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Evaluation of cement degradation induced by the metabolic products of two fungal strains

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Summary. During their metabolism, microorganisms can produce acids able to bring about the degradation of cement. Two acid-producing alkalophilic fungal strains have been isolated from soil, a strain of *Aspergillus niger* which produces gluconic and oxalic acid and of *Mycelia sterila* which produces gluconic and malic acid. After eleven months of contact the acids produced by *Aspergillus* dissolved portlandite with a low leaching of calcium, increased the cement porosity by 11.4%, and reduced the bending strength by 78%. The second strain is responsible for a significant dissolution of portlandite with a leaching of calcium of 4.2% of the initial content, an increase of the porosity of 11%, and a loss of the bending strength of 62%. Direct contact of mycelia with the cement surface is not necessary for effective dissolution to take place. Low pH and a high temperature favor the production of acids.
Key words. Radioactive waste; cement; degradation; microorganisms; organic acids.

Introduction

Waste repositories can be situated in geological formations where microorganisms capable of degrading complex organic materials are present[21]. As part of a program studying the long-term stability of the materials used for nuclear waste disposal, the biodegradability of cement (a coating matrix for the low and intermediate level waste) was determined.

Cements are mineral materials, primarily hydrated calcium silicate C–S–H and portlandite ($Ca(OH)_2$) for the ordinary Portland cement. Their biodegradation is due to the products of microbial metabolism and thus is an indirect effect of microbial growth. Examples of deterioration of stones or cement have often been described[20], mainly in sewer systems but also on building structures. In the sewer systems, degradation is due to the activity of autotrophic sulphur-oxidizing bacteria[14–16]. Hetero-

trophic microorganisms can also induce degradation[11,13] by excreting carboxylic acids during the decomposition of organic matter.

Microorganisms able to grow near cement (in alkaline conditions) and to excrete organic acids have been isolated from soil. In an experimental system two fungal strains have been brought into contact with the cement. In this paper physico-chemical changes of cement samples which had been in contact with fungal cultures for eleven months are reported and compared with the effect of the acids alone.

The influence of culture parameters such as pH, temperature, dissolved O_2 and glucose consumption, and incubation time has been measured using a fractional factorial design[4,8]. The effect on cement degradation of soil bacteria and of bacteria found in deep groundwater is described elsewhere[17].

Materials and methods

Microbial strains and cultures. The two fungal strains used were isolated from a natural soil sample taken under an oak litter, and have been identified as *Aspergillus niger* and a non-spore-forming *Mycelia sterila.* The culture medium is described by Perfettini[17] and contains glucose (111 mmol/dm^3) as carbon source. The initial pH is 5.6.

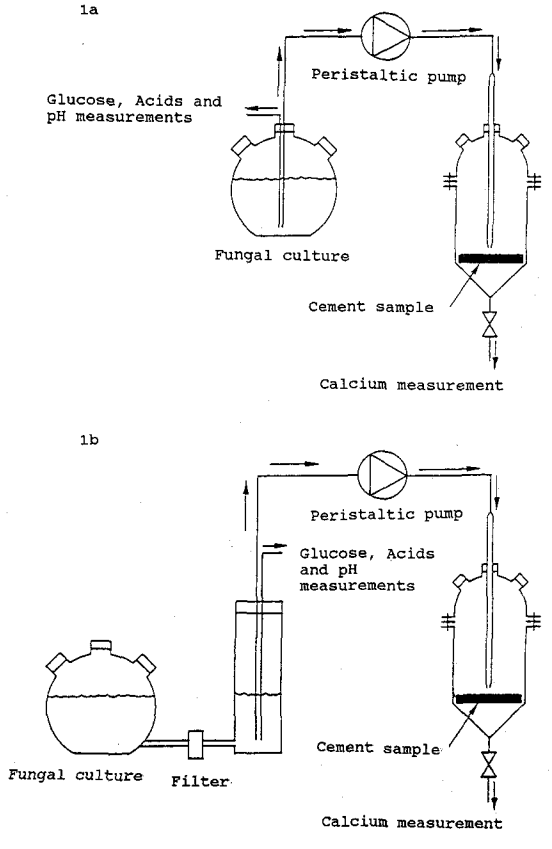

Figure 1. Experimental setup to bring the fungal culture (*a*) or organic acids (*b*) in contact with cement samples.

Experimental systems. Figures 1 a and 1 b show the experimental setup used to test the effect of the microbial cultures with (1 a) or without (1 b) mycelia in contact with the cement samples.

Cement samples. The samples used are disks (thickness 4 mm; diameter 70 mm) made of ordinary Portland cement that contains about 15% w/w of portlandite $Ca(OH)_2$.

Analysis of substrates and products. Acids generated from the fungal decomposition of glucose were determined in the medium by high-performance liquid chromatography[10] in order to quantify the corrosive species. The pH of the medium and the consumption of glucose, using an enzymatic method, were determined at regular time intervals.

Physico-chemical analyses. The effect of microbial cultures on cement were studied as follows:

1) Amount of calcium released: the medium that had passed through the cement was periodically collected and its calcium content was measured by atomic absorption spectrometry;

2) Physico-chemical modifications of cement were evaluated by:

- determination of volume and size of the pores as measured after a full desiccation by intrusion of mercury;
- determination of portlandite content by a thermogravimetric method;
- determination of bending strength (three points methods);
- identification of some constituents like portlandite, ettringite, and C–S–H by X-ray diffraction[13]. For the last test, the samples were powdered and crushed (0.063 μm).

Fractional factorial design and mathematical model. The use of the fractional factorial design allows the modeling of the experimental response obtained in a defined experimental environment. This was a pH in the range of 4 to 8.5 and a temperature between 15 and 37 °C. The mathematical modeling was performed using the REGMU program from the Ecole de Chimie de Marseille (France).

Results and discussion

Alteration induced by the acids and the mycelia

Microbial growth and acid production. The two fungal strains utilize glucose as a substrate and produce organic acids as metabolites. Organic acids are well known to solubilize mineral elements such as Ca, Si, Al, and Fe from rock[1,3].

– *A. niger*: The pH of the medium dropped from 5.6 to 1.8 during growth on glucose. The acids produced were mainly gluconic and oxalic acid. The total amount of acids produced during eleven months was 32 mmoles, with 5.8 mmoles of gluconic acid and 19.6 mmoles of oxalic acid. Other acids were produced only in negligible amounts.

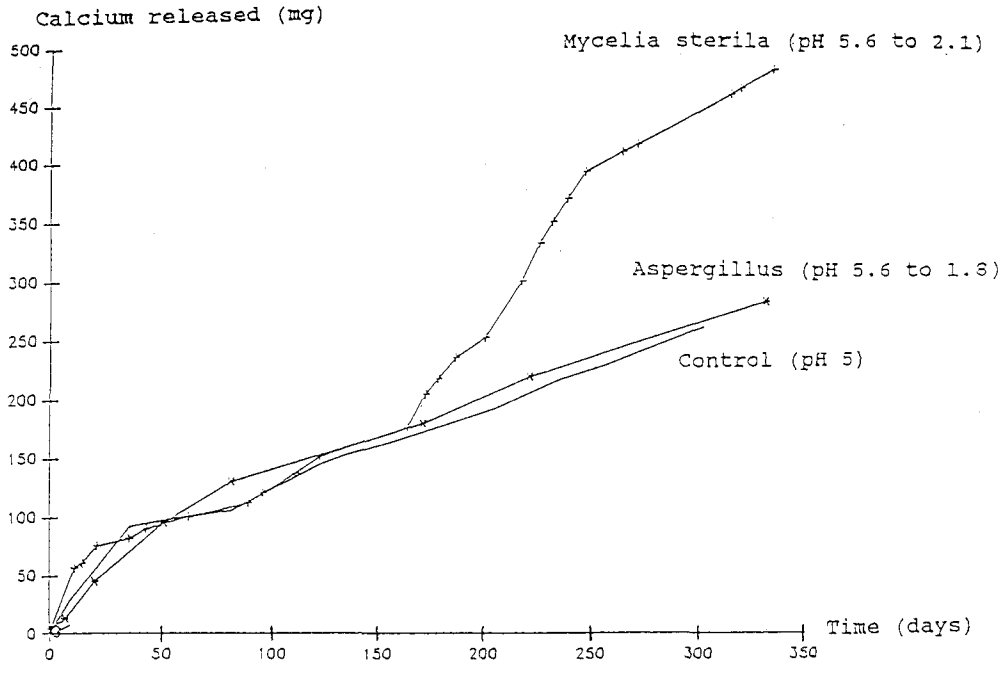

Figure. 2. Release of calcium from the cement samples in contact with two fungal strains. Control uninoculated at pH 5. The change in pH during the time indicated is given in brackets.

– *M. sterila*: The pH of the medium dropped from 5.6 to 2.1 during growth. The main acids produced were gluconic and malic acid. Of a total of 25.8 mmoles of organic acids received by the cement sample, 10.2 mmoles were of malic acid and 5.6 mmoles of gluconic acid. With the first experimental system used, the mycelia grew directly on the surface of the cement samples. Development of *A. niger* was more pronounced than *M. sterila* under the same conditions.

Calcium release. The release of calcium induced by the organic acids was compared to a control with uninoculated culture medium (pH 5) lacking organic acids and microorganisms (fig. 2). The culture medium caused 2.2% of the initial value of calcium to be leached over a ten-month period. In contrast, samples in contact with *M. sterila* lost 4.2% and those in contact with *A. niger* 2.5% of their initial calcium content. As Berthelin observed earlier[1,2] the quantity of calcium released is not related to the quantity of acids produced, 25.8 mmoles leached 4.2% of Ca, 32 mmoles only 2.6%. The solubilization of the various elements was related more to the nature of the acids than to their concentration.

Physico-chemical changes of the cement samples

Portlandite content. Thermogravimetric analysis allows the measurement of the portlandite by following the weight loss of the cement sample between 420 and 480 °C. In this range, the $Ca(OH)_2$ is split into CaO and H_2O. The sample in contact with the *A. niger* contained 2.2% w/w of portlandite after 11 months corresponding to a loss of 85.3% compared with the control of 15% w/w.

Table 1. Calcium balance for the samples in contact with fungal cultures. Experimental time: 11 months.

	Aspergillus niger	*Mycelia sterila*
Ca initial (mg)	11 510	11 512
Ca leached (mg)	283	483
(%)	2.46	4.19
Ca issued from the $Ca(OH)_2$ dissolution (mg)	2 070	1 920

In the sample in contact with *M. sterila* and its acids 4% w/w of portlandite remained indicating a loss of 73.3%. These results can not be directly related to the calcium released from the samples. In a calcium balance (table 1), with *A. niger* strain, 13.7% of the calcium released from the portlandite is leached; with *M. sterila*, it amounts to 25.2%. The difference is due to the fact that with *Aspergillus* calcium precipitates mainly as calcium oxalate in the pores. With *Mycelia*, the calcium salts of gluconic and malic acid are readily soluble and thus more easily leached.

Change in porosity. The total pore volume measured by intrusion of mercury can be divided into various size classes depending on the pore family. An increase in the number of pores of the size range $0.9 > d > 0.6$ µm corresponds to a dissolution of portlandite crystals[18]. The porosity of the samples in contact with the fungal cultures compared with the porosity of the control are given in table 2.

The contact with the organic acids and mycelium of *A. niger* caused an increase of the total porosity of the sam-

Table 2. Porosity of the cement samples in contact with two fungal cultures. The porosity is expressed as the percentage of pore volume compared with the total solid and pore volume. Experimental time: 11 months.

Pore diameter	Pore volume (%)		
	Mycelia st.	*Aspergillus*	Control
1) d > 0.9 μm	3.02	1.86	0.73
2) 0.9 > d > 0.06 μm	9.01	10.70	1.53
3) 0.06 > d > 0.009 μm	12.06	11.36	11.04
4) d < 0.009 μm	6.81	6.94	6.57
Total porosity	30.90	30.86	19.86

ple of + 11.4% mainly due to an increase of 9.2% in number of pores in the size range 0.06 to 0.9 μm. This confirms the thermogravimetric measurements that indicate an excessive dissolution of portlandite. For the sample in contact with *M. sterila*, the total porosity increased 11%. As with *Aspergillus* the number of pores with a diameter between 0.06 and 0.9 μm increased, but not to the same extent. Furthermore the amount of large pores increased by 2.3%, possibly due to the microcracking generated by the dessication of silica and alumina gels, gels that are ultimately products of decalcification [19].

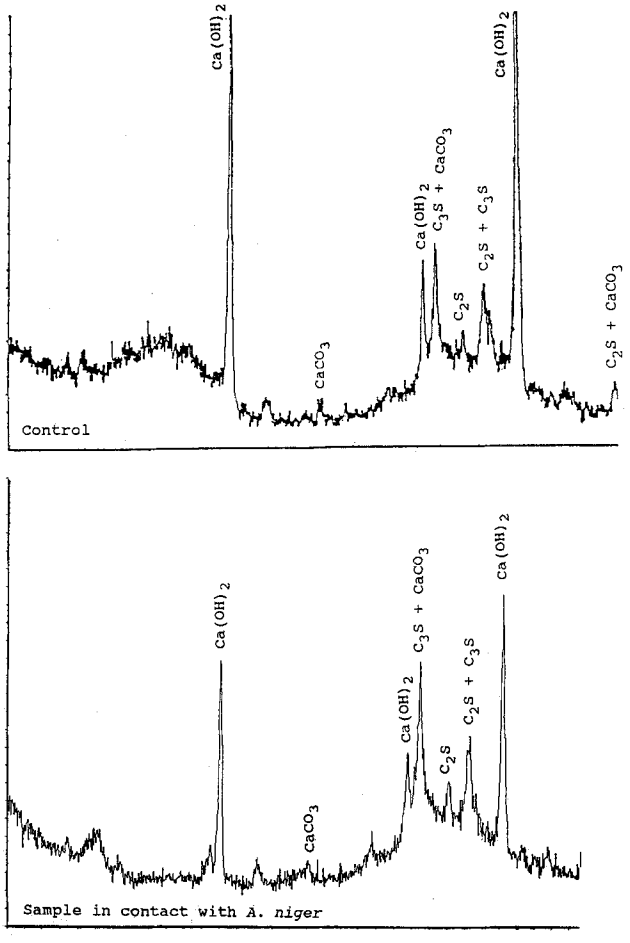

Figure 3. X-ray spectra of control and of sample in contact with *A. niger* Experimental time: 11 months.

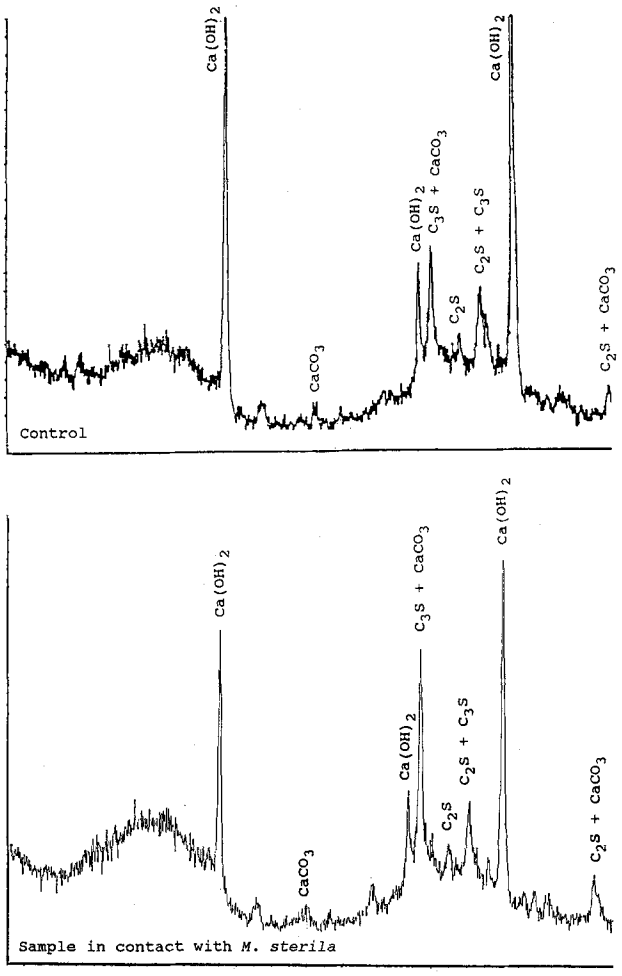

Figure 4. X-ray spectra of control and of sample in contact with *M. sterila*. Experimental time: 11 months.

The increase in the porosity has important indirect effects: penetration of aggressive species into the cement and the resulting corrosion are greatly facilitated.

X-ray diffraction. The X-ray diffraction spectra for the sample in contact with *A. niger* and *M. sterila* for 11 months are given in figures 3 and 4, respectively. Compared with the spectrum for the control sample (no contact with microorganisms, acids or culture medium), we can easily recognize changes in the peaks of the portlandite, the calcium silicate (C_2S and C_3S), the C–S–H and others. The method used is qualitative as well as semi-quantitative.

The sample in contact with *A. niger* generating gluconic and oxalic acids shows a decrease in the portlandite crystals but no change for the other crystals. Some calcium carbonate crystals appear, due to carbon dioxide excreted as a metabolic product of the fungus. The sample in contact with *M. sterila* producing gluconic and malic acid shows similar changes with regard to the reference, i.e. a heavy loss of portlandite and the formation of calcium carbonate. The portlandite peaks are less pronounced than with *Aspergillus*.

Table 3. Bending strength expressed in Newton (measured by the three point method) of cement samples in contact with the microbial strains.

Time of contact (months)	Fungal strains *Aspergillus*	*Mycelia sterila*	Control
0			460
1	420	–	
2	380	420	
4	205	350	
8	180	–	
11	100	180	

Bending strength. The bending strength is expressed by the strength which, applied on three points to the surface of the sample, causes its breakage. When brought into contact with the oxalic and gluconic acid produced by *A. niger* and overgrown with the mycelium the cement shows a drastic loss of bending strength of 78% (table 3). The structural and chemical modifications induced by *M. sterila* in the cement lead to a smaller loss of the bending strength of 62%, parallel to the the loss of portlandite.

Changes induced by fungal cultures compared with organic acids alone

To distinguish between effects of growth of the organisms on the cement and the effect of the acids produced the experimental setup was modified (fig. 1 b).

The results for an 8-month period are presented in table 4. No clear differences are obvious: in the case of *Aspergillus*, growth of the mycelium on the cement had a greater corrosive effect than the acids alone while with *Mycelia* the reverse was observed when looking at changes in porosity and portlandite dissolution. This is perhaps due to the fact that *Aspergillus* grows more easily on the cement surface than *Mycelia.*

Modeling of acid production according to environmental parameters

The change in cement may be due mainly to the action of the organic acids released. For this reason the effect of environmental parameters on acid production was determined for both fungal strains at various pH and temperatures: pH 4.0, 5.6, 6.2, 7.0, 8.5; temperatures 15, 20, 26, 30, 37 °C (cultivation in liquid media).

Glucose consumption (Glc), consumption of dissolved oxygen (O_2), pH and temperature were measured and the results analyzed by multilinear regression. The following relationships were found for *A. niger* (fig. 5):

$$(\text{Acids}) = 32.635 + 1.058\ T - 1.425\ Glc - 0.809\ pH \times T - 0.296\ pH \times Glc - 0.103\ T \times Glc - 0.003\ t \times Glc + 0.01\ t \times O_2 - 0.036\ Glc \times O_2 + 2.253\ pH^2 - 0.023\ T^2 + 0.026\ Glc^2$$

for *M. sterila* (fig. 6):

$$(\text{Acids}) = -61.606 - 0.618\ pH + 0.564\ Glc + 0.681\ t + 0.026\ pH \times O_2 + 0.010\ Glc \times T - 0.031\ Gl \times cO_2 - 0.001\ Glc \times t + 0.012\ Glc^2$$

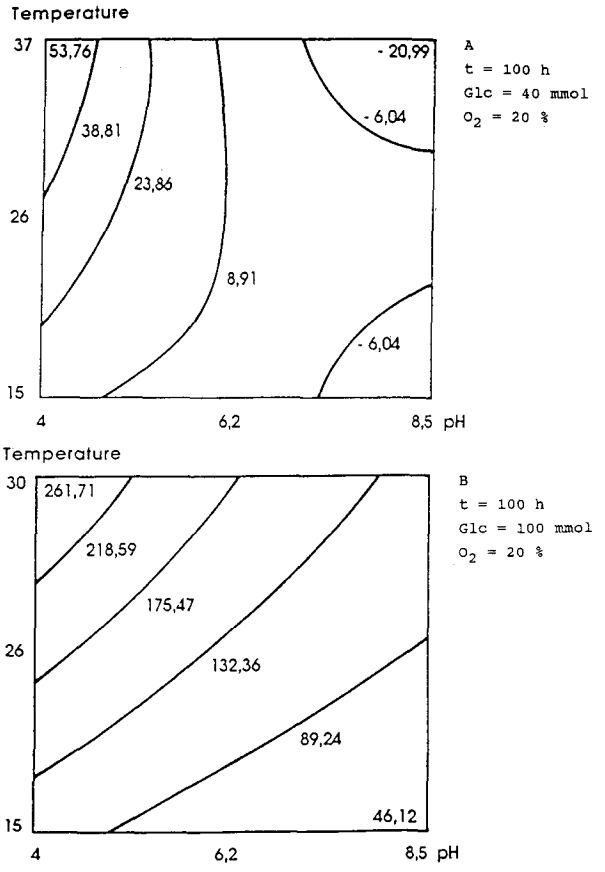

Figure 5. Calculation and graphical representation of the production (mmol) of organic acids as a function of glucose concentration, temperature and pH by *A. niger*. Curves indicate isoproduction at the level given in mmoles/h. Calculated negative values mean zero production.

These relationships indicate for *A. niger* that glucose concentration, pH and temperature have a direct effect on acid production. Maximal production occurred at low pH and high temperature as indicated by the levels written on the graph. For *M. sterila* acid production is dependent on the temperature and glucose concentration, while a change in the pH has only a negligible effect.

Considering the waste repository conditions, it is known that irradiation increases temperature thus stimulating acid production. In contrast the immediate vicinity of cement is alkaline. Acids produced in the environment, where the pH is lower, can diffuse to the cement and have a corrosive effect. At present we do not know that

Table 4. Changes in cement by microbial cultures and organic acids respectively. Experimental time: 8 months. +: presence of mycelium; –: absence of mycelium; $Ca(OH)_2$ (%) = portlandite content w/w; Calcium release = % of the initial Ca content; ND = not determined.

	Control	*Mycelia sterila*		*Aspergillus niger*	
		+	–	+	–
Total porosity (%)	19.87	26.49	30.73	30.14	24.70
$Ca(OH)_2$ (%)	15.00	5.80	7.75	6.39	2.31
Calcium release (%)	–	3.55	3.28	1.02	1.61
Bending strength (N)	460	ND	ND	100	175

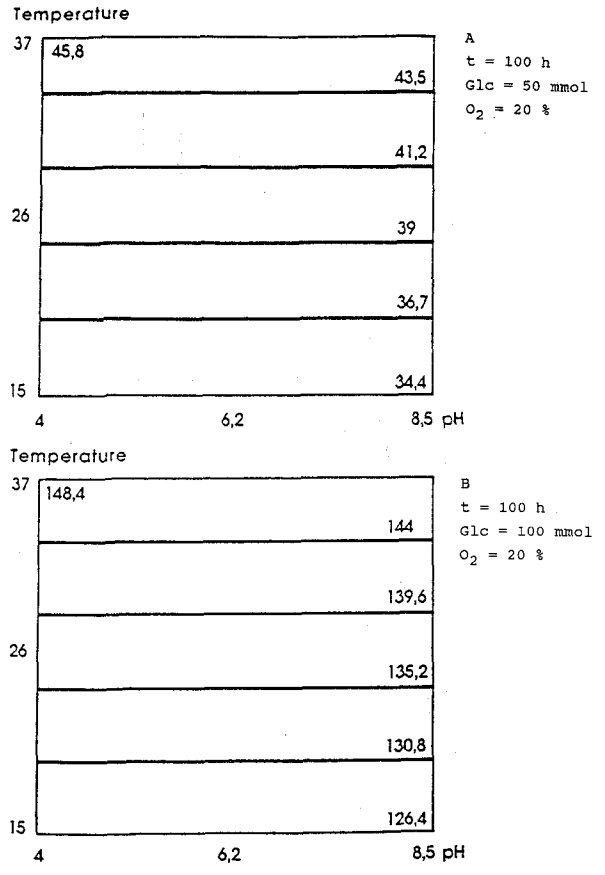

Figure 6. Calculation and graphical representation of the production (mmol) of organic acids as a function of glucose concentration, temperature and pH by *M. sterila*. Curves indicate isoproduction at the level given in mmoles/h.

changes in acid production occur beyond the experimental domain tested, and it would not be surprising to find alkaline conditions combined with high acid production at a suitable temperature.

Conclusions

In soil, groundwater can transport organic or inorganic matter and microorganisms far from their initial site[5]. Organic acids produced by heterotrophic microorganisms are known to be able to generate physico-chemical modifications in the cement[1-3]. In the laboratory, an experimental setup was used to simulate the running of groundwater on cement samples. We established that organic acids can solubilize mineral elements like Ca, and perhaps therefore others such as Si, Al and Fe; however, the nature of the acid is more important than its concentration[1,2,12].

The two strains tested produced both gluconic acid and a second acid, oxalic acid for *A. niger*, and malic acid for *M. sterila*. The combination of gluconic and oxalic acid causes the solubilization of portlandite $Ca(OH)_2$ and probably a C–S–H decalcification but with low calcium leaching, since the calcium oxalate solubility product is lower than the calcium hydroxide one. The calcium hydroxide dissolution results in the formation of new pores with a diameter of 0.06–0.9 µm. The loss of portlandite is apparently responsible for the decrease in bending strength of the material. This can also be attributed to the C–S–H decalcification. The growth of mycelium of *Aspergillus* on the cement seems to favor corrosion, perhaps because the production of acids occurs within the pores.

The mixture of gluconic and malic acid as produced by *M. sterila* effects an important portlandite dissolution, in addition to the calcium leaching. An increase in the porosity and a loss of the bending strength were recorded. Growth of this strain on concrete does not stimulate the corrosion process.

The modeling of acid production according to culture parameters shows that maximal production is obtained at acidic pH and high temperature. A transport of acids towards the cement is a fact which must be taken into consideration in the prediction of changes of the surrounding environmental conditions of the waste-packing matrix.

In this study, the effect of acids produced through aerobic microbial metabolism on the stability of cement has been determined. It has to be kept in mind that also in anaerobic conditions, bacteria as well as fungi may produce other kinds of acids than those tested here[5].

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Microbial degradation of bitumen

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Summary. A blown bitumen Mexphalte R 90/40 with a high content of saturated hydrocarbons was degraded by several microorganisms to the same extent. In batch cultures of *Saccharomycopsis lipolytica*, maximal biodegradation was estimated to be about 9% w/w, $3.2 \cdot 10^{-3}$ g/cm^2 and $3.1 \cdot 10^{-3}$ cm of degraded bitumen. The Mexphalte R 90/40 degradation rate was closely coupled to biofilm formation. The microbial activity concerned predominantly the oxidation of saturated hydrocarbons. A direct distillation bitumen 80/100 with a low content of saturated hydrocarbons and a high content of aromatic hydrocarbons and resins was more resistant to biodegradation.
Key words. Biodegradation; bitumen; radioactive waste.

Introduction

Bitumen is a residue of successive crude oil treatments. It is a mixture of high molecular weight hydrocarbons with such a complex chemical composition that it is still undefined. Because of their theoretical long term stability, bitumen materials are used in many countries[1–3] to immobilize low and intermediate level wastes. The bitumenized wastes are then disposed of in deep geological repositories.

Although the composition of bitumen is relatively complex and undefined, its organic content makes it susceptible to biodegradation. It is known that many microorganisms have the ability to degrade petroleum hydrocarbons[2, 7, 11, 16] although few studies have included the microbial degradation of bitumen, even when it has been shown that bituminous materials are subject to microbial attack and deterioration[3–5, 9, 10]. The presence in a repository of a large microflora which can undertake such geochemical processes[8] could affect the long-term stability of bitumen and the integrity of bitumenized wastes.

Rates of bitumen biodegradation depend on the chemical composition of the bitumen substrate, the metabolic specificity of the microorganisms involved and a large number of biological and physico-chemical parameters liable to come into play in a disposal site. Among the methods used to assess biodegradation rates, the measurement of CO_2 production is one of the most commonly used[3, 13].

Since the rates of oxidative degradation processes of hydrocarbons are greater than the ones of anaerobic decomposition[2], our experiments were therefore carried out under aerobic conditions.

In order to evaluate the microbial degradation of bitumen, investigations were made a) to quantify the rate of biodegradation from the CO_2 production, b) to show the possible alterations of bituminous material through chemical transformations such as the oxidation processes, c) to compare the activities of microorganisms from the point of view of metabolic specificity, and d) to determine whether the chemical nature of bitumen can affect