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Subterranean microorganisms and radioactive waste disposal in Sweden

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Abstract

In 1987, microbiology became a part of the Swedish scientific program for the safe disposal of high level nuclear waste (HLW). The goal of the microbiology program is to understand how subterranean microorganisms will interact with the performance of a future HLW repository. The Swedish research program on subterranean microbiology has mainly been performed at two sites in granitic rock aquifers at depths ranging from 70 m down to 1240 m, the Stripa research mine in the middle of Sweden and the Äspö Hard Rock Laboratory (HRL) situated on the south eastern coast of Sweden. Some work has also been performed in cooperation with other national or international research groups in Sweden, Canada and at the natural analogue sites Oklo in Gabon and Maqarin in Jordan. The following conclusions are drawn. There is a very high probability of the existence of a deep subterranean biosphere in granitic rock. The documented presence of a deep biosphere implies that relevant microbial reactions should be included in the performance assessment for a HLW repository. A HLW repository will be situated in a subterranean biosphere that is independent of solar energy and photosynthetically produced organic carbon. The ultimate limitation for an active microbial life will be the availability of hydrogen as energy source over time, and hydrogen has indeed been found in most deep groundwaters. Sulphide producing microorganisms are active in environments typical for a Swedish HLW repository, and the potential for microbial corrosion of the copper canisters must be considered. The bentonite buffer around the copper canisters will be a hostile environment for most microbes due to the combination of radiation, heat and low water availability. Discrete microbial species can cope with each of these constraints, and it is theoretically possible that sulphide producing microbes may be active inside a buffer, although the experiments conducted thus far have shown the opposite. Microorganisms have the capability to enzymatically recombine radiolysis oxidants formed by radiation of water. It has earlier been concluded that the migration of radionuclides due to sorption on microorganisms can be neglected. The influence of microbially produced complexing agents remains to be studied at realistic conditions in deep groundwater. Microorganisms have been found in natural alkaline groundwaters, but it could not be conclusively demonstrated that they were in situ viable and growing, rather than just transported there from neutral groundwater. A possible hypothesis based on the obtained results from investigations of natural alkaline groundwaters is that fresh concrete may be a bit too extreme for active life even for the most adaptable microbe – but this remains to be demonstrated. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Bacteria; Bentonite; Gas; Hydrogen; Methanogens; Redox

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1. Introduction

Radioactive waste in Sweden arises mainly from the production of nuclear power. Some waste also comes from research, hospitals and industry. The bulk of radionuclides produced in a nuclear power reactor remains in the spent fuel elements, characterised as high level radioactive waste (HLW). Radioactivity will decay with time, but some long-lived radionuclides will make the HLW hazardous for a very long time. The spent fuel elements will be encapsulated in copper–steel canisters and placed in deposition holes in tunnels at an envisaged depth of about 500 m (Fig. 1). The amount of spent fuel in a canister and the distances between the canisters in the repository are chosen so that the peak temperature will reach about 80°C at the warmest location on the canister surface. The restriction in temperature is mainly there to guarantee the long time performance of the bentonite. The low solubility of the spent fuel matrix, the copper canister, the bentonite buffer and the depth of emplacement in stable host rock are the main barriers to protect man from the radionuclides.

In 1987, microbiology became a part of the Swedish scientific program for the safe disposal of HLW. The goal of the microbiology program is to understand how subterranean microorganisms will interact with the performance of a future HLW repository. The Swedish research program on subterranean microbiology (Pedersen, 1996, 1997b) has mainly been performed at two sites in granitic rock aquifers at depths ranging from 70 m down to 1240 m; the Stripa research mine in the middle of Sweden (Ekendahl and Pedersen, 1994; Ekendahl et al., 1994; Pedersen and Ekendahl, 1992a) and the Äspö Hard Rock Laboratory (HRL) situated on the south eastern coast of Sweden (Pedersen, 1997a; Pedersen and Ekendahl, 1990, 1992b; Pedersen et al., 1996b, 1997b,c). The Äspö HRL has been constructed as a part of the development of the Swedish concept for deep geological disposal of spent nuclear fuel and the work has been divided into three phases; the pre-investigation (1986–1990), the construction (1990–1995) and the operating (1995–) phases. Some work has also been performed in cooperation with other national or international research groups in Canada (Stroes-Gascoyne et al., 1997) and at the natural analogue sites Oklo in Gabon (Pedersen et al., 1996a,c) and Maqarin in Jordan (Pedersen et al., 1997a).

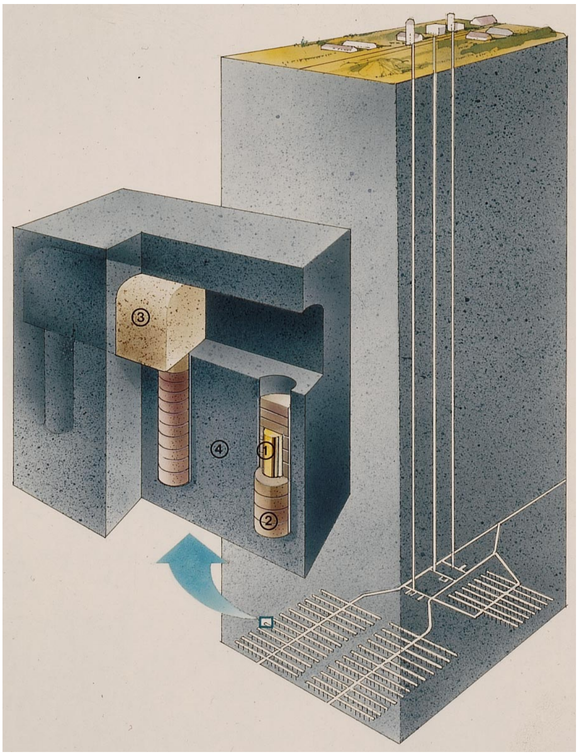


Fig. 1. Schematic drawing of a deep repository. A system of tunnels with vertical deposition holes is built at a depth of about 500 m. The spent fuel assemblies are encapsulated in copper canisters. The canisters are placed in the holes, where they are embedded in bentonite clay. Multiple barriers will protect the spent fuel in the deep repository. (1) Copper canister. The canister isolates the fuel from groundwater contact. The fuel itself is in solid form and has very low solubility. (2) Blocks of bentonite clay. The clay prevents groundwater flow around the canister and protects it against minor movements in the rock. The diffusivity of radionuclides is very low in bentonite, which will prevent radionuclide migration in case of a failing canister. (3) A tunnel backfill. A mixture of sand and bentonite, or crushed rock, fills up the tunnels. (4) A rock barrier. The rock offers a durable environment, both mechanically and chemically. It also acts as a filter for radionuclides possibly released to the groundwater.

2. Research tasks

Seven research tasks were identified by Pedersen (1996) that are of importance for the performance assessment of microorganisms in radioactive waste disposal.

1. *The subterranean biosphere*. Is there a deep subterranean biosphere and how does it sustain its life processes? What energy sources and fluxes of energy will be available for microorganisms in and around a HLW repository?
2. *Microbial production and consumption of gases*. Will bacterial production and consumption of gases like carbon dioxide, hydrogen, nitrogen and methane influence the performance of repositories?
3. *Microbial reducing activity*. Will bacterial oxygen consumption significantly contribute to oxygen removal from a HLW and to what extent may bacterial production of reduced compounds such as organic material, methane, sulphide and ferrous iron contribute to keeping the repository host rock reduced?
4. *Microbial corrosion of copper*. Bacterial corrosion of the copper canisters, if any, will be a result of sulphide production. Two important questions arise. Can sulphide producing bacteria survive and produce sulphide in the bentonite surrounding the canisters? Can bacterial sulphide production in the surrounding rock exceed a performance safety limit?
5. *Microbial recombination of radiolysis products*. Will bacterial recombination of radiolysis products significantly contribute to the removal of unwanted oxidised molecules such as oxygen?
6. *Microbial influence on radionuclide migration*. To what extent, if any, can bacterial dissolution of immobilised radionuclides and production of complexing agents increase radionuclide migration rates?
7. *Alkaliphilic microbes and concrete*. Do relevant microorganisms survive at pH equivalent to that of repository concrete and can they possibly influence repository performance by concrete degrading activities such as acid production?

The following sections summarise the state-of-the-art in the Swedish program for the above arrayed research tasks.

3. The subterranean biosphere

3.1. Evidence for the existence of a deep subterranean biosphere

Diverse and active populations of microorganisms have been observed in most subsurface and subseafloor environments investigated (Bachofen, 1997), including granitic rock aquifers at depths ranging from 10 m down to 1500 m (Pedersen, 1997a). Documentation of in situ activity of microbial populations in deep granitic rock groundwaters suggests that the microbes present are active at low, but significant levels (Ekendahl and Pedersen, 1994; Pedersen and Ekendahl, 1990, 1992a,b).

Igneous rocks are too hot when formed to host life of any kind. Therefore, observed life in hard rock must have entered after cooling and fracturing of the rock mass. The drilling and excavation to access microbial ecosystems in hard rock are vigorous operations. It can be argued that the observed life there is a contamination artefact of the access operations (Pedersen, 1993a) and not a true deep biosphere present before drilling. The risk of microbial contamination of the aquifers by drill water used to cool the drill and transport the drill cuttings out of the borehole during drilling is obvious, and investigations were therefore undertaken to study this risk. Samples were collected in the Äspö Hard Rock Laboratory (HRL) tunnel during drilling of boreholes for geological, hydrological and hydrogeochemical characterisation of designated experimental rock volumes. 16S rRNA gene sequencing, total count and culturing methods were used to investigate whether a lasting microbial contamination due to the drilling occurred. Samples were collected from the drill water source, the drilling equipment and from the drilled boreholes. This work is described in detail by Pedersen et al. (1997c).

Proving that certain species of microorganisms found in the drilled boreholes are intrinsic and not introduced during drilling is extremely difficult.

Instead, the opposite situation was easier to investigate, i.e. testing if a known contaminating microbial population does or does not develop in deep granitic aquifers during and after drilling (Pedersen et al., 1997c). The 600 m long tubing used for drill water supply constituted a source of bacterial contamination to the rest of the drilling equipment and the boreholes. Nevertheless, using molecular, total count and culturing methods, it was shown that although large numbers of contaminating bacteria were introduced into the boreholes during drilling, they did not become established in the aquifers at detectable levels. Therefore, it seems reasonable to conclude that we find no evidence for lasting microbial contamination of boreholes drilled in granitic rock. The reason for this is the inability of foreign microbes to adapt to the prevailing oligotrophic, reducing, anaerobic and low temperature environmental conditions in deep granitic aquifers. We have recently described two new species isolated from fractures deep under Äspö HRL: *Desulfovibrio aespoeensis* (Motamedi and Pedersen, 1998) and *Metanobacterium subterraneum* (Kotelnikova et al., 1998). These two species are adapted to life under the conditions prevailing where they were isolated from, i.e. they are most probably intrinsic. Recent findings of bacterial fossils in a granitic aquifer 207 m below ground at Äspö (Pedersen et al., 1997b) support the hypothesis of a deep and intrinsic biosphere in hard rock aquifers.

3.2. Subterranean microbes and biogeochemical processes

The subterranean biosphere, concluded above to exist, may influence the geochemical situation in many different ways. A full understanding of deep subterranean environments cannot, therefore, be achieved until microbial processes are included in models, theories and interpretations of results. This is because microbes catalyse many reactions that, for kinetic reasons, are very slow or not possible at low temperature and pressure. One obvious example is the bacterial reduction of sulphate to sulphide in anoxic waters. Another example is that bacterial activity usually influences the redox potential in the environment. If, for instance, the environment is rich in Fe(III) and organic matter, iron reducing bacteria will dominate, produce Fe(II) and carbon dioxide in large quantities, and the resulting redox potential will be controlled by Fe(II) at or below approximately -100 mV. A third example is methane producing archaea which typically coexist syntrophically with hydrogen producing bacteria that ferment organic material. If the hydrogen concentration increases too much, the decomposition of organic material by these bacteria stops. As methanogens produce methane from hydrogen and carbon dioxide, they remove hydrogen from the environment and the bacterial decomposition of organic material can continue.

Microbial decomposition and production of organic material depend on the sources of energy and electron acceptors present. Organic carbon, reduced inorganic molecules or hydrogen are possible energy sources in subterranean environments. During microbial oxidation of these energy sources the microbes use electron acceptors in a certain order according to Fig. 2. First oxygen is used, thereafter follows the utilisation of nitrate, manganese, iron, sulphate, sulphur and carbon dioxide. Simultaneously, fermentative processes supply the respiring microbes with hydrogen and short organic acids. As the solubility of oxygen in water is low and because oxygen is the preferred electron acceptor by many microbes utilising organic compounds in shallow groundwater, anaerobic reduced environments and processes usually dominate at depth in the subterranean environment. Microorganisms have the capability to reduce important groundwater components such as sulphate to sulphide and to produce and consume gases. The documented presence of a deep biosphere implies that relevant microbial reactions should be included in the performance assessment for a HLW repository.

3.3. The hydrogen dependent subterranean biosphere hypothesis

Groundwater at depths of 500 m in Sweden can be very old and ages of 10 000 years are not unusual. This poses a conceptual problem for the deep subterranean biosphere. What ultimate

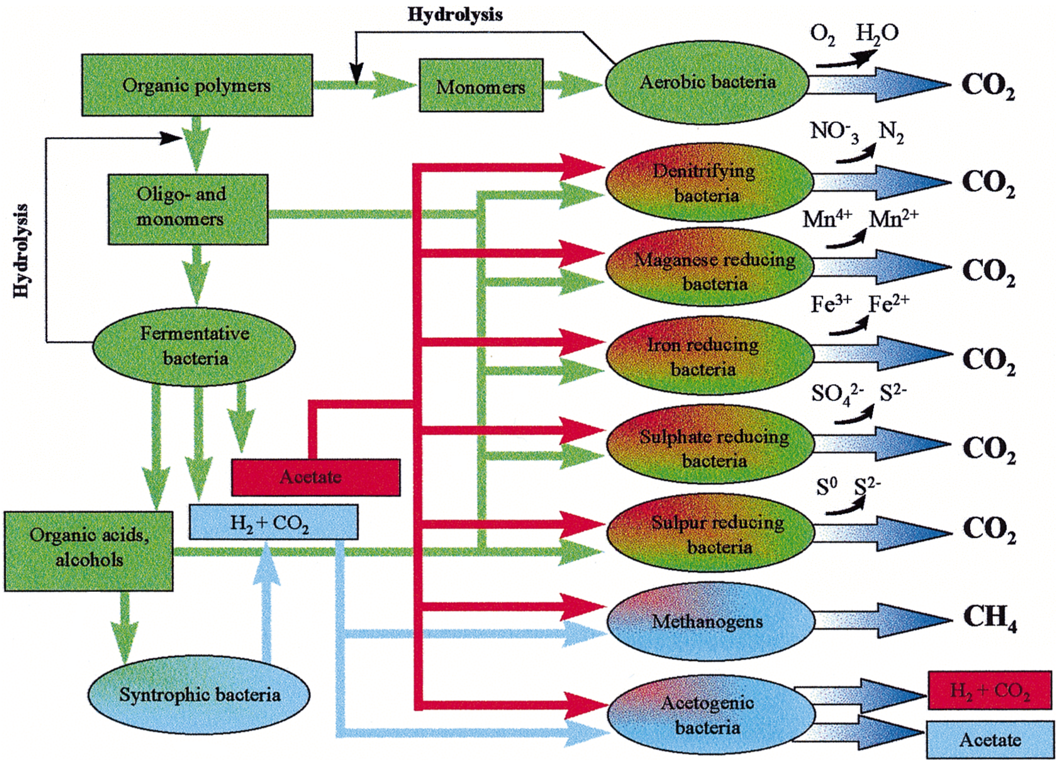


Fig. 2. The degradation of organic carbon can occur via a number of different metabolic pathways, characterised by the principal electron acceptor in the carbon oxidation reaction. A range of significant groundwater compounds is formed or consumed during this process. Of great importance for HLW disposal is the production of hydrogen sulphide, a potential copper corrodant and the turnover of gases such as carbon dioxide, hydrogen and methane.

energy source is it using? Organic carbon from the sun-driven surface ecosystem would not last for so long. Typical values at depth are one or a couple of milligrams dissolved organic carbon per litre groundwater (Pedersen and Karlsson, 1995). Any energy source at this depth must be renewable. Throughout our work results have indicated the presence of autotrophic microorganisms in the studied deep granitic rock environments that utilise hydrogen as a source of energy. Therefore, a hypothesis of a hydrogen driven biosphere in deep granitic aquifers, described in Fig. 3, has been suggested (Pedersen, 1993b, 1997a; Pedersen and Albinsson, 1992). The organism base for this biosphere is suggested to constitute acetogenic bacteria that have the capability of reacting hydrogen with carbon dioxide to acetate (homoacetogens) and methanogens that yield methane from hydrogen and carbon dioxide (autotrophic methanogens) or from acetate produced by homoacetogens (acetoclastic methanogens). A similar hypothesis has been published for deep basaltic rock aquifers (Stevens and McKinley, 1995). One of the aims of our recent studies has, therefore, been to collect evidence for a hydrogen driven deep biosphere in deep granitic aquifers, and has focused on acetogenic bacteria and methanogens as the autotrophic base for such a biosphere. Distribution, numbers and physiological diversity of homoacetogens and methanogens in deep granitic rock aquifers at the Äspö HRL were investigated using a variety of methods. The results showed that methanogens and homoacetogens are present and are metabolically active in the Äspö HRL groundwaters at depths down to 450 m (Kotelnikova and Pedersen, 1997, 1998). Pure cultures of autotrophic, rod-shaped methanogens were isolated and one of them could be described

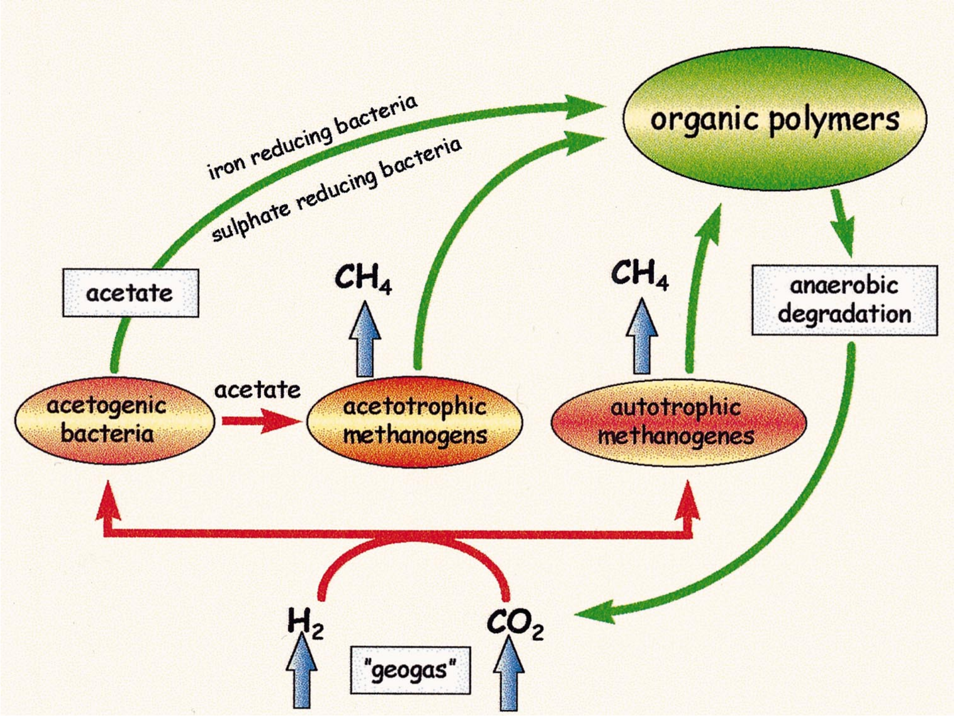


Fig. 3. The deep hydrogen driven biosphere hypothesis, illustrated by its carbon cycle. At relevant temperature and water availability conditions, subterranean microorganisms theoretically are capable of performing a life cycle that is independent of sun-driven ecosystems. Hydrogen and carbon dioxide from the deep crust of earth or organic carbon from sedimentary deposits can be used as energy and carbon sources. Phosphorus is available in minerals like apatite and nitrogen for proteins, nucleic acids, etc. can be obtained via nitrogen fixation; this gas is predominant in most groundwaters (see Table 1 later).

as a new species *Methanobacterium subterraneum* (Kotelnikova et al., 1998).

3.4. The deep hydrogen driven biosphere

Until recently, it has been a general concept that all life on Earth depends on the sun via photosynthesis; including most of the geothermal life forms found in deep sea trenches as they use oxygen for the oxidation of reduced inorganic compounds (almost all oxygen on earth is produced via photosynthesis). Our results now suggest that a deep subterranean granitic biosphere exists, driven by the energy available in hydrogen formed through radiolysis, mineral reactions or by volcanic activity (Fig. 3). Knowledge about this biosphere has just begun to emerge and is expanding the spatial borders for life from a thin layer on the surface of the planet Earth and in the seas to a several kilometre thick biosphere reaching deep below the ground surface and the sea floor. If this theory holds, life may have been present and active deep down in Earth for a very long time and it cannot be excluded that the place for the origin of life was a deep subterranean igneous rock aquifer environment (probably hot with a high pressure) rather than a surface environment. A HLW repository will be situated in a subterranean biosphere that is independent of solar energy and photosynthetically produced organic carbon. The only ultimate limitation will be the availability of hydrogen over time.

3.4.1. Performance assessment relation for the deep subterranean biosphere

The HLW environment and the surrounding rock will not be sterile. Microorganisms will, at various rates, be active in biogeochemical processes of which several do not occur without them. They may influence the performance of a HLW repository in negative, neutral and positive ways. Six major influence areas are discussed below.

4. Microbial production and consumption of gases

4.1. Dissolved gas in groundwater

The content of gases in deep granitic groundwater that can be produced or consumed by microorganisms has been analysed by us earlier, but the method used did not separate hydrogen from helium (Table 1). The deep biosphere hypothesis depends totally on the presence of hydrogen in deep groundwater. We have, therefore, recently invested significant work in the measurement of dissolved gases in deep granitic rock environments, now including hydrogen. The technique developed is described in detail by Pedersen (1997c). Recent results on dissolved gas, analysed with this method, have been obtained for some boreholes in the Äspö tunnel and the results are presented in Table 1. It can be seen that the total amount of gas found differs at most three times between boreholes and sites. The sensitivity and reproducibility of the new gas extraction and analysis methods are good and hydrogen could be detected. Each of the gases found is discussed in more detail below, from a microbiological perspective.

Nitrogen is by far the most dominant gas in all samples analysed (Table 1). Some of the nitrogen may have been dissolved from air in rain and surface waters that become groundwater with time, but the solubility of nitrogen at 10°C and atmospheric pressure is 19.6 ml l^{-1}. Most of the nitrogen values in Table 1 exceed this solubility limit and other sources of dissolved nitrogen in groundwater must exist as well. Nitrogen can be used by nitrogen fixing bacteria as a source of nitrogen and many produce nitrogen from nitrate during

Table 1
The content of nitrogen, hydrogen, helium and carbon-containing gases and the total volumes of gas extracted from groundwater samples of the Stripa borehole V2, the Laxemar borehole KLX01 and the Äspö boreholes KR0012, 13 and 15 (Pedersen, 1993b; Pedersen and Ekendahl, 1992a,b), KA3005, KA3010 and KA3110 (Pedersen, 1997c)

Boreholes	Sampling depth (m)	N_2 (μl l^{-1})	H_2 (μl l^{-1})	He (μl l^{-1})	CO (μl l^{-1})	CO_2 (μl l^{-1})	CH_4 (μl l^{-1})	C_2H_6 (μl l^{-1})	C_2H_{2-4} (μl l^{-1})[a]	Total gas (μl l^{-1})
Stripa										
V2	799–807	25000	n.a.[b]	<10	<1	32	245	0.3	<0.1	25277
V2	812–821	31000	n.a.	<10	<1	11	170	0.6	<0.1	31181
V2	970–1240	24500	n.a.	<10	<1	10	290	2.9	<0.1	24803
Laxemar										
KLX01	830–841	46500	n.a.	4600	0.5	460	26	<0.1	<0.1	51586
KLX01	910–921	37000	n.a.	3500	0.1	500	27	<0.1	<0.1	41027
KLX01	999–1078	18000	n.a.	2450	0.7	1600	31	<0.1	<0.1	22082
Äspö HRL										
KR0012	68	22000	n.a.	40	0.1	6050	1030	<0.1	<0.1	29120
KR0013	68	25000	n.a.	110	0.2	9640	1970	<0.1	<0.1	36720
KR0015	68	22000	n.a.	64	0.1	15037	4070	<0.1	<0.1	41171
KA3005/2[c]	400	25930	1.68	1757	<1	1082	1715	<0.1	<0.1	32300
KA3005/4	400	26661	0.11	3809	<1	2100	1849	<0.1	<0.1	34419
KA3010/2	400	40626	30.96	7946	1.4	142	55	<0.1	<0.1	48801
KA3110/1	414	14861	14.50	448	<1	1832	925	<0.1	<0.1	18080

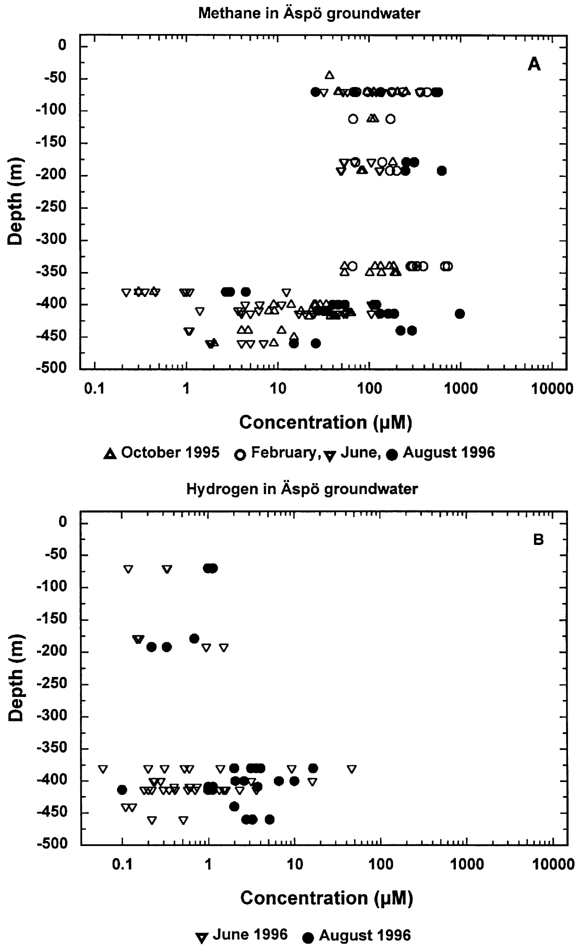
[a] The content of $C_2H_2+C_2H_4$.

[b] Not analysed.

[c] Number after slash denotes sampled borehole section.

an anaerobic respiration process called denitrification. Microbial processes could contribute to the pool of dissolved nitrogen in groundwater through denitrification processes, but it is unknown if this occurs in sufficient amounts to explain the excess of dissolved nitrogen. Helium is a noble gas and is not produced or consumed by microorganisms.

All living and active organisms expel carbon dioxide from their degradation of organic material and many microorganisms and all plants and algae can transform carbon dioxide to organic carbon. The concentration of this gas may, therefore, be influenced by microorganisms which may affect the carbonate system, pH and mineral precipitation and dissolution. The suggested deep biosphere hypothesis requires hydrogen as its energy base. Hydrogen is expected to act as an inert gas in most geochemical systems and it is therefore usually overlooked and not analysed. Some data on hydrogen in hard rock were published earlier (Sherwood Lollar et al., 1993a,b). From 2.2 up to 1574 µM hydrogen in groundwater from Canadian shield and Fennoscandian shield rocks was found. The origin of such hydrogen can vary. Most granitic rocks show a low but significant radioactivity which can generate hydrogen by radiolysis of water. Anaerobic mineral reactions (e.g. anaerobic corrosion of iron) will also create hydrogen (Stevens and McKinley, 1995). Finally, deep volcanic gases contain hydrogen. Screening the Äspö HRL groundwater for hydrogen with a simple 'closed bottle head space' method revealed significant amounts of hydrogen in most samples analysed (Fig. 4). All together, the gas analysis results (Table 1, Fig. 4) show that hydrogen is present and, consequently: there is an energy base available for the deep biosphere.


Methane in Äspö groundwater

Hydrogen in Äspö groundwater

Fig. 4. Data from repeated sampling expeditions on the concentration of hydrogen and methane in Äspö groundwater, measured in the head space of closed water samples and recalculated to dissolved hydrogen and methane.

Methane occurs frequently in subterranean environments all over the globe. Evidence for an ongoing methane generating process in deep Swedish granite has been published (Flodén and Söderberg, 1994; Söderberg and Flodén, 1991, 1992). Pockmarks in Baltic sea sediments were found, indicating gas eruption from fracture systems in the underlying granite, mainly of methane. From 1.3 up to 18576 µM of methane in groundwater from Canadian shield and Fennoscandian shield rocks have been published. Concentrations from 1 up to 181 µM of methane in Swedish groundwater have been published previously (Table 1). Recent data indicate up to 720 µM of methane down to 440 m depth at Äspö HRL (Fig. 4). The stable carbon isotope profile is commonly used as an indication of a biogenic origin of the methane. Some results on the $^{13}C/^{12}C$ signatures indicate biogenic origin of the Äspö methane (Banwart et al., 1996).

4.1.1. Performance assessment relation for microbial gas transformations

The deep biosphere organisms will tend to keep a HLW repository reduced as long as hydrogen is available. Organic material will be produced from carbon dioxide with hydrogen as the energy source and eventually degrade again to carbon dioxide. Oxygen that appears in a repository during closure or via radiolysis will probably be rapidly consumed by microorganisms that oxidise methane and hydrogen (and available organic material as well). Such microbial gas transformations may be beneficial for the HLW repository performance.

5. Microbial reducing activity

The present concept for disposal of nuclear fuel waste (Fig. 1) includes a scenario with oxygen trapped in bentonite clay, backfills, in pockets of the surrounding rock and dissolved in groundwater. This oxygen is detrimental for the copper canisters and will increase the mobility of several long-lived radionuclides. It has been calculated that oxygen will have disappeared within 300 years time due to inorganic reactions (Wersin et al., 1994). Several anaerobic microorganisms utilise hydrogen efficiently and many more can use hydrogen and methane if oxygen is available. Adding the catalytic abilities of microorganisms to the modelling of oxygen disappearance may therefore reduce the inorganic 300 years scenario to an organic scenario lasting not more than months or a couple of years.

The ability of microorganisms to buffer against an oxidising disturbance in bentonite, backfill and the deep environment is a rather overlooked possibility until now. The presence of an active and diversified microbiota at repository depths is well documented, as is the reducing capacity of microorganisms in surface environments. The major potential redox buffers are methane and organic carbon. Additionally, hydrogen, sulphide and ferrous iron can contribute but these compounds generally appear in much lower concentrations than methane and organic carbon.

A variety of bacteria, the methanotrophs, oxidise methane readily with oxygen, utilising it as an electron donor for energy generation and as a sole source of carbon. Most of these bacteria are aerobes and they are widespread in natural soils and water. They are also of a diversity of morphological types, seemingly related only in their ability to oxidise methane. They are found wherever stable sources of methane are present. There is some evidence that, although methane oxidisers are obligate aerobes, they are sensitive to oxygen and prefer microaerophilic habitats for development. They are therefore often found concentrated in a narrow band between anaerobic and aerobic zones were methane meets an oxygenated system. Such environments will be common in future repositories during the open phase and for some time after closure. Once established, this group of bacteria will be active as long as there is oxygen present for the oxidation of methane and they will most probably react all available methane with remaining oxygen after closure. Consequently, a deep repository will rapidly become anoxic after closure if methane is in excess. Recent DNA results on the microbial diversity during drilling of boreholes in the Äspö HRL tunnel revealed 16S rRNA sequences closely related to methanotrophs (Pedersen et al., 1996b). One molecule of methane (CH_4) contains eight electrons that can be used to reduce two molecules of oxygen (O_2). In the worst case scenario, there will be approximately 250 μM dissolved oxygen in groundwater close to the repository which can be balanced by 125 μM methane. Consulting Fig. 4 reveals that the standing concentration of methane in many groundwaters can reduce all oxygen assuming a 1:1 mixing ratio. The time needed for this process depends on the bacterial activity, but it will probably take much less than a year as most microbes work very fast when given a possibility to proliferate.

It has been demonstrated that microbial organic carbon oxidation is responsible for keeping shallow groundwater reduced (Banwart et al., 1996). It has also been shown (preliminary data from Linköping University, not published) that the content of TOC decreases with depth at Äspö from some 10–20 $\mathrm{mg\,l^{-1}}$ in shallow ground water down to about 1 $\mathrm{mg\,l^{-1}}$ at 400 m depth. The per cent humic and fulvic acids of TOC decreased from 90% to 30–40% and the per cent hydrophilic acids increased with depth. It was also found that the organic concentration correlated with the concentration of chloride. The traditional model on the fate of organic carbon suggests degradation of humic and fulvic acids with depth, but the deep biosphere hypothesis suggests that the organic carbon is produced at depth with hydrogen as the energy source. Except for humic substances, most of the organic carbon present will be readily oxidised if oxygen is present.

5.1. Performance assessment relation for microbial reducing activity

The presence of active microorganisms in a HLW repository cannot be avoided and may be very beneficial for obtaining reducing conditions rapidly there. Microbial activity will thereby have a positive influence on the performance of a HLW repository and reduce the risk for oxygenic copper corrosion of the canisters.

6. Microbial corrosion of copper

Corrosion is an important process to consider in the performance assessment of a radioactive waste repository for at least two reasons. The first is obvious; if canisters are used, they are an absolute barrier to radionuclide dispersal, for as long as they remain intact. Copper–steel canisters are considered in the present Swedish spent fuel concept and especially the outer copper canister is an important protective barrier. A second reason for an interest in corrosion is gas generation. Gaseous compounds are mainly of interest in performance assessment because, if generated at a high enough rate, they may form a separate gas phase that exerts a pressure on the construction and aids the dispersion of contaminants.

The only components of groundwater that will corrode copper are oxygen and sulphide ions. Oxygen reacts with copper to form copper oxides. Sulphide ions react to form copper sulphides and hydrogen. Microbial corrosion of copper was treated extensively by Pedersen and Karlsson (1995) and will not be discussed here. It was concluded that at least two limiting factors have to be considered: (1) the supply of substrate and (2) the question of whether the reaction of sulphate reduction can occur in the bentonite or only outside. Considering the first factor, recent studies have pointed to the possibility that other substances such as methane and hydrogen may act as electron donors in addition to organic material.

Direct and indirect evidence for the presence and activity of sulphate reducing bacteria in deep geological formations are reported by Laaksoharju et al. (1995) and by Pedersen et al. (1996b, 1997c). Obviously, the anticipated risk for sulphide production at disposal depths is relevant and must be accounted for in the safety performance assessment. Two scenarios must be assessed. In the first scenario, sulphate reducing bacteria will grow in the surrounding geological formation and produce hydrogen sulphide that must diffuse through the buffer to corrode the copper canister. This case has been thoroughly discussed by Pedersen and Karlsson (1995). It was concluded that if sulphide is generated by microbes outside the buffer, somewhere in the near-field, it will have to be transported to the buffer surface and diffuse to the canister. The transport resistance at the interface between buffer and flowing water in rock fractures was concluded to be efficient, and less corrosion will occur if the sulphide production occurs away from the canister surface compared with if it occurs in the buffer. In the second scenario, sulphate reducing activity will occur inside the bentonite and although the slow diffusion of hydrogen in compacted bentonite is an important barrier which will considerably limit sulphide production and corrosion, the actual sulphide production rate must be assessed.

In a first approach, a full scale nuclear fuel waste disposal container experiment was carried out 240 m below ground in the underground granitic rock research laboratory in Canada (Stroes-Gascoyne et al., 1997). An electric heater was surrounded by buffer material composed of sand and bentonite clay and provided heat equivalent to what is anticipated in a Canadian type nuclear fuel waste repository. During the experiment, the heat caused a mass transport of water and gradients of moisture content developed in the buffer ranging from 13% closest to the heater to 24% at the rock wall of the deposition hole. Upon decommissioning after 2.5 years, microorganisms could be cultured from all samples having a moisture content above 15%, but not in samples with a moisture content below 15%, corresponding to a water activity (a_w) of approximately 0.96. The results suggested that a nuclear fuel waste buffer will be populated by active microorganisms only if the moisture content is above a value where free water is available for active life, i.e. $a_w \geq 0.96$.

In a second approach, sodium bentonite (MX-80) was inoculated with two species of sulphate reducing bacteria and compacted to three different densities, 1.5, 1.8 and 2.0 g cm^{-3} by means of a hydraulic press and incubated at 30°C (Motamedi et al., 1996). These densities correspond to water activities of 1.0, 0.99 and 0.96, respectively. All samples were incubated at 30°C for 1 or 60 days. The amount of water available in the bentonite significantly influenced the survival of the studied sulphate reducing bacteria. Both strains were 100% non-viable after 1 day at the lowest a_w studied, 0.96. The dry conditions at this density of 2 g cm^{-3} effectively killed 100 million sulphate reducing bacteria per gram bentonite in less than 24 h. The best survival was observed in the bentonite with an a_w of 1.0, but the survival differed markedly between the species. About 10% of the initial population of *D. baculatum* survived for 60 days, but *Desulfovibrio sp* did not survive at all after this time. Limitation in nutrients and energy sources, accumulation of hydrogen sulphide and interference of the redox potential may add constraints to a closed batch system like the one used here. Therefore, this work has been followed by field experiments and additional laboratory tests that are in progress and will be reported towards the end of 1998.

6.1. Performance assessment relation for microbial corrosion

Microbial corrosion is an important process to consider in the performance assessment of a radioactive waste repository because the canisters used are an absolute barrier to radionuclide dispersal, for as long as they remain intact. Copper–steel canisters are considered in the present Swedish spent fuel concept, and especially the outer copper canister is an important protective barrier. Sulphide producing bacteria are present and active at HLW repository depth and possible places and extent of their production must be considered.

7. Microbial recombination of radiolysis products

In the event of high level waste (spent fuel) becoming exposed to groundwater, ionising radiation, for example alpha particles emanating from exposed HLW, can split the water molecules and thereby produce hydrogen and oxidising species (oxygen and hydrogen peroxide). The case of microbial recombination of radiolysis products was thoroughly discussed by Pedersen and Karlsson (1995) and will not be discussed here.

7.1. Performance assessment relation for recombination of radiolysis products

Microbial recombination of radiolysis products will add to inorganic processes and, as it is a catalytic process, microbial recombination will be continuous.

8. Microbial influence on radionuclide migration

Dissolution and transport with the groundwater is by far the most important migration mechanism for radionuclides, if released from an underground nuclear waste repository (Francis, 1990). Deep groundwaters in Swedish bed-rock are usually anoxic and reduced with a pH around 7. These factors are critical for safe function of a repository. This is because the mobility of many radionuclides depends on the pH and redox potential of the system and many of them take very insoluble forms at high pH and low redox potentials. The bed-rock surrounding a repository is expected to sorb escaping radionuclides in its porous matrix and thereby retard migration from the repository. The retardation may be negatively affected if there are particles or compounds in the groundwater that sorb radionuclides more strongly than the rock. This effect will increase with the concentration of particles and their ability to attract and bind the radionuclides. Consequently, the content of microorganisms and microbial complexing agents constitute important factors in the evaluation of how radionuclides may travel to the surface biosphere. The content of microorganisms and microbial complexing agents constitute important factors in the evaluation of how radionuclides may travel to the surface biosphere.

The presence of microorganisms can influence the groundwater transport of radionuclides in different ways. Free-living microorganisms constitute mobile suspended particles which may have a radionuclide sorbing capacity higher than that of the surrounding rock (Pedersen and Albinsson, 1991). Radionuclide transport will then proceed faster with than without microorganisms. On the other hand, if the majority of the microorganisms are growing in biofilms on fracture surfaces, transport of radionuclides may be reduced. Finally, microbial production of complexing agents and other metabolites can affect speciation and thus mobility of radionuclides independently of whether microorganisms are attached or not. Microbial production of complexing agents and other metabolites can affect speciation and thus mobility of radionuclides.

8.1. Performance assessment relation for microbial influence on radionuclide migration

Dissolution and transport with the groundwater is by far the most important migration mechanism for radionuclides, if released from an underground HLW repository. Microorganisms and microbial complexing agents may constitute important factors in the assessment of how radionuclides may travel to the surface biosphere.

9. Alkaliphilic microbes and concrete

Many radioactive waste repository concepts envisage the use of large quantities of cement and cement-based materials which will create environments with initial pH values of up to 13.5. The natural springs of the Maqarin area in NW Jordan contain highly alkaline groundwater, with pH values as high as 12.9, occurring within an organic-rich marl formation (Khoury et al., 1992). This environment is, therefore, regarded as a natural analogue for the study of processes that may take place in the hyperalkaline parts of low and intermediate radioactive waste repositories (Miller et al., 1994). An earlier investigation has indicated the presence of microorganisms in the alkaline groundwater of Maqarin by the use of culturing techniques for the assessment of numbers and types of bacteria (Coombs et al., 1994). A diversified microbial population was found with a pH tolerance in cultures up to the highest pH studied, pH 11. Pedersen et al. (1997a) describe how molecular methods, microscopy, culturing techniques and chemical analysis were used in an attempt to study the culturability and diversity of microbial populations detected in the hyperalkaline ground water of Maqarin. Microorganisms were found in all of the Maqarin groundwaters, but it could not be conclusively demonstrated that they were viable and growing in situ, rather than just transported there from neutral groundwater. The diversity of the microorganisms found was similar to what has been detected with the 16S rRNA gene sequencing method earlier, but none of the sequences found were typical for known alkaliphilic organisms. A possible hypothesis based on the obtained results is that the majority of the Maqarin springs investigated may be a bit too extreme for active life, even for the most adaptable microbe –but this remains to be demonstrated.

9.1. Performance assessment relation for alkaliphilic microbes and concrete

Some types of bacteria produce acids in their metabolism and may, therefore, be corrosive to concrete if they can survive the extreme pH.

References

Bachofen, R., 1997. Proc. 1996 Int. Symp. on Subsurface Microbiology (ISSM-96), Davos, Switzerland, 15–21 September, Bachofen, R. (Ed.), FEMS Microbiol. Rev. 20, 179–638.

Banwart, S., Tullborg, E.-L., Pedersen, K., Gustafsson, E., Laaksoharju, M., Nilsson, A.-C., Wallin, B., Wikberg, P., 1996. Organic carbon oxidation induced by largescale shallow water intrusion into a vertical fracture zone at the Äspö Hard Rock Laboratory (Sweden). J. Cont. Hydrol. 21, 115–125.

Coombs, P., Gardner, S.J., Rochelle, C.A., West, J.M., 1994. Interner Bericht 94-37, Nagra, Weetingen.

Ekendahl, S., Pedersen, K., 1994. Carbon transformations by attached bacterial populations in granitic ground water from deep crystalline bed-rock of the Stripa research mine. Microbiol. 140, 1565–1573.

Ekendahl, S., Arlinger, J., Ståhl, F., Pedersen, K., 1994. Characterization of attached bacterial populations in deep granitic groundwater from the Stripa research mine with 16S-rRNA gene sequencing technique and scanning electron microscopy. Microbiol. 140, 1575–1583.

Flodén, T., Söderberg, P., 1994. Shallow gas traps and gas migrations models in crystalline bedrock areas offshore Sweden. Baltica 8, 50–56.

Francis, A.J., 1990. Microbial dissolution and stabilization of toxic metals and radionuclides in mixed wastes. Experientia 46, 840–851.

Khoury, H.N., Salameh, E., Clark, I.P., Fritz, P., Milodowski, A.E., Cave, M.R., Bajjali, W., Alexander, W.R., 1992. A natural analogue of high pH cement pore waters from the Maqarin area of northern Jordan II: Introduction to the site. J. Geochem. Explor. 46, 117–132.

Kotelnikova, S., Pedersen, K., 1997. Edvidence for methanogenic Archaea and homoacetagenic *Bacteria* in deep granitic rock aquifers. FEMS Microbiol. Rev. 20, 339–349.

Kotelnikova, S., Pedersen, K., 1998. Distribution and activity of methanogens and homoacetogens in deep granitic aquifers at Äspö Hard Rock Laboratory, Sweden. FEMS Microbiol. Ecol. 26, 121–134.

Kotelnikova, S., Macario, J.L., Pedersen, K., 1998. *Methanobacterium subterraneum* sp. nov., a new alkaliphilic, eurythermic and halotolorant methanogen isolated from deep granitic groundwater. Int. J. Syst. Bacteriol. 48, 357–367.

Laaksoharju, M., Pedersen, K., Rhen, I., Skåman, C., Tullborg, E.-L., Wallin, B., Wikberg, W., 1995. Sulphate reduction in the Äspö HRL tunnel. SKB Technical Report 95-25. Swedish Nuclear Fuel and Waste Management Co., Stockholm.

Miller, W., Alexander, R., Chapman, N., McKinley, I., Smellie, J., 1994. Natural analogue studies in the geological disposal of radioactive waste. Studies in Environmental Science 57. Elsevier, London.

Motamedi, M., Pedersen, K., 1998. *Desulfovibrio aespoeensis* sp. nov.: a mesophilic sulphate-reducing bacterium from deep ground water at Äspö Hard Rock Laboratory, Sweden. Int. J. Syst. Bacteriol. 48, 311–315.

Motamedi, M., Karland, O., Pedersen, K., 1996. Survival of sulfate reducing bacteria at different water activities in compacted bentonite. FEMS Microbiol. Lett. 141, 83–87.

Pedersen, K., 1993a. The deep subterranean biosphere. Earth-Sci. Rev. 34, 243–260.

Pedersen, K., 1993b. Bacterial processes in nuclear waste disposal. Microbiol. Eur. 1, 18–23.

Pedersen, K., 1996. Investigations of subterranean bacteria in deep crystalline bedrock and their importance for the disposal of nuclear waste. Can. J. Microbiol. 42, 382–391.

Pedersen, K., 1997a. Microbial life in granitic rock. FEMS Microbiol. Rev. 20, 399–414.

Pedersen, K., 1997b. The microbiology of radioactive waste disposal. In: Wolfram, J.H., Rogers, R.D., Gazso, L.G. (Eds), Microbial Degradation Processes in Radioactive Waste Repository and in Nuclear Fuel Storage Areas. Kluwer Academic, Dordrecht, pp. 189–209.

Pedersen, K., 1997c. Investigations of subterranean microorganisms and their importance for performance assessment of radioactive waste disposal. Results and conclusions achieved during the period 1995 to 1997. SKB Technical Report 97-22. Swedish Nuclear Fuel and Waste Management Co., Stockholm.

Pedersen, K., Albinsson, Y., 1991. Effect of cell number, pH and lanthanide concentration on the sorption of promethium by *Shewanella putrefaciens*. Radiochem. Acta 54, 91–95.

Pedersen, K., Albinsson, Y., 1992. Possible effects of bacteria on trace element migration in crystalline bed-rock. Radiochim. Acta 58/59, 365–369.

Pedersen, K., Ekendahl, S., 1990. Distribution and activity of bacteria in deep granitic groundwaters of southeastern Sweden. Microb. Ecol. 20, 37–52.

Pedersen, K., Ekendahl, S.. 1992a. Incorporation of CO_2 and introduced organic compounds by bacterial populations in groundwater from the deep crystalline bedrock of the Stripa mine. J. Gen. Microbiol. 138, 369–376.

Pedersen, K., Ekendahl, S., 1992b. Assimilation of CO_2 and introduced organic compounds by bacterial communities in ground water from Southeastern Sweden deep crystalline bedrock. Microb. Ecol. 22, 1–14.

Pedersen, K., Karlsson, F., 1995. Investigations of subterranean microorganisms – their importance for performance assessment of radioactive waste disposal. SKB Technical Report 95-10. Swedish Nuclear Fuel and Waste Management Co., Stockholm.

Pedersen, K., Allard, B., Arlinger, J., Bruetsch, R., Degueldre, C., Hallbeck, L., Laaksoharju, M., Lutz, M., Pettersson, C., 1996a. Bacteria, colloids and organic carbon in groundwater at the Bangombé site in the Oklo area. SKB Technical Report 96-01. Swedish Nuclear Fuel and Waste Management Co., Stockholm.

Pedersen, K., Arlinger, J., Ekendahl, S., Hallbeck, L., 1996b. 16S rRNA gene diversity of attached and unattached groundwater bacteria along the Access tunnel to the Äspö Hard Rock Laboratory, Sweden. FEMS Microbiol. Ecol. 19, 249–262.

Pedersen, K., Arlinger, J., Hallbeck, L., Pettersson, C., 1996c. Diversity and distribution of subterranean bacteria in groundwater at Oklo in Gabon, Africa, as determined by 16S rRNA gene sequencing technology. Mol. Ecol. 5, 427–436.

Pedersen, K., Arlinger, J., Erlandson, A.-C., Hallbeck, L., 1997a. Culturability and 16S rRNA gene diversity of microorganisms in the hyperalkaline groundwater of Maqarin, Jordan. In: Pedersen, K. (Ed.), Investigations of subterranean microorganisms and their importance for performance assessment of radioactive waste disposal. Results and conclusions achieved during the period 1995 to 1997. SKB Technical Report 97-22. Swedish Nuclear Fuel and Waste Management Co., Stockholm, pp. 239–261.

Pedersen, K., Ekendahl, S., Tullborg, E.-L., Furnes, H., Thorseth, I.-G., Tumyr, O., 1997b. Evidence of ancient life at 207 m depth in a granitic aquifer. Geol. 25, 827–830.

Pedersen, K., Hallbeck, L., Arlinger, J., Erlandson, A.-C., Jahromi, N., 1997c. Investigation of the potential for microbial contamination of deep granitic aquifers during drilling using 16S rRNA gene sequencing and culturing methods. J. Microbiol. Met. 30, 179–192.

Sherwood Lollar, B., Frape, S.K., Fritz, P., Macko, S.A., Welhan, J.A., Blomqvist, R., Lahermo, P.W., 1993a. Evidence for bacterially generated hydrocarbon gas in Canadian shield and Fennoscandian shield rocks. Geochim. Cosmochim. Acta 57, 5073–5085.

Sherwood Lollar, B., Frape, S.K., Weise, S.M., Fritz, P., Macko, S.A., Welhan, J.A., 1993b. Abiogenic methanogenesis in crystalline rocks. Geochim. Cosmochim. Acta 57, 5087–5097.

Söderberg, P., Flodén, T., 1991. Pockmark development along a deep crustal structure in the northern Stockholm Archipelago, Baltic Sea. Beitreiben Meereskunde 62, 79–102.

Söderberg, P., Flodén, T., 1992. Gas seepages, gas eruptions and degassing structures in the seafloor along the Strömma tectonic lineament in the crystalline Stockholm Archipelago, east Sweden. Continental Shelf Res. 12, 1157–1171.

Stevens, T.O., McKinley, J.P., 1995. Lithoautotrophic microbial ecosystem in deep basalt aquifers. Science 270, 450–453.

Stroes-Gascoyne, S., Pedersen, K., Daumas, S., Hamon, C., Jahromi, N., Haveman, S., Delaney, T.-L., Dekeyser, K., 1997. Occurrence and identification of viable microbes in compacted clay based buffer material designed for use in a nuclear fuel waste disposal vault. Can. J. Microbiol. 43, 1133–1146.

Wersin, P., Spahiu, K., Bruno, J., 1994. Time evolution of dissolved oxygen and redox conditions in a HLW repository. SKB Technical Report 94-02. Swedish Nuclear Fuel and Waste Management Co., Stockholm, pp. 1–32.