The microbiology programme for UK Nirex

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Summary. The Nirex Safety Assessment Research Programme (NSARP) considers the effect of microbial action on the repository near field. The upper limit of growth for natural soil organisms appears to be pH 12.25. Environmental conditions in the repository will probably allow slow growth particularly on damp wastes. Experiments using packed columns of waste and concrete have shown that an extremely high pH is not conducive to rapid microbial growth. However, viable populations can exist within regions adjacent to the concrete, e.g. where a surface film coats the concrete. Carbon dioxide and methane will be produced by microbial action within the repository but actual rates of production will be lower than that in a domestic landfill. The cellulosic fraction of waste is the main determinant of cell growth. It appears to be the sugar acids arising from alkaline degradation of cellulose which cause enhanced plutonium solubility. The potentially beneficial reduction of chemically derived polyhydroxy acids by the microorganisms is possible. A mathematical model has been constructed to describe the main features of biological action in the repository.

Key words. Repository; radioactive waste; microbiology; alkaline; modelling.

Introduction

Overview

The performance of a radioactive waste repository will be determined by the chemistry of both the waste and the materials of construction. Although there was no direct experimental data relating to this environment, literature studies by a number of groups[7,12–15] pointed to the potential of microbiology to alter the near field environment.

Initially these groups studied the microbiology of potential sites worldwide[9,20] but recently more attention has been directed to microbiology of the waste form itself. Experimental work concentrated on the influence of environmental factors on pure cultures and limited consideration was given to the effects of repository structure and chemistry on the biology. Simple mathematical models described some features of microbial metabolism in a repository and the possible influence of this on repository integrity[11,19].

The scope of research programmes has been bounded by the detailed design and location of the repository. The disposal policy of UK Nirex involves the multibarrier concept, incorporating both physical and chemical barriers to the migration of radioelements. The waste is initially placed in a cementitious matrix within a metal or concrete container. This primary containment is lodged in an underground excavation which is packed with a cementitious backfill. This keeps the near field environment alkaline for many thousands of years, thus ensuring that most radionuclides remain chemically immobile. A further level of protection is provided by the geology and hydrology of the site which is chosen to avoid substantial water movement to the biosphere.

Scope of the Nirex microbiology programme

The current microbiological studies for the Nirex Safety Assessment Research Programme (NSARP) consider the effect of microbial action on the repository near field, i.e. within the waste and repository backfill. These investigations were initiated in 1986 and have followed a similar course to the programmes elsewhere, notably those in Switzerland, Canada and the USA. However, the Nirex programme has concentrated on the microbiology of the waste form itself, using mixed cultures and representative waste held under conditions typical of the proposed repository. This has provided information which is directly relevant to the real situation in which consortia of organisms will be in an alkaline environment, created by cementitious material. Standard mixtures of organic materials which simulate two typical LLW and ILW wastes have been used throughout these studies (Appendix).

There are four interrelated components within the programme. Experimental work on the survival and overall metabolism of microorganisms under representative repository conditions; the generation of gas, principally methane and carbon dioxide; work on aqueous phase chemistry and finally the development of a computer-based description of the microbiology within a repository.

Microbial survival and metabolism

Microbial screening

The repository will form a very different microenvironment from that normally imposed on soil microorganisms. However, the organic component of the waste is a potential nutrient for cell growth. It is anticipated that a mixed population of microorganisms capable of exploiting this will be selected from the diversity of organisms in the local biosphere.

Environments which have been subject to selective pressures similar to the repository (e.g. alkaline sites) are generally expected to contain a richer diversity of tolerant organisms. Nevertheless, even normal, fertile soil will

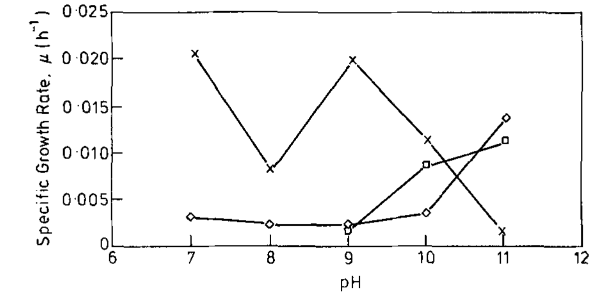

Figure 1. The effect of pH on the specific growth rate of three microbial strains, typical of those isolated from the natural environment: EPC10.55 (◇), ILPA7V (□) and LPF3 (×); determined at 25 °C over 120 h in Mineral Salts Medium (MHM).

contain a number of potential extremophiles (i.e., organisms which grow best under conditions of high pH, temperature, salinity or other atypical environmental conditions). Samples obtained from a number of relevant geological sites, waste forms and fertile soils have been used as inocula into selective nutrient media. The effects of pH and nutrients on the growth of organisms present in these environments has been investigated. Around 300 individual strains of bacteria, fungi and actinomycetes were studied in detail as pure cultures. Figure 1 illustrates the pH of optimum growth for a typical alkalotolerant and two alkalophilic organisms which have been isolated at Harwell. Figure 2 summarises the growth optimum data from 47 of the fastest growing isolates using glucose as substrate. The number of organisms able to grow pH 12 is limited and an upper limit to growth appears to be pH 12.25.

Growth at alkaline pH is not dependent on the type of soluble carbon substrate, and figure 3 illustrates the ability of a typical pure strain to grow on volatile fatty acids or glucose. A novel screening technique using microtitre plates was developed to measure the pH optimum for a large number of organisms on a range of substrates[4]. These data indicated that for the majority of organisms

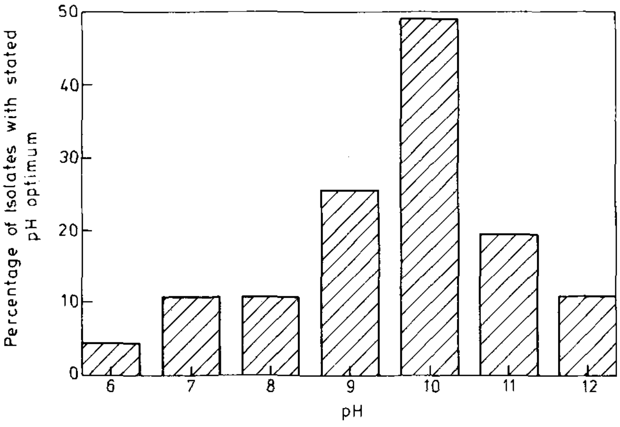

Figure 2. Distribution of optimum pH for growth of the 47 fastest growing isolates studied in the Nirex programme.

isolated so far, the pH for optimum growth was pH 6.5–9.5. Cellulose, in the form of paper, wood and cotton, is judged to be the most likely source of easily metabolised carbon in a typical low level radioactive waste. Representative isolates from the microbial screening have been grown successfully on a number of celluloses including shredded paper tissue, balled milled paper, cellulose powder (Whatman CF-11), microcrystalline cellulose (Avicel) and carboxymethyl cellulose. These polymers will be broken down by enzymes produced by the organisms to give the directly metabolisable compounds such as glucose. However, chemically derived organic acids and soluble sugar derivatives can be expected in an alkaline waste. Preliminary experiments with analogues of these polyhydroxy acids such as gluconic and saccharic acid have illustrated that individual cells will grow on these intermediates.

These data confirm that in the normal environment, there are organisms which are capable of growing on the organic component of the waste at the high pH envisaged in the repository. The upper limit to growth appears to be pH 12.25.

Growth under defined conditions

Most natural degradation of organic material results from the sequential action of a number of individual type of microorganisms on the intermediates which arise from the initial attack on the primary substrate. Consequently, having established that a variety of organisms might be present even at high pH, the majority of the experimental work has been undertaken with naturally derived mixed cultures. In general, fertile soil has been taken from a single site which has not been specifically stressed to enrich it in alkalotolerant species. In addition, inocula obtained from the adventitious growth of organisms on waste and other non-sterile materials under study have been employed.

All waste material contains a natural microflora which is stimulated into growth if the appropriate conditions are created. However, in order to accelerate the production of observable changes in the waste, soil has been used as the universal inoculum for mixed microbial populations. Initial testing has involved batch experiments in which gas production has been used as a convenient marker of microbial action. Individual conditions have been studied in more detail using continuously leached column systems and dynamic arrangements such as the Gradostat.

Batch studies in small bottles

Broad screening of environmental factors has been performed using large numbers of simple batch tests, set up in sealed 100-ml bottles with similar quantities of soil and waste. The effect of the more important environmental conditions such as water content of the waste, the main inorganic nutrients, the form of the carbon source and the pH have been studied in detail. Where possible tap

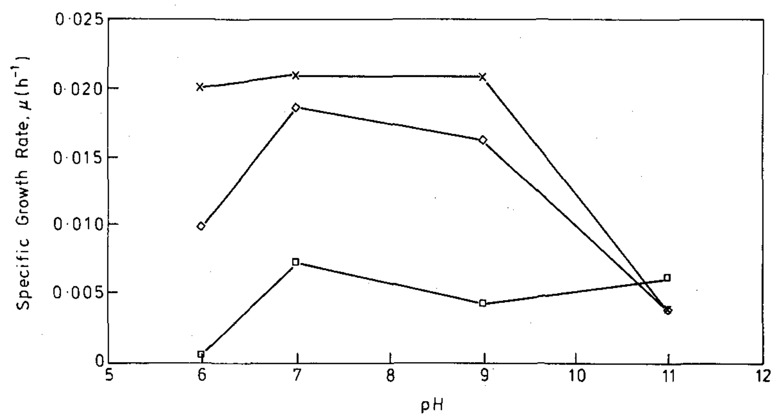

Figure 3. Effect of pH on specific growth rate for *Pseudomonas aeruginosa* NCIB 10938; determined at 30 °C over 120 h in Mineral Salts Medium (MHM) containing 50 mM organic buffer; acetate (-◇-), formate (-□-) or glucose (-×-) as carbon source at 1 g/l.

water or simple salt mixtures have been used. The progress of microbial action has been followed by measuring the gas pressure in the vessel as an indication of the volume of new gas generation but periodically bottles have been destructively tested for individual microbial products.

In such closed systems all metabolites are retained and the gaseous end products are monitored. Comparisons have been made between the aerobic start up condition and the longer term anaerobic situation in the repository. This has been simulated by running parallel experiments in which the head space is initially air and others in which anaerobic conditions were created by purging the headspace with nitrogen.

a) *Water activity*. Water is essential for metabolic activity in a living organism. The amount of free water available to the cell is influenced by the local environment including adsorption and the presence of solutes. Water may be bound up in the solid waste or its activity may be reduced by its hydration of polar molecules and ions. Although the initiation of microbial action may be inhibited in very dry conditions, the processes involved in respiration and energy utilisation produce water as a by-product. Thus once started, microbial action will be self-sustaining.

Multiple samples of waste (1 g) and soil (0.5 g) were incubated at room temperature with various amounts of water ranging up 60 ml in bottles sealed with a gas tight rubber plug. The samples ranged in appearance from superficially dry through damp material to a slurry. Even in damp waste with no fluid water apparent in the bottle, there was a consumption of gas from the head space (fig. 4). These bottles were initially aerobic so that the loss is attributed to removal of oxygen and partial dissolution of the respired carbon dioxide. The headspace gas was monitored towards the end of this experiment. Samples containing the most water had produced methane, indicating that they had become anaerobic and were producing new gas to compensate for the loss of oxygen.

b) *Inorganic salts*. The second requirement for microbial growth is a suitable nutrient mixture. Using an amount of water which was known to facilitate growth, bottles were set up containing waste (1 g), soil (2 g) and 20 ml distilled water with controlled amounts of inorganic supplements. Experiments under initially aerobic and initially anaerobic conditions were incubated in parallel at ambient temperature.

The samples set up under air experienced a steady drop in pressure over the first 20–30 days (fig. 5) which was shown to be associated with oxygen consumption. The subsequent rise after 30 days has been shown to be the result of carbon dioxide production. Nitrate supplementation produced the most substantial increase in gas formation (and by implication microbial action). The alternative source of added nitrogen is ammonium ion and this too increased activity as did added magnesium and potassium. This would indicate that under normal circumstances, the waste degradation is limited by available nitrogen and intracellular cations.

The anaerobic bottles did not show the substantial drop in gas pressure associated with respiration in the aerobic bottles. However, net gas production began after 25 days, with nitrate supplementation showing the greatest benefit (fig. 6). In contrast to aerobic conditions, sulphate appeared to enhance gas formation with ammonium, magnesium and potassium having positive, though less obvious benefit than noted above. This may indicate that sulphate-reducing organisms were active in the sulphate-supplemented sample. Gas analysis of the headspace showed that methane, hydrogen and carbon dioxide had been generated in the bottles.

Since growth on the wastes appears to be nutrient-limited, it is to be expected that compaction of the waste would increase the concentration of the key nutrients and hence improve growth rates.

c) *Organic nutrients*. The availability of a suitable carbon source is the prerequisite for significant microbial action in the waste. Although cellulose is likely to be the major

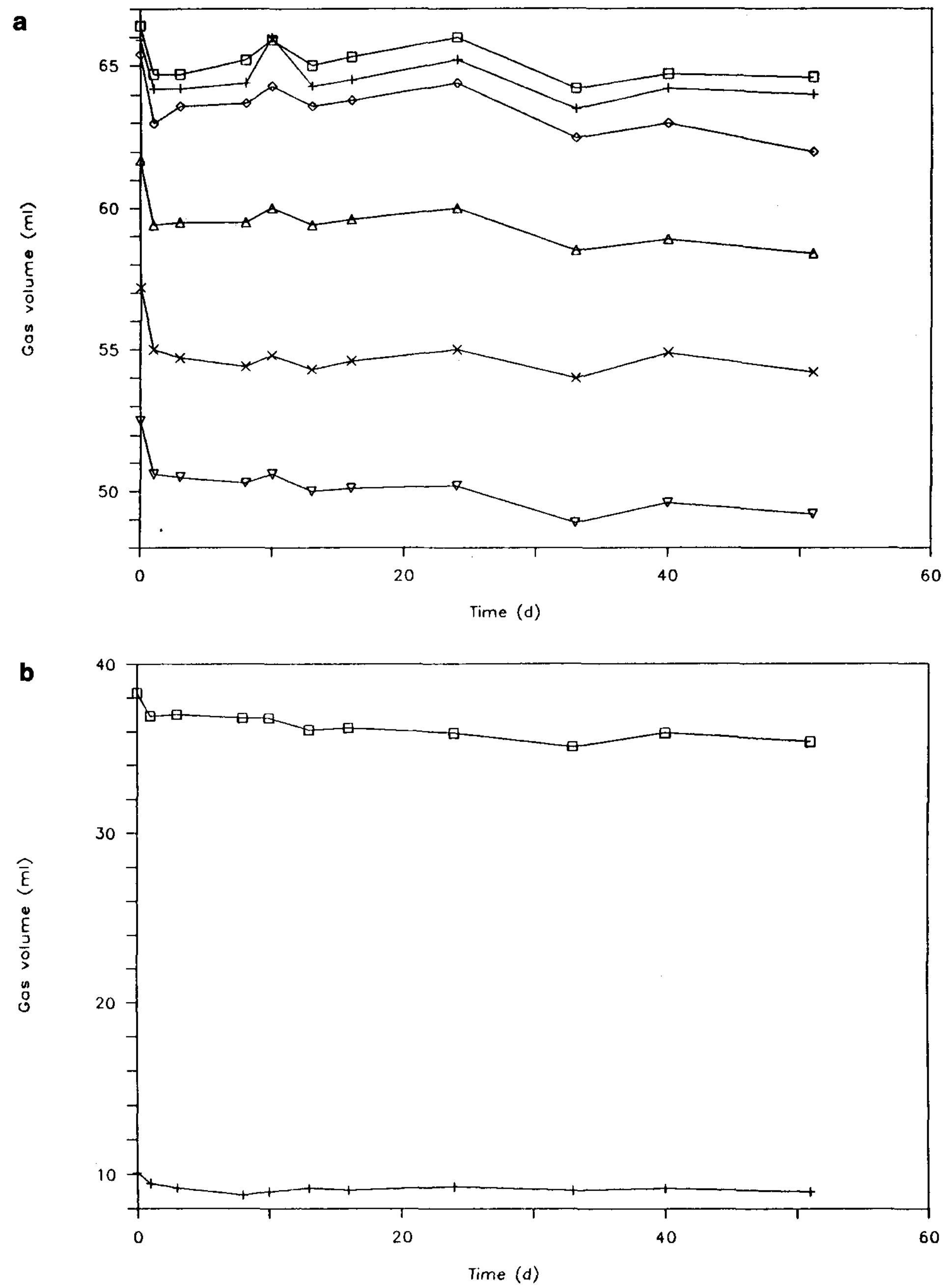

Figure 4, a and b. The effect on subsequent gas production of adding increasing quantities of water (0–60 ml) to a mixture of waste (1 g) and soil (0.5 g) sealed in a 100-ml bottle and held at ambient temperature. a) □, 0 ml water added; +, 0.5 ml; ◇, 1.0 ml; △, 5.0 ml; ×, 10 ml; ▽, 20 ml. b) □, 30 ml water added; +, 60 ml.

source of metabolisable carbon, it will be the soluble compounds derived from this which will be acted on directly by the cells. Thus the intermediates of both chemical and biochemical action are relevant to describing the microbial development of the repository. The speed with which soluble intermediates are broken down by the cells will determine their equilibrium concentration in the system.

Bottles containing waste (1 g) and soil (2 g) were set up as in the previous section, but the water was supplemented with various biodegradable compounds which might arise in organic waste. Easily metabolised, soluble substrates such as acetate and glucose increased gas production substantially (fig. 7). Carboxymethyl cellulose, a chemically modified, water soluble polymer seemed to delay the initial onset of gas generation. However, this too eventually showed evidence of microbial action, presumably as the relevant enzymes were induced. Acetate supplementation showed a marked effect, reflecting its central role in metabolism, particularly under anaerobic

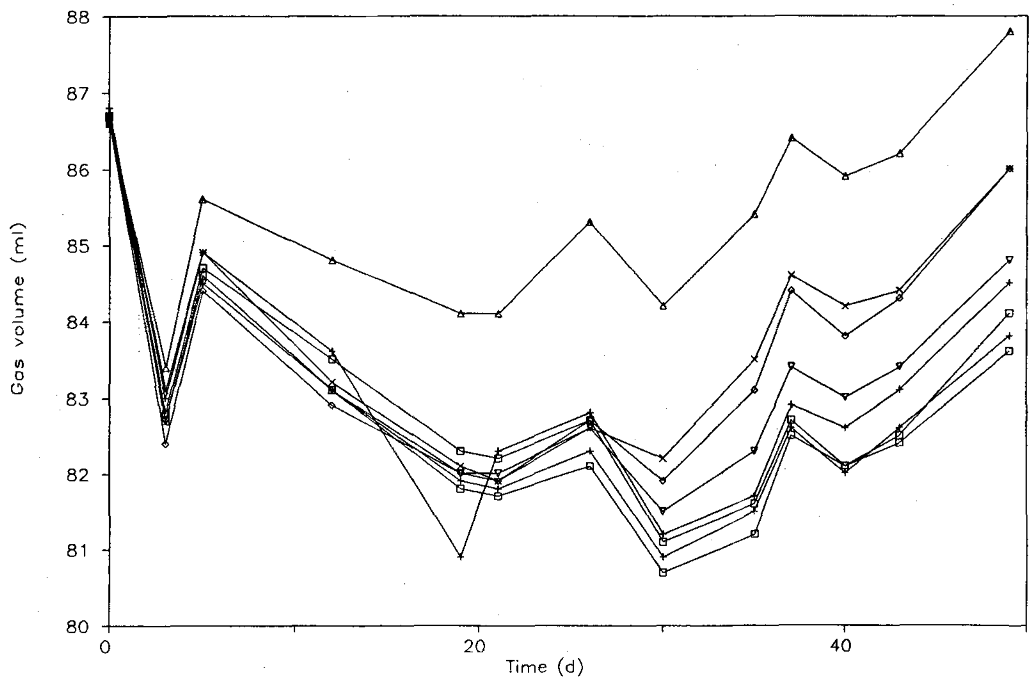

Figure 5. The effect on subsequent gas production of adding a solution containing inorganic nutrient salts (20 ml) to a mixture of waste (1 g) and soil (2 g) sealed in a 100-ml bottle and held at ambient temperature. Initial conditions aerobic. Addition of: □, water only; +, 15 mM phosphate; ◇, 46 mM ammonium; △, 50 mM nitrate; ×, 20 mM magnesium; ▽, 67 mM potassium; □, 10 mM calcium; +, 10 mM sulphate.

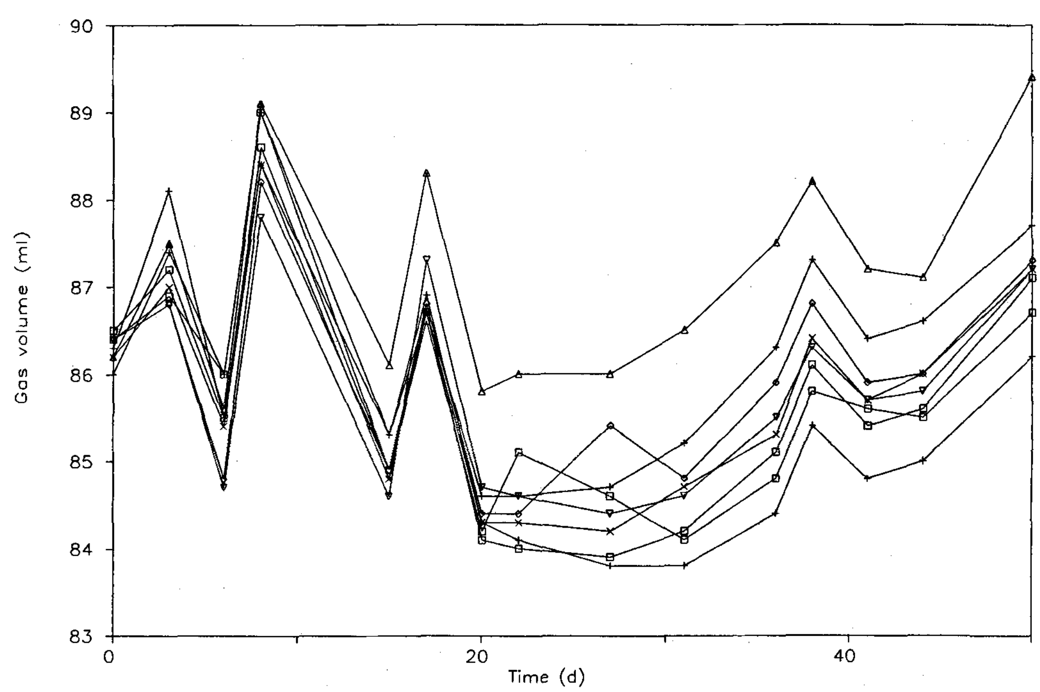

Figure 6. The effect on subsequent gas production of adding a solution containing inorganic nutrient salts (20 ml) to a mixture of waste (1 g) and soil (2 g) sealed in a 100-ml bottle and held at ambient temperature. Initial conditions anaerobic, i.e. headspace purged with nitrogen. Addition of: □, water only; +, 15 mM phosphate; ◇, 46 mM ammonium; △, 50 mM nitrate; ×, 20 mM magnesium; ▽, 67 mM potassium; □, 10 mM calcium; +, 10 mM sulphate.

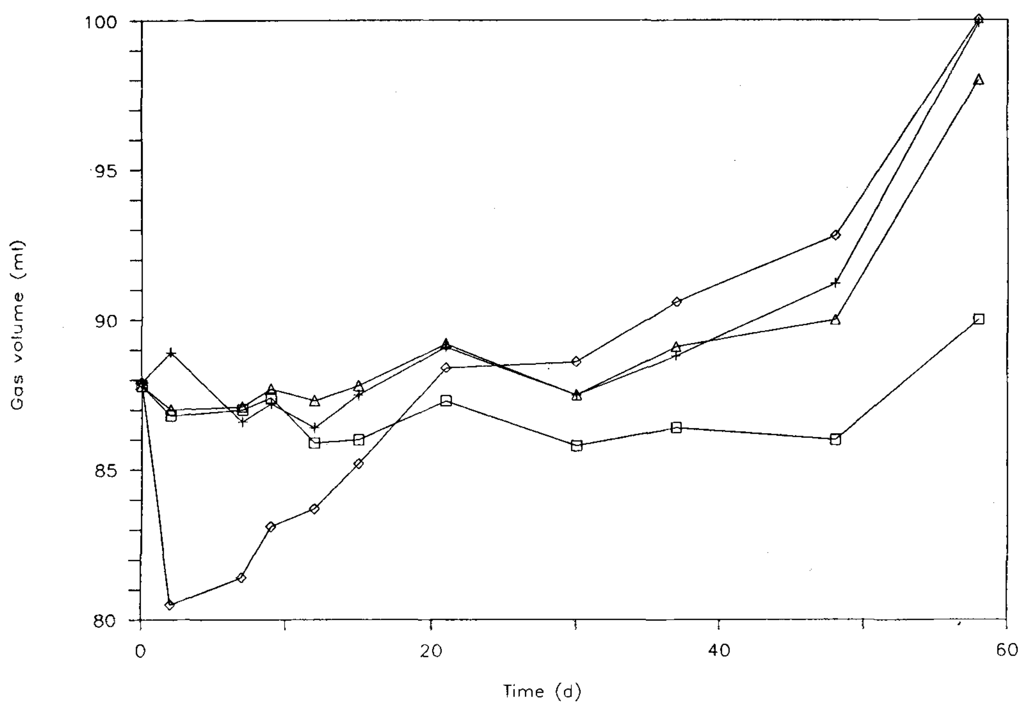

Figure 7. The effect on subsequent gas production of adding a solution (20 ml) containing organic nutrient to a mixture of waste (1 g) and soil (2 g) sealed in a 100-ml bottle and held at ambient temperature. Additions of either 10 mM glucose (+), 30 mM acetate (◇), 1% carboxymethyl cellulose (CMC) (□), water only (△). Initial conditions aerobic.

conditions. Analysis of the head space gas under these conditions indicated that it contained 7% methane derived from the organic acid.

d) *Alkalinity.* A final series of bottle tests were undertaken to measure gas production from a series of waste samples soaked in various 10 mM buffer solutions. In general gas production increased with buffer pH up to mildly alkaline values (fig. 8). The very marked drop in gas volume at the highest pH probably results from the adsorption of the carbon dioxide produced by the initial microbial action. The apparent pH optimum will be increased by the alkaline buffers which prevent the fall in local pH and the resultant souring of the waste by organic acids.

These screening experiments confirmed that, although the waste may not be of the optimum to sustain microbial action, the environmental conditions are sufficient to allow slow growth which may be accelerated by common supplements. Microbial action can be found on waste which is only damp so that some degradation may be expected in stored materials as well as buried wastes. Compaction may be expected to increase the concentration of limiting nutrients but may decrease availability.

Flow-through columns

The effect of construction materials on microbial action is being studied in a column arrangement in which liquid passes through packed beds of the solids. Two general designs have been employed; one in which the liquid phase is recycled, the other where it is continuously removed and replenished with fresh liquid. Neither design lends itself to the use of gas production as a measure of microbial action and so this must be inferred from analysis of the leachate. The percolated columns can provide a constant supply of leachate but the recycle columns can only offer a limited number of samples before the reservoir is depleted.

The columns are a convenient format for studying aqueous phase chemistry since the leachate can be constantly interrogated to determine pH, redox potential, and effects on the solubility of radionuclides through chelating agents. Physical factors such as voidage and compaction can be seen directly and it is possible to visually inspect the waste for signs of surface growth.

a) *Recycle columns.* Three sets of recycled columns have been used at Harwell. In the first series liquid was circulated through clay and concrete in various combinations. Even when a nutrient solution containing 0.1 mg/ml glucose, 0.025 mg/ml ammonium nitrate and 100 mM phosphate was passed through these columns, a strong microbial population was not sustained and the pH of the eluant rose. Once it reached pH 12, all microbial activity was inhibited. A second set of recycled columns contained combinations of a simulated ILW (Intermediate Level radioactive Waste), supplemented with cellulose and typical samples of crushed OPC (Ordinary Portland Cement) and SRPC (Sulphate Resistant Portland Cement). Basal microbiological mineral salts solution was recycled through each column and the leachates were

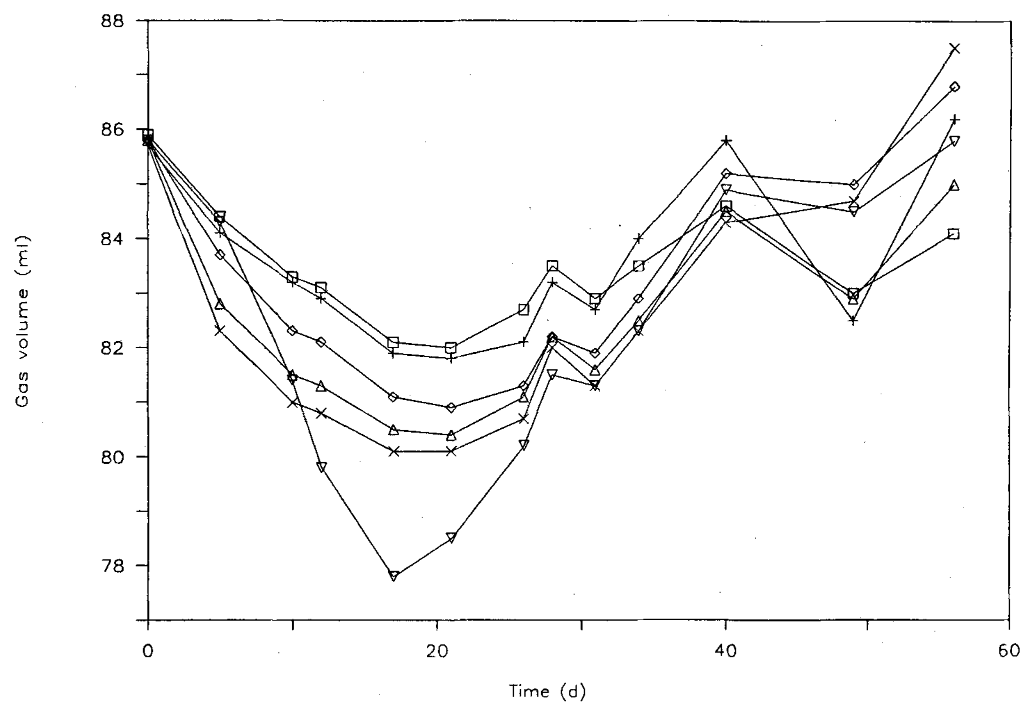

Figure 8. The effect on subsequent gas production of adding a solution (20 ml) containing organic buffer salts (10 mM) to a mixture of waste (1 g) and soil (2 g) sealed in a 100-ml bottle and held at ambient temperature. Initial pH of buffers: +, 6.9 (BES); ◇, 7.6 (EPPS); △, 8.7; ×, 9.8 (CHES); ▽, 12.4 (CAPS); □, water only. At the end of the experiment all pH values were around neutrality.

monitored for number of live cells and leachate pH. These columns were run for 35 days and were kept in the dark to prevent growth of phototrophic organisms. During this experiment, the pH did not rise above 11 so microbial activity was to be expected. The columns containing waste alone stabilised at a pH of 7, those containing waste and OPC maintained pHs between 8 and 10, while those containing waste and SRPC had high pHs between 9.5 and 11. In general the addition of ammonium nitrate increased the number of viable cells in solution and resulted in an equilibrium pH 2 units lower than the basal salts solution.

The final series of columns were of similar design but used either basal mineral salts medium or distilled water as eluant. A control column containing sterilised waste was included to allow the effects of chemical degradation to be assessed. These experiments ran for 98 days and the leachates were monitored for free microbial cells, pH, plutonium solubility in the leachate and final microbial count in the waste[5]. The details of these data will be discussed later.

b) *Percolated single pass columns.* Breakdown of the cement and growth of microorganisms in the pump tubing tended to block the recycle columns and so the once-through columns were assessed as an alternative design. A particular advantage of the single pass column is that they can be used in sequence to study the effects of adjacent environments on microbial action. This has proved particularly convenient in studying the effect of concrete leachate on waste and vice versa. The details of the studies with these columns are covered under the discussion of aqueous chemistry in the section 'Microbiology and aqueous phase chemistry'.

Gradostat

The single pass columns have been developed into a more sophisticated arrangement in which countercurrent flows of liquid are used to generate gradients of particular conditions. This Gradostat concept[21] establishes spacially fixed, stable gradients in a series of linked fermentation vessels with two dissimilar liquids flowing countercurrent into the two-end vessels. It is well suited to allow microbial action on soluble substrates to be studied in detail.

This approach was first tested with a concentration gradient of soluble substrate against a gradient of redox potential. Particular microbial populations were established at specific positions in the gradients where conditions were optimum for their metabolism. A more complex gradient has recently been set up in which tap water is used to leach an inoculum of soil in waste, held in a cage at one end of the system. This establishes a gradient of soluble organic nutrients in one direction through the Gradostat. An opposing gradient of inorganic alkalinity is established by a counterflow of water through a bed of crushed concrete. The entire system is maintained under anaerobic conditions using oxygen-free nitrogen. This provides a spacial model of the interaction between the organic material in the waste and the alkalinity in the backfill or the grout material.

Table 1. Microbial counts (microorganisms/ml) obtained for a Gradostat containing caged LLW in vessel 5 and cement leachate in vessel 1

Vessel No.	Microbial counts/ml of solution Aerobic microbes	Anaerobic microbes	pH
Tap water	2.5×10^5	2.7×10^4	7.9
Cement leachate	1.07×10^4	0	11.4
1	1.3×10^3	0	10.9
2	3.9×10^4	1.3×10^3	9.7
3	5.4×10^5	1.8×10^5	8.8
4	1.93×10^6	6.3×10^5	8.2
5	4.8×10^5	1.9×10^5	8.3
Distilled water	3.16×10^4	1×10^3	6.2

The spread plate technique was used to determine colony forming units on tryptone/soya agar.

The pH gradient (table 1) had become established within 10 days. In the region of the concrete column the pH was 10.8 and fell to pH 8 in the last vessel before the waste. The numbers of both aerobic and anaerobic organisms decreased across the vessels moving from the waste in vessel 5 to the concrete next to vessel 1 (table 1). No soluble carbohydrate was detected in any vessel suggesting that any free organic material was rapidly scavenged by the population of organisms. Although the Gradostat was initially inoculated with a very diverse population of organisms in soil, after 42 days only 8 microbial species were present in measurable numbers within the 5 vessels. Three of these isolates survived the conditions at the high pH end of the Gradostat.
The overall conclusions drawn from the column experiments is that an extremely high pH is not conducive to rapid microbial growth so waste which is in direct contact with raw cement is unlikely to degrade. However, viable populations can exist within regions adjacent to the concrete where the pH is still alkaline but below 11–12. Such regions can form if for instance a surface film coats the concrete.

Microbial gas generation

Background

The conversion of solid substrates into gaseous end products is one of the most obvious signs of microbial action since the volume change is so dramatic. The gases produced will depend on the redox potential of the environment and the particular organisms present. In general aerobic conditions will give rise to carbon dioxide through respiration of the carbon compounds using oxygen as the terminal electron acceptor. Under anaerobic conditions alternative electron acceptors are used and so a variety of gaseous products can arise. Most commonly, methane and carbon dioxide are formed in equimolar amounts but gases such as hydrogen sulphide and hydrogen may also be produced. A typical domestic landfill, once it becomes anaerobic, can produce 400 m^3 of gas per t of dry waste at a rate of 2–20 m^3/t/year[1]. This represents half the theoretical gas yield based on the carbon content of the waste. In addition sites which have a high sulphate content can support populations of sulphate-reducing bacteria and so give rise to quantities of hydrogen sulphide in the produced gas.
Under the extremely alkaline environment of an engineered repository, some of the carbon dioxide will dissolve and contribute to carbonation of the concrete. The repository design already allows for the production of gas by metal corrosion and biologically produced gases will be similar in character. Thus the microbiology is again modulating chemical effects which will take place in a predictable manner.

Gas production during tests

The experimental work described above has used gas production as a convenient measure of microbial action. These studies clearly show the transition of initial aerobic conditions to anaerobic conditions as respiration consumes the oxygen (e.g. fig. 8). Carbon dioxide is the main gas produced soon after the waste is sealed with the inoculum but methane and carbon dioxide are both produced once the redox potential of the system is reduced.
Unlike isolated organisms mixed populations do not appear to liberate much hydrogen. This is probably an indication that hydrogen itself is used as a substrate by some of the species present. This observation has implications for the fate of the hydrogen produced by anaerobic corrosion. Methanogens are routinely grown in a 4:1 hydrogen/carbon dioxide atmosphere, converting this to methane. Thus microbial utilisation of hydrogen in a repository may decrease the steady-state concentration of the gas and enhance further corrosion. The mathematical model in the 'Microbial modelling' section has been designed to deal with this situation.
The present studies indicate that both carbon dioxide and methane will be produced by microbial action within the repository. Carbon dioxide will dissolve to a limited extent so methane will be the principal component of the produced gas. It is likely that hydrogen concentrations will be depressed by microbial action and that this will further elevate methane levels. Actual rates of production will be lower than that in a domestic landfill due to the more extreme pH.

Microbiology and aqueous phase chemistry

Background

Microorganisms generally act on soluble compounds so, although the primary substrate may be solid or gaseous, microbial action will always have an important influence on the aqueous environment in which it takes place. Organics will enter solution either as a result of chemical degradation or through biological degradation of materials as a result of enzymic action. In addition, soluble intermediates of metabolism will appear in solution. Most microbial degradation involves a sequence of processes in which the end-products of one organism become the substrate for a second. Compounds will accumulate before the degradative population begins to grow and will then be reduced to an equilibrium level as microbial consumption balances rate of production of fresh soluble substrate. The modelling described in the next section of this paper is based on this dynamic situation.

The soluble organic compounds arising from these three sources can influence near-field aqueous chemistry in several ways. The acids produced by partial oxidation of carbon compounds will increase solubility of critical radionuclides by decreasing the local pH. In addition, some of the intermediates may form complexes with the radioelements and enhance solubility. On the other hand, microbial action could result in degradation of organic complexing agents which would normally accumulate as a result of chemical degradation of the waste. Organic acid production may enhance corrosion rates while sulphide generation may also influence the chemistry of metal attack. The biomass itself may have an indirect influence on the chemistry by coating surface with an impermeable slime which prevents the concrete buffering the pore water adequately. The biomass may, however, have a beneficial effect in adsorbing normally mobile ions onto the polymeric compounds upon its surface.

Radionuclide solubility

The solubility of actinides in alkaline concrete pore water is extremely low, and any significant increase would still be below the detection limit of normal chemical analysis. Thus it is not feasible to use a non-radioactive model for solution studies, and plutonium solubility has been adopted as the most sensitive test of complexation. These tests have been performed by the Waste Disposal Group at Harwell by adding known amounts of plutonium to the aqueous samples. The leachates are adjusted to pH 12.5 prior to testing and the data are compared with a control sample obtained from a sterile concrete leachate.

Table 2. Plutonium solubility (nM) in samples of leachate from recirculating columns

Column description	Time (days) 8	21	45	98
OPC + PCM + water	–	–	–	3919
SRPC + PCM + water	–	–	–	1625
OPC + PCM + CMM + N	–	–	–	0.56
OPC + PCM + CMM	11	350	1140	0.39
PCM + water	905	2.3	2	1651
OPC + PCM + CMM + N (sterile)	71	4.6	–	0.27
SRPC + PCM + CMM	–	–	–	8.2
PCM + CMM + N	9.1	18.1	–	0.95
PCM + CMM	–	7.9	–	0.7
SRPC + PCM + CMM + N	–	–	–	–

Columns contained 30 g of shredded waste (simulated PCM component of ILW), with or without Ordinary Portland Cement (OPC) or Sulphate Resistant Portland Cement (SRPC). Fluid circulated through the columns was either water or a minimal basal salts medium (CMM). Column 6 was autoclaved and had no vegetative cells within it, initially. For definition of PCM and CMM see Appendix.

Representative samples of leachate from the experimental systems described above have been tested to establish whether the solubility of plutonium is enhanced by organic compounds in the solution. Plutonium solubility data from recycle and single pass columns is given in tables 2 and 4. The corresponding pH values for these leachates prior to testing are given in tables 3 and 5. After 98 days recycle, columns containing LLW and either OPC or SRPC leached with tap water, gave solu-

Table 3. pH of leachate from recirculating columns

Column description	Time course (days) 3	10	18	31	45	98
OPC + PCM + water	11.3	12.8	12.5	12.7	12.5	12.0
SRPC + PCM + water	11.3	12.8	12.5	12.9	12.5	12.1
OPC + PCM + CMM + N	11.5	ND	8.5	8.3	7.6	7.5
OPC + PCM + CMM	11.5	12.6	12.5	12.5	12.8	11.9
PCM + water	7.1	8.5	7.7	7.8	8.0	7.4
OPC + PCM + CMM + N	8.9	9.8	11.7	12.2	ND	10.7
SRPC + PCM + CMM	11.5	ND	ND	ND	ND	10.0
PCM + CMM + N	6.8	6.7	6.4	6.3	6.0	6.1
PCM + CMM	6.7	7.1	7.1	7.2	7.1	7.5
SRPC + PCM + CMM + N	10.7	10.2	12.5	12.5	ND	ND

Columns contained 30 g of shredded waste (simulated PCM component of ILW), with or without Ordinary Portland Cement (OPC) or Sulphate Resistant Portland Cement (SRPC). Fluid circulated through the columns was either water or a minimal basal salts medium (CMM). Column 6 was autoclaved and had no vegetative cells within it, initially. For definition of PCM and CMM see Appendix.

Table 4. Plutonium solubility (nM) in samples of leachate from columns

Column description	Time (days) 25	87	149
Aged LLW (AW)	0.113	4900	0.28
AW + OPC	0.386	1.23	1.08
Fresh LLW (FW)	0.39	0.23	0.2
FW + OPC	0.09	0.3	0.55
OPC	0.065	0.04	0.12
AW + OPC leachate (OL)	0.16	0.167	0.11
FW + OPC leachate	0.58	–	0.065
FW leachate – OPC	0.049	0.19	2.46
OL – FW – OL	0.136	0.152	0.03

Columns contained 30 g of shredded waste (either fresh (FW) or aged simulants of LLW), with or without crushed Ordinary Portland Cement (OPC). Water was passed through the columns in the sequence indicated. For definition of LLW see Appendix.

Table 5. pH of leachate from columns

Column description	Time (days) 10	25	60	87	149
Aged LLW (AW)	6.8	6.8	7.6	7.0	7.4
AW + OPC	10.5	9.4	7.8	7.1	7.5
Fresh LLW (FW)	6.4	7.0	8.0	7.0	7.5
FW + OPC	7.0	8.5	8.1	7.5	7.5
OPC	10.9	10.5	9.6	8.0	8.1
AW + OPC leachate (OL)	10.9	10.8	7.8	6.9	7.0
FW + OPC leachate	8.5	11.0	10.6	10.6	10.6
FW leachate – OPC	10.8	8.4	7.9	7.8	7.6
OL – FW – OL	11.0	11.2	11.3	11.1	11.2

Columns contained 30 g of shredded waste (either fresh (FW) or aged simulants of LLW), with or without crushed Ordinary Portland Cement (OPC). Water was passed through the columns in the sequence indicated. For definition of LLW see Appendix.

tions which increased plutonium solubility by over 3 orders of magnitude (i.e., from 0.5 nM to 3900 nM). These leachates had pH values of 12, suggesting that there were no active cells left in the system and that this enhanced solubility arose from chemical degradation of the waste and residual biomass. Although there is considerable scatter on these initial results, columns in which pH values remained low and biological activity was evident, did not enhance plutonium solubility to the same extent (tables 2 and 3). The generally low solubility of samples obtained from the single pass columns listed in table 4 was also associated with the maintenance of a moderate pH and hence low degradation and possibly established microbial populations. The data are not yet sufficient to draw unambiguous conclusions regarding microbial effects on actinide solubility but there is no evidence that microbial action makes the solubility worse than that due to chemical action.

Repository pH

Organic acids in the range C_1 to C_7 are produced by microbial action on organic waste. These partial oxidation products will tend to depress the pH which is normally imposed by the concrete. This phenomenon is illustrated by the relatively neutral pH of the leachates emerging from the mixed waste/concrete columns covered by table 5. It should also be noted that the dissolution of carbon dioxide in the pore water to produce carbonate will also contribute to a reduction of the alkalinity. This effect will not be removed by further microbial action, while that arising from the organic acids will be constantly suppressed through the metabolism of individual organisms in the mixed population.

Surface effects

In a nutrient-limited environment there is a selective advantage for organisms growing on a surface compared with those in free solution. Not only do nutrients concentrate at interfaces, but the attached organisms are well placed to recycle intermediates liberated by adjacent cells. Biofilm formation is normally associated with the secretion of a slime or mucilage which assists in attaching the cells and restricts the loss of nutrients by impeding diffusion. It was noted in the initial screening of alkalitolerant organisms that a common survival strategy was the over-production of these slimes. In general these are rich in acidic polysaccharides and maintain a lower microenvironment pH than is found in the bulk phase. The survival of microorganisms within[6] and upon concrete[10] has been reported elsewhere. Although no direct data are available at present, the fact that successful microbial colonisation of concrete surfaces in column experiments seems to prevent the pH of the liquid phase rising, suggests that some protective coating may be generated. If this occurred within the repository, it would allow insulated pockets of mild conditions to develop. These would be bounded by high pH regions so the barrier concept will still be maintained. However, the build-up of acid may weaken the local matrix, resulting in stress fractures.

Waste composition

The present studies have concentrated on LLW and the PCM (Plutonium Contaminated Material) component of ILW. The composition of these wastes (Appendix) will differ but it is the cellulosic component of the organic fraction which will be the main determinant of microbial action. LLW is likely to contain the greater proportion of cellulosic materials and PCM a higher proportion of less degradable plastics. Consequently both chemical and biological action will give rise to greater changes in the solution chemistry within the LLW.

Identification and lifetime of organic intermediates

The initial work on waste degradation concentrated on the principal organic acids which might be expected to leach from degraded organic waste. Gas chromatographic analysis of leachates from anaerobic columns has shown concentrations of 0.07–0.7 mM of the principal organic acids, ethanoic, propanoic and n-butanoic. There were other volatile acids present in smaller amounts. However, the enhanced plutonium solubility found in leachates can not be accounted for by the presence of the simple volatile fatty acids from C_1 to C_7.
The greatest enhancement in plutonium solubility is associated with cellulosic waste[3]. It is known that alkaline hydrolysis of cellulose under anaerobic conditions can produce highly complexing saccharinic acids, particularly if there are high concentrations of calcium ions in the solution[16]. Attention has now been focused on these polyhydroxy sugar acids and work is in hand to determine their biodegradability. It should be emphasised that these strong complexants are generated chemically and there is no evidence that large amounts of microbially derived complexants have accumulated in the experimental systems.
Microbial action will clearly affect the aqueous phase chemistry where organic material is present in the waste. The cellulosic fraction is the main determinant of cell growth and biodegradation of the polymers. Although organic acid generation is a factor in changing the local environment, it appears to be the sugar acids arising from alkaline degradation of cellulose which cause enhanced plutonium solubility. The potentially beneficial reduction of chemically derived polyhydroxy acids by the microorganisms is possible. Slime formation resulting from biofilm growth on the concrete surface may prevent the maintenance of a high pH in local pockets within the matrix.

Microbial modelling

Background

A computer-based mathematical model of microbial metabolism is necessary if all the interrelated factors are

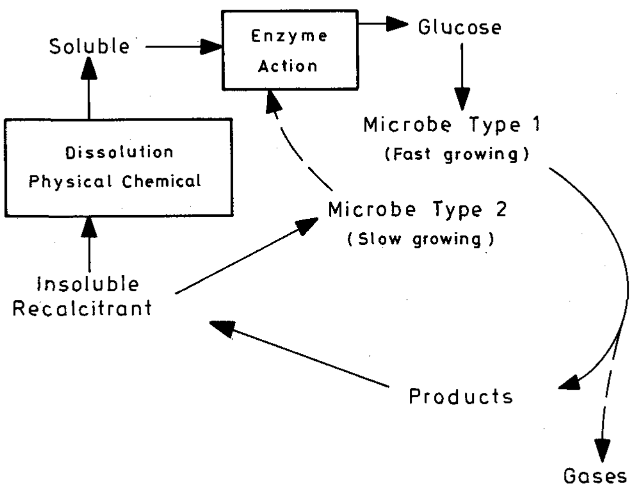

Figure 9. Model of the microbial system involving soluble, insoluble and recalcitrant organic wastes.

to be incorporated in an assessment of the repository performance. Such a model should allow rates of production and the accumulation of products to be predicted and hence the evolution of the near field to be described using measurable parameters. A model aimed at the specific needs of the Nirex programme has been developed and is being validated and refined. However, it is difficult for short-term experiments to encompass the time scale of the planned repository and so existing natural analogues have been sought. Domestic landfills give a pointer to the evolution of the microbiology in a LLW repository. Batch growth data derived from such systems have been used to describe the relationship between microbial metabolism, biomass, product formation and inhibition of metabolism. Anaerobic waste digesters provide information on nutrient flux [2, 17] and natural soil systems [18] give the relationships involved in cycling of organic and inorganic materials in a restricted environment.

The current model

The model assumes that cellulose is the main carbon source for microbial growth. Decomposition of the cellulose gives rise to metabolically active biomass, inactive recalcitrant biomass, soluble metabolic products, chemical intermediates from cellulose breakdown and gases (fig. 9). The rate of appearance and disappearance of these products can be modelled over the lifetime of the repository using simple mathematical functions. The way in which the available carbon partitions between each category as the redox potential alters can be predicted. At present no account is taken of novel products arising from microbial metabolism or of the more recalcitrant organics in the waste such as the plastics and rubber. However, experimental evidence indicates that these may only exercise a minor influence on the medium term performance of the repository. The model predicts that microbial activity is likely to occur over the first 200–400 years of the repository lifetime. It will be possible to extend the model to cover the slowly degrading plastics and any important secondary metabolites which are identified as crucial to actinide solubility.

Validation work is in progress using data from the experimental systems described in previous sections. At present it is possible to model both aerobic and anaerobic growth and to model the evolution of one to the other as the microbial growth depletes the oxygen (fig. 10). The programme deals with the coexistence of several microbial types and the dynamic generation of intermediates at different stages of the degradation process.

Of particular importance has been the need to estimate maintenance energies needed to sustain basic metabolic functions in a non-growing cell. The energetic state of a cell can be estimated by measurement of the adenylate energy charge (AEC). This has been determined by a novel method in which the ratio of the high energy forms of adenine (i.e. ATP, ADP, AMP) in cells is measured by extraction and HPLC analysis. Figure 11 shows that for a pure culture, as the available source of nutrient (glucose) is depleted, biomass production ceases and the energy charge drops to a low, but finite value as the cells senesce. This value is independent of growth rate and may be used in an assessment of microbial status in the repository.

The structure of the present mathematical model which has been developed for Nirex appears to predict the general features which are intuitively expected in a developing microbial population. It illustrates that intermediate compounds will build up in the waste until growth of the next organism needed for sequential degradation is initiated. The soluble compounds in the pore water and the mixture of microorganisms present in the waste will vary with time and sustain biological activity over a prolonged period. Present estimates suggest that microbial action may persist for up to 400 years. There is scope to improve the model to deal with environmental factors such as temperature and pH and to introduce other energy sources such as hydrogen.

Summary of achievements

The programme has shown that microbial action is possible within a repository, even at the highly alkaline pH and low water activity predicted from the design. Representative waste handled in the open laboratory and soils from a variety of geological sites are a source of microorganisms which can survive these conditions. When combustible LLW and ILW is buried underground it will be degraded microbiologically in a similar manner to domestic landfill waste. The products of this degradation will be gases, soluble organic compounds, insoluble organics and cellular biomass.

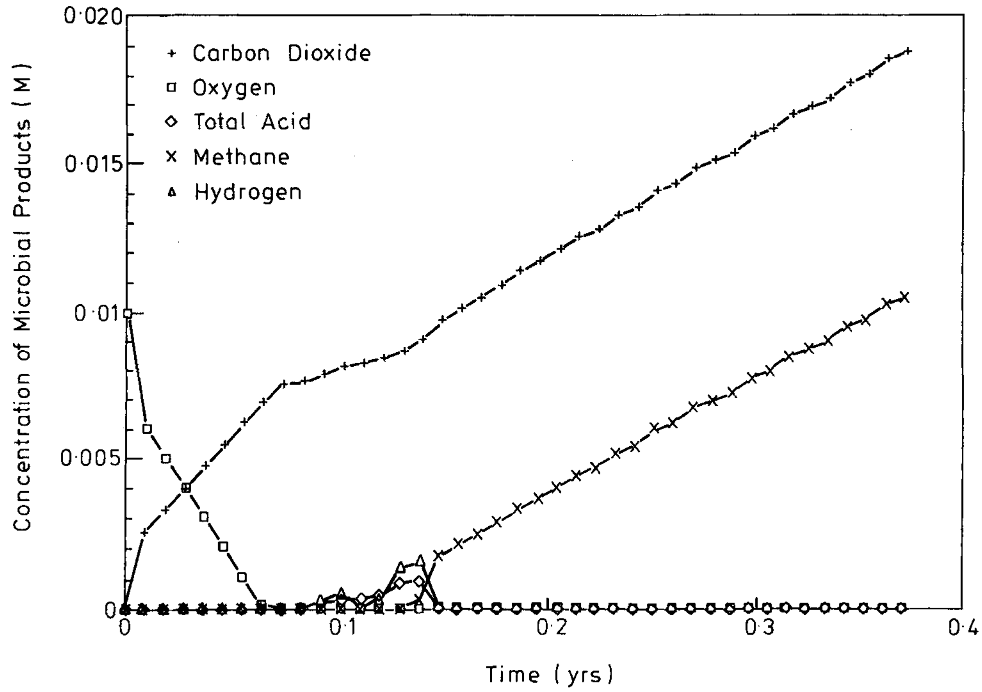

Figure 10. Short time scale simulation of development of repository from aerobic to anaerobic conditions.

Leachates from organic waste enhance the solubility of plutonium by over three orders of magnitude compared to cement leachate. The simple organic acids and alcohol found in microbial leachates do not account for this effect, and it is presumed from work elsewhere [3, 8, 16] that small amounts of chemically derived sugar acids are the main complexants.

A computer simulation of microbial action in a repository has been developed and provided with data from natural analogues such as domestic landfill and laboratory-based experiments with simulated LLW and ILW. This model simulates correctly the distribution of carbon compounds between the gas phase, solution, recalcitrant solids and cellular biomass. This suggests that microbial action will persist for up to 400 years after closure of the repository.

The main issues for future work will be to determine the extent to which chemical degradation products might be destroyed by microbial action and to assess how significant biofilm formation might be in masking the alkaline features of concrete. The development of the mathematical model to deal with a wider range of environmental factors is planned.

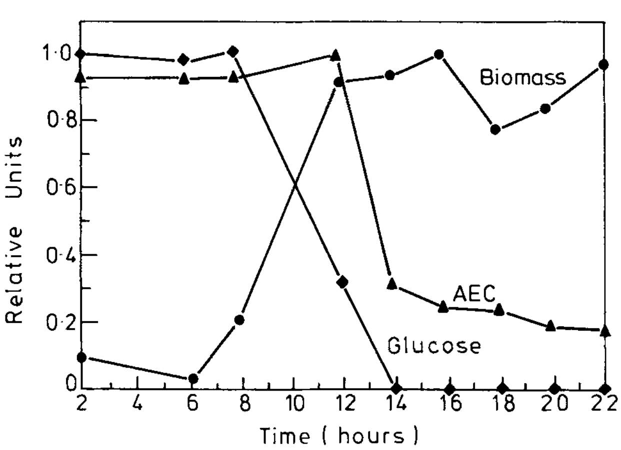

Figure 11. Growth rate for *B. subtilis*, showing relationship between biomass, glucose concentration and adenylate energy charge (AEC).

Appendix

All soil samples were taken from a single site of fertile soil on the Harwell site. This soil is mildly alkaline since it is on a chalk subsoil. Unless specified all experiments are conducted at laboratory ambient temperature which is normally around 20 °C.

Two organic, non-radioactive waste simulants have been used. Each uses materials obtained as standard stores items on the Harwell site and are regarded as typical of those found in actual radioactive waste.

Composition of the PCM component of ILW and Aged Waste (AW):

Shredded;

Cellulose (tissue)	37%
PVC	35%

Polythene	14%
Hypalon	7%
Neoprene	7%

Composition of LLW simulant and Fresh Waste (FW):

Shredded; cellulose (tissue)	37%
Cellulose (wood)	30%
Polythene	12%
PVC	12%
Latex	6%
Neoprene	3%

Basal mineral salts or Column Minimal Medium (CMM) solutions are prepared from:

Dipotassium hydrogen phosphate	1.0 g
Potassium dihydrogen phosphate	0.43 g
Magnesium sulphate, hydrated	0.1 g
Ammonium sulphate	1.0 g
Trace element solution	5 ml
Distilled water up to 1 l.	

Trace elements made from;

Ferrous sulphate, hydrated	0.5 g
Zinc sulphate, hydrated	0.5 g
Manganese sulphate, hydrated	0.5 g
0.1 N sulphuric acid	10 ml
Distilled water up to 1 l.	

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