5 Buckley, L. P., Glegg, B. C., and Oldham, W. K., Microbial activity on bituminized radioactive waste. Radioact. Waste Manag. nucl. Fuel Cycle *6* (1985) 19–36.
6 Dostalek, M., Munk, V., and Volfova, O., Cultivation of the yeast *Candida lipolytica* on hydrocarbons. I. Degradation of n-alkanes in batch fermentation of gas oil. Biotechnol. Bioeng. *10* (1968) 33–43.
7 Herbes, S. E., Rates of microbial transformation of polycyclic aromatic hydrocarbons in water and sediments in the vicinity of a coal-coking wastewater discharge. Appl. envir. Microbiol. *41* (1981) 180–209.
8 McKinley, I. G., West, J. M., and Grogan, H. A., An Analytical Overview of the Consequences of Microbial Activity in a Swiss HLW Repository. NAGRA report 85-43, 1985.
9 Munoz Cebrian, J. M., Microbial deterioration of bituminous materials: selection of microorganisms with specific metabolic capacity on hydrocarbons, in: Biodeterioration 5, p. 472–483. Eds T. A. Oxley and S. Barry. John Wiley Sons Ltd, London 1983.
10 Oudot, J., Le bilan du carbone dans une expérience de biodégradation bactérienne d'un pétrole brut. Envir. Pollut. *20* (1979) 177–187.
11 Oudot, J., Rates of microbial degradation of petroleum components as determined by computerized capillary gas chromatography and computerized mass spectrometry. Mar. envir. Res. *13* (1984) 277–302.
12 Pritchard, P. H., Ventulo, R. M., and Suflita, J. M., The microbial degradation of diesel oil in multistage continuous culture systems, in: Proceedings of the 3rd Int. Biodegradation Symposium, p. 67–78. Eds J. M. Sharpley and A. M. Kaplan. Applied Science Publishers Ltd, London 1976.
13 Roffey, R., and Hjalmarsson, K., Microbial processes in the final repositor the silo part. Theoretical approach and preliminary experiments on the biodegradation of bitumen. Part. 1. FOA-C-40172-B4 (1983).
14 Rontani, J. F., Bosser-Joulak, F., Rambeloarisoa, E., Bertrand, J. C., Giusti, G., and Faure, R., Analytical study of asthart crude oil asphaltenes biodegradation. Chemosphere *14* (1985) 1413–1422.
15 Walker, J. D., Colwell, R. R., and Petrakis, L., Biodegradation rates of components of petroleum. Can. J. Microbiol. *22* (1976) 1209–1213.
16 Westlake, D. W. S., Jobson, A., Philippe, R., and Cool, F. D., Biodegradability and crude oil composition. Can. J. Microbiol. *20* (1974) 915–928.
17 Wolf, M., Mikrobieller Abbau von Bitumen. NAGRA report 89-14, 1989.
18 Wyndham, R. C., and Costerton, J. W., In vitro microbial degradation of bituminous hydrocarbons and in situ colonization of bitumen surfaces within the Athabasca oil sands deposit. Appl. envir. Microbiol. *41* (1981) 791–800.
19 Zobell, C. E., and Molecke, M. A., Survey of microbial degradation of asphalts with notes on relationships to nuclear waste management. Sand (1978) 78–1371.

0014-4754/91/060533-07$1.50 + 0.20/0

Biodegradation of bitumen used for nuclear waste disposal

R. Roffey and A. Norqvist

Swedish Defence Research Establishment, Department of NBC-Defence, S-901 82 Umeå (Sweden)

Summary. Studies have been carried out to test microbial degradation of bitumen used for encapsulating radioactive waste in Sweden. Microorganisms have been isolated that degrade bitumen. In long-term tests under conditions simulating those in the silo part of the final repository for low- and intermediate-level radioactive waste, both aerobic and anaerobic degradation of bitumen has been found, equivalent to 0.6–1.5 µmoles CO_2/month · mg bitumen and 1.1–1.5 µmoles CO_2/month · mg bitumen, respectively.
Key words. Microorganism; bacteria; bitumen; biodegradation; gas production; oil degradation; hydrocarbons; radioactive waste.

Introduction

In Sweden some of the low- and intermediate-level radioactive waste from nuclear power plants will be encapsulated in bitumen, put into steel drums and placed in mined rock caverns 50 m below the Baltic sea bed. After the repository is finally closed it will be filled with water. The question has been raised whether microorganisms can degrade bitumen under the prevailing conditions and contribute to increased gas production. Apart from this direct production due to microbial decomposition of bitumen, it is also assumed that the microbial activity may decrease the pH so that hydrogen-evolving corrosion will occur with large amounts of gas being produced. For safety reasons it is therefore important to estimate the microbial activity in the repository and the rate of microbial degradation of bitumen[1–9,15–17,20]. This paper summarizes experimental investigations carried out in Sweden on aerobic and anaerobic degradation of bitumen used for encapsulation of radioactive waste[11].

The literature available shows that the knowledge is very limited on anaerobic degradation of hydrocarbons including asphaltenes (bitumen). Biochemical pathways are in most cases unknown. Most studies are limited to single compounds and not concerned with complex mixtures like bitumen. It has been claimed that anaerobic degradation of asphalt occurs, e.g., anaerobic conversion of thiophene and other sulphur-bearing compounds of petroleum (asphaltenes, polysulphides, heavy residues) with production of H_2S has been demonstrated[6].

The growth of microorganisms on the surface of bitumen has been shown using electron microscopy[2,13,19]. A

Table 1. Model systems used for long-term studies of biodegradation of bitumen

System	Type of water	Gas phase	pH
I	Minimalmed-2[a]	Air	7.0
II	Groundwater[b]	Air	7.24
III	Groundwater	Argon[d]	7.24
IV	Groundwater, concrete-leached[c]	Air	11.5

The vessels of all four systems were filled with glass beads covered with bitumen (1.8 g) resulting in a bitumen-coated surface of 2 m^2 and overlayed with 2500 ml of water as specified.
[a]Minimal medium-2[11]. [b]Groundwater from Forsmark. [c]Groundwater as in b) but used to leach concrete before added to the system. [d]Argon is used to flush the system to prevent oxygen from entering. In the beginning the system was flushed once a week, but as that was not sufficient to prevent oxygen from entering the system it was later continuously flushed with a low flow rate of argon so that a constant overpressure was maintained.

number of bacterial cultures were isolated with the ability to grow on different fractions of bitumen. While in one study the asphaltic fraction was the only one that could not support growth[19], in another all fractions of bitumen were degraded; however some isolates degraded only certain fractions[4].

Experimental studies on biodegradation of bitumen

For experimental studies, bacteria were isolated and enriched from several environments where petroleum-degrading microorganisms were known to be present[11]. The experimental systems consisted of glass cylinders between two plates of stainless steel (diameter 11.4 cm, height 30 cm), containing a bottom layer of glass beads (1 mm diameter) covered with bitumen (1.8 g bitumen/ 2 m^2) a liquid layer consisting of Minimal medium or groundwater, and a gasphase (air or argon). The vessels were inoculated with bacteria from enrichment cultures or sediment samples from rock caverns used for storing heavy fuel oil. Four such systems were kept at room-temperature in the dark. When samples were taken (100 ml) this volume was replaced by sterile groundwater. The experimental system is described in table 1.
Aerobic bacteria were counted by spread-plate technique with Tryptone-Glucose-Extract-Agar. Sulphate-reducing bacteria were estimated by the Most-Probable-Number (MPN) procedure in Postgate's medium B[10]. The MPN was also used to estimate hydrocarbon-utilizing microorganisms. Radiorespirometry was carried out for activity measurements using ^{14}C labeled compounds[11]

Results

The degradation of bitumen was followed in the four vessels over one year, and samples were analyzed every month. The results are presented as mean values for the whole period in table 2. No major differences are seen between the number of aerobic and oil-degrading bacteria, but the counts were slightly higher in system I with culture medium and IV with high pH (table 2). A pH

Table 2. Summary of mean values of parameters measured in model systems during one year. For the oil-degrading activity the first number gives the value for aerobic, the second for anaerobic incubation of samples with the labeled compound. Systems I to IV are as in table 1.

Parameter	System I	II	III	IV
pH	8.9	8.7	8.6	9.8
O_2 (mg/1)	5.8	7.3	0.9	5.4
SO_4 (mg/1)	390	249	281	158
Fungi/ml O	0	0	0	
Aerob/ml	8×10^4	2×10^4	8×10^3	1×10^5
Oil-d/ml	3×10^4	8×10^3	1×10^3	1×10^4
SuReBa/ml	0	0	0	0
BNP-HN (a)	8.2	3.1	4.4	0.5
" -HND	10.1/6.2	2.4/1.6	3.6/3.5	0.6/0.6
" -C	29.6/27.8	4.4/1.7	13.1/15.3	0.4/0.3
" -P	33.8/35.6	1.4/0.9	4.8/9.8	0.5/0.3
" -N	2.9/1.7	1.6/0.6	1.1/0.5	0.3/0.4
" -A	4.1/1.6	1.9/0.5	1.2/0.2	0.1/0.2
HA-CO_2 (b)	8.8	16.6	22.5	43.1
HA-I	7.2	6.2	8.2	6.8
SO_4^{2-} red. (c)	2×10^{-9}	6×10^{-10}	1×10^{-9}	2×10^{-10}

(a) BNP = biodegradation potential. Percentage of ^{14}C-labeled hydrocarbons degraded to ^{14}C-CO_2 during incubation for two weeks. Abbreviations are as follows: HN, hexadecane + naphthalene; HND, hexadecane + naphthalene + decane; C, cyclohexyldodecane; P, pristane; N, naphthalene; and A, anthracene.
(b) Heterotrophic activity. Percentage of ^{14}C-labeled glutamic acid degraded to ^{14}C-CO_2 (CO_2) or incorporated into cells (% I) during incubation for eight hours.
(c) Sulphate-reducing activity. Moles of ^{35}S-H_2S produced per mol ^{35}S-labeled SO_4^{2-} hour ml of sample.

around 10 did not inhibit growth of the types of microorganisms present. At the beginning a few fungi were found but later no fungi were detected. Sulphate-reducing bacteria were absent in the water of the systems.
Heterotrophic activity as measured by uptake and degradation of labeled glutamic acid (table 2) was present in all systems and no major changes were seen over the period. Small variations in the number of aerobic bacteria might have been a result of the variation in oxygen concentration during the period.
Analysis of the biodegradation potential showed that in all systems microorganisms were present that degraded decane, hexadecane, cyclohexyldodecane, pristane, naphthalene and anthracene (table 2). These compounds were chosen as model substrates for bitumen in order to test for the hydrocarbon degrading activity. Cyclohexyldodecane and pristane are the most easily degraded hydrocarbons used. Hydrocarbon-degrading activity was highest in the system containing Minimal medium-2. The activity was lowest in the alkaline system but still present at a pH between 10 and 10.5. Consistent with the variation in the amount of oil-degrading bacteria, the biodegradation potential also varied. The levels of dissolved oxygen did not differ much in the systems and only a slightly higher value in system II is observed, which might be due to a lack of nutrients in the system. In spite of the flushing of vessel III with argon, the concentration of dissolved oxygen in this system was not zero. For accurate measurements of the anaerobic degradation, samples were incubated in separate vessels under

Table 3. Mean CO_2 production during aerobic incubation of bitumen with water samples from model systems. Systems I to IV as in table 1, incubation during 5 months at room temperature.

System	μmoles CO_2/month · mg bitumen found in Gasphase	Liquid + gas
I	2.8–5.9	6.9–7.9
II	0.4–0.7	0.8–1.8
III	0–0.5	1.1–1.6
IV	0	0.6–1.5

Table 4. Mean CO_2 production during anaerobic incubation of bitumen with water samples from model systems. Systems I to IV as in table 1.

System	μmoles CO_2/month · mg bitumen found in Gasphase	Liquid + gas
I	0.6–0.7	0–2.1
II	0	0
III	0.5–0.9	1.6–2.0
IV	0	1.1–1.5

strict anaerobic conditions. No significant difference in the hydrocarbon-degrading capacity was observed[11], demonstrating real anaerobic hydrocarbon-degrading activity.

For a more exact quantification, samples of water from the four model systems were transferred to 10-ml serum bottles containing 1 g of bitumen-covered glass beads with an area of 10 cm^2 and 1 mg of bitumen. The amount of CO_2 produced during a 5-month aerobic incubation period was estimated by GC (table 3). The results of similar anaerobic incubations (4 months) are summarized in table 4.

The results indicate that anaerobic production of CO_2 from bitumen occurred in three of the systems. Aerobic systems yielded somewhat higher production rates of CO_2 than the anaerobic ones.

Discussion

Recently several studies have been published, demonstrating that various aromatic compounds including hydrocarbons are degraded under strict anaerobic conditions[6, 12, 14, 18]. In earlier work it has often been questioned whether strict anaerobic conditions prevailed during the experiments.

From our studies it must be assumed that anaerobic degradation of bitumen will take place in the repository and over the long time to be considered (up to 500 years), it may be of great significance.

The experiments demonstrate that aerobic degradation occurs at a rate of 0.6–1.5 μmoles CO_2/month · mg bitumen or 7.2–18 mmoles CO_2/year · m^2 bitumen in system IV under conditions simulating those in the repository (table 3). Furthermore, anaerobic degradation may take place in some cases at lower rates of CO_2 production, yielding values from 1.1–1.5 μmoles CO_2/month · mg bitumen or 13–18 mmoles CO_2/year · m^2 bitumen for system IV (table 4).

It is concluded that bitumen will be degraded under conditions similar to those in the repository under aerobic as well as anaerobic conditions and it seems very difficult to forsee what might happen in the final repository during a long time span. It would thus be preferable to use a nonbiodegradable material for encapsulating the radioactive waste.

Acknowledgement. We would like to express our thanks to Dr Karin Hjalmarsson for her valuable contributions in the initial part of the project, to Eva Larsson and Susann Haraldsson for skillful technical assistance and to Maria Westling and Marianne Loge for typing the manuscript. This work was possible due to financial support from the Swedish Nuclear Fuel and Waste Management Co, Stockholm, Sweden.

1 Barnhart, B. J., Campbell, E. W., Martinez, E., Caldwell, D. E., and Hallett, R., Potential microbial impact on transuranic wastes under conditions expected in the waste isolation pilot plant (WIPP), March 15-June 15 LA-7918-PR, progress report 1980.
2 Brunner, C., Wolf, M., and Bachofen, R., Enrichment of bitumen degrading microorganisms. FEMS Microbiol. Lett. *43* (1987) 337–344.
3 Buckley, L. P., Clegg, B. C., and Oldham, W. K., Microbial activity on bituminized radioactive waste. Radioact. Waste Manag. nucl. Fuel Cycle *6* (1985) 19–36.
4 Forrester, P. I., Pearson, D., and Roth, J., Microbial degradation of Athabasca bitumen, in: Microbial Enhancement of Oil Recovery, pp. 156–161. Eds J. E. Zajic, D. G. Cooper and T. R. Jack. Pennwell Publ., Tulsa, Oklahoma 1983.
5 Francis, A. J., Microbial transformation of low level radioactive waste, IAEA Environmental migration of long lived radionuclides, pp. 415–429. IAEA-SM-257/72 1982.
6 Kurita, S., Endo, T., Nakamura, H., Yagi, T., and Tamiya, N., Decomposition of some organic sulfur compounds in petroleum by anaerobic bacteria. Appl. Microbiol. *17* (1971) 185–195.
7 Mayfield, C. I., and Barker, J. F., An evaluation of the microbiological activities and possible consequences in a fuel waste disposal vault. A literature review. Atomic Energy of Canada Ltd TR-139 1982.
8 McKinley, I. G., Van Dorp, F., and West, J. M., Geochemical constraints on the microbial contamination of a deep geological repository for HLW. Eidg. Institut für Reaktorforschung, Rep TM-45-84-11 1984.
9 Molecke, M. A., Degradation of transuranic-contaminated wastes under geological isolation conditions. International Symposium on the management of alpha-contaminated wastes, June 2–6. IAEA-SM-246/37: 653–666 (1980).
10 Postgate, J. R., The Sulphate-Reducing Bacteria. Cambridge University Press, Cambridge, U.K. 1979.
11 Roffey, R., Hjalmarsson, K., and Norqvist, A., Microbial degradation of bitumen used for encapsulating radioactive waste. Swedish Defence Research Establishment, Umeå Sweden. FOA report C 40238-4.9 (1987)
12 Sahm, H., Brunner, H. and Schoberth, S. M., Anaerobic degradation of halogenated aromatic compounds. Microb. Ecol. *12* (1986) 147–153.
13 Schoenen, D., and Tuschewitski, G. J., Microbial colonization of moistered bitumen and high-grade steel plates revealed by scanning electron micrographs. Zbl. Bakt. Hyg., I Abt. Orig. B *176* (1982) 116–123.
14 Sleat, R., and Robinson, J. P., The bacteriology of anaerobic degradation of aromatic compounds. J. appl. Bact. *57* (1984) 381–394.
15 West, J. M., McKinley, I. G., and Chapman, N. A., Microbes in deep geological systems and their possible influence on radioactive waste disposal. Radioact. Waste Manag. nucl. Fuel Cycle *3* (1982) 1–15.
16 West, J. M., McKinley, I. G., and Christofi, N., Geomicrobiology and its relevance in nuclear waste disposal. An annotated bibliography. Rep. Inst. geol. Sci. ENPU 82-8 1982.
17 West, J. M., Christofi, N., and McKinley, I. G., An overview of recent microbiological research relevant to the geological disposal of nuclear waste. Radioact. Waste Manag. nucl. Fuel Cycle *6* (1985) 79–95.

18 Williams, R. J., and Evans, W. C., The metabolism of benzoate by *Moraxella* species through anaerobic nitrate respiration. Biochem. J. *148* (1975) 1–10.
19 Wyndham, R. C., and Costerton, J. W., Hererotrophic potentials and hydrocarbon biodegradation potentials of sediment microorganisms within the Athabasca oil sand deposit. Appl. environ. Microbiol. *41* (1981) 783–790.
20 Zobell, C. E., and Molecke, M. A., Survey of microbial degradation of asphalts with notes on relationship to nuclear waste disposal. Sandia National Laboratories SAND-78-1371 (1978).

0014-4754/91/060539-04$1.50 + 0.20/0

Microbial degradation of bitumen

M. Wolf and R. Bachofen

Institute of Plant Biology, University of Zürich, Zollikerstr. 107, CH-8008 Zürich (Switzerland)

Summary. Bitumen is commonly employed as a matrix for the long-term storage of low and intermediate level radioactive waste. As bitumen can be degraded by microbial activity, it is of great significance to determine the rates at which it may occur in nuclear waste repositories.
Experiments have been carried out under optimal culture conditions using bitumen with a highly increased surface area. The potential of different microbial consortia to degrade bitumen has been examined. The investigations showed clearly that bitumen-degrading organisms are ubiquitous. In general the organisms formed biofilms on the accessible substrate surface area. Under oxic culture conditions a bitumen degradation rate of 20–50 g bitumen $\cdot$ $m^{-2} \cdot y^{-1}$ leading to a CO_2 liberation of 15–40 l was observed. Anoxic conditions yielded a 100 times smaller degradation rate of 0.2–0.6 g bitumen $\cdot$ $m^{-2} \cdot y^{-1}$ and a CO_2 production of 0.15–0.45 l.
Based on linear extrapolation the experimentally determined degradation rates would lead to a 25–70% deterioration of the bitumen matrix under oxic and 0.3–0.8% under anoxic conditions within 1000 years.
Key words. Bitumen; microbial activity; biofilm; degradation; gas production.

Introduction

Bitumen has been used by man since early times and prehistorical findings document various applications such as glue or mastic as surface protection material or for tightening. The presence of asphalt mines has been documented for ancient middle eastern civilizations and the material has been used e.g. to isolate water pipes for the gardens in Ninive or for proof coating the ark of Noah. Historical details are given by Hellmuth[18] and Poll[20]. Todays' use of bitumen is mainly in the construction industry, for road pavements, for protection of roofs and pipes and in the automobile industry[13]. In most recent times bitumen is also used for the disposal of radioactive wastes, particularly for the solidification of low- and intermediate level radioactive wastes. This technology is documented in various publications[12,13,22].
Bitumen and bitumen-like substances e.g. tar, pitch or asphalt, may last for long time periods[18], as seen in remains in the caves of Lascaux in France (around 15 000 years)[12] or as components of crude oil (10^8 years)[26]. However, under certain conditions rapid degradation has also been observed. As early as 1935 microbial degradation of bitumen was described[20] and the phenomenon was investigated later in more detail[6,15,16,21,24,33,39].
To determine the long-term stability of the bitumen matrix used for the solidification of the waste radionuclides it is of great importance to quantify the microbial degradation and to investigate the environmental conditions which support microbial activity on bitumen as the substrate.
The mechanical, physical and chemical properties of the type of bitumen used in nuclear waste disposal, e.g. leaching, radiolysis and thermolysis, aging and change in consistency have been investigated in detail[4,10,13,17,19,22,23,27,31,32,34,38]. However, a possible influence of microorganisms on the stability of the bitumen matrix has been considered only in recent years although as described earlier microbial degradation of bitumen has been known for a long time[11,13,40]. No clear quantitative data were available which would allow an estimation of the long-term stability of bitumen at the sites of the waste disposal. In the present paper a quantification of the degradation rate of the water-insoluble and recalcitrant substrate bitumen is presented and growth on bitumen characterized. Since knowledge of the environmental conditions in a waste repository is minimal and since these conditions may change during the storage period, the experiments were carried out under optimal conditions. At the beginning, after closure of the repositories, the conditions will be oxic turning later to anoxic. Degradation studies therefore have been carried out for both conditions.