

Description of the legal entity and its profile versus the tasks in the proposal

Microbial Analytics Sweden AB (MICANS) is a small enterprise founded 2005 by scientists at Göteborg University, Sweden. MICANS perform applied and environmental research projects with expertise in microbiology, molecular biology and analytical chemistry. MICANS is at present staffed with 11 coworkers; one professor, two PhD, five B.Sc., two analytical biochemists and one laboratory engineer. MICANS research projects deal mainly with microbial life in deep groundwater, microbial influence on engineered barriers, biodegradation of contaminants in groundwater, and microbially induced corrosion. MICANS operate 450 m² modern laboratories that are well equipped for the research tasks MICANS will carry out in this proposal.

Curriculum vitae of person primarily responsible for the research activities

Karsten Pedersen obtained his PhD degree 1982 at Department of Microbiology, University of Gothenburg, Sweden with a thesis on microbial biofilms in seawater. His present affiliations are adjunct professor of Geomicrobiology at the department of Civil and Environmental Engineering, Chalmers University of Technology and CEO at Microbial Analytics Sweden AB, www.micans.se. Pedersen has 30 years of experience in undergraduate and graduate teaching at Gothenburg University. He has supervised 23 undergraduates in their research tasks and 11 graduate (Ph.D.) students, taught 2700 undergraduate lecture hours and has been director of studies for 7 years. Since 1987, his research interests are on microbial ecosystems that dwell underground in deep Scandinavian bedrock aquifers. Diversity, activity and distribution of microorganisms in the deep underground are main themes that have been split up in many different research activities over the years. He has presently published more than 100 papers, reviews and book chapters in peer-reviewed international scientific press and he has written many reports for national and international research agencies. He was chairman for the European Science Foundation (ESF) Scientific program on groundwater pollution, GPoll, duration 1998-2001. He takes on ad hoc peer-review tasks for a wide range of scientific journal and is on the editorial board for several journals. He was contracted as expert and have written 150 articles on microbiology and reviewed 370 articles for the Swedish lexicon "Nationalencyklopedin". His research has over the years been frequently presented in newspapers and magazines, radio and TV.

Recent publications

1. Pedersen K, Bengtsson A, Edlund J, Eriksson L. (2014) Sulphate-controlled diversity of subterranean microbial communities over depth in deep groundwater with opposing gradients of sulphate and methane. *Geomicrobiology Journal* on-line first (DOI: [10.1080/01490451.2013.879508](https://doi.org/10.1080/01490451.2013.879508))
2. Pedersen K. (2014) Microbial life in terrestrial hard rock environments. In *Microbial Life of the Deep Biosphere*. Edited by Kallmeyer J. and Wagner D. De Gruyter, Berlin. pp 63-81.
3. Pedersen K, Bengtsson A, Edlund J, Rabe L, Hazen T, Chakraborty R, Goodwin L, Shapiro N. (2014) Complete genome sequence of the subsurface, mesophilic sulfatereducing bacterium *Desulfovibrio aespoeensis* Asp-2. *Genome Announcement* 2, e00509-e00514.
4. Pedersen K. (2013) Metabolic activity of subterranean microbial communities in deep granitic groundwater supplemented with methane and H₂. *ISME J* 7:839-49.
5. Pedersen, K. (2012), Influence of H₂ and O₂ on sulphate-reducing activity of a subterranean community and the coupled response in redox potential, *FEMS Microbiology Ecology*, 82 653-665.
6. Pedersen, K. (2012), Subterranean microbial populations metabolize hydrogen and acetate under in situ conditions in granitic groundwater at 450 m depth in the Åspö Hard Rock Laboratory, Sweden, *FEMS Microbiology Ecology*, 81 217-229.
7. Hallbeck, L. and Pedersen, K. (2012) Hallbeck, L. and Pedersen, K. 2012, Culture-dependent comparison of microbial diversity in deep granitic groundwater from two sites considered for a Swedish final repository of spent nuclear fuel, *FEMS Microbiology Ecology*, 81 66-77.

8. Krawczyk-Bärsch, E., Lünsdorf, H., Pedersen, K., Arnold, T., Bok, F., Steudtner, R., Lehtinen, A. and Brendler, V. (2012) Immobilization of uranium in biofilm microorganisms exposed to groundwater seeps over granitic rock tunnel walls in Olkiluoto, Finland, *Geochimica et Cosmochimica Acta*, 96 94-104.
9. Jägevall S., Rabe L. and Pedersen K. (2011) Abundance and diversity of biofilms in natural and artificial aquifers of the Äspö Hard Rock Laboratory, Sweden. *Microbial Ecology* 61, 410-422.
10. Kennedy, C. B. Gault, A. G. Fortin, D. Clark, I. D. Pedersen, K. Scott, S. D. Ferris, F. G. (2010) Carbon isotope fractionation by circumneutral iron-oxidizing bacteria. *Geology* 28, 1087-1090
11. Masurat, P. Eriksson, S. and Pedersen, K. (2010) Evidence for indigenous sulphate-reducing bacteria in commercial MX-80 Wyoming bentonite. *Applied Clay Science* 47, 51-57
12. Masurat, P. Eriksson, S. and Pedersen, K. (2010) Microbial sulphide production in compacted Wyoming bentonite MX-80 under in situ conditions relevant to a repository for high-level radioactive waste. *Applied Clay Science* 47, 58-64
13. Moll M. Glorius, M. Johnsson, A. Schäfer, M. Budzikiewicz, H. Karsten Pedersen, K. Bernhard, G. (2010) Neptunium(V) complexation by natural pyoverdins and related model compounds. *Radiochimica Acta* 98, 571-576
14. Pedersen, K. (2010) Analysis of copper corrosion in compacted bentonite clay as a function of clay density and growth conditions for sulfate-reducing bacteria. *Journal of Applied Microbiology* 108, 1094-1104
15. Eydal, H S C. Jägevall, S. Hermansson, M. and Pedersen, K. (2009) Bacteriophage lytic to *Desulfovibrio aespoeensis* isolated from deep groundwater. *The ISME journal* (3) 1139-1147
16. Johnsson, A. Ödegaard-Jensen, A. Skarnemark, G. Pedersen, K. (2009) Leaching of spent nuclear fuel in the presence of siderophores. *Journal of Radioanalytical Nuclear Chemistry* 279, 619-626.
17. Hallbeck L. Pedersen K. (2008) Characterization of microbial processes in deep aquifers of the Fennoscandian Shield. *Applied Geochemistry* 23, 1796-1819
18. Johnsson, A. Ödegaard-Jensen, A. Jakobsson, A.-M. Ekberg, C. and Pedersen, K. (2008) Bioligand-mediated partitioning of radionuclides to the aqueous phase. *Journal of Radioanalytical and Nuclear Chemistry* 277, 637.
19. Kyle, J E. Eydal, H S C. Ferris, F G. and Pedersen, K. (2008) Viruses in granitic groundwater from 69 to 450 m depth of the Äspö hard rock laboratory, Sweden. *The ISME Journal* 2, 571-574
20. Kyle, J E. Ferris, F G. and Pedersen, K. (2008) Virus Mineralization at low pH in the Rio Tinto, Spain. *Geomicrobiology Journal* 25, 338-345.
21. Moll, H. Glorius, M. Bernhard, G. Johnsson, A. Pedersen, K. Schäfer, M. and Budzikiewicz, H. (2008) Characterization of pyoverdins secreted by a subsurface strain of *Pseudomonas fluorescens* and their interactions with uranium(VI). *Geomicrobiology Journal* 25, 157-166
22. Moll, H. Johnsson, A. Schäfer, M. Pedersen, K. Budzikiewicz, K. and Bernhard, G. (2008) Curium(III) complexation with pyoverdins secreted by a groundwater strain of *Pseudomonas fluorescens*. *Biometals* 21, 219-228
23. Pedersen, K. Arlinger, J. Hallbeck, A. Hallbeck, L. Eriksson, S. and Johansson, J. (2008) Numbers, biomass and cultivable diversity of microbial populations relate to depth and borehole specific conditions in groundwater from 3 to 450 m depth in Olkiluoto, Finland. *The ISME journal* 2, 760-775

Five relevant publications

Pedersen K., Bengsson A., Edlund J. and Eriksson L. (2014) Sulphate-controlled diversity of subterranean microbial communities over depth in deep groundwater with opposing gradients of sulphate and methane. *Geomicrobiology Journal* DOI: 101080/014904512013879508

Pedersen K. (2013) Metabolic activity of subterranean microbial communities in deep granitic groundwater supplemented with methane and H₂. *ISME J* 7, 839-849.

Pedersen K. (2012) Influence of H₂ and O₂ on sulphate-reducing activity of a subterranean community and the coupled response in redox potential. *FEMS Microbiology Ecology* 82, 653-665.

Pedersen K. (2010) Analysis of copper corrosion in compacted bentonite clay as a function of clay density and growth conditions for sulfate-reducing bacteria. *Journal of Applied Microbiology* 108, 1094-1104.

Masurat P., Eriksson S. and Pedersen K. (2010) Microbial sulphide production in compacted Wyoming bentonite MX-80 under in situ conditions relevant to a repository for high-level radioactive waste. *Applied Clay Science* 47, 58-64.

Five relevant projects

1. The sulphate reduction experiment (SURE) project (2009-2014, Posiva Oy, Finland). Sulphide produced by microbial sulphate reduction under anaerobic conditions may corrode the copper canisters used for final disposal of spent nuclear fuel (SNF). The sulphate reduction experiment (SURE) program seeks a better understanding of the processes underlying sulphide production (i.e., sulphate reduction) and involves the chemical characterization and detailed microbiological investigation of groundwater from several depths in the ONKALO tunnel. Three consecutive experiments have been performed and the last experiment report is in preparation. The two first experiments are presented in the following papers: Pedersen K, Bengtsson A, Edlund J, Eriksson L. (2014) Sulphate-controlled diversity of subterranean microbial communities over depth in deep groundwater with opposing gradients of sulphate and methane. *Geomicrobiology Journal* on-line first (DOI: [10.1080/01490451.2013.879508](https://doi.org/10.1080/01490451.2013.879508)); Pedersen K. (2013) Metabolic activity of subterranean microbial communities in deep granitic groundwater supplemented with methane and H₂. *ISME J* 7:839-49.
2. Microbiology of the Prototype repository at Äspö Hard Rock Laboratory (2006 -2012, Swedish Nuclear Fuel and Waste management Co) The Prototype repository is an international project to build and study a full-scale model of the planned Swedish final repository for spent nuclear fuel. The Prototype consists of two sections with four and two full-scale copper canisters, respectively. In 2011, the outer section with two canisters (nos. 5 and 6) was excavated and microbiology was studied and compared with earlier monitoring results. The microbiology results are presented in the following report: Arlinger, J. Bengtsson, A. Edlund, J. Eriksson, L. Johansson, J. Lydmark, S. Rabe, L. Pedersen K. (2013) Prototype repository – Microbes in the retrieved outer section *SKB report R-13-16*, pp. 1-40. Stockholm: Swedish Nuclear Fuel & Waste management CO.
3. The Microbe project (1999 – 2009, Swedish Nuclear Fuel and Waste management Co), investigated four specific microbial process areas of importance for proper repository functions, namely: microbial effects on the chemical stability of deep groundwater environments, bio-mobilization of radionuclides, bio-immobilization of radionuclides, and microbially induced corrosion of copper. The outcome of this 10 years research initiative is summarized in the following report: Pedersen K. 2013. The MICROBE project. Achievements of a 10-year research programme. *SKB report R-13-49*, pp. 1-38. Stockholm: Swedish Nuclear Fuel & Waste management CO.
4. Influence of varying growth conditions on gas and sulfide production by bacteria enriched from water standing between the HADES gallery concrete liners in Mol, Belgium (2010-2012, NIRAS/ONDRAF). Report information: Persson, J., A. Bengtsson, J. Johansson, A. Pääjärvi, L. Rabe, K. Pedersen (2013) Influence of varying growth conditions on activity of nitrate- and sulphate-reducing bacteria isolated from the HADES underground laboratory, ONDRAF/NIRAS report NIROND-TR 2013-11(E).
5. Microbially induced copper corrosion in bentonite (2008-2010, Swedish Nuclear Fuel and Waste management Co). This project aimed to investigate the relationships between sulfate-reducing bacteria (SRB), growth conditions, bentonite densities and copper sulfide generation under circumstances relevant to underground, high-level radioactive waste repositories. The work is presented in the following publication: Pedersen, K. (2010) Analysis of copper corrosion in compacted bentonite clay as a function of clay density and growth conditions for sulfate-reducing bacteria. *Journal of Applied Microbiology* 108, 1094-1104.

Description of infrastructure and technical equipment

MICANS has a very well equipped laboratory for the purpose of the research tasks. The laboratory has three gas chromatographs, and one mass spectrometer, HPLC, fluorometer, fluorescent microscopes, anaerobic boxes and all items needed for cultivation of anaerobic

microorganisms such as a gas mixing bench and anaerobic tube systems. A complete system for investigations of microbial activity in compacted buffers and clays comprising 24 titanium test cells with load meters has been constructed and is available for the project. Work with radioisotopes such as ^{35}S , ^{14}C and ^3H is done in an isotope laboratory with scintillation instrument and a 2D-autoradiography instrument. Microelectrode equipment for redox, sulphide, oxygen and pH from Unisense A/S are available. Basic equipment for all types of microbiology and analytical chemistry is at hand. MICANS have a complete set-up for DNA technology work such as polymerase chain reaction (PCR) amplifier, quantitative PCR, temporal gradient gel electrophoresis (TTGE), cloning, digital gel documentation system, laminar air flow box, and -85 C freezers. For field work, MICANS has set-up independent, remotely controlled biofilm flow cell circulation systems that communicate under in situ pressure and chemistry with aquifers in granitic rock of the hard rock laboratories In ONKALO, Finland and Äspö, Sweden.