Working Report 2007-20

Microbiology of Transitional Groundwater of the Porous Overburden and Underlying Shallow Fractured Bedrock Aquifers in Olkiluoto, Finland

October 2005 – January 2006

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May 2007

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ABSTRACT

The subsurface biosphere on Earth appears to be far more expansive and metabolically and phylogenetically complex than previously thought. A diverse suite of subsurface environments has been reported to support microbial ecosystems, extending from a few meters below the surface to several thousand meters. The discovery of a deep biosphere will have several important implications for underground repositories for radioactive wastes. The main potential effects of microorganisms in the context of a KBS-3 type repository for spent fuel in the bedrock of Olkiluoto are:

- Oxygen reduction and maintenance of anoxic and reduced conditions.
- Bio-immobilisation and bio-mobilisation of radionuclides, and the effects from microbial metabolism on radionuclide mobility.
- Sulphate reduction to sulphide and the risk for copper sulphide corrosion.

The main objective of this study was to characterize the geochemistry, biomass and microbial diversity of shallow subsurface groundwater at Olkiluoto, from 4.0 m down to 14.9 m. This objective also permitted the determination of whether or not there is any transition in the shallow depths at Olkiluoto to microbial conditions associated with the deep subsurface.

This was the second investigation that covered both shallow and some moderately deep groundwater microbiology in Olkiluoto. The analysis of microbiology is very important for proper understanding of the evolution of geochemical processes in and around the underground research facility ONKALO being constructed at Olkiluoto by Posiva since autumn 2004, as well as for the planned KBS-3 type spent fuel repository at Olkiluoto. There are several conclusions from this investigation that are of importance for ONKALO. The following present day conclusions can be drawn. Continued investigations will update and test them.

- The shallow biosphere was dominated by oxygen consuming microorganisms that block oxygen migration to deeper groundwater. This effect was most pronounced during the summer and fall seasons.
- There was a clear seasonal variation in the populations of microorganisms in shallow Olkiluoto groundwater.
- The shallow groundwater of Olkiluoto was generally anaerobic all the way up to the water table in the fall 2005, but oxygenated to about 25 m depth in the spring 2004 season. This interesting trend will need to be confirmed by more sampling. Oxygen was present in 3 of the samples in 2005, though at a very low level.
- The data obtained suggest that the transient between the shallow and deep biospheres in olkiluoto occurs at a very shallow depth, typically at 15-25 m.

Keywords: Microbiology, shallow groundwaters, deep groundwaters

MAAKERROKSESSA JA KALLIOPERÄSSÄ LIIKKUVAN POHJAVEDEN MIKROBIOLOGISET TUTKIMUKSET OLKILUODOSSA LOKAKUUSSA 2005-TAMMIKUUSSA 2006

TIIVISTELMÄ

Kallioperän biosfääri näyttää olevan paljon aikaisemmin luultua laajempi ja monimutkaisempi. Maanalaisia ympäristöjä muutamien metrien syvyydeltä aina useiden satojen metrien syvyyksiin on kuvattu mikrobiologisten ekosysteemien tukena. Syvän biosfäärin löytyminen vaikuttaa useilla tärkeillä tavoilla käytetyn ydinpolttoaineen loppusijoitustilan rakentamiseen. Tärkeimmät mikrobiologiset vaikutukset Olkiluodon kallioperään rakennettavaan KBS-3 tyyppisen loppusijoitustilan ominaisuuksiin ovat:

- hapen pelkistyminen ja aerobisten sekä pelkistävien olosuhteiden säilyminen
- radionuklidien bio-kiinnittyminen ja –liikkuminen sekä mikrobiologisen aineenvaihdunnan vaikutus radionuklidien liikkuvuuteen
- sulfaatin pelkistyminen sulfidiksi ja kupari-sulfidi korroosion mahdollisuus

Tämän tutkimuksen päätavoitteena oli Olkiluodon kallion pinta osissa ja maaperässä liikkuvan pohjaveden geokemiallisen ja mikrobiologisen moninaisuuden tutkiminen. Pohjavesinäytteet kerättiin syvyysväliltä 4 m-14.9 m. Tämän tutkimuksen avulla voitiin määrittää myös mahdollisia mikrobiologisia muutoksia siirryttäessä matalista kalliopohjavesistä syviin kalliopohjavesiin.

Tämä on toinen tutkimus, jossa tutkitaan samanaikaisesti sekä matalien että syvien pohjavesien mikrobiologiaa. Mikrobiologisten tutkimusten tekeminen ja tulosten tulkinta on erittäin tärkeää, jotta pohjaveden geokemiallisia prosesseja ymmärretään riittävästi niin rakennettavan tutkimustilan ONKALOn sisällä, että sen ympäristössä sekä myöhemmin varsinaisessa loppusijoitustilassa. Mikrobiologiaan liittyy useita johtopäätöksiä, jotka ovat erittäin tärkeitä ONKALOn rakentamisen ja myöhemmin itse loppusijoituslaitoksen kannalta. Nykyiset olettamukset on listattu seuraavassa ja niitä päivitetään sekä testataan mikrobiologisten tutkimusten edetessä.

- Matalien kalliopohjavesien alueella happea kuluttavat mikro-organismit dominoivat ja estävät hapen kulun syvemmälle kallioperään. Happea kuluttavien mikro-organismien toiminta korostuu etenkin kesällä ja syksyllä.
- Olkiluodon matalien kalliopohjavesien alueella oli selkeä vuodenajoista johtuva vaihtelu nähtävillä mikro-organismien populaatioissa.
- Olkiluodon matala kalliopohjavesi oli kokonaisuudessaan anaerobista syksyllä 2005, kun taas keväällä 2004 se oli hapettunut 25 metrin syvyydelle saakka. Tämä mielenkiintoinen trendi tulee kuitenkin varmentaa uusilla näytteenotoilla.

Vuonna 2005 happea esiintyi todella pieninä määrinä ainoastaan kolmessa näytteessä.

- Saatujen tulosten perusteella voidaan sanoa, että matalan ja syvän kalliopohjaveden muutosvyöhyke sijaitsee hyvin matalalla, tyypillisesti 15-20 metrissä.

Avainsanat: matala ja syvä kalliopohjavesi, microbitutkimus

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1 INTRODUCTION

The subsurface biosphere on Earth appears to be far more expansive and metabolically and phylogenetically complex than previously thought (Amend and Teske, 2005). A diverse suite of subsurface environments has been reported to support microbial ecosystems, extending from a few meters below the surface to thousands of meters underground (Pedersen, 2000). The discovery of a deep biosphere will have several important implications for underground repositories for radioactive wastes (Pedersen, 2002). The main effects of microorganisms in the context of a KBS-3 type repository for spent fuel in the bedrock of Olkiluoto are:

- Oxygen reduction and maintenance of anoxic and reduced conditions.
- Bio-immobilisation and bio-mobilisation of radionuclides, and the effects from microbial metabolism on radionuclide mobility.
- Sulphate reduction to sulphide and the risk for copper sulphide corrosion.

At Olkiluoto, investigations have been ongoing to establish the baselines of geochemical and microbial conditions in the subsurface prior to the construction of ONKALO (Haveman et al, 1998, 1999, 2000; Haveman and Pedersen, 2002). Those have mainly focused on the deep subsurface at depths down to about 900 m. A first investigation of nine shallow boreholes (4-24.5 m) in Olkiluoto was performed in spring 2004 (Pedersen, 2006). This shallow subsurface was found to be the transitional zone between an oxic and an anoxic environment. This zone contained dissolved oxygen in the groundwater and could be clearly distinguished from the deeper groundwater with respect to chemistry and microbiology. The shallow subsurface is expected to experience the greatest amount of disturbance during construction of the ONKALO underground research facility and later the construction of a spent fuel repository at Olkiluoto. This disturbance will likely alter the flow and composition of the surface recharge area and may subsequently impact conditions in the deep subsurface. Such disturbances must be researched and understood in order to fully assess the safety and performance of the repository.

The first investigation of shallow groundwater in Olkiluoto was performed during spring 2004, soon after snow melt and before the summer temperatures raised the temperature in the ground. The second shallow groundwater study reported here was preformed during the fall, in early October 2005. The main objective of this study was to continue to characterize the geochemistry, biomass and microbial diversity of shallow subsurface groundwater at Olkiluoto. It was also deemed important to explore if the situation in shallow groundwater during fall was different compared to the spring situation. To accomplish the objectives of the present study, five groundwater observation tubes in the overburden and five shallow boreholes in the bedrock at Olkiluoto were sampled. Chemistry, pH, the reduction-oxidation potential and oxygen concentration of the water samples were analyzed. Microbial biomass was investigated using acridine orange direct counts (AODC) giving the total numbers of cells, adenosine triphosphate (ATP) extractions for bio-volume and cultivable heterotrophic aerobic bacteria (CHAB) as a measure of aerobic biomass. Microbial metabolic diversity of the aquifers was investigated by determining the most probable number (MPN) of the following metabolic groups; nitrate reducing bacteria (NRB), iron reducing bacteria (IRB), manganese reducing bacteria (MRB), sulphate reducing bacteria (SRB),

autotrophic acetogens (AA) heterotrophic acetogens (HA), autotrophic methanogens (AM), heterotrophic methanogens (HM) and methane oxidizing bacteria (MOB).

2 MATERIALS AND METHODS

2.1 Groundwater observation tubes and shallow boreholes

2.1.1 Site descriptions

The sites sampled at Olkiluoto (PVP1, PVP4A, PVP13, PVP14, PVP20, PR1, PP2, PP9, PP36 and PP39) penetrated groundwater in either the overburden (PVP, groundwater observation tubes) or water conducting fractures in bedrock (PR, PP, shallow boreholes) **(**Figure 2-1, Table 2-1). Overburden extends down to approximately 13 m and is composed of sand and silt with an organic soil layer approximately 0.8 m thick. Bedrock groundwater samples extended from 4 m up to 15 m. The local bedrock at Olkiluoto is Precambrian in age, is composed of metamorphic rocks (dominantly migmatitic gneisses) and intruded by igneous rocks (pegmatitic granites). Local land use above the aquifers ranges from undisturbed forest to open areas cleared for repository construction.

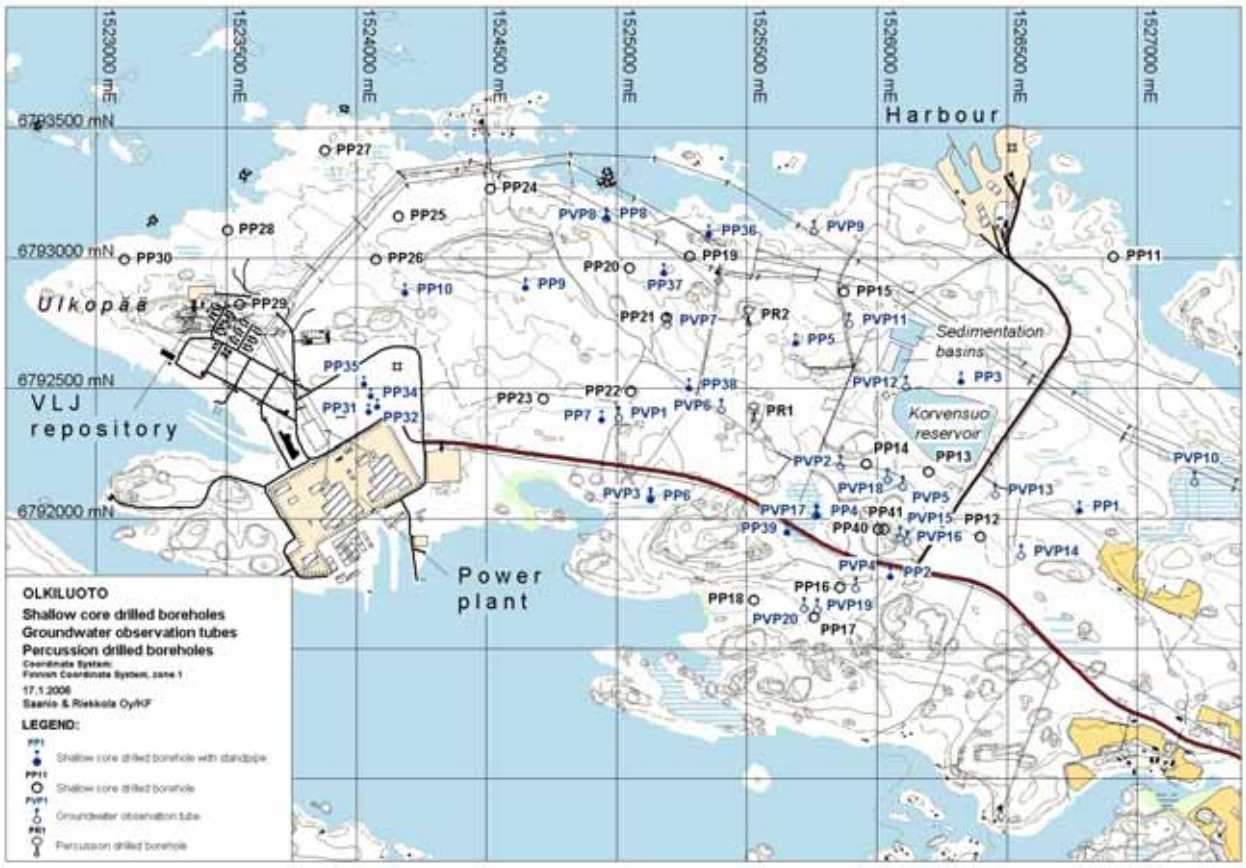

Figure 2-1. *Map showing the sampling points for groundwater observation tubes and shallow boreholes.*

Table 2-1. *Sampling data for the groundwater observation tubes and shallow boreholes. Boreholes equipped with the nitrogen packer (see 2.1.3) are indicated with (packer).*

Borehole	Measured borehole depth (m)	Pump and sampler level (m)	Date and time	Ground-water level (m)	Yield (L/min)	Notes
PVP1	3.90	2.50	2005-10-11/07.40	0.69	2.8	
			2005-10-11/09.50	1.19	2.4	brown water
			2005-10-11/12.10	1.01	2.0	sampling
PVP4A	10.20	5.0	2005-10-12/07.40	1.14	4.8	
(packer)			2005-10-12/09.55		4.5	
			2005-10-12/12.10			sampling
PVP13	5.60	4.0	2005-10-12/10.40	1.51	1.06	roily water
			2005-10-12/10.40	1.87	0.80	clear water
			2005-10-12/10.40	1.99	0.85	sampling
PVP14	9.0	5.0	2005-10-14/07.45	2.29	5.2	
(packer)			2005-10-14/08.50		5.2	
			2005-10-14/09.35		5.2	sampling
PVP20	12.80	5.0	2005-10-13/07.53	0.71	0.4	
(packer)			2005-10-13/09.20		0.4	
			2005-10-13/09.30			sampling
PR1	6.0	4.0	2005-10-10/10.35	3.47	4.8	
			2005-10-10/10.35	3.84	4.5	
			2005-10-10/10.35	3.85	4.5	clear water
			2005-10-10/10.35	3.78	4.5	sampling
PP2	14.90	5.0	2005-10-12/08.00	2.05	4.8	
			2005-10-12/08.40	3.93	4.8	
			2005-10-12/09.30			sampling
PP9	14.70	6.0	2005-10-13/12.25	1.31	0.43	
			2005-10-13/08.35	3.95	0.44	roily water
			2005-10-13/12.55	4.15	0.40	sampling
PP36	12.05	6.0	2005-10-10/09.30	4.31	7.2	
(packer)			2005-10-10/12.05			
			2005-10-10/13.05			clear water
			2005-10-10/13.40			sampling
PP39	14.10	6.0	2005-10-11/08.30	1.32	0.4	
(packer)			2005-10-11/10.00		0.4	roily water
			2005-10-11/12.50		0.4	sampling

2.1.2 Field measurements

All sample sites were pumped with an immersed borehole pump at least 1.5 h prior to any field measurements or sample retrieval (Table 2-1). The pump with tubing was sterilised for about 2 hours in an 10 ppm chlorine dioxide solution (XINIX FreeBact 20), in a 100 L plastic barrel. The pumps were soaked in the chlorine dioxide solution and the solution was also pumped through the tubing. Field measurements were performed in a 1 L container at the surface while groundwater was being pumped. The measurements and sampling for chemistry were preformed at the end of the pumping period (Table 2-1). The temperature conditions in the groundwater was measured with a pIONeer 10 portable pH meter equipped with a pHC5977 cartrode-combined pH electrode, pH range 0 – 14 pH (±0.5 at zero) temperature range -10 – 110 C (±0.3 C) (Radiometer, Labora, Stockholm). REDOX was measured with a pIONeer 10 portable pH meter equipped with a MC3187Pt-combined platinum electrode with an Ag/AgCl reference system, range -2000 – 2000 mV (±0.01 % of reading) (Radiometer). Dissolved oxygen concentration was measured with two different electrodes: 1) a pIONeer 20 portable oxygen meter equipped with a DOX20T-T oxygen probe with a concentration range 1-20 mg/L (0-200% ±1%) (Radiometer), 2) a HQ10 Hach Portable LDO^{tm} Dissolved Oxygen Meter, Cat No. 51815-00 (HACH). The probes were calibrated *in situ* as per manufacturer's instructions.

2.1.3 Packer control tests with nitrogen

During the spring 2004 sampling, oxygen was detected in all groundwater observation tubes and shallow boreholes. It was not possible to determine if the sampling procedure introduced this oxygen, or if it actually was present in the groundwater that entered the groundwater observation tubes or the boreholes. Therefore, an inflatable packer that allowed passage of sampling tubes plus the wire for the pump was constructed. Nitrogen was flushed through half of the number of boreholes that was sampled at a rate of about 1 Litre nitrogen gas per minute. The flushing was started before pumping. In that way, the gas above the water level was replaced with nitrogen during the lowering of the groundwater level that occurred when pumping (Table 2-1). Thereby, oxygen could not enter the sampled groundwater from the atmosphere in the borehole or the groundwater observation tube.

Figure 2-2. *Groundwater from PP36 is transferred from the SOLINST sampler to various microbiology analysis tubes by the team from Microbial Analytics Sweden AB.*

2.1.4 Sample collection

Groundwater samples were collected using a SOLINST Model 425 Discrete Interval Sampler (Solinst Ltd., Canada) immediately after the pumping period was finished and the pump was hoisted up from the sampling point (Figure 2-2). The sampled depth coincided with the depth of the borehole pump (Table 2-1). Two different diameter samplers (26 mm and 51 mm) were used depending on the diameter of the observation tube or borehole used. Prior to sampling, all of the exterior and interior fittings of the SOLINST were sterilized with a 20 ppm chlorine dioxide solution (XINIX FreeBact 20) and rinsed with sterile, autoclaved MilliQ water to eliminate microbial contamination to groundwater. To collect *in situ* groundwater from the required depth interval, the sampler was kept pressurized to 2 bars with N_2 gas until it was at depth, then de-gassed (vented at the surface) allowing the ambient water in, and lastly re-pressurized once the sampler was full prior to surface retrieval. Water from the sampler was then dispensed to the various containers for the analyses described below. Pressurizing the sampler to a pressure that was double or more compared to the highest water pressure experienced by the sampler ensured that the sampler was closed until it reached the sample depth.

2.1.5 Sterilisation control of borehole pumps

The sterilisation procedure of the pumps was tested by pumping of sterile water after a completed sterilisation. The pumps were soaked as described above for 2 hours. The barrel was washed with 500 ml MilliQ water. The adapter used for microbiology sampling was installed on the orifice of the pump tube. 2 L MilliQ was then pumped

through the system. Thereafter 10 L MilliQ was filled in the barrel and pumped (1 L min^{-1}) out to waste through the sampling adapter. The adapter was removed after two minutes and the remaining 8 L were pumped out (8 L min^{-1}). This procedure simulated the pumping of a borehole before starting the sampling (See Table 2-1). The adapter was flushed with 1 L sterile MilliQ water and it was then mounted again. Finally, 5 L sterile MilliQ water was pumped through the tubing, after which a full sampling for microbiology was performed according to the procedures used in the field.

2.1.6 Comparison of sampling with the SOLINST sampler or with the borehole pump

During sampling of overburden holes it was noticed that the water sampled with the SOLINST sampler in some cases was slightly more turbid than the water sampled with the borehole pump. This effect was not observed in the bedrock holes. It was assumed that the hoisting up of the pump and the lowering of the SOLINST sampler might have caused some hydrodynamic disturbance that increased the concentration of suspended material in the borehole. Therefore, a comparison was made in the PVP20 groundwater observation tube between sampling for microbiology, first with the borehole pump and thereafter with the SOLINST sampler.

2.1.7 Groundwater chemistry

Water samples were transferred from the investigation site to the TVO (Teollisuuden Voima Oy) laboratory directly after sampling. The chemical analyses were performed by TVO (Teollisuuden Voima Oy) laboratory according to their protocols, or as a subcontracting from external laboratories.

Groundwater samples for laboratory analysis were collected during pumping prior to stop of the pump (Table 2-1) in a 5 L plastic canister (Br^-, Cl^-, F^-, SO_4^{2-}, S_{tot}, pH and conductivity), 1 L glass bottles (alkalinity, acidity, DIC/DOC) and 1 L acid-washed (nitric acid) glass bottles for metals. Groundwater samples for sulphide analysis were collected into three Winkler bottles (100 ml). Water samples were partly filtered with a membrane filter (0.45 µm), bottled, and preserving chemicals were added to part of the samples according to Table 2-2. Analysis methods, detection limits and uncertainties of the measurements are presented in Table 3-3.

2.1.8 Winkler titration of oxygen

Oxygen was analysed in the laboratory using a modified Winkler method as described elsewhere (Carritt and Carpenter, 1966).

Table 2-2. *Pre-treatment of the groundwater samples.*

Parameter	Container (L)	N_2- shielding / Filtering	Preserving chemicals and details	Laboratory
Conductivity, density, pH, NH_4	1 x 0.5 HDPE	-/-	-	TVO
Alkalinity, Acidity	1 x 0.5 Duran bottle	x/x	Sampling with titration sampler.	TVO
S^{2-}	3 x 0.1 measuring bottle	x/x	0.5 mL $Zn(Ac)_2$ and 0.5 mL 0.1 M NaOH. Sampling with sampler.	TVO
Cl, Br, SO_4, S_{tot}	1 x 0.25 HDPE	-/x		TVO
F	1 x 0.25 HDPE	-/x		TVO
Fe^{2+}, Fe_{tot}	6 x 0.05 measuring bottle	x/x	Addition of Ferrozine reagent to Fe^{2+} samples in nitrogen atmosphere. Sampling with sampler.	TVO
Sodium fluorescein	1 x 0.05 measuring bottle	-/x		TVO
DIC/DOC	1 x 0.05 brown glass bottle	-/x	Sampling with sampler.	TVO
Na, Ca, K, Mg, Fe, Mn, SiO_2	1 x 0.25 PE, acid washed	-/x	2.5 mL conc. HNO_3 / 250 mL	TVO
PO_4	1 x 0.25 HDPE	-/x	2.5 ml 4M H_2SO_4/ 250 ml	TVO
Sr, B_{tot}	1 x 0.1 HDPE, acid washed	-/x	1 mL suprapur HNO_3 / 100 mL	VTT
N_{tot}, NO_2, NO_3	1 x 0.25 HDPE	-/x		Rauman ymp.lab.
H-2, O-18	1 x 0.125 Nalgene bottle	-/-	Bottle is filled to the brim.	GTK
H-3	1 x 0.25 glass bottle	-/-		The Netherlands
C-13 / C-14	1 x 0.2 brown class bottle	-/x		Uppsala
Sr-87 / Sr-86	1 x 0.125 Nalgene, acid washed	-/-		GTK
Rn-222	1 x 0.01 Ultimagold solution bottle	-/-	Sampling time is written down.	STUK
S-34 (SO_4), O-18 (SO_4)	1 x Nalgene	-/-	Sample volume depends on SO_4-concentration. $Zn(Ac)_2$ is added to sample according to Posiva water sampling quide.	Waterloo
Uranium, U-238	1 x 1 PE, HCl-washed	-/x	50 mL conc. HCl / 1L. Filters are sent to HYRL for analyses.	HYRL
Uranium, U-234/U-238	1 x 1 PE, HCl-washed	-/x	50 mL conc. HCl / 1L. Filters are sent to HYRL for analyses.	HYRL

PE = Polyethylene, HDPE= High density polyethylene

Laboratories:

TVO	Teollisuuden Voima Oy
VTT	VTT Technical Research Centre of Finland
Rauman ymp.lab.	Rauman ympäristölaboratorio
Uppsala	Ångström-laboratory, University of Uppsala, Sweden
GTK	Geological Survey of Finland
Waterloo	Environmental Isotope Lab, University of Waterloo, Canada
The Netherlands	Centre for isotope research, Groningen, The Netherlands
STUK	Radiation and Nuclear Safety Authority, Helsinki, Finland
HYRL	Department of Radiochemistry, University of Helsinki, Finland

***Table 2-3**. Methods and detection limits for groundwater chemistry*

PARAMETER	APPARATUS AND METHOD	DETECTION LIMIT	UNCERTAINTY OF THE MEASUREMENT
pH	pH meter ISO-10532		± 0.05
Conductivity	Conductivity analyser SFS-EN-27888	5 µS/cm	5%
Density	Posiva water sampling guide /1		± 0.001 g/cm^3
Sodium fluorescein	Fluorometry	1 µg/l	15 µg/l: 0.8% 200 µg/l: 1.2% 275 µg/l: 0.4%
Alkalinity	Titration/Posiva water sampling guide /1	0.05 mmol/L	± 5%
Acidity	Titration/Posiva water sampling guide /1	0.05 mmol/L	± 10%
DOC/DIC	SFS-EN 1484	0.1 mg/L	
Fe_{tot}	Spectrophotometry/ Posiva water sampling guide /1	0.01 mg/L	± 5%
Fe^{2+}	Spectrophotometry/ Posiva water sampling guide/1	0.01mg/L	± 5%
Fe_{tot},, Mn	ICP/OES	0.002 mg/L	
K, Na		0.5 mg/L	
SiO_2		0.01 mg/L	
Ca		0.1 mg/L	
Mg		0.02 mg/L	
Sr	ICP-MS	0.5 µg/L	
B_{tot}		2 µg/L	
Cl	Titration/Posiva water sampling guide/1	5 mg/L	± 2.5%
Br	IC, conductivity detector. SFS-EN ISO 10304-1	0.5 mg/L	± 4.2%
F	ISE/ Posiva water sampling guide/1	0.1 mg/L	± 5%
PO_4	Spectrophotometer SFS-EN 1189	0.012 mg/L	±24%
S^{2-}	Spectrophotometer SFS 3038	0.01 mg/L	0.07 mg/L: 36% 0.17 mg/L: 17% 0.53 mg/L: 10%
SO_4	IC, conductivity detector. SFS-EN ISO 10304-1	1.25 mg/L	±3,2%
S_{tot}	H_2O_2 oxidation +IC	0.2 mg/L	
NH_4	Spectrophotometer SFS 3032	0.002 mg/L	± 4%
Total nitrogen, N_{tot}	HPLC SFS3031	0.20 mg/L	
Nitrate, NO_3	HPLC Internal method n:o 10	3.0 mg/L	3.0-5.0 mg/L: 12% >5.0 mg/L: 7%
Nitrite, NO_2	Spectrophotometer SFS3029:1976	0.010 mg/L	0.010-0.10 mg/L: 10% >0.10 mg/L: 8%
^{18}O	MS		< 0.1‰
^{18}O (SO_4)	MS		0.5‰

***Table 2-4** (continued). Methods and detection limits for groundwater chemistry*

^{3}H	Electrical enrichment + home made Proportional Gas counter (PGC) detection method	0.2 TU	100±2, 20±0.5 and 1.00±0.10 TU
^{2}H	MS		1‰
^{13}C (DIC)	MS	0.3 pM	0.05‰
^{14}C (DIC)	AMS		0.1 pM
$^{86}Sr/^{87}Sr$	MS		0.003‰
^{34}S (SO_4)	MS	0.1 mBq/L	0.2‰
Rn-222	Liquid scintillation counting / 2		5-10%
U(tot) ja U-234/U-238	Alfaspectrometer ASTM D3648-95, 1995	0.2 mBq/L	

References

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2 Salonen L. and Hukkanen H., Advantaged of low-background liquid scintillation alpha-spectrometry and pulse shape analysis in measuring 222Rn, uranium and 226Ra in groundwater samples, Journal of Radioanalytical and Nuclear Chemistry, Vol 226, Nos 1-2, 1997.

2.2 Deep boreholes

2.2.1 List of packer equipped boreholes

Three deeper, packer equipped boreholes were sampled as listed in Table 2-5.

***Table 2-5.** The sampled deep packer equipped boreholes and depths.*

Borehole	Sample date	Depth (m)
OL-KR-8	051025	77-84
OL-KR-32	060111	50-52
OL-KR-33	060114	95-107

2.3 Microbiological analyses

2.3.1 Biomass – total counts

Total cell numbers were determined using the acridine orange direct count method (AODC) (Hobbie et al, 1977; Pedersen and Ekendahl, 1990). The three first filled tubes were used. From each tube 1 ml were filtered onto a black polycarbonate filter (0.22 µm) and stained with 0.2 mL acridine orange solution (10 µL/ml) for six minutes and then rinsed with double distilled water (DDW). On each filter, 30 sight field areas of 10^4 μm^2 were counted using a Nikon inverted epifluorescence microscope with a blue filter (530 nm). Two filters were prepared per tube. The results were calculated from an average of six filters from three tubes for each groundwater sample.

2.3.2 Cultivable heterotrophic aerobic bacteria

A plate count medium was constructed based on earlier work (Pedersen and Ekendahl, 1990; Pedersen et al, 1997). It was prepared as follows: Per litre of medium: peptone,

0.5 g; yeast extract, 0.5 g; starch (soluble), 0.25 g; sodium acetate, 0.25 g; $CaCl_2$ x $2H_2O$, 0.2 g; K_2HPO_4, 0.1 g; NaCl, 10 g, trace element solution according to Table 3-6, 1 ml (Non-chelated trace element mixture); agar, 15 g. pH was adjusted to 7.0. The medium was autoclaved at 121 °C for 20 minutes, cooled to 50 °C and poured in 15 ml portions in sterile Petri dishes. The produced agar plates were allowed to dry for about 12 hours and then put in plastic bags until use. Dilution tubes were prepared as follows: 18 ml Hungate tubes were supplied with 10 ml the following solution and autoclaved: NaCl, 10 g; K_2HPO_4, 0.1 g.

Groundwater from the sampling points in Table 2-1 was diluted in 10 times increments. From each dilution tube, 0.1 ml was spread with sterile glass loops on an agar plate produced as described above.

Table 2-6. *Non-chelated trace element solution for cultivable heterotrophic aerobic bacteria.*

Component	Amount
Double distilled H_2O	987 mL
HCl (25%=7,7M)	12,5 mL
$FeSO_4*7H_2O$	2,1 g
H_3BO_4	30 mg
$MnCl_2*4H_2O$	100 mg
$CoCl_2*6H_2O$	190 mg
$NiCl_2*6H_2O$	24 mg
$CuCl_2*2H_2O$	2 mg
$ZnSO_4*7H_2O$	144 mg
$Na_2MoO_4*2H_2O$	36 mg

2.3.3 Biomass – ATP

Groundwater for ATP extraction and analysis was collected in triplicate from one sample tube and transferred into a sterile 15 mL Falcon centrifuge tube. Extraction and analysis of ATP concentrations were based on the methods by Lundin *et al*. (1986) and Lundin (2000). ATP was extracted from 100 µL aliquots of groundwater within an hour of collection using 100 µL BS-buffer (BioThema ATP Biomass Kit HS, Sweden). After extraction, 100 µL of the ATP extract was mixed with 400 µL of HS-buffer (BioThema ATP Biomass Kit HS, Sweden) in a FB12/Sirius luminometer (Berthold, Germany). The total volume of groundwater in the sample then became 50 µl. Prior to each sample measurement, light emission of the 400 µL HS-buffer without sample addition was allowed to diminish to less than 50 relative light units per second (rlu/s) in the luminometer. After addition of the ATP extracted sample, light emission was immediately determined using FB/Sirius PC Software quick measurement (Berthold, Germany). To account for attenuating effects from the sample on the enzymatic reactions (e.g. salinity, dissolved metals, sulphide), and to calibrate the luminometer readings, 10 µL of internal ATP-standard (0.1 µmole/L) was added to the extracted sample in the luminometer and light emission was measured again. All light emission

measurements were performed three times. The equation used to calculate ATP-concentration in the sample was based on the average of three measurements as follows:

$$\text{pmol ATP / sample} = \frac{I_{smp} - I_{bkg}}{I_{(std + smp - bkg)} - I_{(smp - bkg)}} \qquad \text{(eq. 1)}$$

Where:
I: *light intensity measured as relative light units*
smp: *sample*
bkg: *background with the HS buffer*
std: *standard*

The amount of ATP per ml groundwater was calculated as:

amol/ml groundwater = pmol ATP/sample (from eq. 1) x SF x DF (eq. 2)

Where:
SF: *shift factor from pmol to amol = 10^6*
DF: *dilution factor to obtain amount of ATP per ml =1000/50*

2.3.4 Preparation of media for most probable numbers of cultivable anaerobic microorganisms

Media for the most probable number of microorganisms (MPN) in groundwater from Olkiluoto were composed based on previously measured chemical data from the site. This allowed artificial media to be composed that most closely resembled *in situ* groundwater chemistry for optimal microbial cultivation (Haveman and Pedersen, 2002). Media for the metabolic groups of nitrate reducing bacteria (NRB), iron reducing bacteria (IRB), manganese reducing bacteria (MRB), sulphate reducing bacteria (SRB), autotrophic acetogens (AA), heterotrophic acetogens (HA), autotrophic methanogens (AM) and heterotrophic methanogens (HM) was autoclaved and dispensed according to the various compositions outlined in Table 3-7 in anaerobically into 27 mL anaerobic aluminium seal tubes (Bellco glass Inc. (www.bellcoglass.com), product number 2048-00150) with butyl rubber stoppers (product number 2048-117800) crimped with aluminium seals (product number 2048-11020).

All culture tubes were flushed with 80/20% N_2/CO_2 gas and then filled with 9 mL of their respective media. For IRB, 1 mL of hydrous ferric oxide (HFO) was added to each culture tube. The HFO was prepared from $FeCl_3$. The final concentration of the iron solution was 0,44 M. For MRB, 2 mL of 135 mM MnO_2 solution (Lovley and Phillips, 1986) was added. The HM media also contained 20 mL/L of 100 g/L NaCOO, 3 mL/L 6470 mM trimethylamine, 4 mL/L methanol and 20 mL/L of a 20g/L solution of $NaCH_3COO$. The HA medium also contained 20 mL/L of 100 g/L NaCOO, 3 mL/L 6470 mM trimethylamine and 4 mL/L methanol. The final pH was adjusted to between 7 to 7.5 with 1 M HCl or 1 M NaOH.

2.3.5 Inoculations and analysis for anaerobic microorganisms

Inoculations for NRB, IRB, MRB, SRB, AA, HA, AM and HM were performed in the laboratory within six hours from sample collection for all boreholes. After inoculating, the headspace of only AA and AM was added with H_2 to an overpressure of 2 bars and all MPN tubes were incubated in the dark at 20 °C for 8 weeks. Analysis of the MPN tubes after incubation was performed by detecting metabolic products. The production of nitrate was determined using a HACH DR/2500, The chromotrophic acid method 10020 for water and waste water (0.2 – 30 mg/L NO_3^--N). The production of ferrous iron by IRB was determined using a HACH DR/2500 spectrophotometer (HACH company, Loveland Colorado, USA) and the 1,10 phenanthroline method (method no. 8146). Ferrous iron at concentrations twice that in the un-inoculated control tubes were taken as positive for IRB. The HACH method 8034 based on periodate oxidation was used in a similar way to determine Mn^{2+} concentrations in MPN tubes for MRB. Detection of SRB was assessed by measuring sulphide production using the $CuSO_4$ method according to Widdel & Bak (1992) on a UV visible spectrophotometer (Ultraspec 2000, Amersham Pharmacia Biotech). Methanogens were detected by the production of CH_4 in the culture tubes headspace by gas chromatography. Acetogens were detected by acetate production using an enzymatic UV method (Enzymatic Bioanalysis kit, Boehringer Mannheim, Germany) using a UV visible spectrophotometer (as per SRB). The MPN procedure results in a scheme with tubes that score positive or negative growth. The results from the analyses were graded positive or negative in comparison to a control. Three dilutions were used to calculate the most probable number of each respective group according to the calculations found in Greenberg *et al.* (1992).

Table 2-7 A-G. *Composition of anaerobic media used for MPN cultivation of different metabolic groups of anaerobic microorganisms. All components were anoxic.*

A) Ready medium	**Metabolic group**[a]				
Component (mL/L)	**NRB**	**IRB / MRB**	**SRB**	**AA & HA**	**AM & HM**
Basal medium (Table B)	925	940	960	860	890
Trace elements (Table C)	1.0	1.0	1.0	--	--
Trace elements (Table D)	--	--	--	10	10
Vitamins (Table E)	1.0	1.0	1.0	--	--
Vitamins (Table F)	--	--	--	10	10
Thiamine stock (Table G)	1.0	1.0	1.0	1.0	1.0
vitamin B_{12} stock (Table G)	1.0	1.0	1.0	1.0	1.0
Fe stock (Table G)	-	--	--	5.0	5.0
Resazurin (Table G)	-	--	1.0	2.0	2.0
cystein hydrochloride (Table G)	-	--	--	10	10
bromoethansulfonic (Table G)	-	--	--	30	--
Selenite-tungstate (Table G)	-	--	1.0	--	--
$NaHCO_3$ (Table G)	30	30	30	60	60
Yeast extract (Table G)	1.0	1.0	--	10	10
$NaCH_3COO$ (Table G)	25	25	--	--	--
Lactate (Table G)	5.0	--	5.0	--	--
KNO_3 (G)	10				
Sodium sulphide (0.2 M)			7.5	10	10

[a]NRB=nitrate reducing bacteria, IRB = iron reducing bacteria, MRB = manganese reducing bacteria, AA = autotrophic acetogens, HA = heterotrophic acetogens, AM = autotrophic methanogens, HM = heterotrophic methanogens.

B) Basal medium	**Metabolic group**[a]			
Component (g)	**NRB, IRB & MRB**	**SRB**	**AA & HA**	**AM & HM**
Double distilled H_2O	1000	1000	1000	1000
NaCl	15	15	15	15
$CaCl_2*2H_2O$	1.0	0.1	0.28	0.28
KCl	0.1	0.5	0.67	0.67
NH_4Cl	1.5	0.25	1.0	1.0
KH_2PO_4	0.6	--	--	--
K_2HPO_4	--	0.2	0.17	0.15
$MgCl_2*6H_2O$	0.1	1.2	5.5	5.5
$MgSO_4*7H_2O$	0.1	--	6.9	6.9
$MnCl_2*4H_2O$	0.005	--	--	--
$Na_2MoO_4*2H_2O$	0.001	--	--	--
Na_2SO_4	--	4.0	--	--

[a]NRB=nitrate reducing bacteria, IRB = iron reducing bacteria, MRB = manganese reducing bacteria, AA = autotrophic acetogens, HA = heterotrophic acetogens, AM = autotrophic methanogens, HM = heterotrophic methanogens.

C) Trace element solution	
Component	**Amount**
Double distilled H_2O	1000 mL
Nitrilotriacetic acid	1500 mg
$Fe(NH_4)_2(SO_4)_2*6H_2O$	200 mg
Na_2SeO_3	200 mg
$CoCl_2*6H_2O$	100 mg
$MnCl_2*4H_2O$	100 mg
$Na_2MoO_4*2H_2O$	100 mg
$Na_2WO_4*2H_2O$	100 mg
$ZnSO_4*7H_2O$	100 mg
$AlCl_3$	40 mg
$NiCl_2*6H_2O$	25 mg
H_3BO_3	10 mg
$CuCl_2*2H_2O$	10 mg

D) Non-chelated trace element	
Component	**Amount**
Double distilled H_2O	987 mL
HCl (25%=7,7M)	12,5 mL
$FeSO_4*7H_2O$	2,1 g
H_3BO_3	30 mg
$MnCl_2*4H_2O$	100 mg
$CoCl_2*6H_2O$	190 mg
$NiCl_2*6H_2O$	24 mg
$CuCl_2*2H_2O$	2 mg
$ZnSO_4*7H_2O$	144 mg
$Na_2MoO_4*2H_2O$	36 mg

E) Vitamin mixture for NRB, IRB, MRB & SRB	
Component	**Amount**
Sodium phosphate buffer 10 mM pH 7.1	100 mL
4-Aminobenzoic acid	4 mg
D(+)-biotin	1 mg
Nicotinic acid	10 mg
Pyridoxine dihydrochloride	15 mg
Calcium D(+)-pantothenate	5 mg

F) Vitamin mixture for AA, HA, AM & HM	
Component	**Amount**
Sodium phosphate buffer 10 mM pH 7.1	100 mL
p-Aminobenzoic acid	10 mg
Nicotinic acid	10 mg
Calcium D(+)-pantothenate	10 mg
Pyridoxine dihydrochloride	10 mg
Riboflavin	10 mg
D(+)-biotin	5 mg
Folic acid	5 mg
DL-6-8-thiotic acid	5 mg

G) Stock solutions	
Component	**Amount**
$NaHCO_3$	84 g/L
Thiamine chloride dihydrochloride in a 25 mM sodium phosphate buffer, pH 3.4	100 mg/L
Cyanocobalamine (B_{12})	50 mg/L
KNO_3	1000 mg/L
$NaCH_3COO$	100g/L
Yeast extract	50 mg/L
$Fe(NH_4)_2(SO_4)_2*6H_2O$,initially dissolved in 0.1 mL concentrated HCl	20 g/L
Resazurin	500 mg/L
Cysteine-HCl	500 mg/L
Bromoethansulfonic (BESA)	35 g/L
Sodium lactate solution	50%

2.3.6 Preparation, inoculations and analysis of aerobic methane oxidizing bacteria

Sets of MPN tubes were performed for samples in a nitrate mineral salts (NMS) medium (Whittenbury et al, 1970). It was prepared as follows: Per litre of medium: KNO_3, 1.0 g; $MgSO_4$ x 7 H_2O, 1 g; $CaCl_2$ x 2 H_2O, 0.2 g; $CuCl_2$ x $2H_2O$; 1 mg; NaCl, 7 g, 1 ml iron solution made of 0.5 g of ferric (III) chloride in 1000 ml DDW; 0.5 ml trace element solution according to Table 3-8, 1 ml; phosphate buffer solution made of 3.6 g Na_2HPO_4 and 1.4 g Na_2HPO_4 in 1000 ml DDW; pH was adjusted to 6.8 - 7.0. Culture conditions were optimised to support the growth of both type I and II methane oxidizing bacteria (MOB) by adding 2 mg/L copper chloride dihydrate. This is because soluble and particulate methane monooxygenase (s/pMMO) common to all known MOB is controlled by a copper inducible regulatory pathway.

MPN inoculations were completed at the 450 m level in the MICROBE laboratory within an hour of sample collection for all boreholes. Five parallel dilution tubes were used for each dilution. All transfers were performed aseptically with new sterile syringes and needles. After each transfer, the tubes were vortexed to gain homogeneity. Control tubes contained nitrate minimal salt medium and 1 mL of filtered groundwater. After inoculation, a filter-sterilized (using 0.2-µm Millipore filters) methane was injected into the headspace of each tube, to 1 Bar overpressure. The tubes were then incubated horizontally in the dark at 20°C. Growth of cells was detected after between 2 and 4 weeks as judged from turbidity compared to negative controls and the concomitant production of CO_2 from methane oxidation in turbid tubes. MPN calculations were done by using a combination of positive tubes in a 3-tube dilution series (15 tubes) according to Greenberg *et al.* (1992). The detection limit was <0.2 cells/mL.

Table 2-8. *Trace element solution for methanotrophic bacteria.*

Component	Amount
DD-H_2O	1000 ml
Na_2 EDTA	500mg
$FeSO_4.7H_2O$	200 mg
$ZnSO_4$ x 7 H2O	10 mg
$MnCl_2x4H_2O$	3 mg
H_3BO_3	30 mg
$CoCl_2.6H_2O$	20 mg
$NiCl_2x6H_2O$	2 mg
$Na_2MoO_4x2H_2O$	3 mg
$CuCl_2*2H_2O$	1 mg

3 RESULTS

3.1 Groundwater observation tubes and shallow boreholes

3.1.1 Field measurements and chemistry

Groundwater retrieval went as expected for all the sites. Shallow groundwater field conditions in the overburden can be found in Table 3-1 and Table 4-2. Oxygen was below detection in all boreholes except for the groundwater observation tube PVP1, and shallow boreholes PP9 and PR1 that showed oxygen values close to the detection limit. These were also the boreholes or groundwater observation tubes with the highest reduction-oxidation (REDOX) potential. In addition PP36 also showed a similar high redox potential though with oxygen below detection limit. All five boreholes with the packer described in section 2.1.3 had oxygen below the detection limit Table 3-1 and Table 4-2. Three of the five boreholes without packer showed traces of oxygen. It seems plausible that some oxygen mixes with the groundwater during sampling in boreholes without the packer. However, more sampling should be performed with the packer to confirm this observation.

Table 3-1. *Field measurements in transitional groundwater of the porous overburden in Olkiluoto, Finland. Sampling was made 10th to 14th of October 2005.*

Measurement	PVP1	PVP4A	PVP13	PVP14	PVP20
Packer installed (see 2.1.3)	No	Yes	No	Yes	Yes
Depth (m)	3.90	10.20	5.60	9.0	12.80
pH in the field	4.95	7.04	7.10	7.19	7.10
Conductivity (µS/cm)	103	764	539	612	582
Temperature (°C)	11.9	9.3	9.8	6.4	7.6
O2 electrode (mg/L)	0.56	< 0.20	< 0.20	< 0.20	-
O2 Winkler (mg/L)	0.08	< 0.05	< 0.05	< 0.05	< 0.05
REDOX mV (E_h)	+356.9	+149.9	+152.5	+150	+136.7

Table 3-2. *Field measurements in transitional groundwater of fractured bedrock aquifers in Olkiluoto, Finland. Sampling was made 10th to 14th of October 2005.*

Measurement	PR1	PP2	PP9	PP36	PPP39
Packer installed (see 2.1.3)	No	No	No	Yes	Yes
Depth (m)	6.0	14.90	14.70	12.05	14.10
pH in the field	5.41	7.19	6.60	5.93	6.75
Conductivity (µS/cm)	121	806	274	224	1485
Temperature (°C)	11.2	7.3	9.9	9.9	12.6
O2 electrode (mg/L)	-	< 0.20	0.61	< 0.20	< 0.20
O2 Winkler (mg/L)	0.08	< 0.05	0.40	< 0.05	< 0.05
REDOX mV	+402	+121.2	+280.4	360.4	+130.5

PVP1 was a very shallow borehole with a brownish-yellow groundwater (Table 2-1) characterised by high a concentration of humic acids and a high concentration of iron (Table 4-4). The pH of the groundwater measured in the field was approximately neutral, with exception for PVP1, PR1 and PP36 that was lower than pH 6.0 (Table 4-1, Table 4-2). Laboratory pH measurements (Table 4-4, Table 4-5) agreed well with the field data. REDOX values ranged from a maximum of +402 mV in PR1 to a minimum of +121.2 mV in PP2. There was no obvious difference in the REDOX potential between overburden and bedrock groundwater. The conductivity ranged from low values at about 100 µS/cm up to 800 µS/cm in both the overburden and the bedrock groundwater. PP39 had the highest conductivity reaching 1485 µS/cm.

The chemical composition of shallow groundwater at Olkiluoto can be found in Table 4-4 and Table 4-5. Major constituents (HCO_3^-, Ca^{2+}, Cl^-, K^+, Mg^{2+}, Na^+, SO_4^{2-}), minor elements (Br^-, F^-), conductivity and total alkalinity are consistent with fresh, pristine groundwater in all of the samples but PP39. Groundwater from site PP39 had elevated concentrations for several components listed consistent with a shield bedrock environment. The concentration of NO_3^- was below detection (3 mg/L) in all samples. Sulphide was only detected in a small amount in PVP1.

Table 3-3. *Results from the sterilisation tests of the borehole pump.*

Measurement[a]	MilliQ in the Onkalo laboratory	Sterile MilliQ water for washing of pumps and samplers	Sterile MilliQ water after pumping and sampling
AODC x 10^4 (cells/mL)	12000 (2200[b])	110 (120)	7000 (3700)
ATP x 10^4 (amol/mL)	9725 (3209)	472 (214)	3351 (86)
CHAB x 10^4 (cells/mL)	-[d]	3 (6)	43 (15)
NRB (cells/mL)	-	-	0.4 (0.1 – 1.7[c])
IRB (cells/mL)	-	-	<0.2
MRB (cells/mL)	-	-	<0.2
SRB (cells/mL)	-	-	<0.2
AA (cells/mL)	-	-	<0.2
HA (cells/mL)	-	-	<0.2
AM (cells/mL)	-	-	<0.2
HM (cells/mL)	-	-	<0.2
MOB (cells/mL)	-	-	-

[a] AODC=acridine orange direct count, ATP=adenosine-tri-phosphate, CHAB= cultivable heterotrophic aerobic bacteria, IRB = iron reducing bacteria, MRB = manganese reducing bacteria, SRB = sulphate reducing bacteria, AA = autotrophic acetogens, HA = heterotrophic acetogens, AM = autotrophic methanogens, HM = heterotrophic methanogens, MOB = methane oxidizing bacteria. [b] Standard deviation, n=6. [c] Lower and upper 95 % confidence limits. [d] not analysed

3.1.2 Control of sterilisation of pumps

The MilliQ water in the ONKALO laboratory showed total number (AODC) that was significantly separated from zero (Table 3-3). ATP confirmed some biomass in this water producing unit, which is expected, as systems like this one are not sterile. However, by the use of cleaning procedures and a UV-lamp in the water tank, MilliQ systems can be kept at a very low bacterial number. The sterilised MilliQ water (by autoclave) had a total number that was not significantly different from zero. The CHAB was not significantly different from zero as well. A very small amount of ATP was detected, but at this concentration level, the ATP analysis is very sensitive to even the smallest contamination. The sterilised pump came directly from the field and had been in use for several years. Still, the sterilisation control showed very good results with values just above zero, but still lower than what was found in the ONKALO laboratory MilliQ system. It can be safely concluded that the sterilisation procedures worked properly. The sampling systems did not cross-contaminate the sampled boreholes or the samples.

3.1.3 Biomass

Estimates of biomass varied markedly between the respective subsurface sites at Olkiluoto (Table 3-6, Table 3-7). Total counts (AODC) indicated a maximum biomass of 250 x 10^4 cells/mL in groundwater from PVP1 and a minimum of 9 x 10^4 cells/mL in PVP14. The cultivable heterotrophic aerobic bacteria (CHAB) varied from 60 up to 1.17 x 10^4 cells/mL, and were generally reversibly correlated with depth. ATP correlated with the AODC and was highest in PVP1 and lowest in PVP14.

3.1.4 MPN of metabolic groups of microorganisms

The total number of cells cultivable by the MPN method ranged between 0.0006 and 1.46 % (Table 3-6, Table 3-7). Nitrate reducing bacteria and acetogens were the most dominant groups in the groundwater samples. Sulphate reducing bacteria, IRB and MRB were generally present in moderate numbers. Autotrophic and heterotrophic methanogens were absent in all but two of the samples. The remaining metabolic groups were often at low cell densities or below detection.

3.1.5 Comparison between sampling with the SOLINST sampler and the borehole pump

All results for microbiology, except MOB, were lower when sampling was done with the borehole pump, compared to when the SOLINST sampler was used (Table 4-6). The largest effect was found for NRB and AA.

Table 3-4. *Laboratory analyses of the chemical composition of transitional groundwater of the porous overburden in Olkiluoto, Finland. Sampling was made 10th to 14th of October 2005.*

Measurement	PVP1	PVP4A	PVP13	PVP14	PVP20
Depth (m)	3.90	10.20	5.60	9.0	12.80
pH in the laboratory	4.91	7.21	7.20	6.99	7.13
Na-fluorescein (µg/L)	<10	<10	<10	<10	<10
Total alkalinity (mmol/L)	0.79	5.50	5.72	5.84	4.34
Carbonate alkalinity (mmol/L)	<0.05	0.49	<0.05	<0.05	<0.05
Total acidity (mmol/L)	2.69	0.44	0.34	0.32	0.40
Conductivity (mS/cm)	0.10	0.80	0.55	0.64	0.60
Dissolved inorg. C mg/L	19.7	64.4	69.9	73.8	56.0
HCO_3^- (mg/L)	48.2	336	349	356	265
Cl^- (mg/L)	7.0	89.3	8.9	13.6	49.9
F^- (mg/L)	0.2	0.5	0.9	1.1	0.5
Br^- (mg/L)	<0.5	<0.5	<0.5	<0.5	<0.5
K^- (mg/L)	1.57	10.6	8.77	7.58	8.31
NO_3^- (mg/L)	<3.0	<3.0	<3.0	<3.0	<3.0
SO_4^{2-} (mg/L)	1.4	47	32	60	31
S^{2-} (mg/L)	0.02	<0.01	<0.01	<0.01	<0.01
S total (mg/L)	4.0	15	11	19	10
Na^+ (mg/L)	6.32	56.9	15.8	35.1	61.1
Fe total (mg/L)	17.9	6.68	0.329	2.37	7.15
Fe^{2+} (mg/L)	14.4	4.89	0.26	1.72	5.70
Ca^{2+} (mg/L)	7.96	160	80.8	103	50.7
Mg^{2+} (mg/L)	3.73	19.3	19.3	11.1	12.4
Mn^{2+} (mg/L)	0.081	1.77	0.94	0.824	0.863
SiO_2 (mg/L)	26.3	24.6	22.4	22.3	22.2
Sr (µg/L)	29	230	47	38	87
TDS (mg/L)	120	750	540	610	510
Charge balance[a] (%)	+25.3	+11.7	-0.79	+2.44	+2.38

[a] Charge balance = (cations-anions)/(cations+anions) x 100

Table 3-5. *Laboratory analyses of the chemical composition of transitional groundwater of fractured bedrock aquifers in Olkiluoto, Finland. Sampling was made 10^{th} to 14^{th} of October 2005.*

Measurement	**PR1**	**PP2**	**PP9**	**PP36**	**PP39**
Depth (m)	6.0	14.90	14.70	12.05	14.10
pH in the laboratory	5.23	7.38	6.77	5.82	6.96
Na-fluorescein (µg/L)	<10	<10	<10	<10	<10
Total alkalinity (mmol/L)	0.50	4.63	2.45	1.07	5.82
Carbonate alkalinity (mmol/L)	<0.05	<0.05	<0.05	<0.05	<0.05
Total acidity (mmol/L)	0.94	0.24	0.37	0.92	0.43
Conductivity (mS/cm)	0.12	0.81	0.27	0.23	1.52
Dissolved inorg. C mg/L	21.5	59.9	32.6	23.7	81.6
HCO_3^- (mg/L)	30.5	282	149	65.3	355
Cl^- (mg/L)	2.9	103	5.7	31.4	191
F^- (mg/L)	0.1	0.6	0.3	0.2	0.4
Br^- (mg/L)	<0.5	<0.5	<0.5	<0.5	1
K^- (mg/L)	1,72	7.81	5.36	3.09	18.7
NO_3^- (mg/L)	<3.0	<3.0	<3.0	<3.0	<3.0
SO_4^{2-} (mg/L)	38	24	27	32	230
S^{2-} (mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01
S total (mg/L)	13	16	8.9	11	77
Na^+ (mg/L)	6.41	46.3	16.9	28.4	240
Fe total (mg/L)	2.04	1.76	0.464	0634	7.43
Fe^{2+} (mg/L)	1.23	1.54	0.32	0.34	2.83
Ca^{2+} (mg/L)	7.96	134	33.6	11.3	55.6
Mg^{2+} (mg/L)	3.95	23.1	10.6	4.49	26.5
Mn^{2+} (mg/L)	0.323	1.12	0.091	0.094	0.656
SiO_2 (mg/L)	13.4	22.4	17.2	16.3	22.7
Sr (µg/L)	25	330	47	55	240
TDS (mg/L)	110	650	270	190	1150
Charge balance[a] (%)	-9.26	+15.1	+3.98	+2.81	+0.69

[a] Charge balance = (cations-anions)/(cations+anions) x 100

Table 3-6. *Total number of cells, ATP concentration and numbers of cultivable of metabolic groups of microorganisms in boreholes penetrating the porous overburden in Olkiluoto, Finland. Sampling was made 10th to 14th of October 2005.*

Measurement[a]	PVP1	PVP4A	PVP13	PVP14	PVP20-S	PVP20-P
Depth (m)	3.90	10.20	5.60	9.0	12.80	12.80
AODC x 10^4 (cells/mL)	250 (67[b])	66 (1.4)	12 (1.7)	9.0 (8)	32 (4.3)	15 (7.6)
ATP x 10^4 (amol/mL)	68.5 (0.60)	3.02 0.41)	7.94 (1.03)	0.36 (0.048)	10.6 (0.77)	7.61 (0.39)
CHAB x 10^4 (cells/mL)	0.0917 (0.0218)	0.124 (0.0059)	0.140 (0.0106)	0.0057 (0,0030)	0.22 (0.042)	0.20 (0.023)
CHAB of AODC (%)	0.037	0.19	1.17	0.063	0.68	1.33
NRB (cells/mL)	80 (30-250[c])	800 (300-2500)	500 (200-1700)	0.4 (0.1-1.7)	130 (50-390)	2 (0.9-8.6)
IRB (cells/mL)	<0.2	0.2 (0.1-1.1)	1.3 (0.5-3.8)	<0.2	2.2 (0.9-5.6)	0.4 (0.1-1.7)
MRB (cells/mL)	1.3 (0.5-3.8)	1.4 (0.6-3.5)	33 (15-77)	5 (2-17)	8.0 (3-25)	2.3 (0.9-8.6)
SRB (cells/mL)	1.3 (0.5-3.8)	<0.2	5 (2-17)	0.4 (0.1-1.5)	5 (2-15)	3 (1-12)
AA (cells/mL)	30 (10-120)	110 (40-300)	160 (60-5300)	5 (2-17)	1600 (600-5300)	170 (70-480)
HA (cells/mL)	30 (10-130)	130 50-390)	900 (300-2300)	3 (1-12)	30 (10-130)	30 (10-120)
AM (cells/mL)	<0.2	<0.2	0.8 (0.3-2.4)	<0.2	<0.2	<0.2
HM (cells/mL)	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
MOB (cells/mL)	5 (2-17)	300 (100-1200)	2.3 (0.9-8.6)	n.a.[d]	2.3 (0.9-8.6)	24 (10-94)
MPN of AODC (%)	0.0006	0.20	1.33	0.015	0.56	0.15

[a] AODC=acridine orange direct count, ATP=adenosine-tri-phosphate, CHAB= cultivable heterotrophic aerobic bacteria, IRB = iron reducing bacteria, MRB = manganese reducing bacteria, SRB = sulphate reducing bacteria, AA = autotrophic acetogens, HA = heterotrophic acetogens, AM = autotrophic methanogens, HM = heterotrophic methanogens, MOB = methane oxidizing bacteria. [b] Standard deviation, n=6. [c] Lower and upper 95 % confidence limits. [d] not analysed

Table 3-7. *Total number of cells, ATP concentration and numbers of cultivable of metabolic groups of microorganisms in boreholes penetrating shallow fractured bedrock aquifers in Olkiluoto, Finland. Sampling was made 10th to 14th of October 2005.*

Measurement[a]	PR1	PP2	PP9	PP36	PP39
Depth (m)	6.0	14.90	14.70	12.05	14.10
AODC x 10^4 (cells/mL)	200 (45[b])	11 (5.3)	20 (4.6)	11 (0.45)	58 (56)
ATP x 10^4 (amol/mL)	26.6 (0.73)	2.51 (0.24)	2.45 (0.18)	2.69 (0.108)	19.8 (1.08)
CHAB x 10^4 (cells/mL)	1.17 (0.35)	0.031 (00.0399)	0.0013 (0.0006)	0.0037 (0.0006)	0.0913 (0.0093)
CHAB of AODC (%)	0.59	0.28	0.0065	0.034	0.16
NRB (cells/mL)	24000 (10000-94000[c])	<0.2	8 (3-25)	1.7 (0.7-4.6)	300 (100-1200)
IRB (cells/mL)	1.3 (0.5-3.8)	<0.2	<0.2	<0.2	1.7 (0.7-4.6)
MRB (cells/mL)	0.8 (0.3-2.4)	<0.2	0.4 (0.1-1.5)	0.8 (0.3-2.4)	140 (60-350)
SRB (cells/mL)	30 (10-120)	2.3 (0.9-8.6)	<0.2	2.3 (0-9-8.6)	26 (12-65)
AA (cells/mL)	50 (20-170)	110 (40-300)	1.1 (0.4-2.9)	50 (20-150)	80 (30-250)
HA (cells/mL)	50 (20-170)	2.3 (0.9-8.6)	0.8 (0.3-2.4)	30 (10-130)	50 (20-170)
AM (cells/mL)	<0.2	<0.2	<0.2	<0.2	3 (1-12)
HM (cells/mL)	<0.2	<0.2	<0.2	<0.2	<0.2
MOB (cells/mL)	5000 (2000-20000)	13 (5-39)	3 (1-12)	2.3 (0.9-8.6)	300 (100-1200)
MPN of AODC (%)	1.46	0.116	0.0067	0.08	0.16

[a] AODC=acridine orange direct count, ATP=adenosine-tri-phosphate, CHAB= cultivable heterotrophic aerobic bacteria, IRB = iron reducing bacteria, MRB = manganese reducing bacteria, SRB = sulphate reducing bacteria, AA = autotrophic acetogens, HA = heterotrophic acetogens, AM = autotrophic methanogens, HM = heterotrophic methanogens, MOB = methane oxidizing bacteria. [b] Standard deviation, n=6. [c] Lower and upper 95 % confidence limits. [d] not analysed

3.2 Deep groundwater

For reference, the microbiology results of three deeper boreholes are presented in Table 4-8). Deep borehole results will be presented and discussed in detail in a separate report.

Table 3-8. *Total number of cells, ATP concentration and numbers of cultivable of metabolic groups of microorganisms in packer equipped boreholes penetrating fractured bedrock aquifers in Olkiluoto, Finland.*

Measurement[a]	OL-KR32	OL-KR8	OL-KR33
Depth (m)	50-54	77-84	95-107
AODC x 10^4 (cells/mL)	2.6 (0.76[b])	13 (2.7)	4 (1.5)
ATP x 10^4 (amol/mL)	2.34 (0.2)	0.69 (0.044)	0.70 (0.049)
CHAB x 10^4 (cells/mL)	0.24 (0.038)	0.050 (0.0021)	0.001 (0.001)
CHAB of AODC (%)	9.23	0.38	0.025
NRB (cells/mL)	1300 (500-3900[c])	110 (40-300)	8 (3-25)
IRB (cells/mL)	24 (10-94)	0.2 (0.1-1.1)	80 (30-250)
MRB (cells/mL)	24 (10-94)	0.4 (0.1-1.7)	22 (9-56)
SRB (cells/mL)	0.4 (0.1-1.7)	3 (1-12)	7 (2-21)
AA (cells/mL)	17 (7-48)	70 (30-210)	24 (10-94)
HA (cells/mL)	13 (5-39)	30 (10-120)	8 (3-25)
AM (cells/mL)	<0.2	<0.2	<0.2
HM (cells/mL)	<0.2	<0.2	<0.2
MPN of AODC (%)	5.3	0.16	0.37

[a] AODC=acridine orange direct count, ATP=adenosine-tri-phosphate, CHAB= cultivable heterotrophic aerobic bacteria, IRB = iron reducing bacteria, MRB = manganese reducing bacteria, SRB = sulphate reducing bacteria, AA = autotrophic acetogens, HA = heterotrophic acetogens, AM = autotrophic methanogens, HM = heterotrophic methanogens, MOB = methane oxidizing bacteria. [b] Standard deviation, n=6. [c] Lower and upper 95 % confidence limits. [d] not analysed

4 DISCUSSION

Two microbiology sampling campaigns have been performed in the shallow groundwater of Olkiluoto. The first was done in May 2004 and the second was completed in October 2005. Methods and techniques used worked well. Several control activities were executed to test and improve the methods. Adjustments of the procedures were performed when judged important. The goal with the adjustments was to establish stable and reproducible procedures.

4.1 Selection of sampled boreholes

The selection of boreholes for the 2004 and the 2005 activities differ. Four boreholes coincide between the 2004 and 2005 activities (PVP1A, PVP4A, PR1 and PP2), but 6 boreholes were new during fall 2005. This change in the selection of boreholes was due to several different reasons. Firstly, some of the boreholes sampled 2004 collapsed (PP8) or were contaminated by bentonite (PVP3A and PVP3B). Others were found to have closely related chemistry (PVP4A and PVP4B). Finally, some of the 2004 boreholes were not accessible due to the ongoing construction of ONKALO. Therefore, a new selection of boreholes was made for the 2005 field activity. Five overburden and 5 shallow rock boreholes were selected showing the largest possible distribution in the content of dissolved solids. They were also selected to ensure that the ONKALO construction would not interfere with future sampling activities. Thereby, it was judged that the field activities repeatedly would return data sets that represent a wide selection of Olkiluoto shallow groundwater environments.

4.2 Sampling procedures in groundwater observation tubes and shallow boreholes

Sampling of groundwater observation tubes and shallow boreholes for microbiology differs in many aspects compared to deep boreholes. The most obvious aspect is that specific fractures in the deep boreholes are isolated with packers and pumped for several weeks before sampling. The shallow sampling points are not equipped with packers and they are only pumped for a couple of hours. The deep boreholes are sampled with the PAVE system, a closed container that collects pressurized samples. This procedure has been followed for the shallow sampling points as well, using the SOLINST borehole sampler. It is optional to use a borehole sampler or to collect samples directly from the pump tubings. However, it had to be demonstrated that the pumps can be sterilised between the boreholes. It was also judged important to test the difference between pumped samples and samples collected using the SOLINST sampler.

The groundwater observation tubes and shallow boreholes were open and in contact with air. It was therefore judged possible that air might have mixed with the sampled water during pumping, as oxygen was found in most samples during the May 2004 investigation. The applied technique to pump the borehole before sampling was to ensure that groundwater with dissolved air, precipitation and dust from the ground surface was removed before a sample is taken. Therefore, groundwater was eventually sampled after 1.5 h or more of pumping in the investigations. A packer system was

tested during the October 2005 investigation to test if this pumping was enough to keep air from contaminating the samples. The results suggest that some oxygen may mix with the water. Oxygen could not be detected in any of the five boreholes with a packer, while three out of five boreholes without a packer showed oxygen. It is concluded that a packer may be needed to hinder oxygen from mixing with the sampled groundwater.

4.2.1 Control of pump sterilisation

The use of chlorine dioxide (XINIX) for pump sterilisation worked very well (2.1.5). The MPN of microorganisms obtained after sterilisation was below detection (0.2 cells/mL) for all analyses except NRB that showed a number just above the detection limit (Table 4-3). The sampling systems did not cross-contaminate the sampled boreholes.

4.2.2 Comparison between sampling with the SOLINST sampler and the borehole pump

The microbiology results were generally lower when sampling with the borehole pump, or equal to the numbers obtained with the SOLINST sampler, except for MOB (Tabel 4-6). The largest difference was found for NRB and AA. During sampling with the SOLINST sampler, it was observed that the groundwater was slightly more turbid after retrieval of the borehole pump and lowering of the SOLINST sampler. The increase in turbidity was only observed in overburden boreholes. This difference was most probably caused by hydrodynamic disturbances during the hoisting of pumps and samplers up and down the borehole. Sediment and colloids suspended in groundwater by disturbance during sampling will certainly have microorganisms attached to it and this will subsequently increase biomass estimates in turbid, compared to non-turbid groundwater. The increased ATP biomass value from PVP20S compared to PVP20P may be attributed to the ATP method extracting from both planktonic and biofilm microorganisms in the turbid water. For total counts, the situation is similar with higher numbers in the SOLINST sample compared to the pump sample. Groundwater from PVP20S was also associated with the detection of the greatest number of metabolic groups. This would again be due to higher numbers of microorganisms in the SOLINST sample caused by addition of sediment particles with attached microorganisms.

In the choice of using the SOLINST for sampling or the pump, it can be argued that the SOLINST gives higher numbers related to particles in the groundwater. This is of course a true result in a sense that those organisms are present and possibly active in the sampled borehole. However, in the same time, the use of the SOLINST introduces an uncertainty in the results, as it is not possible to reproducibly cause turbidity in the boreholes. For comparative purposes, it is better to sample from the borehole pump in the coming field activities. Else, overburden boreholes may overestimate the planktonic cell numbers compared to the rock samples that was free from turbidity caused by sampling activities in the borehole.

It has been demonstrated that attached microorganisms in deep groundwater environments outnumber planktonic microorganisms significantly (Pedersen and Ekendahl, 1992a-b). However, it can be safely assumed that the planktonic numbers and diversity reflects the numbers and diversity of attached microorganisms. High activity and growth of attached microorganisms will result in an increase in the numbers of

microorganisms that slough off by hydrodynamic forces or migrate from growing colonies. The investigations of shallow groundwater aim at understanding seasonal variation and the future impact of ONKALO on the biogeochemistry. Therefore, it is judged more important to apply reproducible methods, than methods that may give highest possible numbers as a result of manipulation of the particle content in the analysed groundwater. The two field activities reported here, May 2004 and October 2005 form a solid method and technology base for the design of future field activities. The only major change for the coming investigations will be that the pump will be sampled directly for microbiology. The SOLINST will still be used, but only for analysis of the gas content and composition in the groundwater. A new method for gas analysis will be implemented during the spring 2006 field activity.

4.3 Comparison of shallow borehole field data and chemistry between spring 2004 and fall 2005

The boreholes had varying lengths under the groundwater level, commonly several meters. The inflowing groundwater to a specific borehole during pumping may, therefore, be of several different origins. For instance, one component may originate from very shallow groundwater layers, while a second one can originate from a deeper inflow location. All this is related to the preferential flow paths of the aquifers in the sampled ground. For consistency, the total, measured, depth of a shallow borehole is used in this report when discussing correlations between analysed parameters and depth.

It is not fruitful to compare data of chemistry between 2004 and 2005, as there were only 4 boreholes coinciding. Chemistry such as salinity, and ionic composition is closely related the sites. However, general physical field measurement data such as temperature, oxygen and possibly REDOX will to a lesser extent be site dependent. This is because the average depths of the analysed boreholes were similar. It was 11.19 metres during the May 2004 activity and 11.14 metres during the October 2005 activity. Comparisons between the spring and fall data show some interesting and expected results.

4.3.1 Temperature

As could be expected, the average temperature was higher in the fall compared to the spring (Figure 4-1). The difference was 2.3 degrees. The increase in temperature is of course an effect from the summer air and ground temperatures being higher than the winter temperatures. The result shows that higher temperatures during summer influence the groundwater temperature at least down to 15 m. Again, this comparison will be more accurate once repeated measurements are preformed in the same borehole in future field activities. The effect from increasing temperatures during summer time is expected to correlate with an increase in the microbial activity, as microbial activity generally increases with increasing temperatures.

4.3.2 Oxygen

There was a striking difference in the oxygen concentrations between the spring and fall seasons. While oxygen reached values above 5 mg/L in the spring time, this gas was below detection in all boreholes in the fall 2005 except three sampling points in which

traces of oxygen were found (Figure 4-2). The results suggest a seasonal effect on the concentration of dissolved oxygen. However, more measurements over time and several years will be needed to be able to draw general conclusions. The most plausible explanation to variation in oxygen is that microbial activity consumes oxygen during the summer time, when the temperature is increasing and the input of organic carbon from plants and trees on the surface is high. Decomposition of this organic material in the soil and litter layers consumes oxygen. This consumption probably continues in the groundwater, where dissolved organic carbon (DOC) that follows recharge is consumed with oxygen as well. During winter and spring, the input of DOC diminishes and the microbial activity decreases with decreasing temperature. Snow melt will result in a large input of oxygenated water with low concentration of DOC to a groundwater system with low microbial activity, resulting in increasing oxygen concentrations in the groundwater. The following summer, microbial consumption will again reduce the oxygen during decomposition of DOC.

The results strongly suggest that microorganisms are responsible for reducing oxygen in recharge water and in shallow groundwater. It has generally been argued that minerals of various kinds, such as ferrous iron and sulphides are responsible for the removal of oxygen. However, such a process would not be seasonally dependent as the temperature difference of 2.3 degrees is too small to explain the observed effect as a chemical process. However, more investigations in time series are needed before a general model of microbial scavenging of oxygen can be safely presented.

4.3.3 Redox

The observed REDOX potential did not differ significantly between spring and fall measurements (Figure 4-3). It averaged at 204 mV during the May 2004 field activity and at 216 mV during the October 2005 field activity. At both occasions, the REDOX values decreased with increasing depth. Comparing REDOX values with other analysed parameters showed an asymptotic correlation with the content of total dissolved solids (TDS) (Figure 4-4). But, TDS did not correlate well with depth (Figure 4-5), so it is not just a depth-REDOX relation we observed. It was also found that the concentration of oxygen had an effect on the obtained REDOX value. Redox electrodes generally give a measure of the will of a system to donate electrons. The higher will the system has to donate electrons, the lower REDOX is obtained. That would explain the correlation with TDS. Increasing the TDS will probably mean increasing the concentration of compounds willing to donate electrodes to the platinum electrode used. When high concentrations of oxygen were present, the REDOX values were higher, compared to groundwater with lower oxygen concentrations. This is in line with oxygen acting as a strong electron acceptor, which will drive the REDOX to more positive values. The shallow groundwater analysed had sulphide concentration at or below detection in all boreholes, except a small measurable content in PVP1. If sulphide was present it would have forced the measured REDOX towards more negative values, than presently registered.

It is well recognized that analysis of REDOX in deep groundwater is very difficult and it may take weeks before stable readings are obtained (Grenthe et al, 1992). The choice of electrode systems may also have a significant influence on the obtained readings. The electrode used in this investigation has been used with good and reproducible results in microaerophilic groundwaters in the Äspö tunnel (Anderson and Pedersen, 2003).

However, here we immersed the electrode in running water from the borehole pumps while in the Äspö research, the electrode was immersed in very slowly flowing groundwater. The higher hydrodynamic turbulence around the electrode in this investigation may have resulted in a more sluggish response compared to the tunnel investigations. Possibly, REDOX measurements should be done with the electrode immersed down in the boreholes in the future.

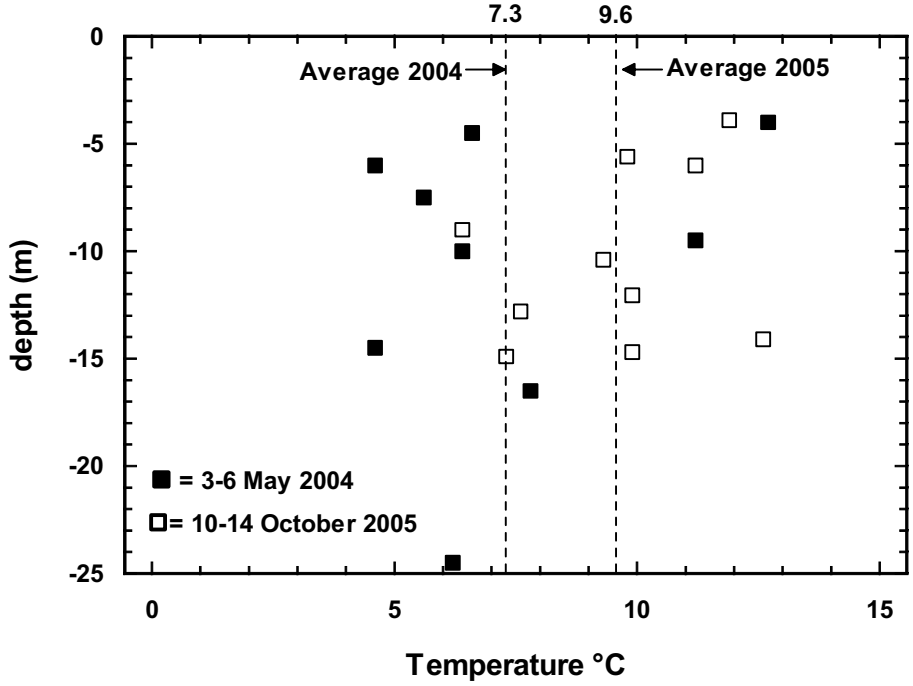

Figure 4-1. *The analysed temperatures over borehole depths in Olkiluoto.*

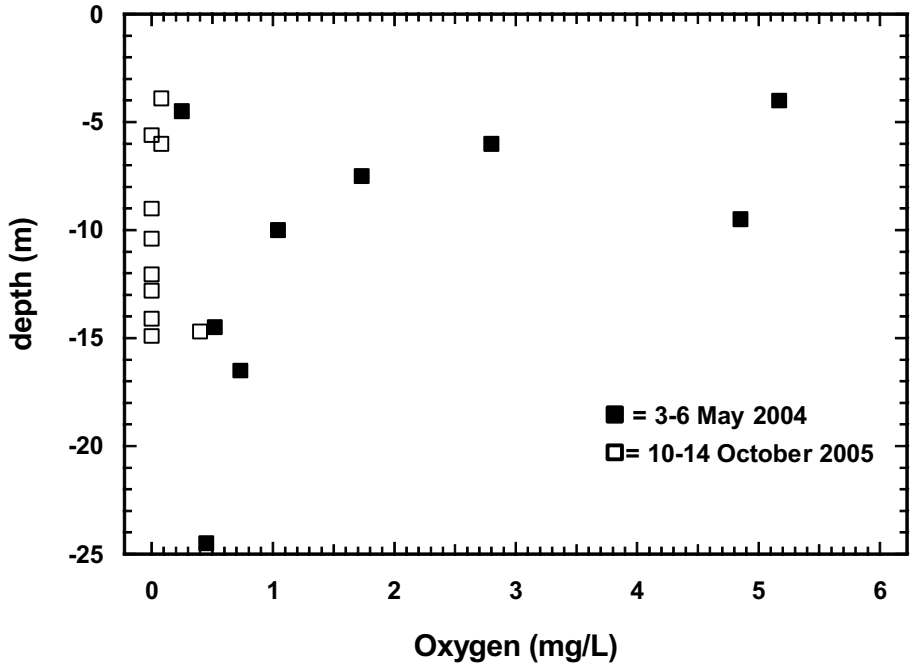

Figure 4-2*. The analysed oxygen concentrations over borehole depths in Olkiluoto.*

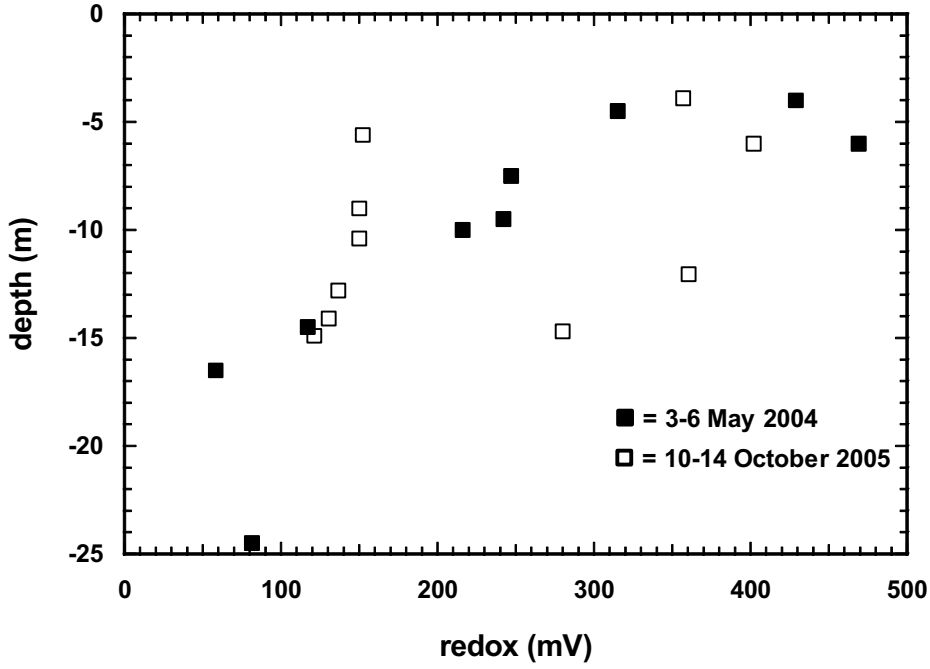

Figure 4-3. *The analysed reduction oxidation potentials over borehole depths in Olkiluoto.*

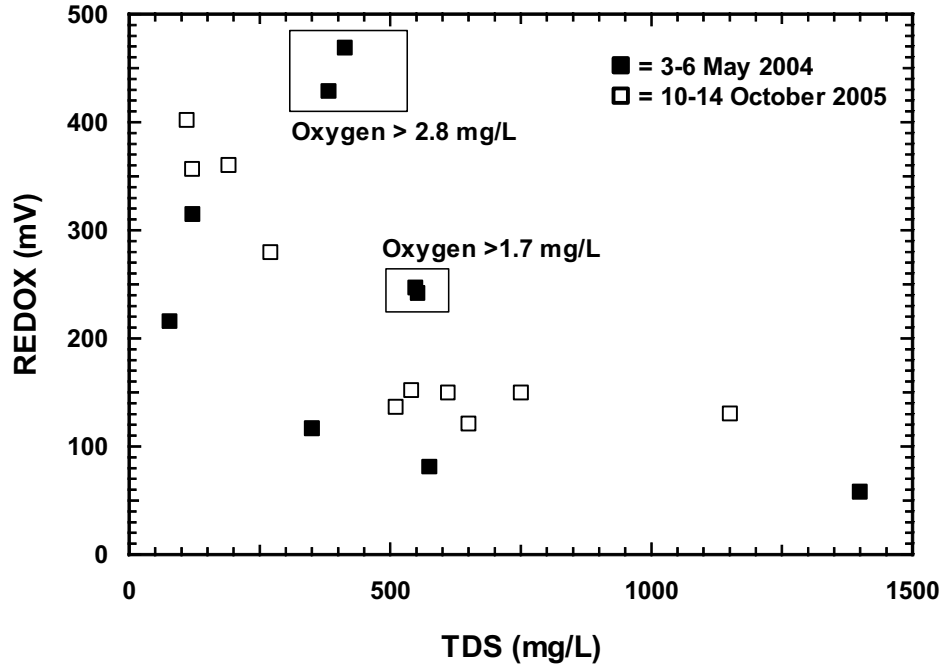

Figure 4-4. *The relation between the observed reduction oxidation (REDOX) potential and the content of total dissolved solids (TDS).*

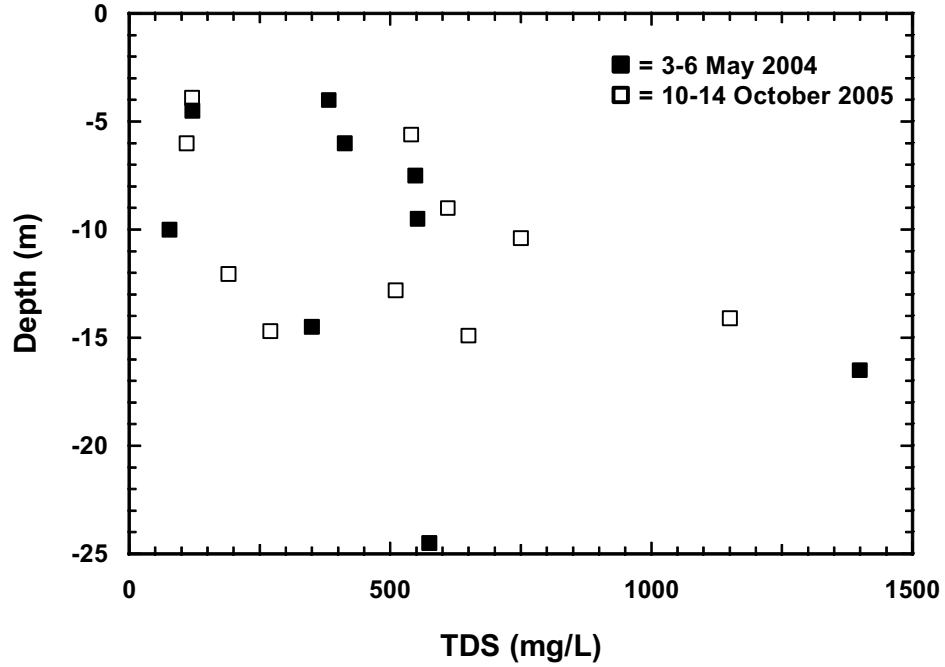

Figure 4-5. *The relation between depth and the content of total dissolved solids (TDS).*

4.4 Comparison of shallow borehole microbiology between spring 2004 and fall 2005

4.4.1 Biomass

The biomass determinations i.e. AODC, ATP and CHAB, revealed decreasing numbers with depth. Three packed off boreholes between 50 and 107 m were also analysed and reported. Overall, the 10 shallow boreholes (Table 3-6, Table 3-7) showed higher biomass values compared to the 3 relatively deeper boreholes (Table 4-8). The CHAB showed large variation between the boreholes and distributed around the values obtained during spring 2004. Although CHAB implies aerobic bacteria, it must be stressed that many bacteria are facultative anaerobes. That is, they prefer to grow with oxygen if this gas is present, but they can also switch metabolism to fermentation and anaerobic respiration with nitrate, iron or manganese (c.f. Figure 4-17). This is neatly demonstrated when plotting CHAB versus NRB, where increasing CHAB correlates with increasing MPN of NRB (Figure 4-7).

The concentration of ATP in living cells is fairly constant over the volume. Therefore, the ATP analysis shows the volume of living biomass. The ATP concentrations were much higher in the overburden and somewhat higher in the shallow bedrock (Figure 4-8) compared to the deep boreholes (Figure 4-9). This result suggests that the shallow boreholes had more living biomass (bio-volume) than the deeper holes. In the shallow boreholes, organisms much larger than bacteria, such as protozoa (unicellular animals), fungi and possibly also small metazoans (tiny, multi-cellular animals) may proliferate, which will significantly increase the ATP over AODC quota. The proximity of the overburden boreholes to the surface biosphere with high concentrations of organic material will support more living biomass compared to deep groundwater, which will further add bio-volume to the ATP measured. The decrease in ATP concentrations and AODC numbers with depth seems to occur over a relatively short distance. The shallowest packer boreholes analysed (OL-KR32, OL-KR8 and OL-KR33) showed AODC and ATP data that lies within the range of those measured down to about 600 meters (Pedersen, 2006). It appears as if the transition from a shallow groundwater biomass signature to a deep groundwater signature in Olkiluoto occurs over a couple of tens of meters at present.

4.4.2 The MPN signature

The MPN determination gives a signature of what metabolic types of microorganisms were present in the examined boreholes. Far from all viable cells, as judged from the percentage of AODC and ATP cultivated (Table 3-6, Table 3-7, Table 3-8), which is a common, well known and accepted result throughout the scientific literature. Many microorganisms just cannot be cultivated. This is not as surprising as it first may seem. There are many animals – fishes, birds, insects and plants on Earth than can only be studied in their natural environment. If we capture them, they will soon die because we do not understand how to give them suitable living conditions. It is the same for many microorganisms and the only way to increase the percentage of cultivated microorganisms is to develop skills to cultivate. This is a continuous process and applied during the site investigations as well as during other investigations by our laboratory.

4.4.3 IRB and MRB

Iron and manganese reducing bacteria oxidize organic carbon with ferric iron and Mn^{4+}. Those are solids that can occur in large amounts in hydro-thermally altered fractures and as secondary mineral precipitates in fractures. The IRB and MRB are generally observed in larger numbers at shallow depths down to a couple of hundred meters (Pedersen, 2001) where the REDOX potential is above or around -200 mV, according to the present site investigation model for the Swedish radioactive waste disposal program. The numbers of IRB and MRB were significantly higher in the fall 2005 results compared to the spring 2004 (Figure 4-10). This is consistent with the absence of oxygen in the shallow groundwater during the fall. Microorganisms that can use iron and manganese in their respiration will increase in numbers when oxygen is absent as was found in the fall. All shallow groundwater contained ferrous iron and Mn^{2+} (Table 3-4, Table 3-5). Those species are produced by IRB and MRB respectively, in their anaerobic respiration (Figure 4-17). Iron and manganese was not analysed during the spring 2004 activity, so comparisons with an oxygenated groundwater situation is not possible. Future investigations will, however, enable such comparisons as a full chemistry analysis is being performed concomitant with microbiology analyses from fall 2005 and onwards. IRB and MRB were also observed in the moderately deep boreholes at relatively high numbers (Table 4-8), which is consistent with present knowledge (Pedersen, 2001).

4.4.4 NRB and SRB

Nitrate reducing bacteria (NRB) are, next to oxygen reducing bacteria, the group that get most energy from oxidation of organic carbon (Figure 4-17). NRB are, therefore, expected to be a common group as long as nitrate is available. They are commonly facultative anaerobes and will switch to an oxygen dependent metabolism if this gas appears (4.4.1). Consequently, they will add reducing catabolic power to “the microbial oxygen barrier”. This analysis was introduced in the fall 2005 activity. NRB was found in all analysed boreholes (Figure 4-11), including the moderately deep ones).

The sulphate reducing bacteria oxidize organic carbon with the sulphur in sulphate to sulphide (Figure 4-17). They are commonly observed in deep groundwater (Pedersen, 2001). High numbers are not unusual as observed earlier in deep Olkiluoto groundwater (Haveman et al, 1999). Low but significant numbers of SRB were observed in all but 2 shallow boreholes (Figure 4-12, Table 4-8). There were more SRB in the shallow groundwater in the anaerobic fall situation compared to the oxygenated spring situation, which is consistent with that SRB are sensitive to oxygen. Sulphide was generally below detection in the shallow groundwater supporting the conclusion that SRB were not active there.

4.4.5 Acetogens

This group of microorganisms produces acetate from one carbon organic compounds or from hydrogen and carbon dioxide (Figure 4-17). They are common in deep groundwater (Kotelnikova and Pedersen, 1998) and they have been detected in all samples at relatively high numbers during the Swedish site investigations. This is consequently a very versatile and common group, represented by a large number of species and genera that have adapted to a wide variety of environmental conditions. The

numbers of AA and HA were more than 10 times higher in the fall compared to the spring. This is again in line with the absence of oxygen and the assumed higher load of DOC to the shallow groundwater system (4.3.2). The acetate produced by those organisms can be utilised by many other microorganisms, including IRB, NRB, SRB and HM. Thereby, the acetogens form a very important base for the microbial ecosystems in groundwater. The AA will in particular be important if hydrogen and carbon dioxide is present. They then form a chemosynthetic base for microbial ecosystems that reduce or even exclude the need of a surface input of DOC, when geological hydrogen is available. The importance of AA for microbial ecosystems in groundwater then increases with depth.

4.4.6 Methanogens

This group of microorganisms produces methane from one carbon organic compounds and from acetate or from hydrogen and carbon dioxide (Figure 4-17). They are commonly detected above the detection limit in deep groundwater (Kotelnikova and Pedersen, 1998). The numbers of AM and HM were below detection in almost all samples in the fall 2005 investigation. Those organisms typically prefer very reduced environments with REDOX potentials approaching -300 mV or lower (Oremland 1988). It is, therefore, in line with our knowledge that methanogens occur sparsely or not at all in shallow groundwater. However, the AM and HM also belong to the group of microorganisms that are most difficult to cultivate. It must, therefore, be kept open that the negative results can be due to problems with the cultivation procedure, although we presently are not aware of any reasons for why the cultivations of methanogens would have failed.

4.4.7 Methane oxidizing bacteria

The MOB oxidise methane with oxygen. They are very versatile in the interface between anoxic, methane containing groundwater and oxygenated environments. For instance, the Äspö HRL tunnel has been demonstrated to harbour high numbers of MOB (Kotelnikova, 2002). They will constitute one very important oxygen scavenging mechanism that will protect a future repository from oxygen. The numbers of MOB did not differ between the seasons. Although the environment was virtually free from oxygen in fall, it is still possible that oxygen was transported downwards in the groundwater via diffusion, convection and recharge. Unless microorganisms continuously reduce oxygen migrating into the groundwater, oxygen concentrations would of course build up. This is not yet proven. Next step is to analyse for methane and other gases in the shallow groundwater. This will be done with a new method during fall 2006. Later it may be doable to analyse *in situ* methane oxidizing rates, and oxygen reduction during different seasons but that will need development and adjustment of several methods for shallow groundwater, applied earlier for deep groundwater (Kotelnikova, 2002).

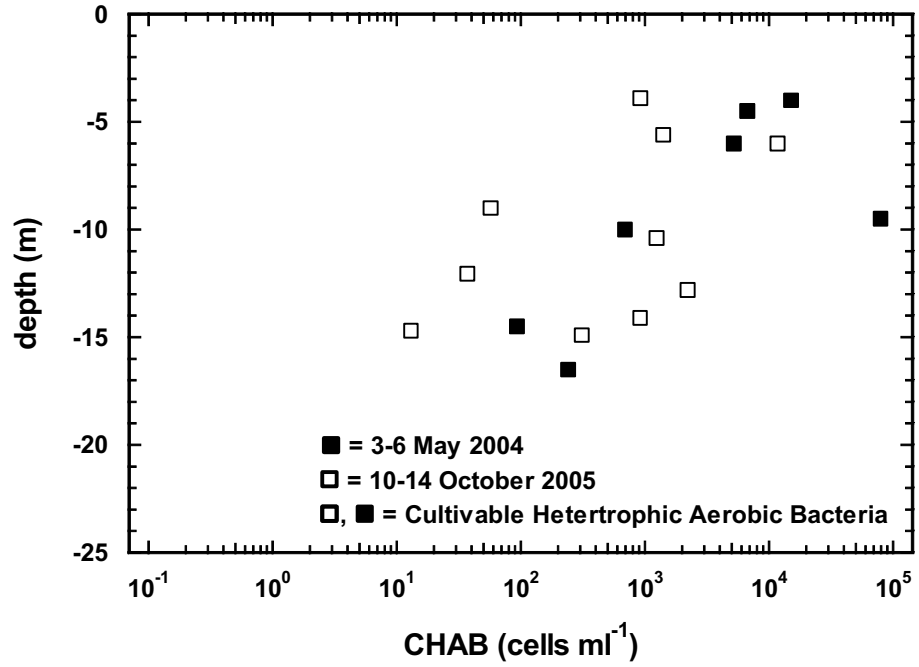

Figure 4-6. *Cultivable, heterotrophic, aerobic bacteria versus depth in shallow Olkiluoto boreholes.*

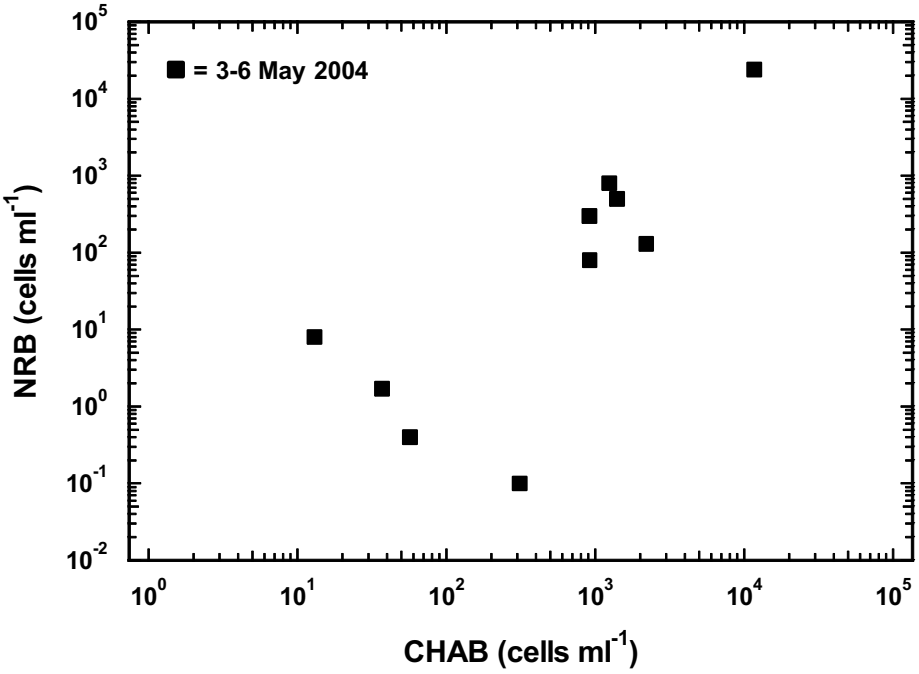

Figure 4-7. *The relation between cultivable, heterotrophic, aerobic bacteria (CHAB) versus most probable numbers (MPN) of nitrate reducing bacteria (NRB) in shallow Olkiluoto boreholes.*

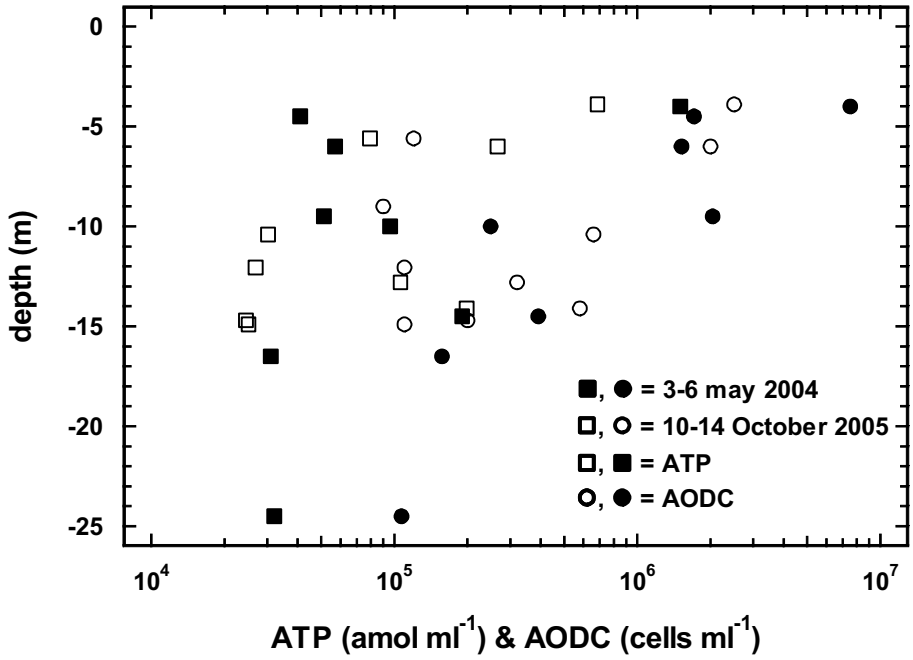

Figure 4-8. *Biomass (ATP) and the total numbers of bacteria (AODC) versus depth in shallow Olkiluoto boreholes.*

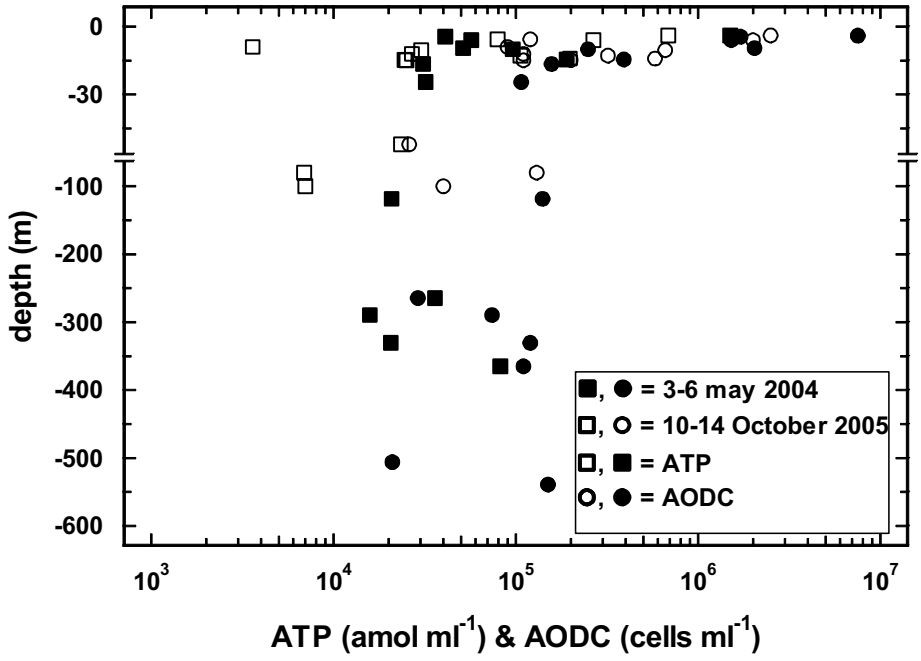

Figure 4-9. *Biomass (ATP) and the total numbers of bacteria (AODC) versus depth in shallow and deep Olkiluoto boreholes.*

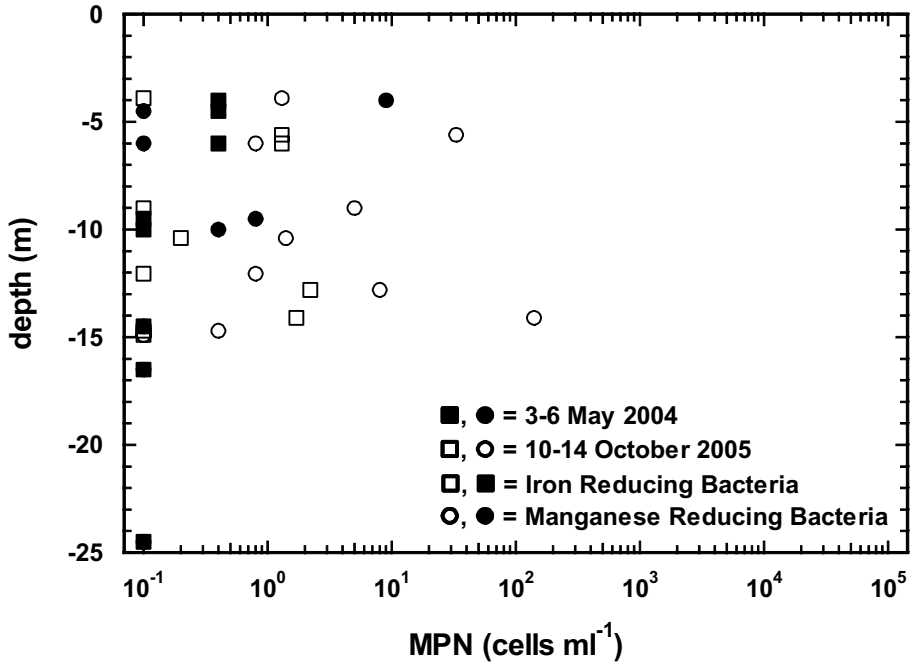

Figure 4-10. *Iron and manganese reducing bacteria (IRB and MRB) versus depth in shallow Olkiluoto boreholes.*

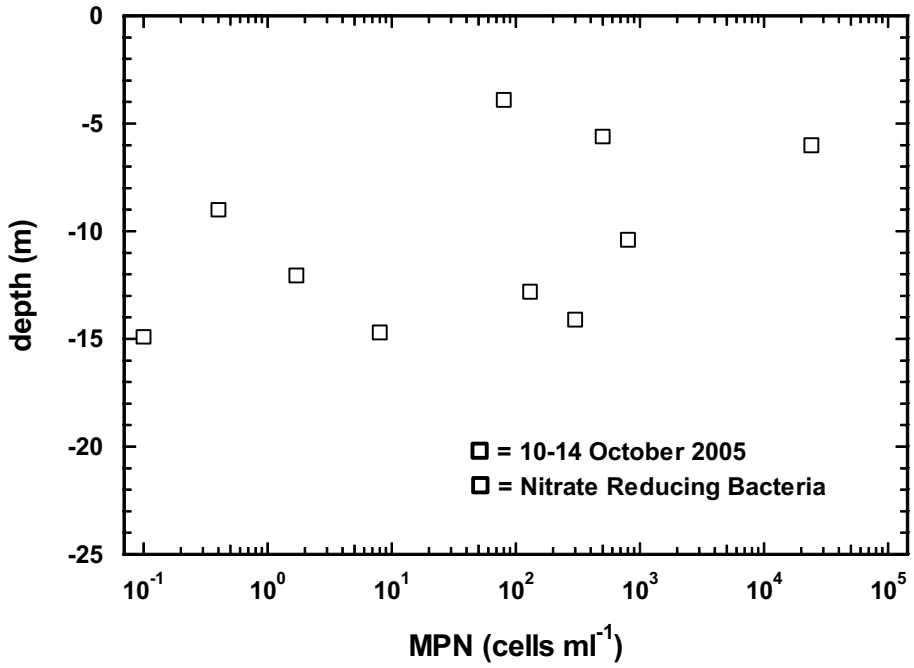

Figure 4-11. *Nitrate reducing bacteria (NRB) versus depth in shallow Olkiluoto boreholes.*

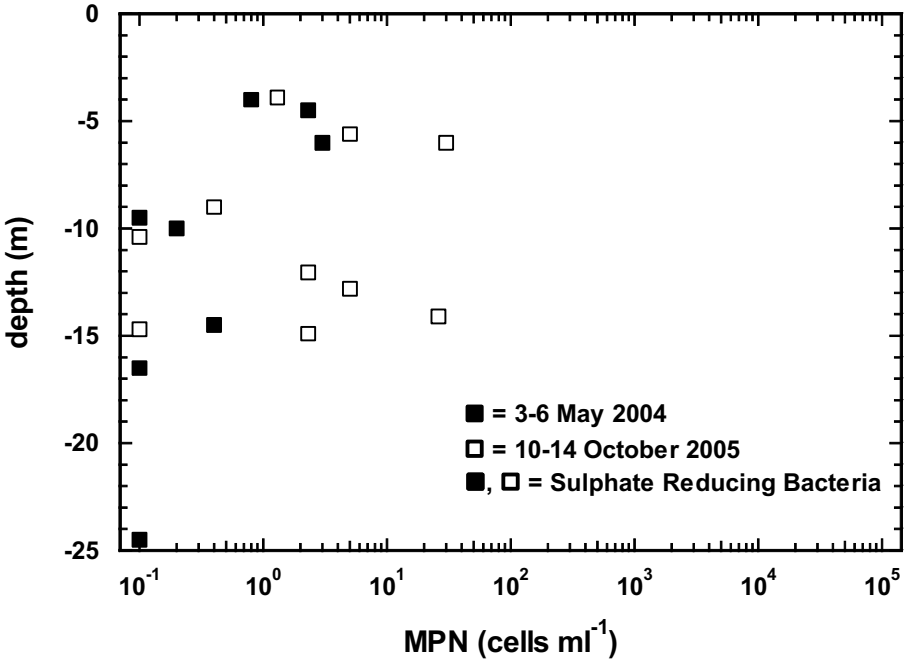

Figure 4-12. *Sulphate reducing bacteria (SRB) versus depth in shallow Olkiluoto boreholes.*

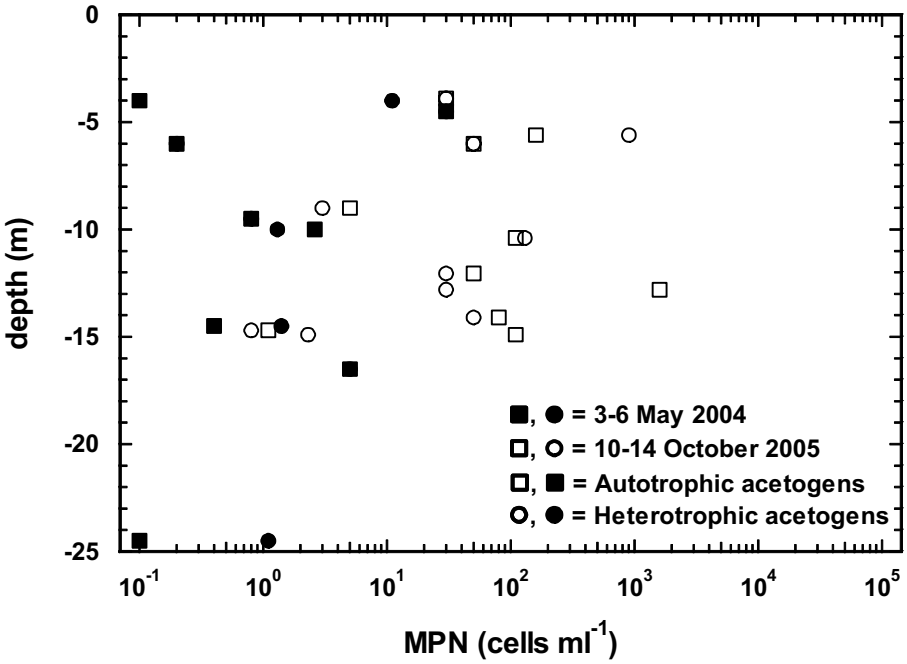

Figure 4-13. *Autotrophic and heterotrophic acetogens (AA and HA) versus depth in shallow Olkiluoto boreholes.*

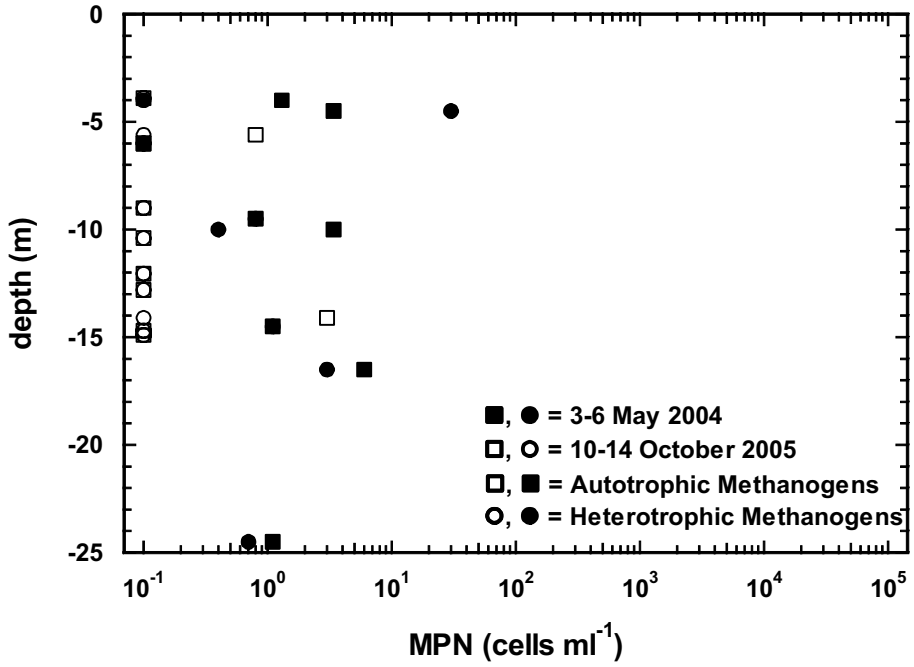

Figure 4-14. *Autotrophic and heterotrophic methanogens (AM and HM) versus depth in shallow Olkiluoto boreholes.*

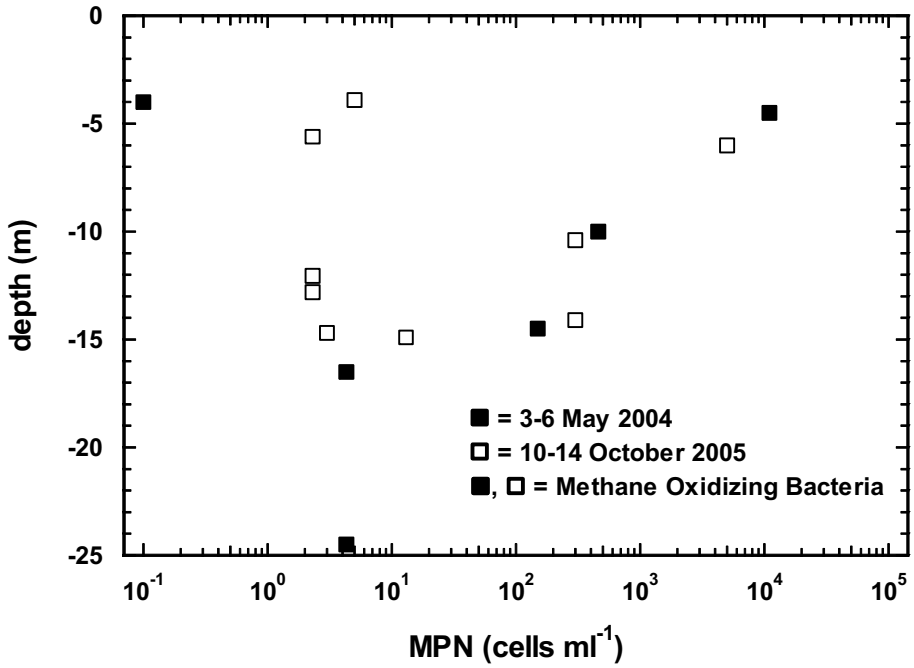

Figure 4-15. *Methane oxidizing bacteria (MOB) versus depth in shallow Olkiluoto boreholes.*

4.4.8 Comparisons of average numbers for shallow borehole microbiology between spring 2004 and fall 2005

The average of each analysed microbiology variable is presented in Figure 4-16, distributed over the season the work was done. Clear and mostly expected differences were found. The average of total numbers of bacteria (AODC) was about 5 times higher in the fall compared to the spring. This is in line with the interpretations done in this report that microbial activity increased over summer and fall. Such an increase will bring up the total numbers. The content of biomass (ATP) showed the opposite trend with about 10 times more average ATP in the spring compared to the fall. This is also expected. A paper in preparation by the Deep Biosphere Laboratory at Göteborg University (Eydal and Pedersen, 2006), shows that microorganisms in aerobic and shallow groundwater are larger that in deeper groundwater. ATP is a measure of bio-volume, which will decrease when an ecosystem changes from aerobic to anaerobic conditions, as did the Olkiluoto groundwater from the spring to the fall season. The number of cultivable aerobic bacteria decreased from spring to fall. As there was oxygen in the spring but not during fall, this is an expected result. The number of aerobes should decrease when oxygen disappears from an ecosystem. The percentage of the AODC that corresponded to the CHAB decreased more than 10 times from spring to fall, which agrees with the discussion above.

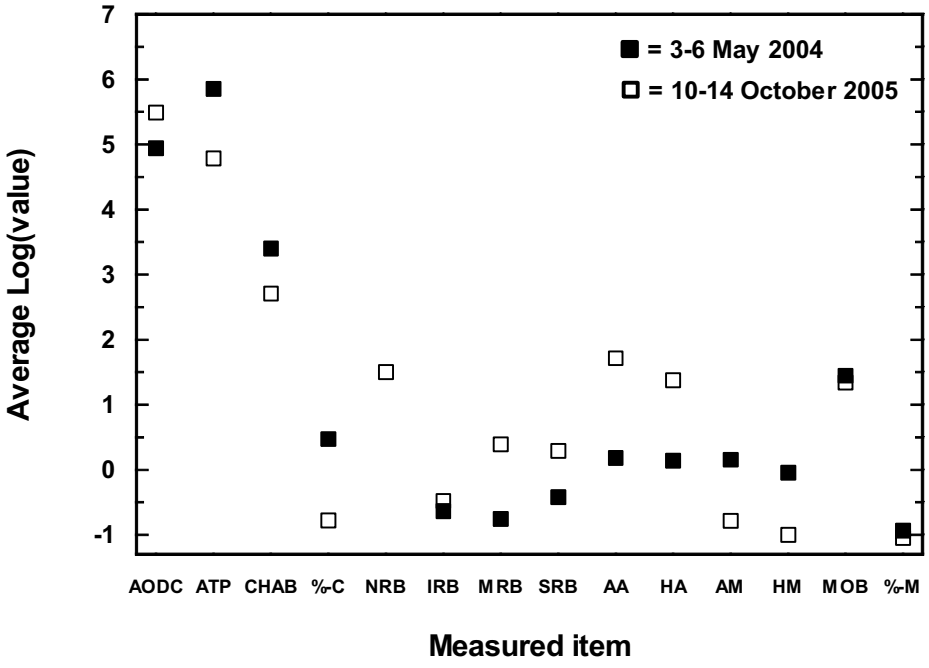

Figure 4-16. *Average numbers distributed over analyses shown in Table 3-6 and Table 3-7.*

NRB were analysed for the first time during fall 2005 and cannot be compared. IRB and in particular MRB increased from spring to fall as did SRB. This agrees again with the groundwater system going anaerobic in the fall, which will favour anaerobic respiration. The acetogens increased most of all analyzed metabolic groups. The very large

versatility and diversity of this group of microorganisms suggest that they will respond faster and stronger than most other groups, which was the case. The AA grows on hydrogen and there are two possible sources for this hydrogen. Firstly, it can be hydrogen that is transported upwards from deep groundwater in which hydrogen is found, sometimes in large concentrations (Pedersen, 2001). Secondly, anaerobic degradation of particulate organic matter via fermentation results in, among many other compounds, hydrogen from synthropic bacteria (Figure 4-17). The methanogens decreased for reasons not clarified at present. More investigations need to be fulfilled before safe conclusions can be made. Finally, it was found that the average of MOB was about similar in the spring and the fall seasons. The percentage organisms of the AODC cultivable with MPN also remained about the same both seasons.

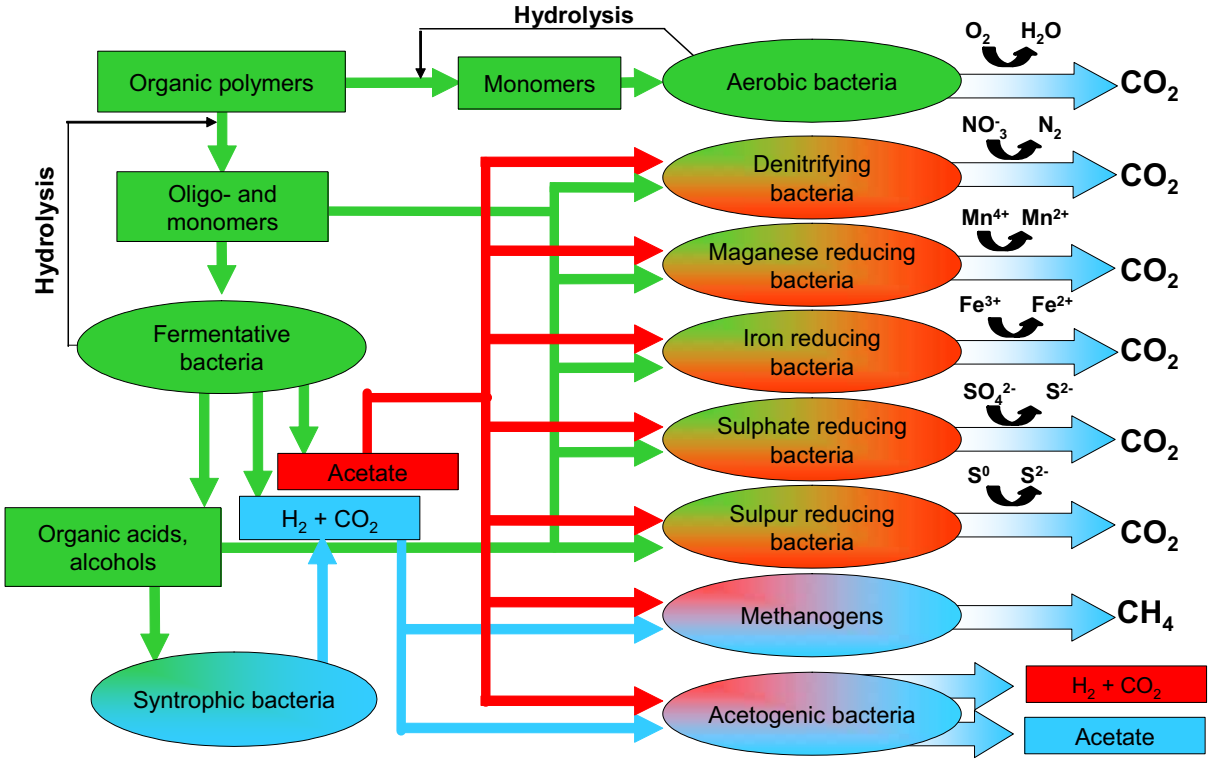

Figure 4-17. *The degradation of organic carbon can occur via a number of different metabolic pathways, characterized by the principal electron acceptor in the carbon oxidation reaction. A range of significant groundwater components and gases are formed or consumed during those microbiological processes.*

4.5 Conclusions of importance for ONKALO

This was the second investigation that covered both shallow and deep groundwater microbiology in Olkiluoto. The analysis of microbiology is very important for proper understanding of the evolution of geochemical processes in and around ONKALO. There are several conclusions from this investigation that are of great importance for ONKALO. The following present day conclusions can be put. Continued investigations will update and test them.

- The shallow biosphere was dominated by oxygen consuming microorganisms that block oxygen migration to deeper groundwater. This effect was most pronounced during the summer and fall season.
- There was a clear seasonal variation in the populations of microorganisms in shallow Olkiluoto groundwater.
- The shallow groundwater of Olkiluoto was generally anaerobic all the way up to the water table in the fall 2005, but oxygenated to about 25 m depth in the spring 2004 season. This interesting trend will need to be confirmed by more sampling. Oxygen was present in 3 of the samples in 2005, though at a very low level.
- The obtained data suggest that the transient between the shallow and deep biospheres in Olkiluoto occurs at a very shallow depth, typically at 15-25 m.

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