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Relevant metabolic processes and limits on chemical conditions leading to methane generation in LLW and ILW

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Publishable Summary

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

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

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

Contents

1 Introduction

Gas generation in final repository conditions can lead to overpressure in the repository, effect engineered barrier materials and enhance migration of water-borne radionuclides or gaseous radionuclides, such as ^{14}C, in the fractures of crystalline bedrock and finally to the biosphere (Figure 1-1). Soluble organic degradation products also have potential to enhance corrosion and form aqueous complexes with radionuclides thus affecting their mobility. Two major processes influencing the gas formation in LLW (low level radioactive waste) and ILW (intermediate level radioactive waste) repositories are related to corrosion of steel and microbial degradation of organic materials. This report considers factors and chemical conditions influencing the gas and especially methane formation in final disposal conditions. Results obtained from unique *in-situ* large-scale Gas Generation Experiment (GGE) in final disposal repository for operational LLW and ILW in Olkiluoto, Finland, are also summarized here.

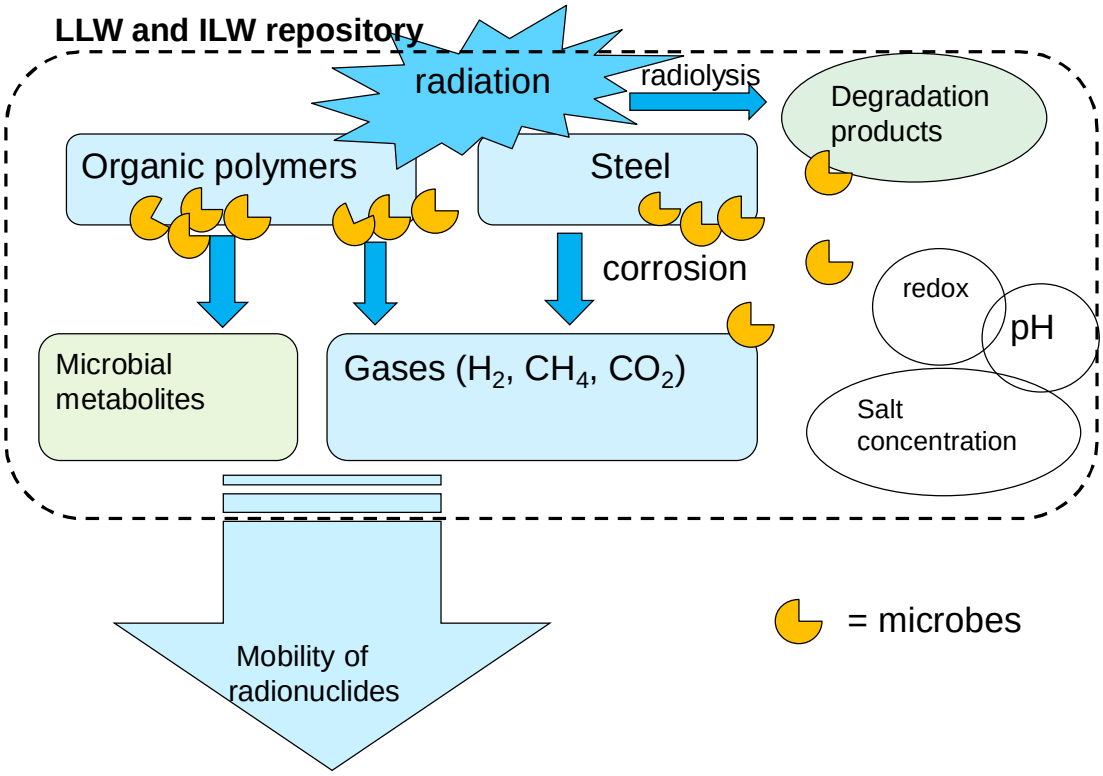

Figure 1-1. Microbial reactions in LLW and ILW repository leading to the gas generation (Figure modified from Vikman et al [1]).

1.1 Low and intermediate level radioactive waste

In addition to high activity level spent nuclear fuel also low (LLW) and intermediate level waste (ILW) is produced during the operation of nuclear power plants. The composition of LLW and ILW in various European countries varies depending on the nuclear power generation, industry

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and research activities as reviewed by Abrahamsen et al. [2]. Wide ranges of organic materials are present in ILW and LLW including bitumen, organic ion exchange resins, different kind of polymers and cellulose-based materials. In Finland LLW is composed mainly of scrap metals and maintenance waste (e.g. protective cloths, fire protection fabrics, plastics) produced during operation and maintenance of nuclear power plants. ILW contains ion exchange resins and evaporation concentrates which are encapsulated to bitumen or to concrete.

1.2 Geological repositories

In Finland, all nuclear waste must be treated, stored and disposed of within the Finnish borders. The Finnish nuclear power companies take care of their own LLW and ILW. Olkiluoto repository for operational LLW and ILW consists of two rock silos constructed at a depth of 60-100 metres inside the bedrock [3]. Compressible LLW is packed into 200-litre carbon steel drums and deposited in the rock silo inside a concrete box. Scrap metal is packed in steel and concrete cases and steel drums. The ILW consisting mainly of ion exchange resins that are solified in bitumen and into steel drums. Drums with ILW are deposited in a rock silo of steel-reinforced concrete. In Loviisa LLW and ILW is disposed in facilities built at a depth of 110 metres. Low-level waste is compressed and packed into 200-litre barrels. ILW is solidified into cement and packed into concrete barrels.

1.3 Gas generation

Gas generation in geological LLW and ILW repositories in anoxic conditions can occur by corrosion of steel, by microbial degradation of organic material and by radiolysis (Table 1-1).

LLW and ILW repositories contain metals present in the radioactive waste itself and from the materials used for disposal containers (e.g. steel drums). Iron is the most common metal but also other metals could be present in steel alloys (e.g. chromium, nickel). In anoxic conditions, the main product of the corrosion process is ferrous hydroxide $Fe(OH)_2$ together with some hydrogen gas H_2. The $Fe(OH)_2$ can further react to form magnetite FeO_4, H_2 and water [4].

Table 1-1. Gas generation processes in LLW and ILW repositories in anoxic condition.

Process	Target of reaction	Reaction	Produced gas
Corrosion	Steel (and other metals) in waste and packaging (e.g. drums)	$Fe\ (s) + 2\ H_2O\ (l) \rightarrow Fe(OH)_2\ (s) + H_2\ (g)$	H_2
Microbial degradation	Organic C (waste material)	$C_{organic} \rightarrow \rightarrow CO_2 + CH_4$	CO_2, CH_4, (H_2)
Radiolysis	Water and some organic molecules in waste packages	$H_2O \rightarrow \rightarrow e^-\ (aq), H^+, OH^-, H\cdot, H_2, H_2O_2, O_2$	H_2

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Microbial degradation of LLW and ILW containing organic carbon results in formation of carbon dioxide and methane (Figure 1-2). In the first phase, complex polymers (e.g. cellulose and hemicellulose) are hydrolysed to basic monomers and oligomers by the hydrolytic extracellular enzymes produced by heterotrophic microbes. These simpler compounds are then fermented to volatile fatty acids VFAs (e.g. formic, acetic, propionic acids), H_2 and CO_2 by the fermentative bacteria (acidogenic bacteria). After that VFAs are transformed into acetate and H_2 by the acetogenic bacteria. H_2, CO_2 and acetic acid transformed to methane by archaeal methanogens. Methane production from acetic acid is carried out by acetoclastic methanogens and from hydrogen and carbon dioxide by hydrogenotrophic methanogens. [5,6,7]

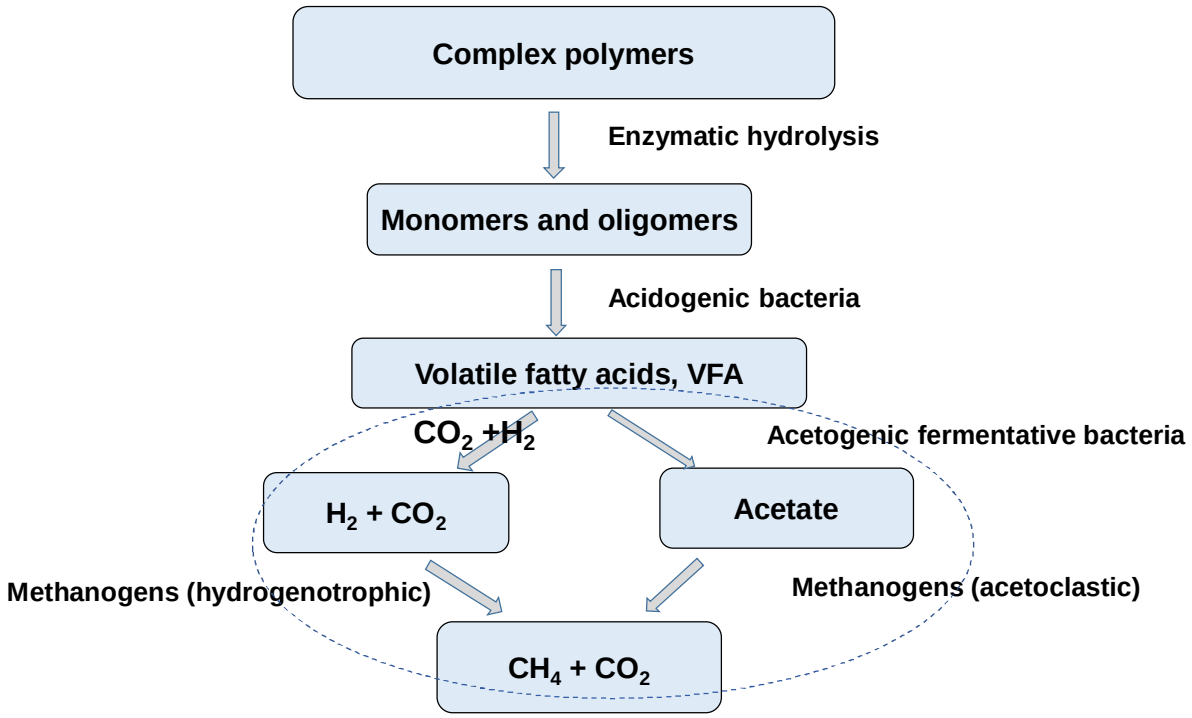

Figure 1-2. Microbial degradation of polymers in anoxic conditions.

Hydrogen gas can also be formed as a result of water radiolysis which is the dissociation process of water molecules by ionizing radiation from the decay of radionuclides. Primary products of water radiolysis include reactive chemicals, such as H_2 and H_2O_2. It is estimated that the gas generation by water radiolysis is so small in VLJ repository conditions in Finland that it is not necessary to take that account in safety assessments [8].

2 Gas Generation Experiment

The Gas Generation Experiment (GGE) has been established in 1997 to examine gas generation from LLW in TVO's final disposal repository for operational LLW and ILW in Olkiluoto, Finland. The aim of the GGE was to study gas generation in LLW repository conditions using

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representative maintenance waste from nuclear power units. The results obtained from the GGE has been used as a basis of the conclusions presented in this report.

2.1 Experimental design

Sixteen carbon-steel waste drums (200 L) were emplaced in a concrete box and enclosed in acid proof steel tank (Figure 2-1). Drums were filled with representative maintenance waste from nuclear power units including paper, cardboard, cotton, polyethylene, polyvinylchloride, polycarbonate, rubber, metals, glass fibre and electrical components. The amount of easily biodegradable cellulose- and hemicellulose-based materials inside the drums varied from 5 to 95 w-%. Before closing GGE was filled with locally sourced untreated river water and is maintained at 8-11°C.The proportion of concrete to cellulose in the GGE (mass ratio 6.5) is lower than in actual repositories [9] that should considerer when results are interpreted.

The GGE has been monitored for generated gas, water chemistry and microbiology. Electrical conductivity, pH, Eh and dissolved oxygen in the tank water are continuously monitored by on-line measurements at the drum lid level. The volume and composition of the released gas is measured at certain time intervals. Water samples were taken at scheduled time intervals and analysed for major anions and cations (e.g. SO_4^{2-}, Fe^{2+}). Chemical parameters were analysed by TVO's laboratory. The experimental design and sampling information are described in more detail by Small et al. [9,10].

Figure 2-1. The gas generation experiment GGE with waste drums and sampling lines for water samples and capsules.

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2.2 Microbiological sampling

Microbiological analyses have been made from water samples taken from different compartments in the GGE (inside drums, in tank water outside of drums). In addition, sample capsules containing steel plate and maintenance waste have been loaded to the experiment (Figure 2-2). In MIND project, samples have been taken from the GGE in October 2015, in February 2017 and in October 2017.

Figure 2-2. Capsules and water samples from GGE.

Biomass for DNA and RNA extractions were concentrated by filtration of 50 to 100 mL water on cellulose acetate filters. DNA was extracted with PowerWater DNA Isolation kit (MoBio Laboratories, USA) and RNA with the PowerWater RNA isolation kit (MoBio Laboratories, USA). Extractions were performed according to the manufacturer's instructions and DNA/RNA was eluated with 100 µL of molecular grade water. RNA samples were translated to complementary DNA (cDNA) as described by Purkamo et al. [11]. The composition of microbial community on water samples and biofilms on maintenance waste and steel plates have been analysed by quantitative PCR (qPCR) (Table 2-1) and amplicon 16S sequencing (454 pyrosequencing or IonTorrent). The sequence reads obtained from sequencing were processed with bioinformatics tools at VTT using the Greengenes reference database 13_8 [12] as the reference. Metabolic functions related to hydrolysis of cellulosic waste in the GGE were predicted using the open-source PICRUSt software [13]. Summary of the microbiological analyses is shown at Table 2-1. In order to quantify the numbers of bacteria, archaea, sulphate reducers and methanogens qPCR was applied (Table 2-2).

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Table 2-1. Summary of the microbiological analyses made from GGE. More detailed information can be found from Vikman et al. [14].

Sample	Parameter	Method
Water	Amount of microbes	Dapi (4',6-diamidino-2-phenylindole) staining + Epifluoresecent microscopy
	Bacteria	qPCR
	Archaea	qPCR
	Methanogens	qPCR
	Sulphate reducers	qPCR
	Nitrate reducers	qPCR
	Composition of bacterial and archaeal community	16S amplicon sequencing (IonTorrent/ 454 pyrosequencing)
	Volatile fatty acids	Capillary electrophoresis
	Microbial metabolites	GC + mass spectrometry
Capsules: Steel plate	Rate of steel corrosion	ISO 8407
	Composition of bacterial and archaeal community	16S amplicon sequencing (IonTorrent/ 454 pyrosequencing)
	Visualization by microscopic measurement	Field Emission Scanning Electron Microscopy (FESEM)
Capsules: Maintenance waste	Composition of bacterial and archaeal community	16S amplicon sequencing (IonTorrent/ 454 pyrosequencing)
	Visualization by microscopic measurement	FESEM

Table 1-2. Summary of the microbial groups and genes studied with qPCR. More detailed information can be found from Vikman et al. [14]

Microbes	Gene region	Reference
Bacteria	bacteria 16S rRNA	Muyzer and Stams, 2008 [14]
Archaea	archaea 16S rRNA	Bano et al. 2004 [15]; Barns et al. 1994 [16]
Sulphate reducers	*dsr*B	Geets et al. 2006 [18]; Wagner et al. 1998 [19]
Methanogens	*mcr*A	Hales et al. 1996 [20]
Nitrate reducers	*narG*	(López-Gutiérrez et al. 2004)[21]

2.3 Main results from Gas Generation Experiment

Significant gas generation in the GGE started after one year of operation and has continued with the rate varying between 0.5 and 1.3 m^3/year. The dominant component in the gas phase has been methane (80-95 volume%). The amount of carbon dioxide has been below detection limit because it has probably reacted with alkaline tank water and precipitated as $CoCO_3$.

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The chemical conditions in the GGE were very heterogenic during the first years of GGE (see 2.3.3) which can be seen especially in pH values. The pH was gradually neutralized below pH 9 that coincides with a doubling of the gas generation rate. The results from the chemical analyses have been reported by Small et al. [9,10]. Microbiological results have been reported by Vikman et al. [14].

2.3.1 Microbial community structure

Microbiological results demonstrate that biodegradable part of LLW (cellulose and hemicellulose) was converted to methane and carbon dioxide as a successive action of a complex microbial consortium. The most significant microbial groups influencing the gas generation in GGE are cellulose and hemicellulose hydrolysing microbes, fermentative microbes and methanogens. Several microbial groups with potential to hydrolyze cellulose and hemicellulose, metabolize sugars to acetate and hydrogen or volatile fatty acids and produce methane were detected and summarized below:

Cellulose and hemicellulose degradation:

- Firmicutes, especially lineages of the Clostridia, majority of cellulose degraders have been shown to belong to the Firmicutes phylum
- Bacterial phyla WWE1 (candidate phulum Cloacimonetes), suggested to have a role in an extracellular cellulose hydrolysis process
- Several genes related to cellulose and hemicellulose degradation (hydrolysis) were detected using bacterial 16S rRNA gene sequences and PICRUSt (e.g. endo-1.4-beta-xylanase and beta-mannosidase)

Fermentative microbes:

- Chloroflexi, a diverse group of microbes including species able to ferment sugar to acetate
- Bacteroidales containing obligate fermentative anaerobes, contain saccharolytic species which can ferment sugars to acetate and succinate
- Syntrophomonas belonging to syntrophic acetogens capable of butyrate and propionate degradation
- Bacterial phyla OD1 (candidate phylum Parcubacteria), associated with fermentation of simple sugars to organic acids

Methane production:

- Methanobacteriales and Methanomicrobiales
- Methanosarcinales, Methanosaeta

The methane-producing step in the anaerobic degradation process is mainly performed by methanogenic archaea. Both acetoclastic and hydrogetrophic methanogens were found in the GGE but the formation of CH_4 from H_2 and CO_2 seemed to be more favourable metabolic route compared to the one utilizing acetate, especially in the beginning of the GGE. All acetoclastic methanogens belong to the order Methanosarcinales that were first detected in 2015 (Figure 2-3). The appearance of acetoclastic methanogens coincided with the acetate consumption and increased gas generation.

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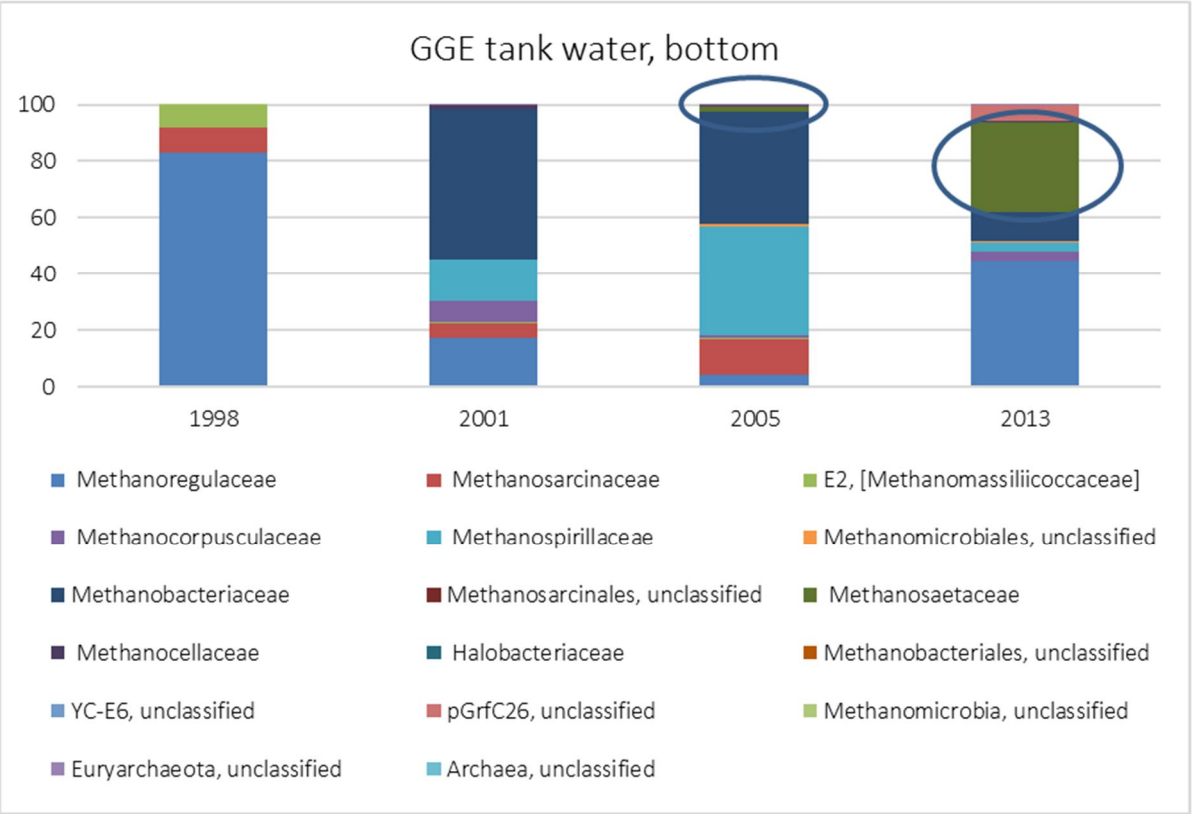

Figure 2-3. Relative abundance of archaeal communities in the GGE presented as taxonomic family-level. Methanosaetaceae were detected in 2005. All species within this family use acetate as their sole source of energy.

2.3.2 Sulphate reducers

SRBs are the most significant microbial group competing with methanogens for the electron donors in the GGE. SRBs are a diverse group of anaerobic archaea and bacteria that can use sulphate as a terminal electron acceptor producing hydrogen sulphide. SBRs belonging to the orders of Desulfobacterales, Desulfovibrionales, Desulfovibrionaceae and Desulfomonadales were detected in the GGE by 16S amplicon sequencing. The qPCR analysis with *dsr*B marker gene revealed that the number of SRBs has remained approximately at the same level at the bottom of the tank and has increased at the lid level of the tank during the operation of the GGE (Figure 2-4).

The relative ratio of SRBs compared to methanogens have decreased during the operation of GGE. The amount of sulphate has been close to the detection limit since 2000 that could indicate that available sulphate is rapidly consumed by SRBs. SRBs can also use other electron acceptors besides sulphate. In addition to competing with methanogens, SRBs can grow syntrophically with them depending on the availability of sulphate [15]. In 2017 ^{35}S tracer method was also used to analyze microbial sulphate reduction in GGE. Sulphate reduction rate in GGE was very low but was rapidly increased when sulphate or sulphate-containing groundwater was added to the system. This indicates that groundwater flow to the LLW repository can influence the gas generation rate.

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SRBs are also known to affect various corrosion mechanisms and cause corrosion of steel in anoxic conditions [20]. SRBs produce the corrosive chemical agent hydrogen sulphide and by consuming excess hydrogen, they are also believed to stimulate corrosion process.

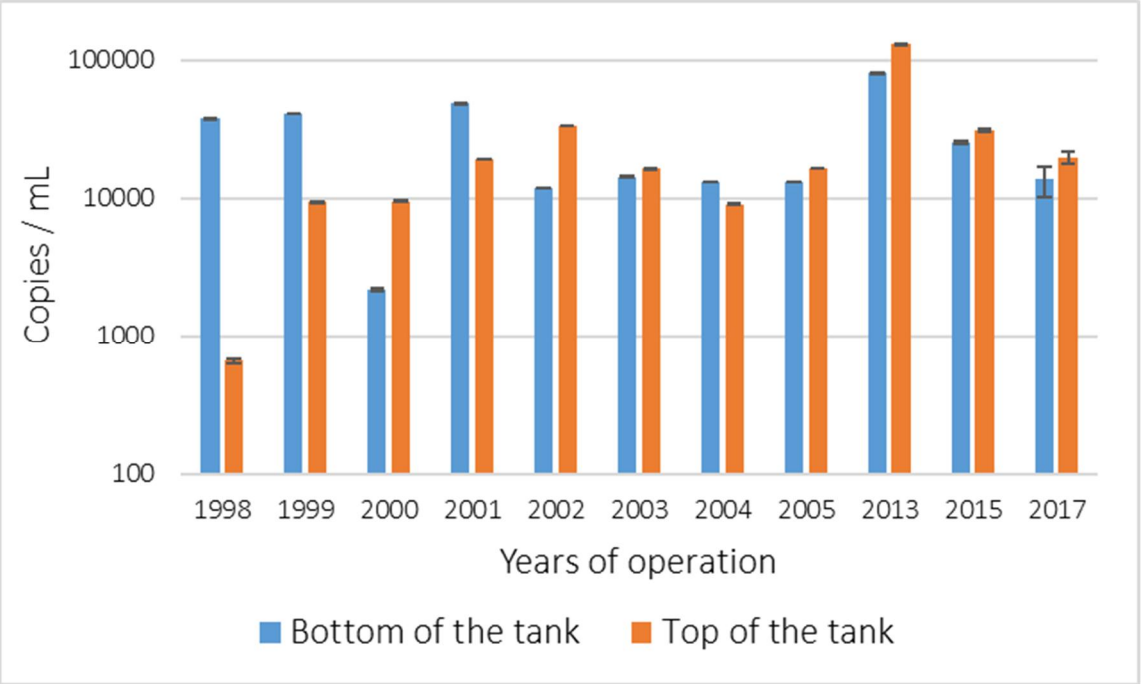

Figure 2-4. DsrB *gene copies in 1 mL of tank water between 1998 and 2017 measured by qPCR. Samples taken from tank water at the bottom and at the lid level of the GGE tank.*

2.3.3 Heterogeneous conditions

The chemical conditions in GGE were very heterogenic during the first years of the GGE [9]. Concrete structures created alkaline environment at the top of the tank but pH was close to neutral at the bottom of the tank and inside the waste drums. In addition, concentration of dissolved organic carbon (DOC) was higher at the bottom of the tank and inside waste drums compared to the top of the tank. Heterogenic conditions created optimal niches for microbial activity that leaded to differences in the microbial abundances in different compartments of the GGE. For example, the amount of methanogens was higher at the bottom of the GGE tank compared to the top of the tank (Figure 2-5).

Microbial metabolites (e.g. CO_2, VFAs) altered chemical conditions in the GGE and by the year of 2013 pH of water was close to neutral also at the bottom of the tank. Because of the more homogeneous conditions, no significant differences were detected in the microbial abundances and community structure between the bottom and the top of the tank (Figure 2-6). However, there were still differences in microbial community structure and activity when samples inside the drums and tank water were compared.

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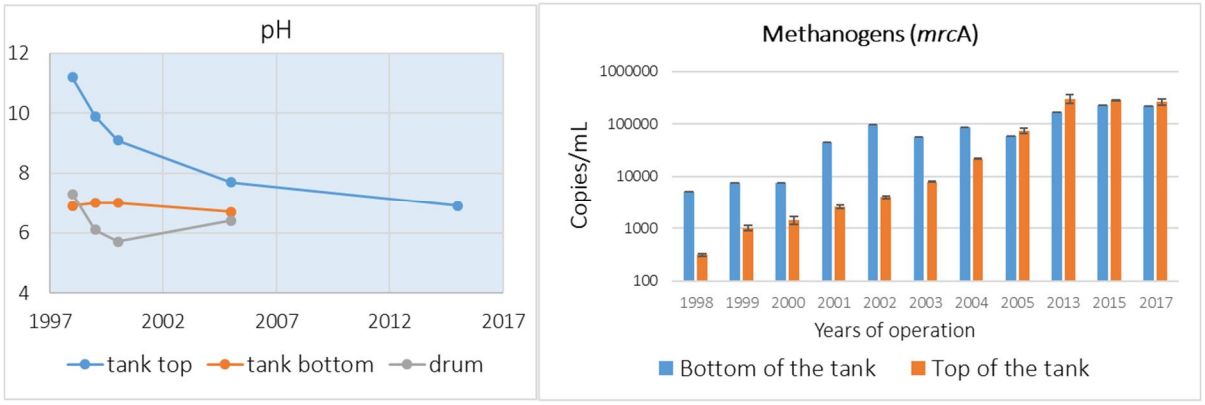

Figure 2-5. The pH and the amount of methanogens at different compartments of the GGE. Mrc*A gene copies in 1 mL of tank water between 1998 and 2017 measured by qPCR. Samples taken from tank water at the bottom and at the lid level of the GGE tank.*

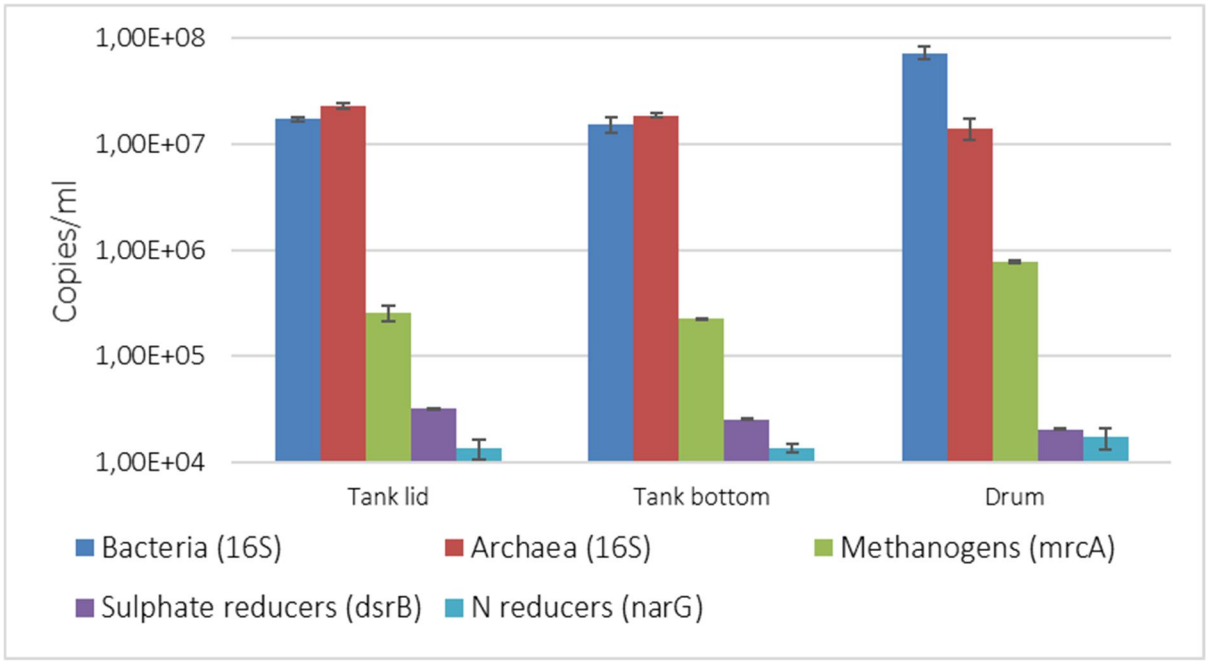

Figure 2-6. Bacterial and archeal 16S, mrc*A,* dsr*B and* nar*G gene copies in 1 mL of tank water in 2015 measured by qPCR. Samples taken from water at the bottom and the top of the GGE tank and from one of the drums.*

2.3.4 Volatile fatty acids and other microbial metabolites

Volatile fatty acids (VFAs) are intermediate products of anaerobic degradation of organic polymers (Figure 1-2). They are organic acids that consist of six or fewer carbon atoms, e.g., acetic acid, propionic acid, butyric acid, isobutyric acid, and isovaleric acid. High VFA concentrations can cause decreasing pH and result in toxic conditions. It has been reported that especially high propionic acid concentration caused significant inhibition of the methanogens [23]. In GGE VFAs were detected in the tank water in the beginning of the experiment as a result of intensive degradation of cellulose and hemicellulose. After that VFA concentrations have decreased as a function of time (Figure 2-7).

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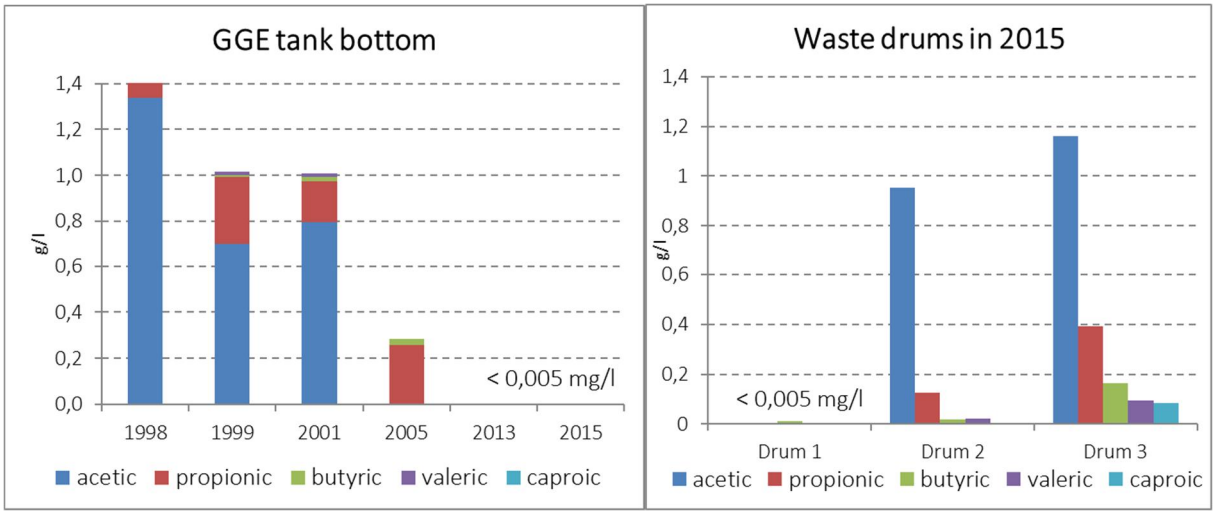

Figure 2-7. VFA concentrations at the bottom of the GGE tank and inside waste drums.

2.3.5 Corrosion of steel

The corrosion of steel in anoxic conditions can lead to the formation of H_2 that may further contribute to gas generation when used as an electron donor in other microbial processes [24]. The corrosion rate of steel has been evaluated by using capsules loaded to the GGE tank in various compartments. Relatively high corrosion rate of steel was observed which indicates also the generation of H_2. The corrosion rate of steel was highest inside the drum containing 95 w-% of cellulose-based LLW. The concentration of soluble ferrous iron Fe^{2+} was also high in water sample taken from this drum indicating dissolution of the carbon steel. One possible reason for more rapid corrosion rate can be the increased microbial activity. The amount of methanogens was one logarithmic unit higher in the drum with 95% of cellulose-and hemicellulose-based LLW than in other drums with less cellulose-and hemicellulose-based LLW.

3 Conclusions on factors influencing gas generation

3.1 Environmental conditions

3.1.1 Water activity

Availability of water is a prerequisite for microbial activity because all biological reactions occur in water. In addition, steel corrosion in anoxic conditions requires water. After the repository closure groundwater flows to the repository gradually saturating waste materials in drums and in other containers. It is estimated that in Olkiluoto, the groundwater fills the L/ILW repository within tens of years after the closure [25].

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3.1.2 Temperature

The key factor influencing microbial growth and activity is temperature [24]. When temperature rises, the rates of enzymatic reactions increase and microbes grow faster. Microbes have optimal temperature range for growth but on the other hand, microbes have been isolated from many extreme environments from hot springs to artic oceans where they are adapted to the existing conditions. The bedrock temperature near the repository facilities in Olkiluoto at the depth of 110 m is about 8–12 degrees [3] which is relatively low temperature but still adequate for microbial activity. Nozhevnikova et al. [27] have isolated methanogenic archaea and acetogenic bacteria from cold environments, and these isolates were able to grow below temperature of 10°C and most of them grow even at 1°C.

3.1.3 pH

In most natural environments, pH is between three and nine, that is the most common pH growth optima for microbes [26]. The use of concrete and cement-based materials in repository structures create alkaline environmental conditions with initial pH level of 12 or higher. High pH is expected to limit microbial activity but diverse range of microbiological processes have been shown to occur also in high pH environments [29]. A wide diversity of haloalkaliphilic bacteria and archaea have been detected e.g. from soda lakes [30]. In order to survive in elevated pH values microbes have developed various adaptations to maintain pH homeostasis and intracellular osmotic pressure. Iron is relatively stable under alkaline conditions due to the formation of a passivation film on its surface [27], and thus high pH reduce corrosion and generation of H_2.

Neutral pH range is typically optimal for methanogens but some species have been isolated with an adaptation to alkaline or acidic pH. For example *Methanocalculus alkaliphilus* from hypersaline soda lake sediments grows at optimum pH of 9.5 [31]. *Methanoregula booneii* has been isolated from an acidic peat bog and has a pH optimum for growth of 5.1 [32].

Microbial metabolites such as CO_2 and VFAs can reduce pH as can be seen in the GGE. This could enhance the microbial living conditions and increase their activity.

3.1.4 Radiation

Radiolytic degradation of organic polymers in repository conditions can generate degradation products that can be utilised by microbial processes and thus influence gas generation. The estimation of the total adsorbed radiation dose of waste and radiation rate is very challenging and discussed in more detailed by Abrahamsen et al. [1].

3.1.5 Heterogeneity

Heterogeneity in the chemical conditions in the repository can create optimal niches (e.g. lower pH, more organic carbon) for microbial activity and can lead to gas generation.

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3.2 Availability of electron donors and acceptors

Microbial activity in repository conditions is controlled by the availability electron donors and acceptors that are needed to generate energy for microbial metabolism. Microbes reduce electron acceptors in a particular order from most energy yielding ones to less energy yielding ones. Typical electron acceptors are sulphate and nitrate (e.g. from bitumen) that can be leached out from waste materials or enter the repository with the infiltrating groundwater.

3.3 Inhibition of methanogenesis

Extensive degradation of organic polymers and the extensive formation of volatile fatty acids reduce the activity of methanogens and gas formation. Other typical chemical compounds that are known to cause toxic effects are ammonia and hydrogen sulphide [9,33]. Sulphide is toxic to several bacterial groups and in addition, methane production can be supressed by the competition of methanogens with SRBs for the common organic and inorganic compounds. Additionally, accumulation of hydrogen and acetate, excess tannins, salts and heavy metals can inhibit methanogenesis.

3.4 Competing microbial groups

Methanogens compete with sulphate and nitrogen reducing microbes for electron donors that in the repository conditions include e.g. H_2 and acetate. Especially the occurrence of SRBs can influence the gas generation rate. Hydrogen is mainly formed as a result of steel corrosion and both compounds are also formed as an intermediate in anaerobic degradation process of polymers. Thermodynamically SRBs can utilize hydrogen and acetate at lower concentrations than methanogens but the presence of sulphate is essential in this competition.

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