

ASSESSMENT OF THE BIODEGRADABILITY OF CONTAINERS FOR LOW AND INTERMEDIATE LEVEL NUCLEAR WASTE

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Abstract

Concrete and reinforced concrete are widely used as engineered barriers (containers) for radioactive waste disposal facilities due to their isolating ability, mechanical stability and low cost. Several types of protective reinforced concrete containers for low and intermediate level waste have been designed in Ukraine. Evaluation of these containers for microbial stability is required according to NRC of Ukraine Regulation No.306.608-96. The research was therefore aimed at studying the degradation of the cement material due to microbiological interaction and the possibility of biodegraded cement as an ideal environment for the growth of other microorganisms under waste disposal conditions to satisfy the regulatory requirements. Results from this study indicated that *Aspergillus niger* induced gluconic and oxalic acids that dissolve portlandite (with a low leaching of calcium) after one year of contact time. This resulted in an increase in porosity, loss in tensile strength biomechanically deteriorated and cracking. XRD analysis identified crystalline precipitates within the biomass on the concrete surface as calcium oxalate dehydrate (weddelite) and calcium oxalate monohydrate (whewellite). The mechanism regarding of the microbiological interaction on the concrete surface can be summarized as follows: Phase 1: Fungi accumulate on the surface of the concrete, thereby degrading the concrete surface by biochemical and biomechanical interactions. When this effect is in the presence of air with available carbon dioxide, the micro fungi reduces the pH of the concrete from >13 to 8.5. During this phase no accumulation were observed in sections where granite aggregates are present. Phase 2: After reducing the pH of the concrete paste during phase 1, and provided that sufficient nutrients, moisture and oxygen are present sulphur oxidizing bacteria start to accumulate on the concrete surface. The result form this study therefore concluded that fungal biogeochemical activity over a long-term period might have negative environmental consequences for the concretes waste containers as fungi (under certain environmental conditions) are able to dissolved the cement matrix resulting in the formation of a biofilm with accumulated structural elements. However, as microbial effects under repository conditions are unknown and difficult to study in a laboratory, no safety assessments conclusions can be made based on results from his study. Long term studies under repository conditions are needed

1. INTRODUCTION

Studying long term behaviour of materials in a nuclear waste disposal facility could include the possible biodegradability of concrete by microbial activity present in the waste site. Degradation of cement surfaces by microbial activity is possible due to the interaction of chemical produced acids (organic and inorganic). Rate of cement degradation will be influenced by the specific acid formation properties of microorganisms, the chemical and mineral composition of concrete components as well as and capillary porous structure of concrete. The latter is extremely important as microorganisms can penetrate into concrete structures through these pores and capillaries [1-5]. In 1997-1998, extensive fungal growth was observed on the walls and building structures of the "Shelter" built over the fourth Unit of the Chernobyl nuclear power plant [6, 7]. Literature indicated that Oxalate-excreting *Aspergillus niger* can form abundant calcium oxalate crystals on concrete surfaces resulting in fungal hyphae [8]. This fungi growth is influenced by the-chemical environment at the interface, e.g., by the concentration of oxygen, salts, pH value, redox potential and conductivity at the concrete interface [9]. The main mechanism regarding the destruction of reinforced concrete containers by bacteria and fungi

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are that these heterotrophic micro-organisms are able to produce organic acids during the assimilation of organic carbon compounds. Organic acids such as lactic, citric, acetic, gluconic, malic and others, which are by-products of metabolism of these micro-organisms, will dissolve concrete matrix.

Sulfur-Oxidizing bacteria (genus *Thiobacillus*) are the microorganisms found on concrete surfaces after the biological degradation of concrete structures. Literature data suggest that, regardless of the initial populations of the various species of thiobacilli in soils, once the process of sulfur oxidation begins, *T. thiooxidans* or *T. ferrooxidans* will eventually become the dominant organisms [10].

The research was aimed at studying the degradation of the cement material due to microbiological interaction and the possibility of biodegraded cement as an ideal environment for the growth of other microorganisms under waste disposal conditions.

2. EXPERIMENTAL

Fungal cultures to study the degradation of concrete in this study were strains of genus *Aspergillus* - *Aspergillus niger* and of genus *Cladosporium* - *Cladosporium cladosporioides* that were isolated from radionuclide-polluted soils in Ukraine. Additionally the Institute of Microbiology and Virology of NAS of Ukraine provided cultures of *Aspergillus niger* van Tieghem strain 42, *Cladosporium cladosporioides* Fresenius de Vries strain 4 and *Thiobacillus* sp. *Thiobacillus* sp strain will be used for studying possible biogenic sulfides distribution and biofilm formation during interaction of sulfate-reducing bacteria (SRB) with mild steel surface.

2.1. Experimental conditions used for cement degradation studies

The agar tablets Czapek-Dox that was used as culture medium for cement degradation experiments had the following composition: NaNO_3 – 3g; KH_2PO_4 – 1g; MgSO_4 ; KCl – 0,5g; FeSO_4 – trace amounts; sucrose – 30g and agar – 20 gram per litre H_2O . This culture medium was poured into sterile Petri dishes (with diameter of 9.5 cm) and cooled down to form a solid surface. After solidification a middle section was removed by a micro-spatula for the placement of concrete samples.

Pieces of concrete from a manufactured intermediate level disposal container (KTZ-3.0) were removed. These concrete samples were repeatedly (three times) sterilized in an autoclave at 120°C for 60 min and then placed in an oven at 100°C for 24 hours. The dried concrete sections were then inserted into the opening in the Czapek-Dox agar in the Petri dish. After the placement there must be a 2 mm gap between the concrete and the agar.

Micromycetes were inoculated 1-2 mm from the cement sample into the sample into the agarised medium. Then the Petri dishes were sealed by “Para film” to prevent drying of the medium. The fungi were then cultured between 25-28°C in order to preserve the vital functions of mycelium. Inoculations were done by removing the grown fungi daily for up to 14 days and visual and microscopic tests were performed at regular intervals

After 14 days the concrete surface was examined using a Polmi-A microscope (Carl Zeiss, Germany). After the microscopic analysis the deterioration of concrete samples by the formation of the fungi colony was studied using a scanning electron microscopy (Philips XL30). Mineralogical and elemental analyses of degraded concrete transformed surfaces were studied using X-ray powder diffraction (DRON-4, SU) and energy dispersive X-ray analysis (EDXA) coupled on a SEM.

2.2. Experimental conditions used for corrosion studies

The Starkey liquid that was used as culture medium for metal corrosion studies induced by *Thiobacillus* sp. consisted of the following compounds that were dissolved in 1 L. of distilled water or dissolved in 1% - 3% (w/v) aqueous NaCl: Dipotassium Hydrogen Phosphate-0,5 g, Ammonium

Chloride-1,0 g, Sodium Sulphate-1,0 g, Calcium chloride dihydrate-0,1 g, Magnesium sulphate heptahydrate-2,0 g and Sodium Lactate 70%-5,0 g

Pieces of concrete from a manufactured intermediate level disposal container (KTZ-3.0) were removed. These concrete samples were repeatedly (three times) sterilized in an autoclave at 120°C for 60 min and then placed in an oven at 100°C for 24 hours. The dried concrete sections were inserted into the opening in the Czapek-Dox agar in the Petri dish. After the placement there must be a 2 mm gap between the concrete and the agar.

The influence of *Thiobacillus* sp. and *Desulfovibrio* sp. on metal corrosion was investigated using concrete samples with reinforcement. The biomineralization and corrosion rate of the mild steel under the influence of *Desulfovibrio* sp. Kyiv-10 strain was studied in a dynamic experiment for 70 days. The Petri dishes were not sealed by “Para film” therefore creating a highly aerobic environment that will accelerate the growth of corrosion-inducing anaerobic microorganisms. Samples were taken after 10, 40, 50, 60 and 70 days and standard methods were used to isolate the microbes. Possible corrosion by sulphuric acid was determined on both the cement and metal in order to determine the differences between biogenic sulphuric acid corrosion and chemical sulphuric acid corrosion. The metal surface was examined using a Polmi-A microscope (Carl Zeiss, Germany) and scanning electron microscopy (Philips XL30). Mineralogical and elemental analyses were studied by energy dispersive X-ray analysis (EDXA) coupled on a SEM. The Biofilm sampled from microcosms after of growth were analyzed for metal content using an atomic absorption spectrophotometer AAS-8500 F (Japan).

3. RESULTS

3.1. Cement degradation studies

The results regarding the growth of the cultures in the agarised medium and onto the concrete surfaces (Fig. 1) after one month are presented in Table 1. Growth was estimated and presented as a 5-point scale. Results indicated that the *Cladosporium cladosporioides* has minimum influence but this is misleading as the growth rate in liquid culture medium for *Cladosporium cladosporioides* is much slower as that of *Aspergillus niger*.

TABLE 1. FUNGI GROWTH ACTIVITY IN THE MODEL SYSTEM

№	Cultures of mycomicetes	Growth		Morphologic characteristics of concrete
		Agarised medium	Concrete	
1.	<i>Aspergillus flavipes</i> – 38	***	*****	Heavy sporification, drops of yellow exudation
2.	<i>Aspergillus niger</i> – 42	*****	*****	Mycelium with heavy sporification, small drops of yellow exudation
3.	<i>Aspergillus versicolor</i> – 26	**	****	Thick mycelium, change of mycelium colour from rosy-cream to yellow-green, creation of exudation
4.	<i>Cladosporium cladosporioides</i> – 3	**	***	Heavy sporification
5.	<i>Cladosporium cladosporioides</i> – 4	**	***	Heavy sporification
6.	<i>Cladosporium cladosporioides</i> – 340	**	***	Heavy sporification

Where: ***** - best; ***** - very good; *** - good; ** - fair.

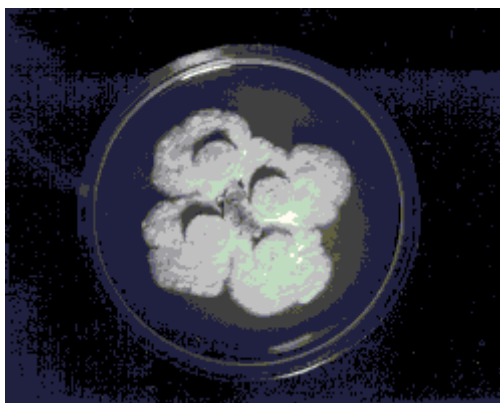


FIG. 1. Growth of *Aspergillus flavipes* – 38 on the surface of concrete samples.

The data presented in Table 1 is based on visual observations and therefore a long-term experiment is needed on to study the interaction on concrete surfaces in order to reveal what species the most active bio destructors of concrete is.

3.2. Chemical transformation of concrete surface

Formation of capillaries within the concrete during the hydration process and the capillary action of water provide a means for penetration of microorganisms into concrete. Petrographic results of the concrete surface after 1 year of exposure to fungi indicate deterioration symptoms such as expansion, discolouration and the formation of cracks.

Analysis of the fungi present on the dissolved concrete surface indicated the accumulation of elements within the fungal biofilm. .

The fungi also leached the chemical elements of the concrete surface into nutrition medium, thereby forming calcium oxalates crystals on the surface of concrete that are in contact with the growing medium. An interesting observation is that the fungi did not colonize around the granite aggregates in the concrete.

The experiment was repeated to study the leaching of chemical elements from concrete by genera *Aspergillus* and *Cladosporium* under submerged conditions. Results indicated that the leaching of chemical elements from concrete surfaces under submerged conditions depends on the growth and metabolites excretion of these fungi species as well as the concrete volume used for the experiments [13].

3.3. Transformation of concrete structure

Transformation of a concrete structure will be influenced by the penetration of the microorganisms into the capillary structure of concrete. The change in total pore volume measured by mercury intrusion indicated in Table 2 that an increase in the number of pores of size $1,0 > d > 0,5 \text{ um}$ was observed. This corresponds with the dissolution of portlandite crystals by the metabolic products generated by the fungi render it soluble for leaching from the concrete structure.

TABLE 2. CHANGE POROSITY OF THE SAMPLES AFTER CONTACT WITH THE FUNGAL CULTURE

Pore diameter	Pore volume (%)		
	<i>Cladosporium cladosporioides</i>	<i>Aspergillus niger</i> -42	Control
$d > 1,0 \text{ um}$	3,12	2,86	1,23
1. $1,0 > d > 0,5 \text{ um}$	10,05	12,45	2,18
2. $0,5 > d > 0,01 \text{ um}$	12,14	11,76	11,00
$d < 0,01 \text{ um}$	6,37	7,08	5,97
Total porosity	31,68	34,15	20,38
Increase of the total porosity, %	11,3	13,77	

The strain of *Cladosporium cladosporioides* that produces gluconic and malic acids is responsible for the dissolution of portlandite thereby increasing the porosity by 11,3%. EDXA spectra of Au/Pd coated concrete samples indicated that crystals associated with hyphae contained calcium. Among the fungal cultures tested, *Aspergillus niger* demonstrated the highest capacity for concrete deterioration as the oxalate-excreting *Aspergillus niger* formed abundant calcium oxalate crystals [calcium oxalate monohydrate ($\text{CaC}_2\text{O}_4 \times \text{H}_2\text{O}$) - whewellite and calcium oxalate dihydrate $\text{CaC}_2\text{O}_4 \times 2\text{H}_2\text{O}$ - weddellite) on the concrete surfaces.

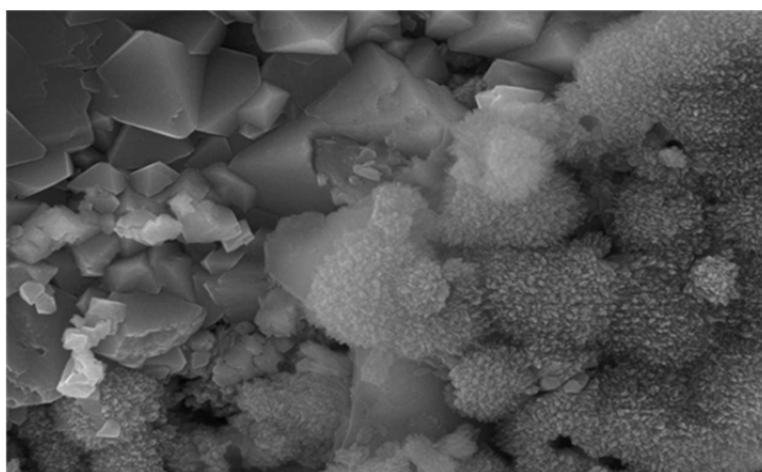


FIG. 2. SEM images of air-dried Au/Pd-coated samples showing deterioration and transformation of concrete.

The fungal-derived calcium oxalate found on the concrete surface has a variety of crystalline forms (tetragonal, bipyramidal, plate-like, rhombohedra or needles). XRD analysis also confirmed the crystalline precipitates within the biomass of the fungi and on the concrete surface as calcium oxalate dehydrate (weddelite) and calcium oxalate monohydrate (whewellite).

Infrared spectroscopy of the *A. niger* biofilm removed from the concrete surface indicate the presence absorption frequency bands (1320, 1630, 1370, 3260, 3080, 3480 cm^{-1}) that are typical for carboxylate groups (C-C, C-H, C-O, C=O, O-H).

The influence of simulated metabolism products of micro-organisms, citric and oxalic acids, on the surface layer of concrete, was also investigated. This was done by putting the concrete samples into respective acid solution (0.0125 N) at room temperature between 8 and 200 hours (depending on the intensity of corrosion effect of various acids). After the testing period the concrete samples were removed and rinsed with distilled water for 24 hours.

Results indicated that within 8 hours in the oxalic acid medium, the concrete surface changed due to the dissolution of the hydrate component of cement paste. This resulted in the decrease of aluminoferrite resulting in a colour change (from brown-red to black) in the clinker zone. The silicate crystals remained unaffected. In the interface between the concrete paste and coarse-grain filler, cracks occurred due to the re-crystallisation. The width of the re-crystallisation zone was between 0.03-0.1 mm.

Results indicated that after 16 hours in oxalic acid the acid had a significant effect on the destruction of the concrete surface. Huge cracks around grains of fine filler material appeared during 24-hour rinsing of the sample in water, small (0.01-0.02 mm) colourless isometric crystals of calcium citrate formed at the surface of the sample. After 48 hours the surface layer of concrete was totally destroyed. The mechanism involved was due to the dissolution of crystallohydrate component in the concrete paste by oxalic acid thereby releasing of grains of filler and clinker into the acid solution.

The results of the influence of 0.025 N solution of citric acid on the surface of concrete indicated that after 20 hours no significant changes were noted in the crystallohydrate mass of the concrete. However, partial decolouration of the allumoferrous clinker grains and the formation of calcium silicate crystals were observed. White unknown sugar-like crystals (0.01-0.02 mm) formed around the edges of the grains of coarse filler material.

Results indicated that after 200 hours in citric solution the crystallohydrate mass remained unaffected. However, complete decolouration of allumoferrous and additional increase in the formation of calcium silicate grains was observed in clinker grains.

3.4. Results of surface degrading using roughness indicator.

Change of properties on the surface of concrete samples is as a result of chemical corrosion.

This degrading rate of surface was measured as mass of material (g) transformed into corrosion products in a unit of time (1 hour) per surface volume (1 m^3). The amount of corrosion products that formed was estimated by measuring the change in surface as a roughness indicator using an instrument measuring a surface relief with the error of 5 μm . This was used as an indication of a relative increasing/decreasing of the surface relief.

Results indicated that irregular (local) corrosion occurred on the concrete surfaces when in contact with citric acid. In certain sections on the concrete surface differences of up to 70 μm and more were measured. The average value of the surface differences was $27.4 \mu\text{m} \pm 7.1 \mu\text{m}$ with a confidence probability of $P=0.95$. With this result it was possible to calculate the length of profile line on the concrete surface and the estimate error during calculations was:

$$l = 6.831^{+0.211}_{-0.206} \text{ mm}$$

Based on the estimate degradation length of 6.0 mm, an elongation value of 11% was calculated. When converting this value into surface area, more than 30% of the concrete surface was corroded away.

The results in this section indicated that quantitative information on corrosion processes on the concrete surface can be made and that the corrosion rate can be determined, especially in cases when concrete samples are porous. Results also indicated that quantitative analysis of the formed crystalline corrosion products are possible, however it was difficult to quantify x-ray-amorphous products even with the use of expensive investigative techniques and the results were not unambiguous. .

3.5. Metal corrosion associated with *Thiobacillus* sp

The research aim was to determine the biogenic sulfides distribution and qualitative composition in the biofilm formed by sulfate-reducing bacteria (SRB) on the mild steel surface..

The interaction of *Thiobacillus* sp. and *Desulfovibrio* sp. with metal were studied in dynamic experiments for 70 days. Qualitative results indicated in Figures 3 and 4 that due to corrosion a biofilm formed by measuring the corrosion potentials of the metallic structure that corroded in the concrete. The observed corrosion process can be described as a result of two electrochemical reactions occurring simultaneously on the metal surface: the metal oxidation (anodic process), which causes an accumulation of electrons, and the reduction (cathodic process) of some species present in the solution, which remove electrons from the metal

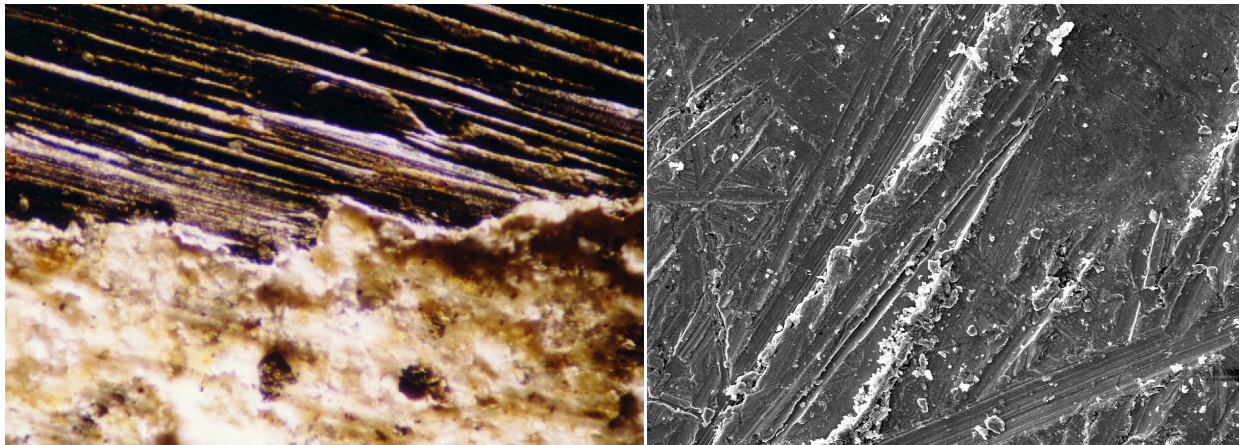


FIG. 3. Corrosion of reinforced concrete.

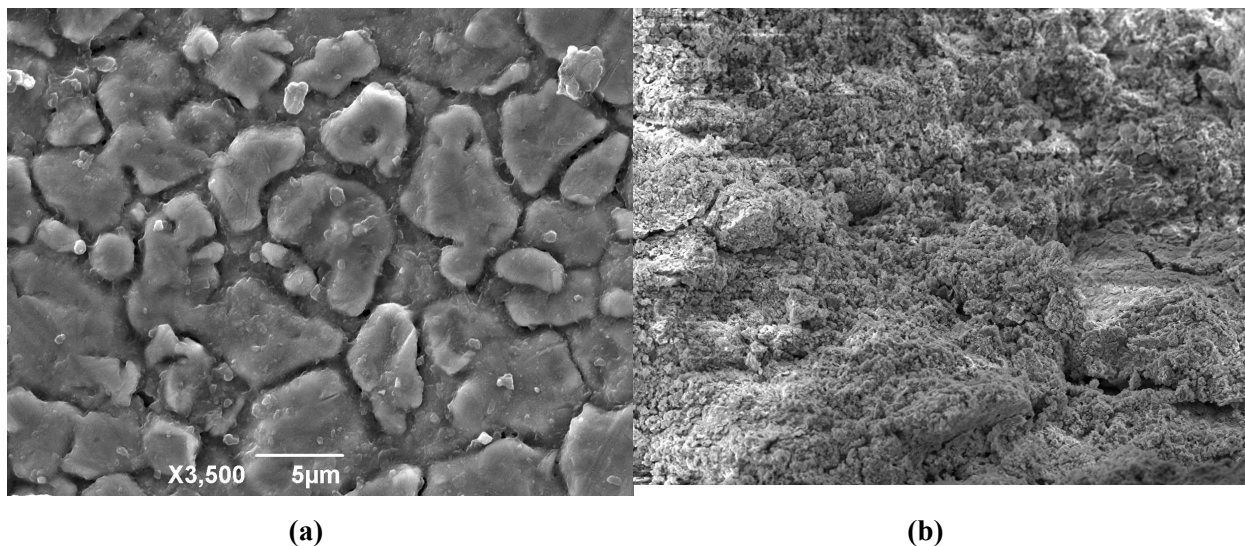


FIG. 4. Corrosion products (a) and biofilm (b) formed on mild steel.

The rate of corrosion of steel reinforcement was estimated on the basis of experimental data. The layer above steel reinforcement was analyzed by electromagnetic technology and the carbonated layer depth was estimated by measurements pH changes using phenolphthalein indicator.

Based on these results, it was estimated that the total lifetime of the *containers* (including the carbonation interval and a decrease of steel reinforcement's area to 50%) will be around 460 years.

4. CONCLUSION

The mechanism regarding of the microbiological interaction on the concrete surface can be summarized as follows:

Phase 1: Fungi accumulate on the surface of the concrete, thereby degrading the concrete surface by biochemical and biomechanical interactions. When this effect is in the presence of air with available carbon dioxide, the micro fungi reduces the pH of the concrete from >13 to 8.5. During this phase no accumulation were observed in sections where granite aggregates are present.

Phase 2: After reducing the pH of the concrete paste during phase 1, and provided that sufficient nutrients, moisture and oxygen are present sulphur oxidizing bacteria start to accumulate on the concrete surface. .

The result from this study therefore concluded that fungal biogeochemical activity over a long-term period might have negative environmental consequences for the concretes waste containers as fungi (under certain environmental conditions) are able to dissolved the cement matrix resulting in the formation of a biofilm with accumulated structural elements..

However, as microbial effects under repository conditions are unknown and difficult to study in a laboratory, no safety assessments conclusions can be made based on results from his study. Long term studies under repository conditions are needed.

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