Microbial influence on the sorption of ^{137}Cs onto materials relevant to the geological disposal of radioactive waste

J. M. West, D. G. Haigh, P. J. Hooker and E. J. Rowe

Fluid Processes Research Group, British Geological Survey, Keyworth, Nottingham NG12 5GG (England)

Summary. A major concern in the geological containment of radioactive wastes is the speed of movement of radionuclides from the repository, after their eventual leaching and release, into the geosphere and finally into the biosphere. Radionuclide sorption onto the host rock is an important retarding mechanism. Experimental evidence shows that the presence of microbes in this environment influences the sorption capabilities of the host rock. Their presence can decrease the amount of retardation of ^{137}Cs, a common radionuclide in radioactive waste, by the solid phase. Sorption methods and data analysis procedures are presented and the implications for radioactive waste disposal assessments are discussed.
Key words. Microbiology; radioactive waste; geology; radionuclide sorption; ^{137}Cs.

Introduction

Radioactive waste can be gaseous, liquid or solid and arises in many different physical forms and concentrates. The safe disposal of these wastes is a major technical issue. Many countries favour disposal of solid wastes in repositories in geological formations to ensure that the waste is isolated from man's environment and from major natural processes or intrusion by man and to provide a shield from the radiation [2,3]. The processes which will affect containment and behaviour of the waste are dominated by the behaviour of groundwater in the host rock and other rock formations above and below a repository. The movement of groundwater through a repository will result in leaching and mobilisation of radionuclides [2,3,12,17].

It is now assumed [4,22,23] that microbes exist in geological formations relevant to the disposal of radioactive waste and that some groups can tolerate the extreme environmental conditions present [3]. Microbes are implicated in biodeterioration of materials that will be used in repository construction and cause geochemical changes in some environments. Microbes mobilise U [13] and take up metals [9,16,21,27], thus they could have a significant role in the mobilisation and transport of radionuclides. The processes involved in radionuclide migration from a repository are complex even without the possible influence of microorganisms. Following failure of the engineered barriers in a repository, retardation of movement of remaining radionuclides will depend on the properties of the host rock. The time to reach the biosphere and the concentrations in which they will arrive are related to the distance and velocity of their movement through the host rock and depend on the geochemistry and physical properties along the migration path [3].

Chapman and McKinley [3] define sorption mechanisms

- as including purely physical processes retarding migration such as molecular filtration, ion exclusion and diffusion into dead-end pores,
- direct chemical reaction with rock surfaces involving physical adsorption, chemical adsorption or direct incorporation into the rock structure,
- and indirect chemical reactions such as precipitation caused by enhanced concentrations at the rock surface.

'Sorption' mechanisms can be measured statically, using batch studies and measuring the partitioning of a radionuclide spike between the solid and liquid phases or dynamically using column experiments where a radionuclide spike is passed through a rock/soil column and the concentration profile in the effluent or the profile in the column is used to give a retardation factor [3].

The influence of microorganisms on such sorption experiments will also give some indication of the significance of microorganisms in the far field. In this paper the effect of sulphate reducing bacterial (SRB) on sorption of ^{137}Cs onto Fullers' Earth (calcium montmorillonite), a potential backfill material, is monitored using a traditional batch sorption method with distribution ratios (Rd) being produced. Sulphate reducing bacteria are of particular interest in waste disposal because they are important in anaerobic corrosion processes [19]; their activity can change the geochemical environment [28]; and their tolerance to repository conditions has been established [24,25].

Materials and methods

The batch sorption method used by West et al. [25] was applied to a study of radiocaesium sorption on Fullers' Earth with an isolate of SRB (*Desulfovibrio desulfuricans*) from Konrad mine (KSRB), West Germany [4].

The methodology consists of contacting the pure rock, and a mixture of rock and microorganisms, with groundwater (filter sterilised through a 0.2-μm filter) spiked with ^{137}Cs under a variety of conditions. Solution to solid ratios remained constant at 10:1 ml/g. Systems were incubated under anaerobic or aerobic atmospheres at room temperature. Five concentrations of ^{137}Cs from 7.3×10^{6} to 7.3×10^{10} M (1 to 10^{-4} ppm) were prepared from standards (Amersham). All tests were run in duplicate and for two equilibration periods (1 and 8 days).

Microbial cultures had been grown in groundwater from the Fullers' Earth quarry in anaerobic conditions for 1 and 1–4 days prior to use in the experiment. An inherent difficulty is the inevitable variation in microbial numbers and the fact that sorption could occur onto actively growing, dormant as well as dead microorganisms. Solid and liquid phases were separated by centrifugation followed by filtration (0.2 μm pore size) since centrifugation alone did not adequately separate the phases. The dry weight of the microbes was ascertained by weighing dried filters before and after filtration which would include the mass of all the organisms (in all conditions) plus any associated organic or inorganic material. Unfortunately this method does not trap particles below 0.2 μm as ultra filtration would have done. Microbial numbers at the beginning and end of the experiment were determined using a standard spread plate technique on Postgates Medium E [19, 26]. Approximately 4×10^6 SRB were introduced into each test from 1-day-old cultures and approximately 3×10^4 from 14-day-old cultures. Numbers were assumed to be unchanged after 1 day's equilibration. No SRB survived after 8 days aerobic equilibration. Anaerobic SRB growth in the groundwater medium had already been established. Figures were not obtained for growth after 8 days.

Samples of rock and groundwater were collected from Baulking Quarry, Fernham, Oxfordshire, England (Brett Bentonite Co). The detailed geology of the area is described by Poole and Kelk [18]. The rock phase used in these experiments was Fullers' Earth (calcium montmorillonite) whose microbial content has already been ascertained. The material was characterised using XRD and grain size analysis [26]. It was treated in three ways: wet sterilised in an autoclave; dry sterilised in a warm air oven; and left unaltered. Sorption was corrected for the variable moisture remaining. Analysis of the groundwater [26] shows high concentrations of Na and Ca, as a result of interaction with the clay via ion exchange. Cs was absent in the groundwater (> 0.05 ppb) and sulphate was the predominant anion.

The kinetics of sorption of ^{137}Cs onto Fuller's Earth was complete after 24 h.

After the allocated equilibration time, the extent of radionuclide sorption was evaluated by measuring the change in ^{137}Cs activity in the aqueous phase by gamma spectrometry using a shielded NaI Gamma detector. The distribution ratios (Rd) were ascertained using the equation described by West et al. [25] and represent the ratio of sorbed to dissolved ^{137}Cs, i.e., $Rd = C_R/C_W$, where C_R is the concentration in the solid phase (g g^{-1}) and C_W is the concentration in the aqueous phase (g ml^{-1}).

Results

Summaries of the calculated distribution ratios (Rd) are given in tables 1–4, the full data sets are presented in West et al. [26].

Table 1. Rd values for aerobic conditions with rock only (ml g^{-1})

Cs conc. (ppm)	1 day equilibration	8 days equilibration
Unchanged rock		
1	157	194
0.1	195	220
0.01	223	294
0.001	193	266
0.0001	232	273
Dry sterilised rock		
1	558 (441)	537 (423)
0.1	682 (539)	788 (622)
0.01	721 (569)	944 (745)
0.001	850 (671)	921 (727)
0.0001	602 (473)	781 (617)
Wet sterilised rock		
1	200 (188)	139 (131)
0.1	247 (232)	149 (140)
0.01	276 (258)	253 (238)
0.001	374 (252)	272 (255)
0.0001	262 (246)	226 (211)

Figures in brackets are values corrected for moisture content equivalent to the unaltered rock.

Table 2. Rd values of anaerobic conditions with rock only (ml g^{-1})

Cs conc. (ppm)	1 day equilibration	8 days equilibration
Unchanged rock		
1	213	141
0.1	319	190
0.01	293	213
0.001	337	218
0.0001	448	218
Dry sterilised rock		
1	710 (560)	481 (379)
0.1	1136 (896)	735 (581)
0.01	1194 (943)	837 (662)
0.001	1182 (934)	817 (645)
0.0001	992 (784)	639 (505)
Wet sterilised rock		
1	172 (162)	139 (131)
0.1	277 (260)	177 (165)
0.01	334 (213)	226 (212)
0.001	276 (258)	235 (221)
0.0001	327 (307)	199 (187)

Figures in brackets are values corrected for moisture content equivalent to the unaltered rock.

By comparing table 1 with 2 and table 3 with 4 it is seen that the ^{137}Cs sorption values onto rock material are similar in the absence of introduced microbes for both aerobic and anaerobic conditions with a slight increase in the anaerobic results. However, high concentrations of Cs give lower sorption values. Unchanged and wet sterilised rock material have similar values but the dry sterilised rock has the highest sorption capacity even when corrected for the loss of moisture (tables 1 and 2).

Consistent results are produced when SRB are added under aerobic conditions, where SRB activity is not favoured, with little change when equilibration time or age of culture is varied (table 3). Values are again high when the rock has been dry sterilised as seen before in table 1. Under anaerobic conditions (table 4), which favour SRB

Table 3. Rd values for aerobic conditions with rock and KSRB (ml g^{-1})

Cs conc. (ppm)	1 day equilibration Age of KSRB (days) 1	14	8 days equilibration Age of KSRB (days) 1	14
Unchanged rock				
1	162	236	174	172
0.1	235	473	225	235
0.01	265	332	256	269
0.001	197	305	245	233
0.0001	279	280	260	233
Dry sterilised rock				
1	623 (492)	558 (441)	584 (395)	578 (452)
0.1	978 (772)	1164 (920)	863 (715)	935 (745)
0.01	1091 (862)	997 (787)	1048 (817)	953 (743)
0.001	958 (757)	2369 (1864)	949 (748)	944 (754)
0.0001	796 (629)	587 (461)	732 (584)	963 (752)
Wet sterilised rock				
1	231 (216)	1669 (1569)	199 (187)	175 (164)
0.1	339 (318)	248 (233)	203 (191)	224 (210)
0.01	428 (402)	316 (296)	225 (211)	245 (229)
0.001	485 (456)	286 (268)	230 (216)	265 (249)
0.0001	272 (255)	316 (296)	245 (229)	236 (222)

Figures in brackets are values corrected for moisture content equivalent to the unaltered rock.

Table 4. Rd values for anaerobic conditions with rock and KSRB (ml $g^{-}1$)

Cs conc. (ppm)	1 day equilibration Age of KSRB (days) 1	14	8 days equilibration Age of KSRB (days) 1	14
Unchanged rock				
1	30	28	26	29
0.1	505	439	483	498
0.01	157	226	244	230
0.001	72	87	105	104
0.0001	829	1075	1097	1293
Dry sterilised rock				
1	139 (110)	116 (91)	108 (85)	113 (89)
0.1	2822 (2229)	2197 (1736)	2197 (1736)	2208 (1744)
0.01	944 (745)	987 (780)	1080 (853)	1346 (1063)
0.001	441 (348)	433 (341)	513 (404)	529 (417)
0.0001	3866 (3055)	4537 (3583)	4316 (3409)	4793 (3786)
Wet sterilised rock				
1	24 (23)	22 (21)	23 (22)	28.(26)
0.1	464 (436)	367 (344)	441 (415)	564 (550)
0.01	255 (239)	211 (198)	228 (214)	313 (294)
0.001	112 (104)	92 (87)	96 (90)	134.(126)
0.0001	1141 (1072)	773 (726)	1313 (1233)	1596 (1500)

Figures in brackets are values corrected for moisture content equivalent to the unaltered rock.

activity, the sorption values are rather erratic, some being higher than the aerobic values and some lower. This is not due to equilibration time nor age of culture. The values for the unchanged and the wet sterilised rock are similar (whether corrected for moisture content or not) whilst those from the dry sterilized rock are much higher. It appears that the presence of SRB in conditions where their activity is favoured has complex effects on ^{137}Cs sorption. There is little change in Rd values for the unchanged rock even after 8 days equilibration (tables 1 and 3). Values for the dry sterilised rock are also similar except for a few anomalogous values in table 3. Values for the wet sterilised rock under aerobic conditions are similar.

From tables 2 and 4 it is obvious that the presence of microbes in anaerobic conditions causes changes in the Rd values. Some values are higher than in the 'rock only' experiments whereas other values are lower. Again this variation is not related to KSRB age nor to equilibration time but rather on Cs concentration.

Discussion

The Rd values obtained suggest that Cs and, under anaerobic conditions, microbes are sorbed onto the rock material. Addition of KSRB to the rock material gives variable Rds when compared to the rock only results. Lower Rds suggest competition for the rock sorption sites between the microbes and Cs ions. Microorganisms are larger than Cs ions and one microbe could cover many sorption sites normally available for Cs, thus confining Cs more to the aqueous phase with a resulting lower Rd. Lower Rds may be related to the formation and action of biofilms [7,15]. Microbial activity increases after adhesion [8]. The metabolic activity of microbes sorbed onto clays has been studied [20]. Once microbes sorb onto a surface in vivo they produce, amongst other things (e.g. pili [5]), a glycocalyx of extracellular polysaccharides [6] which eventually form a biofilm enabling permanent microbial adhesion and which also maintains an optimum environment for growth. Under stress conditions, glycocalyx formation is promoted [6,10,11]. The low nutrient conditions given in the experiment may have acted similarly as a stressed environment. It is possible that the slightly varying KSRB inoculation sizes have resulted in varying amounts of biofilm. KSRB as gram-negative bacteria bind metals from solution into their walls [1]. This would lower the amount of Cs in the aqueous solution with resulting variations in Rds dependent on the amount of biofilm formation.

Conclusions

Several broad findings can be extrapolated from the data presented. Since wet sterilised and unaltered rock show little difference the microbes present in the rock naturally do not appear to influence sorption. However the presence of KSRB in anaerobic conditions influences irregularly the partition of Cs, possibly in an active process. Variations and fluctuations in Rd do not occur in aerobic conditions. The age of the cultures as well as a prolonged equilibration time do not influence sorption characteristics. Dry sterilised rock with or without SRB has a higher sorption capactiy than unaltered or wet sterilised rock, probably as a result of changes in the montmorillonite layer structure.

Clearly, these findings have important implications for anaerobic batch sorption experiments routinely used for assessing the sorption capacity of potential host rocks for radioactive waste repositories. Microbial contamination will lead to anomalous results, this has also to be taken into consideration for field tests where migration of a radioactive tracer is studied.

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