-simplified-model-of-gas-generation-from-radioactive-wastes/))
- Swift, B.T. 2016b. User Guide for SMOGG Version 7.0: A Simplified Model of Gas Generation from Radioactive Wastes. RWM007409, AMEC/204651/002, Issue 2. (Available at [rwm.nda.gov.uk/publication/user-guide-for-smogg-version-7-0-a-simplified-model-of-gas-generation-from-radioactive-waste/](http://rwm.nda.gov.uk/publication/user-guide-for-smogg-version-7-0-a-simplified-model-of-gas-generation-from-radioactive-waste/))
- Tournassat, C., P. Alt-Epping, E.C. Gaucher, T. Gimmi, O.X. Leupin and P. Wersin. 2011. Biogeochemical processes in a clay formation in situ experiment: Part F –reactive transport modelling. Applied Geochemistry, 26, pp 1009–1022. (Available at <https://doi.org/10.1016/j.apgeochem.2011.03.009>)
- Tullborg, E.L., J. Smellie, A.-Ch. Nilsson, M.J. Gimeno, L.F. Auqué, V. Brüchert and J. Molinero. 2010. SR-Site – sulfide content in the groundwater at Forsmark. SKB Report TR-10-39. (Available at [skb.se/upload/publications/pdf/TR-10-39.pdf](http://skb.se/upload/publications/pdf/TR-10-39.pdf))
- Van Dyke, M.I. and A.J. McCarthy. 2002. Molecular biological detection and characterization of Clostridium populations in municipal landfill sites. Applied Environmental Microbiology, 68, p 2049-2053. (Available at [doi.org/10.1128/AEM.68.4.2049-2053.2002](http://doi.org/10.1128/AEM.68.4.2049-2053.2002))

- Vikman, M., H. Miettinen, E. Sohlberg, M. Matuszewicz and M. Olin. 2018. Microbial diversity in bentonite buffer of aged bentonite buffer experiment. MIND Project Deliverable D2.6, EC Grant Agreement 661880. (Available at [mind15.eu/deliverables/](http://mind15.eu/deliverables/))
- Vikman, M., K. Marjamaa, M. Nykyri, J.S. Small, H. Miettinen, L. Heikinheimo, T. Haavisto and M. Itävaara. 2019. The biogeochemistry of gas generation from low-level nuclear waste: Microbiological characterization during 18 years study under in situ conditions. *Applied Geochemistry*, 105, p 55-67. (Available at [doi.org/10.1016/j.apgeochem.2019.04.002](https://doi.org/10.1016/j.apgeochem.2019.04.002))
- Wersin, P., L.H. Johnson, B. Schwyn, U. Berner, E. Curti. 2003. Redox Conditions in the Near Field of a Repository for SF/HLW and ILW in Opalinus Clay. NAGRA TR 02-13. (Available at [nagra.ch/data/documents/database/dokumente/\\$default/Default%20Folder/Publikationen/NTBs%202001-2010/e\\_ntb02-13.pdf](http://nagra.ch/data/documents/database/dokumente/$default/Default%20Folder/Publikationen/NTBs%202001-2010/e_ntb02-13.pdf))
- Wersin, P., O.X. Leupin, S. Mettler, E. Gaucher, U. Mäder, P. De Cannière, A. Vinsot, H.E. Gabler, T. Kunimaro, K. Kiho and L. Eichinger. 2011. Biogeochemical processes in a clay formation in situ experiment: part A – overview, experimental design and water data of an experiment in the Opalinus Clay at the Mont Terri underground research laboratory, Switzerland. *Applied Geochemistry*, 26, pp 931–953. (Available at [doi.org/10.1016/j.apgeochem.2011.03.004](https://doi.org/10.1016/j.apgeochem.2011.03.004))
- Wolfaardt, G.M. and D.R. Korber. 2012. Near-field microbiological considerations relevant to a deep geological repository for used nuclear fuel – state of science review. NWMO TR-2012-02. Toronto, ON. (Available at [www.nwmo.ca](http://www.nwmo.ca))
- Wu, L., D. Guo, M. Li, J.M. Joseph, J.J. Noel, P.G. Keech, J.C. Wren. 2017. Inverse crevice corrosion of carbon steel: effect of solution volume to surface area. *Journal of The Electrochemical Society*, 164, pp 539–553. (Available at doi: 10.1149/2.0511709jes)
- Yang, C., J. Samper, J. Molinero and M. Bonilla. 2007. Modelling geochemical and microbial consumption of dissolved oxygen after backfilling a high level radioactive waste repository *Journal of Contaminant Hydrology*, 93, pp 130–148. (Available at [doi.org/10.1016/j.jconhyd.2007.01.008](https://doi.org/10.1016/j.jconhyd.2007.01.008))
- Zhang, G. 2001. Nonisothermal hydrobiogeochemical models in porous media. Ph. D. dissertation, University of La Coruña, Spain 324 pp.