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Microorganisms and Their Influence on Radionuclide Migration in Igneous Rock Environments

Karsten Pedersen*

Göteborg University, Department of Cell and Molecular Biology, Microbiology, Box 462, 405 30 Göteborg, Sweden

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Microorganisms interact with their surroundings and in some cases they greatly modify the characteristics of their environment. Several such interactions may have a significant influence on the behaviour of radionuclides possibly escaping from underground radioactive waste repositories. Microbes can mobilise trace elements. Unattached microbes may act as large colloids, transporting radionuclides on their cell surfaces with the groundwater flow. Many microbes produce ligands that can mobilise trace elements from solid phases and that can inhibit trace element sorption to solid phases. Bacterial species from the deep subsurface have demonstrated a significant effect on the mobilization of ^{59}Fe(III), ^{147}Pm(III), ^{234}Th(IV) and ^{241}Am(III) under varying redox conditions. The extent of bacterial immobilisation of radionuclides has been investigated under *in situ* conditions. Experiments have demonstrated this effect with ^{60}Co, ^{147}Pm, ^{234}Th, ^{237}Np, and ^{232}U. A large group of microbes catalyse the formation of iron oxides from dissolved ferrous iron in groundwater that reaches an oxidising environment. Such biological iron oxide systems (BIOS) will have a retardation effect on many radionuclides. Microorganisms execute an important influence on the chemical situation in groundwater. Especially, they may catalyse reactions that stabilise the redox potential in groundwater at a low and, therefore, beneficial level for a radioactive waste repository.

1. Introduction

A microbe is an organism that has all it needs for a complete life cycle in one single cell. Many microbial species may under appropriate circumstances form multi-cellular structures, sometimes with morphological and metabolic differentiations between the different cells making up a bio-structure. Growth by cell division is the most common way of microbe propagation. The microbes outnumber multi-cellular organisms on our planet. They are characterised by their almost infinite ability to bio-degrade and bio-synthesize naturally occurring compounds in the environment. Microbial reactions control, partly or fully, the chemical composition of many, very different environments such as sediments, soils, lakes and groundwater. They also have the ability to adapt to extreme environments such as alkaline and acidic groundwater.[1, 2]

Microbes occur either as unattached, planktonic cells passively floating or actively swimming in the free water phase, or are attached to solid surfaces. Any solid surface, living or dead, immersed in a water phase with living microbes is predestined to develop biofilms i.e. films of life that cover the surface. Microbes from the water phase become attached to the surface and, if growth conditions are appropriate, they start to grow and divide, in an attached mode.

All microbial life is based on metabolic processes driven by chemical energy extracted from the oxidation of inorganic or organic compounds. The oxidative harvesting of energy requires an electron acceptor that is concomitantly reduced. This process is called respiration when a reducible external electron acceptor is used (e.g. oxygen, nitrogen, sulphur, iron or manganese), and fermentation when the electrons are shuffled around between the degradation products of the original energy source (e.g. sugar to alcohol and carbon dioxide). The microbes certainly must obey the laws of thermodynamics, but they are utterly sophisticated lawyers that understand to use every single, constructive combination of those laws for making a good living of all or almost nothing.

2. Microbial Processes and Radionuclides

Microbial processes can significantly alter the mobility of radionuclides in the environment. Multi-disciplinary research combining microbial physiology, ecology and molecular biology with nuclear chemistry, geochemistry and geology plays an important role in the exploration of the processes described in this paper.

Figure 1 summarises microbial processes that can influence radionuclide speciation and thereby their migration behaviour. Microbial processes will act immobilising or mobilising, depending on the type of process and the state of the microbes. Microbes in biofilms will, with exception for those who produce complexing agents, be immobilising. Planktonic cells, that bio-sorb or bio-accumulate radionuclides, will act mobilising on radionuclides. The processes can have a direct or indirect action on radionuclide transport in the geosphere. A direct action involves contact between a microbe and the

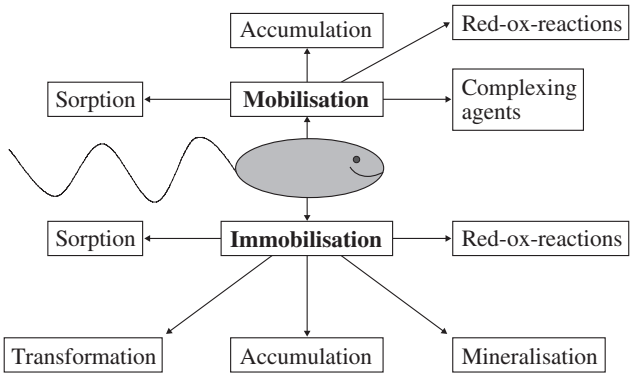

Figure 1. Schematic view over how microorganisms interact with their surroundings and how they may influence the behaviour of radionuclides possibly escaping from underground radioactive waste repositories.

*Corresponding author. E-mail: karsten.pedersen@gmm.gu.se. FAX: +46-31-773-2578.

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radionuclide with a resulting change in its speciation. Indirect action is caused by changes in the environment generated by microbial metabolism, which in turn influences radionuclide behaviour. Finally, all microbial processes except bio-sorption require an active, energy driven metabolism. The modelling of microbial processes, therefore, must include a proper understanding of microbial energy turnover rates in deep rock aquifers. The processes in Figure 1 are discussed in some detail below, with emphasis on their importance for understanding geosphere retention phenomena in the safety assessments of radioactive waste disposal. Table 1 outlines some important features of the discussed processes.

TABLE 1: Microbial Processes Can Influence Retention of Radionuclides Directly or Indirectly in Several Different Ways

Microbial processes that influence radionuclide migration	Microbes in this process are in the following state(s):		This action of this microbial process on radionuclides is:		This process requires an active microbial energy driven metabolism:	
	planktonic	biofilm	direct	Indirect	yes	no
Immobilisation processes						
Bio-sorption		×	×			×
Bio-accumulation		×	×		×	
Bio-transformation	×	×	×		×	
Bio-mineralisation	×	×		×	×	
Metabolic red-ox reactions	×	×		×	×	
Mobilisation processes						
Bio-sorption	×		×			×
Bio-accumulation	×		×		×	
Production of complexing agents	×	×	×		×	

The most important variables for such processes are the state of attachment, are the microbes attached or unattached, and whether the microbes are active with a metabolism that turns over energy, or dormant and inactive.

3. Bio-Sorption

The term bio-sorption is used to describe the metabolism-independent sorption of heavy metals and radionuclides to biomass, i.e. microbial cells. Bio-sorption can be summarized as the sorption and accumulation of trace elements to the surface of microbial cells. Both living and dead biomass are capable of bio-sorption and ligands involved in metal binding include carboxyl, amine, hydroxyl, phosphate and sulfhydryl reactive groups on the cell wall.[3]

Microbes have the largest surface area to volume ratio of any independent life form due to their extremely small size, typically centring around 1 μm^3. Fitting of experimental data to Langmuir sorption isotherms gives information on the sorption capacity of microbes that can be cultured on the laboratory. Values for equilibrium sorption constants and sorption capacity have been established for the two major types of bacterial cells, those with Gram-positive and Gram-negative cell walls.[4] Distribution ratios at varying pH, cell number and concentration of the lanthanide Promethium have also been determined.[5]

The typical volume of a groundwater bacterium is in the range of a part of 1 μm^3 up to a couple of μm^3. Numbers of planktonic microbes in deep groundwater vary in the range of 10^6 to 10^9 cells L^{-1} of groundwater.[6–8] Bio-sorbing planktonic microbes can be regarded as large colloidal particles and are dealt with elsewhere in this paper. However, microbes in biofilms are different. They have been reported to reach 10^{11} cells m^2 in Fennoscandian shield rock groundwater.[7, 8] Biofilm microorganisms commonly excrete extra-cellular material supporting attachment and also the three dimensional shape of a growing biofilm. As this extra-cellular material is organic in its nature, it will add a bio-sorption capacity to the cell's surfaces. In conclusion, bio-sorption to planktonic microbes will act mobilising on radionuclides, while bio-sorption to biofilms will act immobilising (Table 1).

Host rocks surrounding hydraulically conductive fractures are considered important barriers to the migration of radionuclides in the far-field. Fluid-rock interfaces can be isolated from one another by the growth of microbial biofilms. In an ongoing study of the adsorptive capacity of *in situ* anaerobic biofilms grown 500 m underground in 'repository' conditions was compared to the capacity of host granite from the same environment. Surfaces were exposed to $^{60}Co(II)$, $^{234}Th(IV)$, $^{237}Np(V)$, $^{147}Pm(III)$, and U(VI) over the period of 6 weeks in a pH neutral anaerobic synthetic groundwater. Adsorption was investigated using radioactive tracers and autoradiography. Results indicate that the trivalent elements did attach better than the other tested elements. Anaerobic biofilms and rock surfaces were found to share similar adsorption capacities but probably have dissimilar complex stability due to the surface functional groups available and competition for those groups. The high ionic strength of the groundwater and the chemical properties of the radionuclide species used potentially reduced the effectiveness of biofilm surfaces as a sorbant. The effect of fracture biofilm coverage on radionuclide adsorption and migration in subsurface environments cannot be ignored. Potential suppression of adsorption by biofilms should be accounted for in performance safety assessment models.

4. Bio-Accumulation

Metals catalyse many metabolic reactions in the microbial cell. Microbes, therefore, have evolved energy dependent uptake systems for physiologically important metals. The import mechanisms of metals and other elements into the cells relate to size and charge of the element in focus. For example, the close similarity of the Cs^+ and K^+ cations dictates that the broad-specificity alkali metal uptake transporters take up both those cations in all microbial groups. The specific mechanisms by which Cs^+ is transported into the cell have been reviewed by Lloyd and Macaskie.[9]

As concluded for bio-sorption, bio-accumulation will have a very different effect on radionuclide retention in planktonic versus biofilm microbes (Table 1). In the planktonic state, the microbe can be regards as a large, living colloid, while the biofilm microbes relate to solid phase retention models. Although some microbes may alter between unattached and attached modes, many may become trapped and fossilised over time.[10] As bio-accumulation is a process that requires energy, it can be expected that this process is biased towards biofilm microbes, because microbial biofilm populations have been found much more metabolically active relative to planktonic microbes.[7]

Up to date, *in situ* experimental data does not exist on the importance of bio-accumulation processes for understanding geosphere retention phenomena in the safety assessments of radioactive waste disposal. The potential of this process is linked to metabolism, and therefore also to the availability of sources of energy for microbial metabolic reactions in deep groundwater, further discussed below.

5. Metabolic Red-Ox Reactions

Microbial energy metabolism requires a reduced electron and energy donor and an oxidised electron acceptor (Table 2). The energy donor can be an organic or an inorganic compound. The electron acceptor is generally an inorganic compound, with exception for fermentation, where the electron donor and electron acceptor is the same organic compound. Electron donors and acceptors can be combined in redox

TABLE 2: The Most Common Energy and Electron Donors and Electron Acceptors in Microbial Metabolism

Organic energy sources and electron donors		Inorganic energy sources and electron donors		Electron acceptors	
Reduced	Oxidised	Reduced	Oxidised	Oxidised	Reduced
Carbohydrates	$\underline{C}O_2$			$\underline{O}_2$	$H_2\underline{O}$
Amino acids	$\underline{C}O_2$	$\underline{N}H_4^+$	$\underline{N}O_3$	$\underline{N}O_3$	$\underline{N}_2$
Organic acids	$\underline{C}O_2$	$\underline{Mn}^{2+}$	$\underline{Mn}^{4+}$	$\underline{Mn}^{4+}$	$\underline{Mn}^{2+}$
Fat	$\underline{C}O_2$	$\underline{Fe}^{2+}$	$\underline{Fe}^{3+}$	$\underline{Fe}^{3+}$	$\underline{Fe}^{2+}$
		$H_2\underline{S}$	$\underline{S}O_4^{2-}$	$\underline{S}O_4^{2-}$	$H_2\underline{S}$
		$\underline{C}H_4$	$\underline{C}O_2$	$\underline{S}^0$	$H_2\underline{S}$
		$\underline{C}O$	$\underline{C}O_2$	$\underline{U}^{6+}$	$\underline{U}^{4+}$
		$\underline{H}_2$	$\underline{H}_2O$	$\underline{C}O_2$	$\underline{C}H_4$

The respective atom that donates or accepts one or several electrons is underlined.

couples according to the difference in free energy. Any redox couple that releases energy via a reaction is a possible source of energy for microbes. The result from microbial harvesting of energy from redox couples is an oxidised donor and a reduced acceptor. Important to notice here is that microbial metabolism generally lowers the redox potential in the environment. Further, some of the reduced electron acceptors (e.g. sulphide), may result in bio-mineralization processes, as discussed next.

A case of special importance is the reduction of oxygen in microbial metabolism. Oxygen is the preferred electron acceptor by many microorganisms, because the free energy available in oxidation of an electron/energy donor is largest when oxygen is used, compared to other acceptors (Table 2). A first indication that microbial oxygen reduction may be of considerable proportions in granitic environments was published after a series of full scale oxygen intrusion experiments into a 70 m deep vertical fracture zone at the Äspö Hard Rock Laboratory (HRL), Sweden.[11] Later, the Michaeles-Menten kinetics was successfully used to model oxygen reduction during a series of field (also at Äspö HRL) and replica laboratory experiments in a detailed scale called REX aiming at the study of O_2 depletion in granitic media.[12] This model describes the effect of the initial oxygen concentration on the oxygen reduction rate. Over a wide range of O_2 concentrations tested *in situ*, the variation of (v) with respect to [O_2] followed the empirical Monod equation (Michaelis-Menten kinetic used for microbial growth):

$$v = V_{max} \frac{[O_2]}{Km_0 + [O_2]}$$

where v is O_2 reduction velocity at a specific O_2 concentration, V_{max} is the maximum rate when the organism is O_2 saturated and Km_0 is the O_2 concentration when the O_2 reduction rate is 1/2 of the maximum. This model was further developed and adapted to experimental data during the REX-project.[12, 13]

The most important conclusions from the REX experiments were:[12]

- Microbes play a substantial role in O_2 reduction in granitic media, and microbial processes implicate a significant added reducing capacity in a repository environment.
- When a surface water containing O_2 encounters the "stationary" groundwater system at depth, there is an important increase in microbiological activities, resulting in O_2 depletion, transformation of organic material to CO_2 and formation of biofilms.
- The time scale for microbial oxygen reduction to nought in typical fractures was estimated to be in the order of a few days.

6. Bio-Mineralisation

Radionuclides can precipitate with microbially generated ligands, e.g. phosphate, sulphide or carbonate. The concentration of free radionuclide (metals) at equilibrium is governed by the solubility product of the metal complex (typically 10^{-20} to 10^{-30}). Most of the metal or radionuclide should therefore be removed from solution if an excess of ligand is supplied. Phosphate concentration in deep groundwater is very low, and this compound is an indispensable and central metabolic energy transport entity. Therefore, it is not likely that microbes excrete phosphate to the environment. Sulphide is the respiration waste product from sulphate reduction, and is commonly found in deep groundwater. With exception for volcanic and hydrothermal areas, it can be safely assumed that dissolved sulphide to a large part is the result of microbial sulphate reduction. Sulphate reducers have been demonstrated common in Fennoscandian groundwater,[7] which support the assumption that sulphate reduction is an important retention factor for radionuclides prone to form solid sulphide compounds. Microbial metabolism results in the production of carbon dioxide from degradation of organic carbon. Locally, such production may give rise to carbonate precipitates, e.g. calcite, with a concomitant co-precipitation of radionuclides.[14]

Organic surfaces and iron oxides have been identified as important factors in radionuclide transport modelling. Several microorganisms oxidise ferrous iron to ferric iron resulting in a mix of organic material (microbes) and iron oxides, here denoted BIOS (Biological Iron Oxides). Such biological iron oxide will have a retardation effect on many radionuclides. Typically, the microbes form stalks and sheaths that increase the volume of the iron oxides from densely packed inorganic oxides to a fluffy rust-like material with water contents of up to 99% or more. The microbes contribute to the exposure of a large oxide area to trace elements flowing by and the organic biological material adds a strong retention capacity in addition to iron oxides. BIOS can be found everywhere along the Äspö HRL tunnel system. This BIOS is mainly produced by the stalk-forming bacterium *Gallionella ferruginea*.[15, 16] BIOS from different sites and with varying age were analysed for the content of rare earth elements (REE).[17] The results showed that the REE concentration analysed in the inflow and outflow of the experimental artificial ditches were more than 1000 times lower compared to a chondrite standard. Ten to twelve weeks old BIOS that had developed in artificial ditches at tunnel length 2200 m adsorbed three orders of magnitude more REE compared to the groundwater. Very old BIOS (4 – 6 years), collected at 1127B m tunnel length had a REE concentration that were about one million times higher compared to the groundwater and 10 to 100 times higher than what is found in typical Äspö/Ävrö rock material. The obtained data clearly demonstrates the excellent REE sorption capacity of BIOS, as first suggested by Ferris et al.[18, 19]

Microbial BIOS and carbonate mineralization processes will have the largest retention impact on radionuclides at sites where there is an outflow of anoxic groundwater to an aerobic environment. This is because the gradients formed when the anoxic groundwater is oxygenised offer an excellent environment for microbial proliferation resulting in development of BIOS and carbonates that co-precipitate and sorb radionuclides. In opposite, sulphide bio-mineralization will occur along the transport routes up to the ground surface.

7. Bio-Transformation

Microbes can catalyse the direct transformation of some radionuclides to less soluble forms via metabolic processes. The most studied process is how sulphate and iron reducing bacteria can use U^{6+} as a surrogate for sulphate[20] and Fe^{3+},[21]

respectively, as electron acceptors (Table 2). It is not known if this process is of significant importance for the safety in radioactive waste. The concentrations of SO_4^{2-} and solid Fe^{3+} phases in most repository environments will be much higher than that of possibly occurring U^{6+}. Therefore, bio-transformation of radionuclides escaping from a waste repository may not be significant, but this remains to be demonstrated under *in situ* conditions.

8. Production of Chelating Compounds

Microbes need metals for their metabolism, just as all multicellular living organisms. Such metals are often available only in small quantities or, as in the case of iron in surface waters, are not bio-available at all due to low solubility under aerobic conditions. Therefore microbes produce various kinds of chelating compounds to increase the bioavailability of essential elements, needed for metabolism. These ligands are not always highly specific, and several of them will also mobilize other elements such as heavy metals and radionuclides. In the process of capturing the metal-ligand complex, microbes sort toxic metals from essential ones and expel the toxic elements back to the environment. The potential for mobilization of radionuclides from repository environments by bacterially produced ligands is unknown and, therefore, a concerning possibility in the safety analysis that should be explored.

In a first study, the action of the chelating compounds introduced above on solid phase material was investigated.[22] Three bacterial species (*Shewanella putrefaciens, Pseudomonas fluorescens* and *Pseudomonas stutzeri)*, isolated from the deep subsurface and a uranium mine shale material for the former uranium mine Ranstad was chosen for this study. The three species were incubated in a chemically defined medium supplemented with tailings material that had been exposed to natural weathering conditions for 30 years having a content of 0.0013% U by weight. Non-leached uranium ore (0.61% U by weight) from the same area were also incubated. *Pseudomonas fluorescens,* managed to mobilize 0.001–0.005% of uranium from the two ores. This release of U was attributed to the production of species specific pyoverdine chelators, as U could not be detected in either sterile controls or in the experiments with the other two bacteria. *P. fluorescens* also doubles the Cr concentration in solution as compared to the sterile controls whereas *P. stutzeri* and *S. putrefaciens* result in a 5–6 fold increase in Cr concentration. The difference in leaching behaviour between the bacteria used in this study is likely to be explained by the production of different chelators, found to be four using HPLC, as described below.

If ligands are produced in a repository environment, it is crucial to study whether the postulated rock-retardation mechanism of released radionuclides can be impaired by microbial ligands. Three bacterial species (*Shewanella putrefaciens, Pseudomonas fluorescens,* and *Pseudomonas stutzeri)*, isolated from the deep subsurface, and four radionuclides (^{59}Fe(III), ^{147}Pm(III), ^{234}Th(IV), and ^{241}Am(III)) were selected for a laboratory study.[23] The microbes were cultured in the laboratory, separated from dissolved compounds that were produced by the microbes and expelled into solution. The separation was performed by centrifugation, and the supernatants were collected. The supernatants were mixed with radionuclide and solid phase (TiO_2 or SiO_2). The pH ranged between 7.5–9.0. This is an interval, within which all investigated radionuclides should adsorb to the added solid mineral phase, provided the system is free from ligands. All three bacterial species produced ligands that were able to complex the radionuclides at various degrees in competition with the solid phase (Figure 2).

Further investigations were accomplished to identify the complexing agents that caused the obtained mobilization.[23] High performance liquid chromatography (HPLC) was used to separate substances in the microbial supernatant for further characterization. Prior to injection into the HPLC the bacterial supernatants were purified and mixed with $^{59}FeCl_3$. The eluate was measured with UV- (435 nm) and scintillation detector in series. The ^{59}Fe identified the retention times for metal complexed substances, which had correlating peaks in the UV-chromatogram. Four Fe-complexing substances were detected in the supernatant from the *P. fluorescens* culture. The results of HPLC analysis with *P. stutzeri* and *S. putrefaciens* showed two peaks and one peak respectively. All substances eluted from the column varied in retention times, indicating that the microbes studied produced several metabolites that have different chelating abilities. Detailed identification of those and other complexing agents will facilitate the search for presence or absence of identified complexing agents in natural repository environments.

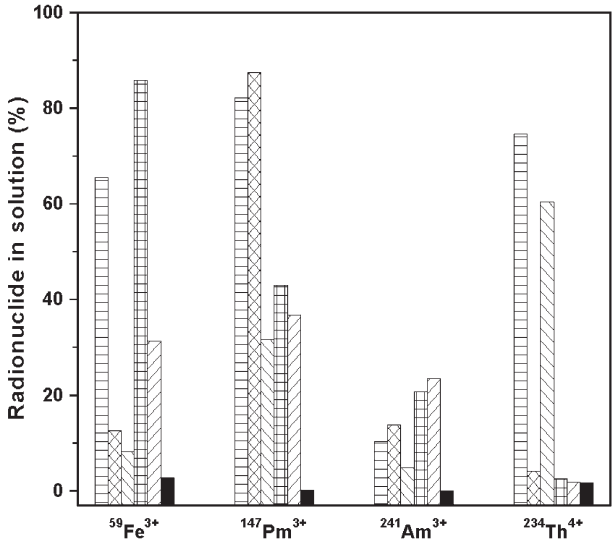

Figure 2. Mobilisation of radionuclides by three different mixes of dissolved compounds that were produced by the microbes and expelled into solution, and two known chelating compounds (Hydroxamate and Catechol) in competition with solid phase SiO_2 at around neutral pH. Bars are, from left to right: *Pseudomonas fluorescens* (pH 8.9); *Pseudomonas stutzeri* (Ph 7.6); *Shewanella putrefaciens* (pH 7.3); Hydroxamate (pH 6.1); Catechol (pH 6.2; Culturing medium (pH 8.4).

The possible production of chelating compounds in deep repository environments is unknown. Therefore, investigations are being launched during 2005 at the MICROBE site 450 m underground at Äspö HRL, Sweden.[24] Microbial biofilms and planktonic microbes will be grown under anaerobic and reduced *in situ* conditions, including pressure, temperature, geochemistry and flow rate. Their possible production of chelating agents will be investigated on solid and dissolved phases of selected radionuclides.

9. Research Needs

The literature on the microbial processes described here is extensive for laboratory research, but sparse when it comes to *in situ* experiments, performed under repository conditions. Microbial processes are controlled by many different factors, of which several are extremely difficult to mimic in the laboratory. Physical parameters such as the high pressure, the composition of gases and the carbonate system, environmental conditions such as anaerobic and reduced systems, and finally, complex ecosystems with multi-species consortia are all very difficult to reproduce in the laboratory. There is a need for in situ experimental conditions and those can be achieved in underground laboratories. The MICROBE laboratory at the 450 m level in the Äspö HRL, Sweden fulfils most of the requirements for in situ investigations.[24] Several research

projects about microbial processes under in situ conditions are under way at MICROBE.

10. Assessments in Radioactive Waste Disposal

Microbial processes are generally not included in various performance assessments of radioactive waste disposal in a quantitative sense, in comparison with how, for example, colloids, sorption and geochemistry are treated. Microbial processes do in general add "new" concepts. Life processes obey the rules of chemistry, thermodynamics, and movement just as any process. The difference is that microbial processes add a metabolic, and therefore, catalytic dimension that is complicated to predict and sometimes difficult to understand. Modelling life processes is consequently more difficult than modelling most non-life processes due to the multi-component nature of the former.

Microbial processes should not be ignored in. Two processes are judged particularly important (Table 3). 1) Microbial production of chelating compounds in the repository environment; and 2) the scavenging of intruding oxygen should be considered. Oxygen reduction was for a long time expected to be solely an inorganic process. New results, reviewed in this paper, have indicated the opposite to be true. Microbial processes will thus be an additional important oxygen scavenging process in repository environments.

TABLE 3: The Importance of Microbial Processes in the Safety Analysis of Radioactive Waste Disposal Can be Ranked According to its Assumed Effect, and the Current Knowledge about the Process

Microbial processes that may influence radionuclide migration	Relative level of current knowledge of *in situ* processes			Ranking 1 = high 2 = medium 3 = low
	high	medium	low	
Immobilisation processes				
Bio-sorption			×	2
Bio-accumulation			×	2
Bio-transformation		×		3
Bio-mineralisation		×		2
Metabolic red-ox reactions		×		1
Mobilisation processes				
Bio-sorption	×			3
Bio-accumulation	×			3
Production of complexing agents			×	1

This will give rough estimate of what processes are more important than other. However, it must be clear that new data from ongoing and future research may change the ranking in the table.

Those two examples demonstrate that overlooking microbial processes may result in models and safety assessments that do not reflect the true situation in future radioactive waste repositories. Conservative assumptions are commonly used to overcome such problems. Therefore, our understanding of geosphere retention phenomena in the context of long-term safety of radioactive waste disposal will benefit from making optimal use of available knowledge on microbial processes in order to reduce such conservatism in future safety assessments.

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