Consequences of sulphate-reducing bacterial growth in a lab-simulated waste disposal regime

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Summary. Experiments are described which investigate corrosion of forged 0.2% carbon steel in the presence of sulphate reducing bacteria (SRB). Cultures of a thermophilic bacterium *Desulfotomaculum nigrificans* were mixed with bentonite and synthetic groundwater to simulate a bacteria-contamined backfill, and placed in contact with carbon steel disc specimens in perspex cells at 50 °C under anaerobic conditions. The rates of corrosion were monitored by electrochemical techniques, together with changes in near field redox potential.
After 340 days the nature and extent of any corrosion was measured and the SRB content of the bentonite determined. Recovery of relatively large numbers of bacteria after about one year incubation in an alkaline (pH 9.5) medium confirmed the pH tolerance of the strain. Enhanced corrosion (three times the rate of the control) occurred in at least two of the five cells that contained SRB despite the nutritionally poor environment which existed in the bentonite gel.
Key words. Sulphate-reducing bacteria; corrosion; nuclear waste.

Introduction

Wyoming bentonite (sodium montmorillonite) is a smectite clay mineral which is considered as a backfill material for high level nuclear waste (HLW) canisters after placement in an excavated repository. Bentonite is stable, has a low permeability, self-healing properties [11] and thus is suitable for this purpose [2, 3, 8, 17].
In a microbiological survey of British bentonite (calcium montmorillonite) a sulphate-reducing bacterium (SRB) was isolated [25] suggesting that there are strains which can survive in this clay environment. SRB have a pH tolerance ranging from below 5 to 9.5 [26] and belong to the most alkali-tolerant bacteria [21]. The upper limit of 9.5 is near to that attained in the moderately alkaline bentonite and synthetic granitic groundwater (SGGW) mixtures chosen as representative of disposal environments [30] for the long-term immersion testing of carbon steel [23].
The ability of SRB to exacerbate the corrosion of carbon steel under anaerobic conditions is well-documented [16], although doubts have been expressed as to whether sufficient nutrients will be present under disposal conditions to sustain their metabolic processes [6, 10].
In this work in situ measurements of electrochemical parameters under anaerobic conditions on 0.2% carbon steel electrodes in contact with a bentonite/SGGW gel containing a sulphate-reducing bacterium are described. To simulate heat generation by HLW within the bentonite and host-rock [7, 14] experiments were done at 50 °C, and thus a thermophilic bacterium (*Desulfotomaculum nigrificans*) was chosen.

Experimental procedures

Strain, media and culture conditions

Desulfotomaculum nigrificans NCIMB 8351 was obtained as a freeze-dried culture from Torry Research Station, Aberdeen. The organism was maintained on medium B and was cultivated on medium C at 50 °C [26].
The organism could not be grown in liquid media above pH 8.2. However the recovery of *D. nigrificans* from the experimental corrosion cells suggests that growth on solid surfaces was an advantage at higher pH values. Several pH gradient method [13] variations were tried but only one proved consistently successful. 17 ml of sterile medium E was inoculated with 0.5 ml exponential culture at 50 °C. When allowed to solidify, 2 ml alkaline agar as given below was layered on top. This served both as the means of pH gradient generation and the overlay to exclude oxygen. The tubes were capped with Subaseal stoppers, the headspace replaced with O_2-free N_2, and the tubes then incubated overnight at 30 °C to allow gradient generation without cell growth. Tubes were subsequently incubated at 50 °C.

Corrosion cell design and electrochemical monitoring

Each of six cells (fig. 1) consisted of a 5 cm I.D., 6 cm O.D., 30-cm long perspex tube which was threaded at one end to receive a carbon steel disc, preweighed to the nearest 0.01 g, prepared from BS 4360 grade 43A carbon steel to a 600-grid surface finish. Five platinum wire loops were sited (fig. 1) at 2-cm incremental positions from the disc face to act as E_h probes. A platinum foil counter electrode and a glass salt bridge to hold a saturated calomel reference electrode (SCE) were also mounted. All electrical contacts and the disc's external surface were insulated with Turco lacquer.
After centrifugation of SRB cells from the culture medium, each batch was resuspended in about 10 ml of the spent medium and mixed with 500 ml of sterile, argon deaerated, synthetic granitic groundwater (SGGW) to approximately 5×10^6 cells ml^{-1} (SGGW composition, table 1). This suspension was added aseptically to an argon-filled conical flask containing 50 g sterile Wyo-

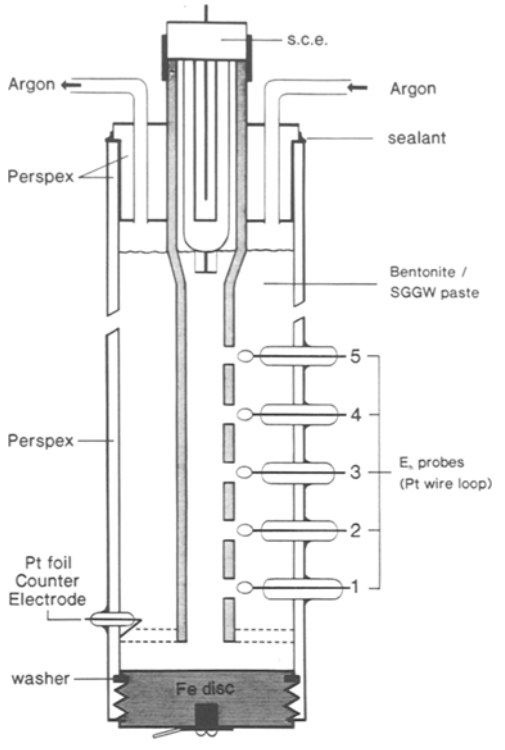

Figure 1. Diagramatic presentation of the corrosion cell used to assess the long-term effect of SRB on the biodeterioration of mild-carbon steel.

ming bentonite powder, re-sealed and thoroughly mixed overnight to produce a thick smooth thixotropic gel. The bentonite/SGGW/SRB mixture was poured into an argon-filled presterilised perspex cell with its carbon steel disc in position and the cell cap fixed on with silicone rubber cement. After the bentonite had formed a firm gel each cell was then placed in a polythene container, ballasted and placed in a thermostat water bath. The carbon steel disc, reference electrode and five E_h probes were connected to a Cristie CD 248 data logger linked to an Epson FX80 print unit to record rest potential (E_{rest}) and redox potential (E_h). The temperature of the water bath was increased in stages to the operating temperature of 50 °C.

Table 1. Composition of synthetic granitic groundwater (SGGW) used in the corrosion cells

Ion	Concentration Meq	mg/l	Added as analytical reagent grade chemical
F^-	0.1	1.9	NaF
Cl^-	1	35.5	$CaCl_2 \cdot 2H_2O$
HCO_3^-	4	244	$NaHCO_3$
SO_4^{2-}	0.5	24	$MgSO_4 \cdot 7H_2O$
SiO_3^{2-}	0.5	19	$Na_2SiO_3 \cdot 5H_2O$
Na^+	4.6	106	F^-, SiO_3^{2-} and HCO_3^-
Ca^{2+}	1	20	$CaCl_2 \cdot 2H_2O$
Mg^{2+}	0.5	6.1	$MgSO_4 \cdot 7H_2O$

pH 9.4

All cells contained SGGW/bentonite slurry with the following variations:

Cell 1: SRB from a 5-day culture. Sterile conditions, prehumidified flowing argon from day 103.

Cell 2: Culture medium from 5-day culture. Sterile conditions.

Cell 3: SRB from an overnight culture. Sterile conditions. Argon as cell 1.

Cell 4: No additions. Sterile conditions, control. Argon as cell 1.

Cell 5: SRB from 5-day and overnight cultures. Non-sterile conditions.

Cell 6: Lag-phase culture (low cell density). Sterile conditions.

Results

Observations on the operating cells (E_{rest}, E_h and R_p)

Changes in corrosion potential (E_{rest}) of the six carbon steel electrodes as a function of exposure time are recorded in figure 2. After some variability during the first week, all except cell 1 generated reasonably stable potentials in the range from −810 to −840 mV/SCE with a temporary excursion to more positive values when the thermostat heater failed (211–213 d). The response from electrode 1 is significantly different from the others after about 83-d exposure, and also from 208 d onwards. Less negative potentials could have been caused by ingress of air, however a stream of sterile prehumidified argon introduced into the headspace of cells 1, 3 and 4 after 103 days had no significant effect on E_{rest} and it is concluded that the gettering action of iron corrosion would have removed any oxygen in the vicinity of the disc electrodes to a very low level. The rest potentials of three carbon steels, obtained during the course of long-term immersion tests are shown in figure 3 for comparison. In this case carbon steel discs were embedded in a 10-cm-thick

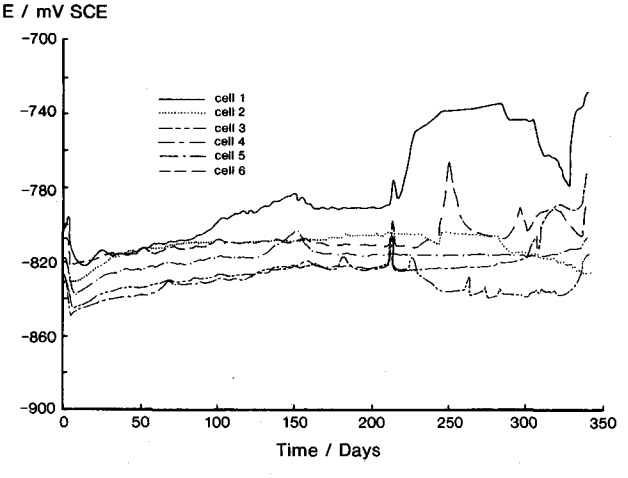

Figure 2. Temporal changes in corrosion potential (E_{rest}) for forged 0.2% carbon steel in corrosion cell systems 1–6 with or without sulphate-reducing bacteria.

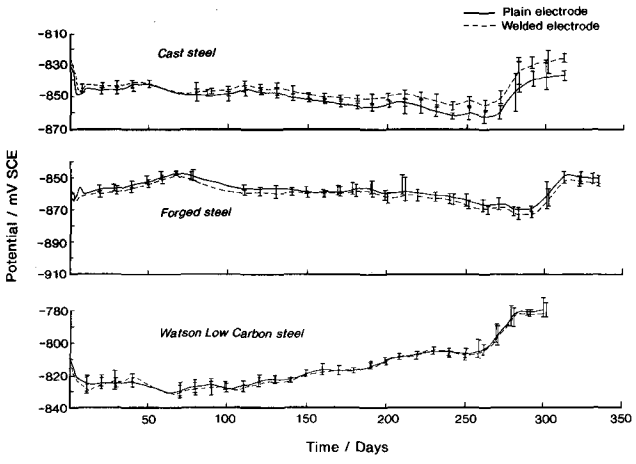

Figure 3. Temporal changes in corrosion potential (E_{rest}) for cast steel, forged steel and Watson low carbon steel incubated in bentonite/SGGW/air.

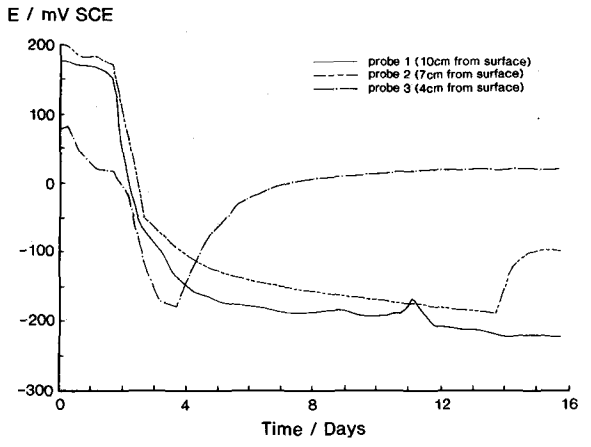

Figure 5. Temporal variation in E_h measured using 3 probes immersed in bentonite/SGGW/air (see text for details).

layer of bentonite/SGGW paste, covered with a 5-cm-depth SGGW and exposed to a blanket of CO_2-free air. E_{rest} exhibited by the cast and forged 0.2% carbon steel coupons are slightly more negative than the microbial electrodes but the low carbon (0.05%) steel shows a similar behaviour with an E_{rest} in the range from -830 to -810 mV/SCE for 200 days exposure before drifting (like cell 1) to more positive values.

The variations in E_h were monitored using the five probes positioned at increasing distances from the carbon steel disc electrodes. Typical data are shown for cell 1 (fig. 4). For the first 39 days of exposure only probes 1–4 were monitored. Later all probes were monitored and E_h recorded every 6 h. For comparison, a separate test was set up with three platinum probes immersed in a bentonite/SGGW slurry at ambient temperature under an air atmosphere at depths of 4, 7 and 10 cm. The results are shown in figure 5 and indicate that the probe with the longest oxygen diffusion path takes up the most negative potential. It might be expected that E_h values in the test cells would become increasingly more negative from probes 5–1, particularly as corrosion of the carbon steel electrodes would consume any O_2 introduced in the bentonite/SGGW slurry near to probe 1. Corrosion cells 1–4 provide evidence that despite considerable variations there was a trend for probes 1 and 2 to have taken up more negative potentials than the others. However, the results for cells 5 and 6 are at odds with expectations. Probe 5 is more negative than probe 1 (data not presented).

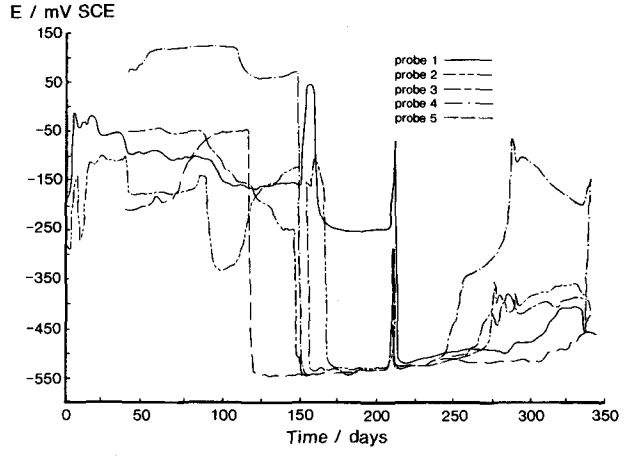

Figure 4. Temporal variation in E_h for corrosion cell 1. Measurements were made using 5 probes at varying distances from the carbon steel surface (see text for details). E_h variations for other corrosion cells are not shown.

Linear polarisation resistance measurements were made at intervals using a galvanostatic method. A small (circa 0.4 μA cm^{-2}) cathodic current was passed through each carbon steel disc to its platinum counter electrode and the change in rest potential (ΔE) generated is measured over a period of 5–10 min. The applied current density (Δi) was adjusted so that $\Delta E < 20$ mV and a stable potential was achieved in a time interval < 10 min. The ratio of $\frac{\Delta E}{\Delta i} = R_p$ has dimensions of ohm cm^2. The corrosion current (i_{corr}) is related to R_p by the following relationship.

$$i_{corr} = \frac{B}{R_p}$$

where

$$B = \frac{b_a b_c}{2.3(b_a + b_c)}$$

and b_a and b_c are the anodic and cathodic Tafel slopes for the reactions involved. Tafel equations are based on the fact that the potential difference across an interface at which an electrochemical reaction is occurring changes linearly with the logarithm of the current density. The R_p

Table 2. Changes in polarisation resistance (R_p) for the six corrosion cells measured over 340 days

Exposure time (days)	R_p values (k ohm cm^2) Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6
11	19.2	26.3	35.5	31.7	33.0	21.6
45	21.3	29.8	43.3	35.1	34.3	12.9
152	9.6	18.5	26.0	20.3	12.3	8.2
178	8.2	16.6	18.7	18.0	18.1	8.0
207	7.3	15.0	17.8	28.5	15.3	8.5
243	1.2	12.6	14.6	16.4	14.5	6.5
270	1.3	10.1	10.8	15.2	14.0	6.3
300	2.6	8.8	8.5	13.0	11.6	5.5
336	1.9	n.m.	13.8	6.1	n.m.	5.1
Time averaged R_p-values over 340 days	9.8	18.1	24.2	21.5	19.5	9.5

*For calculation of R_p see text; n.m. = not measured.

Table 3. Comparison R_p data for cast, forged (0.2% carbon) and low carbon steel coupons from long-term immersion tests

Exposure time (days)	R_p (k ohm cm^2) Cast	Forged	Low carbon
60	14	23	12
100	13	22	13
150	13	22	9.5
200	12.9	22	8.5
250	13.8	22	7.5
310	12.8	23	7.0
350	12	20	7.0

results obtained for the six cells are shown in table 2 together with the time-averaged values. It is interesting to note the decrease in R_p that occurred between 207 and 243 days exposure for cell 1, which implies an increasing corrosion rate, coincided with the movement of E_{rest} also shown by this electrode in figure 2. For comparison, R_p values obtained during the long-term immersion test previously referred to are given in table 3. The values obtained for cells 2–5 (table 2) are not dissimilar to those for the cast and forged steel coupons in table 3. However there are clear indications of some additional changes occurring in cell 1 and to a lesser extent in cell 6.

Observations on the dismantled cells

After 340 days the cells were removed. By this time the bentonite had shrunk and cracked allowing channels to form from the top to near the bottom of most tubes. In some instances E_h probes had become isolated from contact with a conducting medium accounting for some of the peculiar readings. Colourations were observed in the bentonite in the form of successive bands of black, green-grey and orange-brown near the carbon steel disc and black laminations, spots and halos in the bulk of the bentonite.

The carbon steel discs were brush cleaned revealing degrees of attack. The corrosion in cell 1 was different from all the others in that the Fe surface had a concentric ring attack and when corrosion products were removed by chemical cleaning the surface was seen to be micropitted. The pit density was ca 200 pits mm^{-2}. Weight loss measurements are given in table 4. Mean corrosion rates expressed as μm year^{-1} have been calculated from the weight loss using the expression:

$$\bar{\Delta}t\ (\text{cm y}^{-1}) = \frac{\text{corrected weight loss (g)}}{\text{disc area (cm}^2) \times \varrho_{Fe}} \cdot \frac{365}{\text{exposure time (d)}}$$

Mean corrosion current (i_{corr}) has been derived from these data and used to estimate a value for the proportionality constant B. The observed mean corrosion rates ($\bar{\Delta}t$) of the carbon steel discs in cells 2, 3 and 5 (table 4) are about twice that of the control in cell 4 and for cells 1 and 6 up to three times the control. Included in table 4 are results from weight loss measurements made on forged 0.2% carbon steel coupons embedded either in 5 cm of bentonite/SGGW at 90 °C for 473 days ($\bar{\Delta}t = 8$ μm y^{-1}) or under a bed of crushed granite for 169 days ($\bar{\Delta}t = 19$ μm y^{-1}). The increased corrosion rate under granite is due to the more permeable nature of this material to oxygen from the air blanket above the immersion vessel. $\bar{\Delta}t$ exhibited by the control cell 4 is of the same order as the granite bed value and may be partially explained by oxygen diffusion through the perspex tube. If oxygen had been present this would have been reflected by movement of E_{rest} to more positive values, by at least 100 mV/SCE [30], than is apparent in figure 2 for this cell. There are no reference data for batch or continuous culture corrosion rates with *D. nigrificans* and the steel used.

Table 4. Weight loss data for carbon steel during 340 days exposure in a bentonite/SGGW environment with or without sulphate-reducing bacteria

Fe disc No	ΔW Initial-Final weights (g)	$\overline{\Delta t}$ (μ m/yr)	$\bar{i}_{corr}$ (μ A cm^{-2})	$\overline{R_p}$ (kohm cm^2)	$\bar{B}$ (mV)
1	1.11 ± 0.1	66.5 ± 6	5.7	9.8	56
2	0.55 ± 0.1	32.9 ± 6	2.8	18.1	51
3	0.48 ± 0.1	28.8 ± 6	2.5	24.2	60
(control) 4	0.32 ± 0.1	19.2 ± 6	1.7	21.5	36
5	0.58 ± 0.1	34.7 ± 6	3.0	19.5	58
6	0.95 ± 0.1	35.9 ± 6	4.9	9.5	47

Bentonite bed (473 days exposure) $\overline{\Delta t}$ 8 μ m/y.
Granite bed (169 days exposure) $\overline{\Delta t}$ 19 μ m/y.

In batch systems of *Desulfovibrio* species over 28 days the highest corrosion rates (approximately 25 μm y^{-1}) were recorded in those media rich in ferrous ions. This is lower than all our measurements except control cell 4 (table 4). Rates were all lower than control cell 4 in media low in ferrous ions (maximum approximately 15 μm y^{-1}). In the severe environment of the bentonite gel corrosion rates were thus significantly higher than under optimal conditions in batch culture.

SRB counts on dismantled cells

At the end of the experiment SRB counts were made for each of the cores from cells 1, 3, 5 and 6 at five levels within the bentonite matrix:

Sample	*Situation within core*
1	0– 1 cm above Fe disc
2	5– 6 cm above Fe disc
3	10–11 cm above Fe disc
4	15–16 cm above Fe disc
5	20–21 cm above Fe disc

Counts of *D. nigrificans* were done using spread plate and the Most Probable Number (MPN) techniques. Dilution series of bentonite gel were made in phosphate-buffered saline (10 mM phosphate buffer/0.85% NaCl/50 mg l^{-1} thiodiglycollate as reductant). Spread plate surface colony counts were made on medium E agar[26], and plates were incubated in anaerobic jars at 50 °C in an atmosphere of H_2/CO_2.

Medium E deeps were inoculated at 50 °C with bentonite gel dilutions and allowed to solidify and overlayed with 1.5% agar/0.01% ascorbic acid/0.01% thiodiglycollic acid to prevent ingress of oxygen. After incubation at 50 °C, the MPN was determined.

Using the plate count method, relatively large numbers of bacteria were recovered in all the cases tested reflecting approximately a 10–100-fold reduction in the initial cell counts. In some cases about 90% cell viability was retained after almost one year of incubation at 50 °C in a pH 9.5 environment. No gradient of organisms was seen in the cores. Clearly *D. nigrificans* could survive the harsh conditions of the experiment, particularly the high pH.

Tolerance of D. nigrificans to extreme pH values

The alkaline agar was prepared by mixing 0.4 M NaOH/40% (w/v) $Na_2CO_3 \cdot 10H_2O$ with an equal volume of 4% (w/v) purified agar (Difco) at 60 °C. The two components were autoclaved separately and used immediately after mixing.

The results (fig. 6) show that the strain grew at higher pH on solid media than in liquid. Regularly the zone of blackening (FeS precipitation) stopped at a pH in the region of 9–10. The pH along the gel was measured by pH paper (Merck) after extrusion of the gel from the tube. To test if the blackening front corresponded to the growth front, thin slices of agar were removed aseptically at positions 1, 2 and 3 as indicated on figure 6 and placed into fresh medium B. Growth from the gel in the fresh medium by blackening was confirmed by epifluorescence microscopy. Cultures from position 1 took two days to show growth, whereas from 2 and 3 growth usually occurred overnight. The growth from gel slices from position 1 is of particular importance because this corresponded to a pH around 11.0. Although no growth was seen, as evidenced by sulphate reduction, sufficient cells were able to survive the high pH to resume active growth

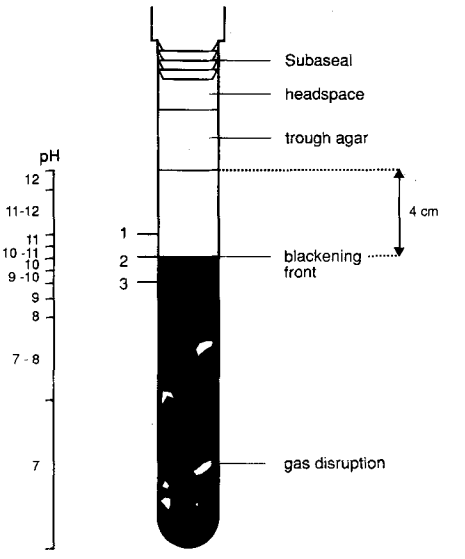

Figure 6. Deep agar pH gradient of *D. nigrificans*. The blackening front corresponds to pH 10 approximately. Positions 1, 2 and 3 are where thin gel slices were removed and used as inoculum into fresh medium B. Position 1 corresponded to pH 11. Gel slices removed as above were SRB inoculum for growth in fresh medium.

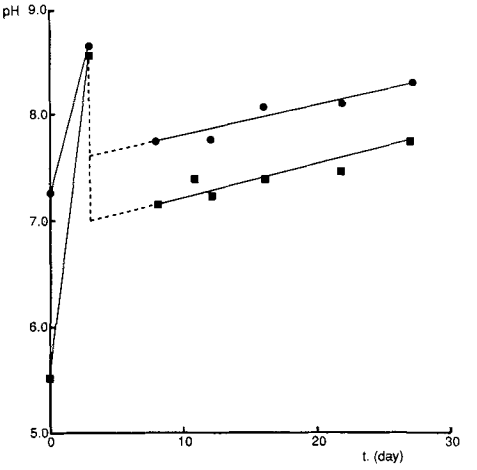

Figure 7. Variation of pH in bentonite/SGGW slurry with time.
● Start pH 9.5. At zero time 0.2 ml 1 M HCl was added reducing the pH to 7.3. After 3 days the pH rose to between 8.6 and 8.7 and another 0.1 ml HCl added. By day 8 the pH was 7.8. No more acid was added but by 27 days the pH increased to 8.4.
■ Start pH 9.2. At zero time 0.38 ml 1 M HCl was added reducing the pH to 5.5. After three days the pH increased to 8.6 and another 0.1 ml acid was added. By day 8 the pH was 7.2. No more acid was added but by 27 days the pH increased to between 7.8 and 7.9.

under more favourable conditions. The tests show that the strain was capable of growth up to and perhaps slightly beyond pH 9.5 in a gel environment.

pH modification of bentonite slurry

Acid was added to bentonite slurries to produce a stable pH at or near to pH 7.0 to allow optimal growth of cells. After a first pH drop (fig. 7) pH always rose sharply. A stable reading was never recorded and the data shown are values taken 2 min after immersion of the electrode into the slurry. This is possibly due to the high ion exchange capacity of the montmorillonite clay, which varies depending on conditions such as pH, concentration of the clay, particle size and clogging of exchange positions. Thus quoted values of cation exchange vary. Grim[15] gives a value of 80–150 milliequivalents (mEq) of NaOH per 100 g, but Na-Wyoming bentonite is not purely of sodium-saturated clay; it has a content of about 30 ppm Ca, 15 ppm Mg and 70 ppm Na in the pore water. Clay-adsorbed Na equals about 60 mEq per 100 g, Ca about 5 and Mg about 3[27].

Discussion

Electrochemical and corrosion measurement

A moist bentonite-clay is normally a highly impermeable barrier but evaporation can lead to drying out, shrinking and cracking. It is considered that this phenomenon was partly responsible for the variable E_h values observed. However, measurements of corrosion potentials (E_{rest}) of the mild steel discs, which remained in contact with the bentonite/SGGW gel, were reasonably consistent. Persistent movement of E_{rest} appears to be related to changes in corrosion rate. Unfortunately there are uncertainties about the measurement of R_p when films of corrosion products/bentonite start to build up on a corroding iron surface. Predictions of corrosion rates from these observations become unreliable[30]. Interestingly enough the proportionality constant B, calculated from i_{corr} and R_p (table 4) is about 50 mV which is an order of magnitude greater than that usually observed from long-term tests with mild steel specimens under a bentonite slurry in the absence of SRB.

The micropitted appearance of the carbon steel disc observed in cell 1, coupled with its enhanced corrosion rate, was significantly different from those in the control and other cells. This seemed to be associated with the presence of SRB in the bentonite/SGGW gel. Differences in corrosion between cell 1 and the other cells, could be related to the non-ideal conditions in the gel which were borderline for SRB growth to initiate. Another complicating factor is that yeast extract in Postgate's medium C, has been shown to inhibit Fe corrosion[1]. Even the trace amounts introduced into the gel preparations could have had a variable delaying influence on the initiation and development of corrosion. Also the duration of the experiment may not be sufficient to allow the semi-protective films of corrosion products to transform into more stable crystallographic forms that are less protective and even cathodic to iron dissolution[20, 29]. Thus after a period of slow corrosion an acceleration of rate might occur.

Microbiological aspects

The incubation at 50 °C, high pH and nutrient-poor conditions for almost one year represent a severe climate for survival. *D. nigrificans* is a spore-former which may influence its survival of harsh conditions[9].

An alternative explanation for starvation survival is the recent finding that SRB generally have a wider range of carbon substrates than was once appreciated[24] and of great significance is the isolation of autotrophic strains[18, 31]. *D. nigrificans* strain has also been shown to grow reproducibly in subcultures with hydrogen and sulphate as the only energy sources[19] which may be important to survival in the carbon-limited bentonite backfill. The evolution of hydrogen from initial oxic canister corrosion and a supply of sulphate from bentonite and/or groundwater from various proposed sites[4, 5, 12, 22] may supply the necessary energy requirements for growth in a repository environment.

In our experiments, however, active growth was not maintained throughout the mass of bentonite. This may simply have been due to lack of available iron in the clay, although iron-rich montmorillonites are known[4] with an iron content of 6.5–10%. As pH is non-restrictive, the obvious growth-limiting factor is available carbon. The inability of the strain to grow on agar plates when CO_2 is excluded from the atmosphere, and also the partial growth on purified agar without carbon sources when CO_2 is supplied implies that the strain is able to obtain some of its cell carbon from CO_2 assimilation. Hydrogenase assays showed that *D. nigrificans* is hydrogenase negative, and that the strain is unable to gain reducing power for sulphate reduction from hydrogen oxidation in contrast to the strain used by Klemps et al.[19].

Attempts to modify the pH of bentonite slurries failed which is attributed to ion exchange capacity. Generally, the higher the valency of the ion, the greater its replacing power and the more difficult it is to displace when already present on the clay. Hydrogen ion is an exception since it behaves like a divalent or trivalent ion[15]. Therefore on acidification of the bentonite slurry, sodium ions are displaced from the clay and replaced by hydrogen ions and the pH of the pore water rises again. In models of montmorillonite crystal structure the high pH is attributed to the $Si(OH)_4$[28] and the pH of the montmorillonite is a sensitive function of the ionic strength.

Conclusions

To model the bentonite-backfilled repository some unusual factors must be considered in an experimental design:

- The extremely low expected groundwater flow rate[12] makes the system resemble a closed batch culture.
- The groundwater-saturated bentonite environment is one of variable nutrient status depending on the individual site.
- The relatively high pH which is maintained by ion exchange is restrictive to organisms.
- Heat generation by the waste requires a thermophilic organism to be used for the experiment.

The tests have shown that the bacteria are able to increase corrosion rates under these conditions. Enhanced corrosion occurred in two of the five cells containing SRB at about three times the rate of the control. Although the recorded rates are low compared to those which can be achieved in the laboratory they seem more realistic considering the conditions likely to be encountered in a bentonite-backfilled repository.

It is plausible that SRB survive in the repository environment because, they have been isolated from bentonite[25]; autotrophic strains and strains with low energy and carbon requirements have been described; thermophilic forms are capable of growth at 50 °C and above; bacteria may be tolerant towards alkali, pressure and nutrients are available in groundwater and/or bentonite which meet the energy requirements (if not carbon) for microbial growth.

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