Modelling of microbial degradation processes: The behaviour of microorganisms in a waste repository

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Summary. In a repository for radioactive disposal the waste material is kept in place by several shells and boundaries to prevent a long term recycling of the material into the environment. Present investigations on various chemical and biological processes can be extrapolated into future centuries only with great uncertainty. Models may therefore be a good tool to forecast processes which may occur within the repository and to estimate whether the barriers present will prevent the leaching of waste material within a given time span.
A mathematical model is described based on an experimental laboratory setup, a microcosm described by West et al.[19–22] simulating in a laboratory system repository conditions for a Swiss L/ILW repository. It includes microbial as well as physico-chemical processes. These simulations indicate that biological processes such as gas formation or proton release should also be included into the safety assessment of the repositories.
Key words. Microbial degradation; modeling; safety analysis; radioactive waste; barrier performance; spatial heterogeneity.

Introduction

Repositories of radioactive materials have to be built so that recycling of deposited material into the biosphere is prevented for as long as its radiation remains above the critical levels for living organisms. Furthermore, it has to be kept in mind that some of the elements are highly toxic and for this reason should not be released into the environment at all. Leaching of radioactive substances into the environment, e.g. into ground water and host rock, will be prevented by a series of different technical and geological barriers which encapsule the waste like layers of onion peel[14,17].
According to our present knowledge, repositories are probably not stable over a time period of several thousand years. The decay of the radioactive material will generate heat, repository material may react chemically and physically within the repository and with the environment. Corrosion processes and chemical transformations of repository materials will occur. To obtain all information available about the relevant processes, the latter have to be studied as causal processes (bottom up), as well as in undefined systems with all interrelations to other processes going on (top down) to reach the optimal solution for the design of the repositories. Since the time scale envisaged in shielding the material from the environment exceeds the life time of many generations of men, predictions concerning the long time behaviour cannot be made from real time experiments. Using models incorporating all available information from experimental work, it might become possible to extrapolate the long time behaviour of such a repository system.
Physical and chemical processes which influence the performance of engineered barriers in L/ILW repositories are well studied. In contrast, microbial processes have been neglected for a long time, although they may be potentially important. Growth and activity of microbes is clearly possible in subsoil environments and thus also under conditions present in such repositories[7,13]. Therefore effects of bacterial growth and metabolism have to be included in a safety analysis of L/ILW repositories. It has been suggested that effects of microbes represent only a subset of thermodynamically possible processes already modelled by the physical and chemical processes and the effect of microbes will be included in the worst case described by physico-chemical models.
Some of the effects of microorganisms on the performance of the model are not easily foreseen and simultaneous processes may lead to opposing results. Microorganisms adsorb and accumulate soluble elements from the environment and thus effectively immobilize toxic ions and prevent leaching by retarding transportation. Biofilms could form an further barrier in addition to the engineered ones. Furthermore excretion of polysaccharides may close pores in the matrix and in the host rock. On the other hand, microbes excrete metabolites which form complexes with insoluble compounds. This will facilitate transport of these substances through barriers and leads to a negative effect in the performance of the barriers. Chelating agents, e.g., are produced at low concentrations of soluble iron, conditions present especially in an alkaline system, e.g., in the proximity of cement.
During the past decades many new species of bacteria have been isolated and described from various natural extreme environments giving evidence of the omnipresence of microorganisms in nature within wide environmental limits. Bacteria are found at high temperatures in hot springs, at extreme pressure in deep oceans or deep soil, in salt brines, or in the presence of toxic elements[5]. Bacteria are not only able to survive but also to grow at extremely low concentrations of compounds necessary for life. Microbial communities as found in nature are mixed cultures and consist of a variety of different species. A single bacterial species may not be able to utilize more than a fraction of the organic substrates available in the environments. However, the presence of the different metabolic types of microorganisms will allow degradation of all the resources available by crossfeeding or

cometabolism. Such a nutritional network system has been evolved in all environments in nature during evolution. When conditions change to favour microbial activities normally silent microbes with many different metabolic capabilities may become established within a very short time. This has been observed with unusual substrates, e.g., when phenol or crude oil has been spilled or for bitumen degradation[18, 23].

Microbes found in a repository may originate from various sources: they may have been present in the host rock formations for long times or have been imported either with the waste matrix, the repository material or by contamination during the filling process of the cavern. In all cases, a multitude of species can be expected to be present and it must be assumed that all microbiological processes known so far will also proceed in a closed repository if the environmental conditions are favourable.

A safety analysis that includes microbial effects in repositories has to answer the following questions and thus modeling of repository behaviour should be able to quantify the answers given[15]:

1. Does microbial activity modify the performance of the repository barriers?
2. If yes, which are the responsible microbial processes?
3. How important are these changes caused by microbial processes for the safety analysis?

Description of the repository and the microcosm experiments

The radioactive waste is solidified in cement, bitumen or plastic material and the mixture is enclosed in steel drums. The backfill of the cavern consists of cement or bentonite, the mechanical lining of concrete. The repository is embedded in a decompressed rock zone within an undisturbed rock formation[14]. Details on the composition of the waste are given by Francis[6].

Groundwater is flowing through the host rock surrounding the repository. Usually, it is assumed that groundwater is slowly moving through the repository itself, thereby importing oxygen, ions and organic substrates into the repository and exporting soluble substances out of it. For thermodynamic reasons a variety of chemical and biological processes compete for the available substrates and chemical species within the repository. Different microbial species will compete for the available substrates.

Microcosm experiments have been developped at the British Geological Survey (BGS) to assess and quantify the importance of microbial processes in a repository. The experiments assume that processes observed in a small laboratory scale are imaging the behaviour of small regions in the repository. Waste material containing concrete and steel blocks was incubated with artificial ground water, inoculated with various bacteria and observed over more than one year[19-22].

The computer model presented here has been constructed to describe the chemical and biological changes occurring in these experiments and to predict changes in the size and activity of the microbial population in the experimental systems.

Concept and complexity of the model

The concept of the work was to start with the most basic form of model which would allow successive refinements to be added at later stages.

At first, the following limitations similar to the models of Grogan and McKinley[15] were assumed:

– All organic waste material will be mineralized completely by microorganisms under the various conditions expected to be present in the repository and the kinetics of the processes is neglected. Three different environmental conditions are assumed to follow in sequence:

1) aerobic respiration, $O_2 > 0$,
2) anaerobic respiration with alternative electron acceptors such as nitrate or sulfate, $O_2 = 0$, SO_4^{-2}, $NO_3^- > 0$,
3) anaerobic, methane production, all electron acceptors (except CO_2) = 0.

– Due to the thermodynamic efficiency for energy transduction of the cells, these processes follow the sequence given, thus energy yield from degradation and thus biomass formation is dependent on the presence of O_2 and possible alternative electron acceptors present[9, 10].

In a further step, kinetic parameters of microbial growth, e.g. growth constants and substrate affinity values for the three environmental conditions given are included. It is assumed that the diffusion rates for the chemical species involved in microbial metabolism are enhanced compared to the metabolic rates. This results in a spatial homogeneity within the repository and treats the system as a homogeneous batch or chemostat culture. The degradation pathways are determined by the electron acceptors present as previously described.

In summary the model describes the overall degradation of organic waste by a non-defined omnipotent microbial community and monitors simultaneously some of the changes in environmental conditions, e.g., electron acceptor used, protons released and gas formation. Degradation depends on the chemical inventory given at the beginning and the thermodynamic conditions present during the processes. For calculations of the latter, standard temperature conditions of 25 °C have been assumed. Products of the microbial metabolism can have great influences on the metabolism itself but also on the chemistry of the environment. Speciation of elements may change causing their mobility to be altered[3]. However, at this stage, only accumulating endproducts have been taken into account. The neutralization capacity of the repository system is not known, thus for the calculation of the chemical species a constant pH is assumed. Protons and electrons used or released are calculated from the stoichiometry of the degradation and compared with a hypothetical buffer capacity of the system.

Mathematics of growth of a microbial community

Growth is described by the widely used Monod model[12], where the growth rate of the microbial population is limited by one or several substrates. In the case of one limiting substrate growth of the microbial population, R_x, is:

$$R_x = \frac{\mu_{max} \cdot S \cdot X}{K_s + S}$$

where X = number (or concentration or mass) of the microbes, S = concentration of the limiting substrate, μ_{max} = maximal specific growth rate, K_s = Monod coefficient, substrate concentration for half-maximal growth rate.

The case for one limiting substrate is easiliy extended to several limiting substrates[12]. For two substrates (subscript 1 and 2) both simultaneously limiting, the growth rate of a microbial population is described as

$$R_x = \frac{k \cdot S_1 \cdot S_2 \cdot X}{(K_1 + S_1) \cdot (K_2 + S_2)}$$

where k = maximal specific growth rate and K_i the Monod coefficient for the substrate S_i.

BlackCell, a simple model for microbial processes in a repository

A guideline during the development of the model was to keep complete flexibility at all stages for future enlargements, refinements and changes. The program offers an open architecture allowing a successive implementation of more processes as well as a wide variation of the inventory of the model system. Starting from a given inventory of a certain chemical composition in the repository and a defined initial oxygen concentration as important environmental factor regulating the degradation path the habitat variables are calculated and compared with the conditions which hold for a certain type of degradation, aerobic or anaerobic respiration, methanogenesis. Limiting concentrations of the electron acceptor cause switching to the next favourable one (sequence $O_2 - NO_3^- - SO_4^{-2} - CO_2$, is given according to values from the literature). The process will stop when all the organic material in the waste is degraded to CO_2 and methane. Sulfide and proton release, and steel corrosion are included in the model.

The loss of energy in the system through the production and the maintenance of microbial biomass, leaving the system as heat energy and the loss of bacteria by leaching or lysis, is simulated by a given death rate of the cells.

As starting conditions for the waste composition the values given by BGS and Nagra are used. The organic substances in the waste material are described by a fixed stoichiometry, as a first approach the values of cellulose are used in the calculations. Different waste compositions can be tested by changing the general chemical formula during a simulation run. $CaSO_4$ concentration in concrete as the source for sulfate has been estimated as 0.4–0.6 moles/kg[2]. Sulfate-resistant concrete in contact with groundwater is degraded about 0.2 mm per year and the pH during the first 1000 years is expected to be around 11–13[1], although niches with lower pH's cannot be excluded.

At the moment the dynamic parameters to model the kinetics of growth and of microbial metabolism must be given in an arbitrary time scale. Not enough experimental data are yet available to correlate a simulated time course with a defined time scale. Furthermore, if it is accepted that a repository is a non-homogeneous system similar overall kinetics may occur in various compartments with very different absolute time scales. Growth parameters are chosen arbitrarily and can be changed at will during simulation.

The pH-buffer system is calculated from the concentrations of carbonate, phosphate, ammonia and sulfide, it is assumed that pH-equilibration in the system is faster than the rates of microbial activities producing or consuming protons. The table gives the description of all the rate variables of the model with their initial values.

Organic waste contains a multitude of different degradable substrates for microbes and at low nutrient concentrations diauxic growth is rarely observed on mixed substrates. Therefore a simultaneous uptake and use of the substrates present was assumed for the calculations, thus treating all organic material essentially as one substrate. We also assume that the microbial activity determined in simulations is not the result of one single species but of a variable community of different microbes. No discrimination between specific biochemical pathways is attempted, the results are treated as the effect of the sum of all microbial processes occurring in the community present.

The environment will be changed by microbial processes, furthermore, non-microbiological processes will greatly influence the microbiological processes as well. Sulfate released from concrete is the main source of sulfate in the system. The rate is determined by a release constant and a saturation constant containing solubilisation and precipitation processes of minerals in aqueous solution. Steel corrosion is primarily restricted to the presence of oxygen when Fe is oxidized to Fe_2O_3[8]:

$$2\,Fe^0 + 1.5\,O_2 \rightarrow Fe_2O_{3(s)}$$

The rate of the corrosion is taken as a constant decreasing the oxygen concentration in a linear function in concurrence with microbial respiration.

Additional carbon sources to the ones available in waste material may be present in or introduced into actual repositories as well as into the experimental microcosms from the environment e.g. from steel corrosion, from bentonite and marl, by groundwater flow through pores

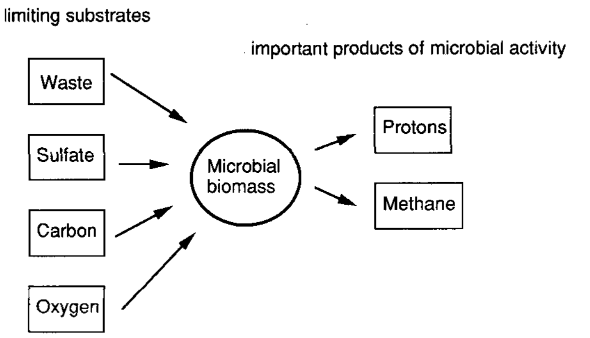

Figure 1. Graphical model containing the relevant environmental factors used acting on the growth of the microorganisms, and effects of microbial metabolism on the environment.

Default conditions for the simulation experiments. The volume of the model container is assumed to be 1 l

Parameter	Dimension	Value
Yield, aerobic condition (1)	g/g substrate	0.5
Yield, sulfate reduction	g/g substrate	0.1
Yield, methane formation	g/g substrate	0.06
Threshold for degradation pathway	molar	0.01
μ_{max}, aerobic conditions (2)	1/t	0.1
μ_{max}, sulfate reduction	1/t	0.05
μ_{max}, methane formation	1/t	0.02
Removal of biomass	1/t	0.001
K_s for waste (3)	molar	10^{-4}
K_s for oxygen	molar	10^{-5}
K_s for sulfate	molar	10^{-6}
Initial amount of waste	g	100
Waste composition, organic C (4)	g%	30
Waste composition, organic H	g%	48
Waste composition, organic O	g%	13
Waste composition, organic N	g%	7
Waste composition, organic P	g%	0.7
Waste composition, organic S	g%	0.07
Waste composition, metal content (as Fe)	g%	1
Initial amount of O_2 (5)	moles	0.01
Initial amount of sulfate in concrete	moles	10
Initial amount of sulfate	moles	0.5

Comments: (1) Yields have been taken and calculated from (11, 16).
(2) μ_{max} have been taken from (11).
(3) K_s have been taken from (16).
(4) Waste composition taken as average of yeast biomass (11).
(5) Oxygen equal to 1 l of air.

and cracks. This is simulated by a flow factor. In all cases, only those environmental state variables which are actually influenced and altered by microbial metabolism are considered in the calculations. Buffer capacity and pH will be dominated by the carbonate and the phosphate system under oxic conditions, under anoxic ones ammonia and sulfide will become additionally important. These equilibration reactions are assumed to be faster than microbial consumption and production of protons, the system thus being in an equilibrium state. In a later refinement of the model acid neutralisation capacity (ANC) and pH changes in the experimental cell may be calculated and the concentration of each chemical species present in water determined. In figure 1 the model is summarized graphically showing the external input factors and the calculated output. The complete set of initial conditions is given in the table.

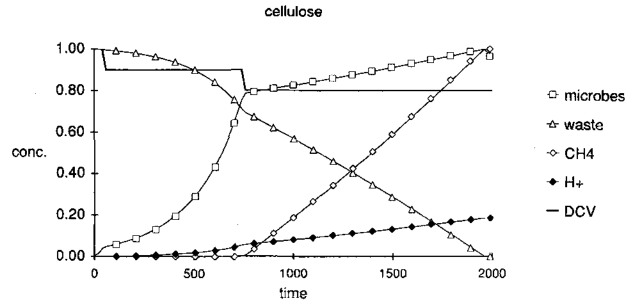

Figure 2. Simulation of microbial growth, gas- and proton production with cellulose as substrate and limited oxygen supply (DCV = degradation condition value, see text).

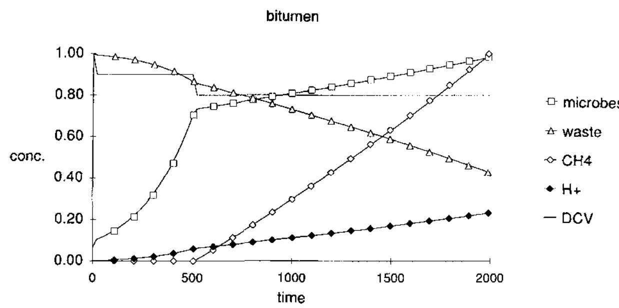

Figure 3. Simulation of microbial growth, gas- and proton production with bitumen as substrate and limited oxygen supply.

Results

Figure 2 demonstrates the simulation of growth, substrate depletion, methane and proton liberation with cellulose as organic substrate. All values are calculated in relative units (0–1) and plotted against an arbitrary time scale. The initial amount of oxygen is used up immediately and the system switches to sulfate reducing conditions (change of filled triangles from the indicator value 1.0 to 0.9, DCV = degradation condition value). Exponential growth of organisms is observed with a decrease in substrate and proton liberation. After 'time 400' all sulfate is reduced and the system changes to methanogenic conditions. Biomass increase through growth is slowed down as well as substrate use, and methane formation begins. Later on when the substrate is depleted the organic carbon is in the form of biomass although lysis causes a certain turnover of carbon to take place. Figure 3 shows the same time course using bitumen as the source of organic carbon. The electron transport rate is determined by the growth constants and since bitumen is a more reduced substrate than cellulose, oxygen consumption is faster and carbon substrate depletion is clearly slower. As the same K_s value was used for the substrate biomass accumulation proceeds at higher rates for the same reason. In both cases the time span of 2000 arbitrary units chosen in figures 2 and 3 is not long enough to reach a complete conversion of the substrate into biomass. As a third example figure 4 shows the bitumen system drawn with unlimiting access of air, as envisaged for parts of the

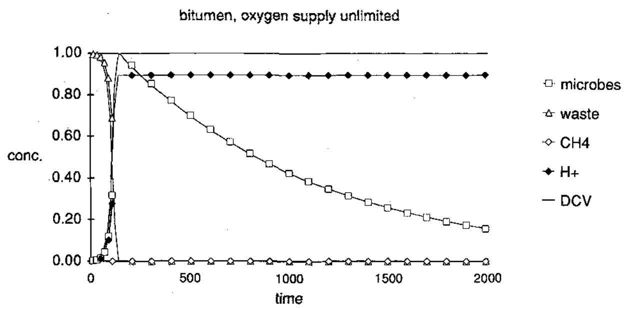

Figure 4. Simulation of microbial growth, gas- and proton production with bitumen as substrate and unlimited oxygen supply.

repository during the filling process. The substrate is depleted rapidly producing, besides CO_2 (not shown), large amounts of protons. After complete degradation biomass has reached a maximum. Its decay is determined by the preset decay rate which includes also lysis and leaching out of the system of the microorganisms.

Discussion

The model presented here is still of a crude nature and should be improved when more experimental observations in laboratory systems of either defined properties or of a Black Box style are available. Of great demand are measurements of the necessary parameters with greater resolution in time and space. This would allow the calibration of the time axis of the simulations from the present arbitrary units to values approaching those of more realistic dimensions and furthermore the calibration of the ordinate with more precise values. But the model itself has also to be improved. To further refine the description of metabolic activities of microorganisms in a repository, spatial heterogeneity must be included into such model calculations. On the one hand a repository is an extremely heterogeneous system, on the other, microbial processes may be extremely rapid compared to transport phenomena within the system and therefore distance criteria will become important. In the extreme case, the spatial arrangement will determine the extent of the microbial effects on the behaviour of the barriers. In such a case, a model assuming homogeneous conditions, as presented here, will completely fail in predicting the performance of the repository. Heterogeneity in closed vessels containing bitumen as substrate has been demonstrated by Wolf and Bachofen[23] showing the simultaneous reduction of sulfate or nitrate and the formation of methane under certain conditions.

The microbes transform the substrates available in a spatially structured system and diffusion processes between the solid phase, the liquid and gaseous one must be included. For such exchange processes, the size of the surface of the different phases becomes important, as well as thickness and structure of the material. Furthermore it must be assumed that the spatial heterogeneity alters with time.

New experimental data not considered so far may also critically change the simulation output. At present, it cannot be decided whether microbes undergo evolutionary processes during growth in a repository over decades or centuries. Changes in metabolic capabilities may follow genetic changes, however, changes in the composition of the microbial community cannot be distinguished from evolutionary changes of a single species. With the new genetic techniques available, such a question could be answered as well as the quantification of a possible exchange of genetic material among different species could be achieved.

Finally radioactive waste in L/ILW repositories may contain recalcitrant materials. At present, the knowledge on microbial degradation of unusual substrates is insufficient to predict whether all organic carbon present will be degraded completely under oxic as well as under anoxic conditions as assumed so far here.

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Microorganisms in nuclear waste disposal. Conclusions

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What is the importance of microorganisms in nuclear waste disposal?

From the various subsoil microbiology programs we know that microorganisms with widely varying activities are present in all kinds of soils and rocks, down to depths of over 500 m; thus it must be concluded that whether autochthonous or brought in during building and filling, microorganisms will be present in any repository.

In contrast to the situation in higher plants and animals where species are thought to be 'habitat bound', archetypal microbial populations for specific environments probably do not exist. While different habitats may have different and location-specific microbial activities, many microorganisms have a world-wide distribution and can be isolated from many different sources. In most situations they may be dormant and show no physiological activity, although they may become active after being brought into the nutrient-rich laboratory test systems. It is therefore not surprising that in laboratory experiments conducted with waste, backfill or rock material, there is no difference in physiological activities whether the system has been inoculated or not. This has been observed e.g. for bitumen degradation, for nuclide adsorption or in black box experiments containing the real components of a repository. It has been known for microorganisms which survive the most hostile environments and stay at conditions which allow absolutely no growth for thousands of years, to suddenly become active when transferred to the conditions to which they were originally adapted; for example it has been demonstrated that thermophiles are present in lake sediments where the temperature has remained at 4 °C for the last 10000 years.

Microbial ecologists agree that probably all natural ecosystems are more or less heterogeneous, macroscopically as well as microscopically. Environmental conditions change in the m to km range as well as in the µm to mm range. At this level of heterogeneity a great number and a broad diversity of niches is created which allows the growth and activity of a wide variety of microorganisms. Often microorganisms form biofilms by attachment on abiotic or biotic surfaces; experimentally, biofilms have been observed even on surfaces of bitumen and concrete. Biofilms are heterogeneous layers of cells mostly of a variety of species and exopolymers, they act as barriers or filters which result in steep chemical gradients across the film, for example pH-gradients across biofilms on concrete.

How do microorganisms change the known processes going on in a repository?

The immediate goal of the various agencies in each country in charge of the safe disposal of waste is to determine whether the presence of microorganisms affects the different physical and chemical processes going on in a repository, either in a positive or in a negative sense, e.g. corrosion of metals or concrete, gas formation from organic material disposed or mobility of elements in ground water and rocks. Although microorganisms cannot change overall thermodynamics, their metabolism may lead to different intermediates promoting indirect effects, e.g. reaction rates may be greatly accelerated, new degradation products may accumulate or the physical environment may be altered. The question is therefore to determine the cases in which microorganisms may be